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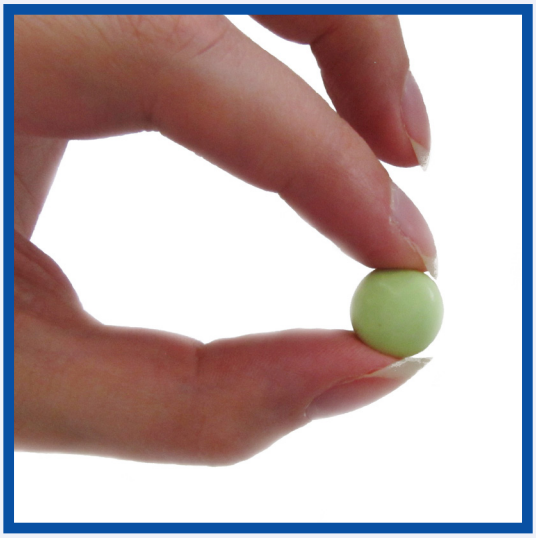
The Scientific Foundation for Herbal Medicinal Products

Agrimoniae herba

Agrimony

2019



E/S/C/O/P

European Scientific Cooperative On Phytotherapy

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E/S/C/O/P
MONOGRAPHS

***The* Scientific Foundation for**
Herbal Medicinal Products

AGRIMONIAE HERBA
Agrimony

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European Scientific Cooperative
On Phytotherapy

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Plant illustrated on the cover: *Agrimonia eupatoria*

FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

AGRIMONIAE HERBA

2019

Agrimony

DEFINITION

Agrimony consists of the dried flowering tops of *Agrimonia eupatoria* L. It contains not less than 2.0 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; Mr 126.1) and calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Agrimony].

CONSTITUENTS

The main characteristic constituents are tannins (3-11%), consisting mainly of proanthocyanidins (condensed tannins) with a small proportion of ellagitannins, of which agrimoniin is the main constituent (0.3-0.5%) [Von Gizycki 1950; Drozd 1983; Granica 2013].

Flavonoids (about 0.9%) [Carnat 1991; Granica 2013] including glycosides of quercetin, the 7-glucosides of luteolin and apigenin [Von Gizycki 1950; Ivanov 1970; Sendra 1972; Gorunovic 1989; Carnat 1991] and 3-glycosides of kaempferol and kaempferide [Bilia 1993a,1993b], as well as triterpenoids such as ursolic acid [Le Men 1995], euscapic acid and the 28-glucosyl esters of euscapic acid and tormentic acid [Bilia 1993b].

Other constituents include phenolic acids [Bucková 1972; Granica 2013], β-sitosterol [Bilia 1993a], polysaccharides (19.5%) [Drozd 1983; Krzaczek 1985], and minerals (7.3-7.9%) [Von Gizycki 1950; Carnat 1991; Szentmihályi 1998].

CLINICAL PARTICULARS

Therapeutic indications

Internal use
Agrimony has been widely documented as a remedy to treat mild diarrhoea [Agrimonia; Blaschek 1993; Dorsch 2002].

External use
Locally as a gargle for inflammation of the oral and pharyngeal mucosa [Gray 1998; Dorsch 2002] and as a compress or rinse to support the healing of wounds [Gorunovic 1989; Blaschek 1993].

Efficacy for these indications is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Internal use

Adults: 3-12 g of dried herb daily, or as an infusion or equivalent preparation [Blaschek 1993; Dorsch 2002]; three times daily, 1-3 mL of a liquid extract (1:1 in 25% ethanol) or 5-10 mL of a tincture (1:5 in 45% ethanol) [Gray 1998].

Children, average daily dose: *1-4 years of age*, 1-2 g; *4-10 years of age*, 2-3 g; *10-16 years of age*, 3-6 g [Agrimonia].

External use
A 10% decoction used several times daily, locally in gargles or externally as a compress or rinse [Dorsch 2002].

Method of administration
For oral administration or external use.

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Duration of administration
No restriction.
If symptoms persist or worsen, medical advice should be sought.

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No human data available. In accordance with general medical practice, the product should not be used during pregnancy or lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None known.

Overdose
None known.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments
A hydroethanolic extract (2.5 g drug/100 mL, ethanol 50% V/V) showed strong elastase-inhibiting activity [Carnat 1991].

A dried hydromethanolic extract (50% V/V) showed no spasmolytic activity on spontaneous or induced contractions of isolated guinea-pig ileum at concentrations up to 800 µg/mL [Izzo 1996].

A decoction (1 mg/ml) stimulated 2-deoxyglucose transport (1.4-fold), glucose oxidation (1.4-fold), glycogenesis (2.0-fold) and lactate release (1.4 fold) in isolated mouse abdominal muscle, comparable to the effects evoked by 0.01 µM insulin. The activity of agrimony on insulin secretion was also evaluated. A decoction (0.25-10 mg/ml) evoked a stepwise 1.9- to 3.8-fold stimulation of insulin secretion from the BRIN-BD11 rat pancreatic B-cell line with no detrimental effect on cell viability. These experiments demonstrated that agrimony has antihyperglycaemic, insulin-like and insulin-releasing activity [Gray 1998].

An infusion (1 g agrimony/200 mL water) demonstrated considerable antioxidant activity. Relative to the reactivity of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as a standard, the Trolox equivalent antioxidant capacity (TEAC) was found to be 3.76 mM [Ivanova 2005].

Aqueous extraction of agrimony aerial parts was performed at four different temperatures in order to determine the optimal extraction temperature for inhibiting the release of hepatitis B surface antigen (HBsAg) from hepatitis B virus-infected HepG2.2.15 cells. Secretion was inhibited dose-dependently and the EC_{50} values for each aqueous extract at 37 °C, 45 °C, 55 °C and 60 °C were 168 µg/mL (extrapolated value), 127.91 ± 4.21 µg/mL, 115.24 ± 4.75 µg/mL and 74.29 ± 41.97 µg/mL respectively. Cell growth was not influenced by treatment with the aqueous extracts at concentrations of 44–132 µg/mL. The inhibitory activity of the extracts on HBsAg secretion varied over the growing season and was the highest at mid-July [Kwon 2005].

An aqueous extract significantly ($p<0.001$) suppressed lipopolysaccharide-induced nitric oxide production in BV2 microglial cells at concentrations of 0.01, 0.1 and 1 mg/ml compared to control. The extract at the same concentrations also significantly ($p<0.05$ to 0.001) suppressed lipopolysaccharide-induced production of the proinflammatory cytokines tumor necrosis factor, interleukin 1 beta and interleukin 6, and inhibited the expression of inducible nitric oxide synthase, in a dose-dependent manner [Bae 2010].

A methanolic extract (80%) and *n*-hexane, BuOH, EtOAc and H_20 fractions significantly attenuated glutamate-induced oxidative stress in HT22 hippocampal cells. Isolated flavonoids also showed a neuroprotective effect on glutamate-induced toxicity in HT22 cells [Lee 2010].

An ethanolic extract (45%; DER 1:2) demonstrated high growth inhibition against *Helicobacter pylori* and *Campylobacter jejuni*. At a dilution of 1:25 the % of inhibition ± SD against *H. pylori* was 97 ± 5 % and against *C. jejuni* was 96 ± 30%. At a dilution of 1:100, results were 5 ± 11 % and 30 ± 37% respectively [Cwikla 2010].

No significant antiadhesion activity (IC_{50} values >35 mg/mL) against *Campylobacter jejuni* was found for a hydroethanolic extract [Bensch 2011].

A lyophilized infusion prepared according to the Pharmacopoeia Bohemoslovaca 4 did not demonstrate cytotoxic activity at the tested concentrations (0.1 mg/mL and 0.05 mg/mL) on a THP-1 cell line after 24 h incubation. The infusion did not alter CAT activity but did decrease the activity of SOD. Good antioxidant activity was shown in the ABTS assay (RC_{50}: 0.79 mg/mL). Further antioxidant properties were shown in a test assessing plasmid DNA protection and the infusion had a lower damage index than the standard rutin [Kuczmannová 2015].

Various extracts were obtained by macerating 30 g of agrimony in 150 mL of a range of solvents, namely ethanol, diethyl ether, water and acetone. The following dried extracts were obtained: 5.42 g of water extract, 0.55 g of diethyl ether extract, 0.76 g of acetone extract and 2.4 g of ethanol extract. The acetone extract (at 250 µg/mL) exhibited the greatest DPPH radical scavenging activity (97.13%), while the water and ethanol extracts also exhibited high antioxidant activity. The strongest antimicrobial activity was detected against gram positive bacteria, with the acetone extract demonstrating the highest activity. BIC_{50} (inhibitory concentration to reduce biofilm coverage by 50%) values for the acetone extract were 4315 µg/mL for *P. mirabilis* and 4469.5 µg/mL for *P. aeruginosa* [Muruzović 2016].

An infusion (20 g agrimony/600mL water, for 15 min) and its ethyl acetate fraction showed considerable antioxidant activity when calculated relative to the reactivity of DPPH as standard, with respective EC_{50} values of 12.80 µg/mL and 4.60 µg/mL. The EC_{50} values for superoxide anion scavenging activity were 3.34 µg/mL for the infusion and 13.59 µg/mL for the fraction. Respective EC_{50} values for hydroxyl radical-scavenging activity were 126.99 µg/mL and 90.97 µg/mL. In experiments assessing cytotoxicity and effects on NO production, RAW 264.7 macrophages were incubated for an hour with various concentrations of the infusion and fraction and then with LPS for 24 hours. Cell viability of the macrophages (measured as % of LPS) was only significantly ($p<0.001$) reduced by the highest concentrations tested of the infusion (770 µg/mL) and fraction (276 µg/mL). LPS-induced increases in nitrite production (% of LPS) were only

significantly ($p<0.001$) inhibited, without detrimental effects to cells, by the infusion at 382 µg/mL and the fraction at 138 µg/mL. In a further experiment assessing NO production, cells were cultured with S-nitroso-N-acetylpenicillamine (SNAP). The infusion significantly ($p<0.05$) inhibited nitrite production (% of SNAP) by 36.67% at 382 µg/mL and the fraction by 29.46% at 138 µg/mL [Santos 2017].

In vivo experiments

After oral administration of an infusion or decoction to rats at a dose equivalent to 3 g dried herb per kg b.w., the volume of urine excreted over the following 6 hours was significantly ($p<0.01$) less than that from rats treated with distilled water, while the amount of uric acid excreted in the urine during the first 4 hours was significantly ($p<0.01$) greater than (more than two-fold) that of rats treated with distilled water, suggesting a uricosuric effect. The amount of urea excreted remained largely unchanged [Giachetti 1986]. Further experiments with rats showed that, while diuresis resulting from distilled water provoked loss of electrolytes, agrimony compensated for electrolyte loss, particularly that of potassium [Giachetti 1989].

Induction of diabetes in mice by treatment with streptozotocin (STZ) led, after 20 days, to reduced body weight ($p<0.01$) and significant increases in fluid and food intake and in plasma glucose (all $p<0.001$ compared to untreated controls). Incorporation of agrimony into the diet (62.5 g/kg in food) and drinking water (2.5 g/litre of a diluted decoction), for 5 days before and subsequent to administration of STZ, counteracted the loss of body weight to some extent and significantly counteracted polydipsia ($p<0.001$), hyperphagia ($p<0.01$) and hyperglycaemia ($p<0.05$) compared to STZ-treated mice receiving a normal diet [Swanston-Flatt 1990; Gray 1998].

A lyophilized aqueous extract (yield 17.1%), containing 8.34 and 11.82 mg/g extract of luteolin 7-glucuronide and apigenin 7-glucuronide respectively, was tested for hepatoprotective activity against chronic ethanol-induced liver injury in rats. Animals were treated orally with the extract at 10, 30, 100 and 300 mg/kg/day. The ethanol-induced increase in ALT was attenuated by the 10 and 30 mg/kg doses, while the increase in AST was attenuated by the 10, 30 and 100 mg/kg doses. Micro- and macrovesicular steatosis and excessive inflammatory cell infiltration were attenuated by the 30 mg/kg dose. The 30 mg/kg dose also had the following effects: prevented the ethanol-induced increase in serum concentrations of TNF-α and IL-6, attenuated the induced increase in CYP2E1 activity, the increase in MDA level and the decrease of GSH content, as well as the increase in hepatic level of TLR4 protein expression. It also attenuated the nuclear levels of p65, a subunit of NF-κB, and prevented the ethanol-induced increases in MyD88, COX-2, and iNOS protein expression [Yoon 2012].

An infusion prepared according to the Pharmacopoeia Bohemoslovaca 4 was provided to male Sprague-Dawley rats (n = 20; 6 months old) for 5 weeks prior to experimental skin flap surgery. The mean vital area of the skin flap 7 days after surgery was 48.7% ± 9.4% in the control group (water only), whereas the agrimony treated rats had a significantly ($p<0.05$) increased flap viability at 58.1% ± 7.7% [Kuczmannová 2015].

The antinociceptive activity of a dried ethanolic extract (500 g aerial parts macerated in a volume of 80% ethanol equal to ten times that of the sample) was investigated in a rat model of cisplatin-induced neuropathy. The effect of the extract (200 mg/kg p.o.) was compared with that of the positive control gabapentin (100 mg/kg i.p.) in various tests. Paw withdrawal duration in response to pin prick was considerably lower in the agrimony group than the cisplatin only control group at week 1 and was sustained at week 4. Gabapentin also showed a lower withdrawal duration than the cisplatin-only control group, but this effect was not sustained at week 4. Similar results were noted in the test of paw-withdrawal threshold in response to mechanically-induced pressure [Lee 2016].

An infusion (20 g agrimony/ 600mL water, for 15 min) and its ethyl acetate fraction were tested for anti-inflammatory and analgesic activity in several assays. In the carrageenan-induced rat paw oedema assay, rats were divided into six groups (n = 6–8): negative control group received water (0.5 µL/g p.o.), positive control group received diclofenac sodium (10 mg/kg i.p.), and test groups received one of two oral doses of the infusion (99.59 mg/kg, D1; 199.18 mg/kg, D2) or the ethyl acetate fraction (18.12 mg/kg, D3; and 36.24 mg/kg, D4) reconstituted in water. The infusion reduced oedema by 43.2% (D1) and 52.2% (D2) and the ethyl acetate fraction reduced it by 34.6% (D3) and 35.4% (D4) compared to the negative control.

Analgesic activity of the preparations was tested using several assays in mice. Neither preparation demonstrated a significant increase in the reaction time of mice in the hot plate test. In contrast, the preparations did inhibit abdominal writhing induced by acetic acid. Mice were divided into six groups (n = 6–8): negative control group received water (0.5 µL/g p.o.), positive control group received diclofenac sodium (10 mg/kg i.p.), and test groups received one of two oral doses of the infusion (199.18 mg/kg, D5 and 398.26 mg/kg, D6) or the ethyl acetate fraction (36.24 mg/kg, D7; 72.48 mg/kg, D8), reconstituted in water. Diclofenac sodium inhibited writhing by 71.3%, while the average number of abdominal constrictions was significantly ($p<0.05$) reduced by D5 (43.5%), D6 (49.8%), D7 (29.2%) and D8 (46.8%) compared to the negative control. Only the D8 dose was tested in the formalin test. It did not show any relevant effect in the early phase of pain response but did reduce time spent licking in the late phase by 32.5% [Santos 2017].

Pharmacological studies in humans

An infusion of the dried aerial parts (1 g in 200 ml water) was given to 19 healthy volunteers (aged 18 to 55 years) twice a day (9 am and 2 pm) for 30 days. Fasting blood samples were taken on days 0 and 30. Significant increases from baseline were found on day 30 for HDL cholesterol ($p<0.05$) and total cholesterol ($p<0.01$), while there were non-significant increases in LDL cholesterol, triglycerides and glucose; all markers remained within reference values. Total antioxidant capacity had significantly ($p<0.001$) increased at day 30. There was a significant ($p<0.05$) decrease in serum levels of the pro-inflammatory cytokine IL-6 at day 30. Plasma levels of adiponectin were not significantly changed at day 30, but were found to be positively correlated with HDL cholesterol levels [Ivanova 2013].

Clinical studies

Patients with cutaneous porphyria (n = 20) were treated orally with an infusion 3-4 times daily (and no other treatment) in an open study. After 15 days substantial improvements in skin eruptions were observed together with decreases in serum iron levels and urinary porphyrins (intensely hyperchromic urine after initial doses, decolorizing to normal over the following days). All the patients showed improvements in general health (appetite, lack of dyspepsia, regularity) and no adverse reactions were reported [Patrascu 1984].

Pharmacokinetic properties

No data available.

Preclinical safety data

Mutagenicity

In the Ames test, using *Salmonella typhimurium* strains TA98

and TA100 with and without metabolic S9 mix activation, a commercial tincture (ethanol 70%, 1:5) [Schimmer 1994] and a methanolic extract [Bilia 1993a] from agrimony did not increase the number of revertants and showed no evidence of mutagenicity.

Clinical safety data

No adverse effects were observed in the clinical study described above, in which 20 patients were treated orally with an infusion of agrimony 3-4 times daily for 15 days Patrascu 1984].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Online Series, 2019
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHAMOMILLAE ROMANAE FLOS	Roman Chamomile Flower	Online Series, 2019
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Online Series, 2018

JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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The second edition of ESCOP Monographs, published as a hardback book in 2003 with a Supplement in 2009, has been widely acclaimed for its authoritative information on the therapeutic uses of herbal medicines. Monographs covering a total of 107 herbal substances include extensive summaries of pharmacological, clinical and toxicological data, and copious references to scientific literature form an important part of each text.

Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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Alchemillae herba

Alchemilla / Lady's Mantle

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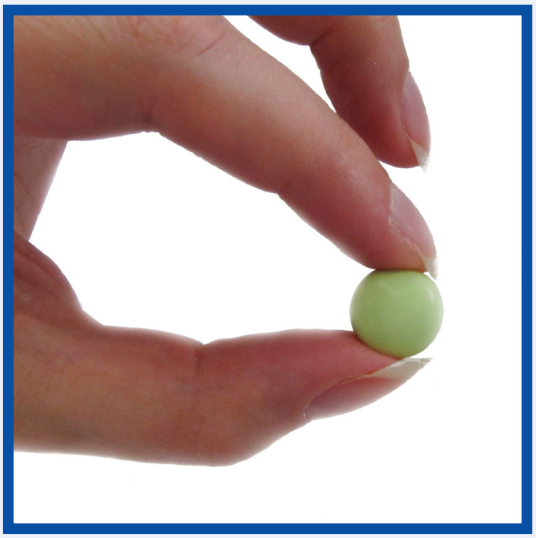

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Plant illustrated on the cover: *Alchemilla vulgaris*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
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Notes for the Reader
Abbreviations
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Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CVI	chronic venous insufficiency
CYP	cytochrome P450
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF	necrosis factor
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ORAC	oxygen radical absorbance capacity
ovx	ovariectomy or ovariectomized
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandine E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

ALCHEMILLAE HERBA

2013

Alchemilla / Lady's Mantle

DEFINITION

Alchemilla consists of the whole or cut, flowering aerial parts of *Alchemilla vulgaris* L. *sensu latiore*. It contains minimum 6.0 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) (dried drug). The material complies with the European Pharmacopoeia [EP].

Note: A synonym for *Alchemilla vulgaris* L. *sensu latiore* as stated in the European Pharmacopoeia definition is *Alchemilla xanthochlora* Rothm. [Blaschek 2009].

CONSTITUENTS

Ellagitannins (5-8%), principally the dimers agrimoniin (3.5-3.8%) and laevigatin F (0.9%) and the monomer pedunculagin (1.2%) as determined by HPLC [Geiger 1990, 1991, 1994; Moeck 2007; Bradley 2006; Blaschek 2009]. Use of the pharmacopoeial method for tannin determination yielded contents of up to 15% in the herb [Lamaison 1990; Schimmer 1992; Scholz 1994; Fraisse 1999].

Flavonol glycosides (2.2-2.5% in leaves, 1.0-1.9% in flowers), mainly quercetin 3-glucuronide, quercetin 3-glucoside (isoquercitrin), 3-rutinoside (rutin) and 3-arabinoside as well as kaempferol 3-(6″-p-coumaryl)-glucoside [Lamaison 1991; Schimmer 1992; D'Agostino 1998; Fraisse 2000; Bradley 2006, Blaschek 2009]. Approximately 3 % leucocyanidin in the flowers [Moeck 2007].

Essential oil, phytosterols and aliphatic hydrocarbons [Schimmer 1992; Bradley 2006].

CLINICAL PARTICULARS

Therapeutic indications

As an adjuvant in non-specific diarrhoea, gastrointestinal complaints and dysmenorrhoea [Schimmer 1992; Scholz 1994; Jänicke 2003; Bradley 2006; Gerlach 2007, Schilcher 2007; Moeck 2007; Blaschek 2009].

Efficacy in these indications is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Adult daily dose:

Internal use:

2-4 g of dried drug as an infusion or equivalent preparation three times daily [Schimmer 1992; Jänicke 2003; Bradley 2006; Moeck 2007; Blaschek 2009].
2-3 ml liquid extract (1:1, 25% ethanol) three times daily [Bradley 2006].

Method of administration

For oral administration.

Duration of administration

No restriction. If diarrhoea persists or worsens, medical advice should be sought.

Contraindications

None known.

Special warnings and special precautions for use

None required.

Interaction with other medicaments and other forms of interaction

None reported.

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Pregnancy and lactation
In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

Due to the high content of tannins Alchemilla has an astringent effect [Moeck 2007; Blaschek 2009].

In vitro experiments

Antibacterial activity
Various aqueous and ethanolic extracts showed antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* [Schimmer 1992].

Astringent activity
The antisecretory activity of a lyophilized hot water extract from *Alchemilla xanthochlora* was examined using isolated rabbit colon. A significant effect could not be demonstrated [Geiger 1994].

The astringency of ellagitannins isolated from *Alchemilla xanthochlora* was determined by haemanalysis with fresh human blood and by their relative affinity to methylene blue. Agrimoniin, pedunculagin and laevigatin F showed the highest activity [Geiger 1991, 1994].

Antioxidant activity
Aqueous and ethanolic extracts of Alchemilla (20 mg powder/10 ml solvent) were tested for their ability to inhibit lipid peroxidation in phosphatidylcholine liposomes by the thiobarbituric acid (TBA) test using butylhydroxytoluene as reference. Inhibition was demonstrated for 3, 10 and 30 µl of the aqueous extract while the ethanolic extract was not active The superoxide anion scavenging activity of the same preparations were tested in the NADH-phenazine methosulfate system. Doses of 10, 30 and 40 µl of the aqueous extract showed a dose-dependent effect [Filipek 1992].

Antioxidant activity was measured by the ABTS assay, and the total polyphenol content was assayed using the Folin-Ciocalteu reagent. An extract from Alchemilla (0.5 g/100 ml water) exhibited higher antioxidant activity expressed as Trolox equivalents (4.79 ± 0.14 mM) than *Ilex paraguariensis* (3.50 ± 0.29 mM) [Kiselova 2006].

A 50 % ethanolic extract demonstrated antioxidant activity by inhibiting H_2O_2-induced cytotoxicity (47.0 ± 3%) and DNA damage (37.2 ± 3.6%) as well as in the DPPH assay (71.8 ± 4.1%) and an Fe^{2+} chelating assay (84.6 ± 3.8%). The same extract decreased bacteriostatic and bactericidal effects of H_2O_2 on *Escherichia coli* and caused an increase in expression of the H_2O_2-inducible gene *katG*, measured by the activity of b-galactosidase in *E. coli* cells [Oktyabrsky 2009].

Anti-inflammatory activity
An aqueous lyophilized extract of Alchemilla did not show inhibitory activity on prostaglandin biosynthesis in bovine seminal vesicle microsomes with ^{14}C-arachidonic acid (reference indometacin). The same extract was tested for its platelet-activating factor (PAF)-induced exocytosis in neutrophils using SAAVNA (0.2 mM) as a substrate and produced an inhibition of 91 ± 4 % [Tunon 1995].

In the hen's egg chorioallantoic membrane (HET-CAM) assay two dry hydroethanolic extracts (lyophilized mother tinctures, not further specified) at a concentration of 500 µg/pellet inhibited membrane irritation by 91 and 100%. The effect was comparable to that of hydrocortisone, phenylbutazone or diclofenac sodium at 50 µg/pellet [Paper 1997].

An Alchemilla fraction consisting of flavone monoglycosides at a concentration of 0.16 mg/ml inhibited 50% of the activity of porcine pancreatic elastase against the substrate N-succinyl-L-alanyl-L-alanine-L-valine-p-nitroanilide (SAAVNA). The inhibitory activity on trypsin and a-chymoptrypsin was less pronounced [Jonadet 1986]. Extracts (25 µg/ml, ethanol 50% V/V) of both flowers and leaves inhibited elastase by 94% [Lamaison 1990].

Antispasmodic activity
A 50% V/V methanolic extract did not show an effect on spontaneous or acetylcholine- or barium chloride-induced contractions of isolated guinea pig ileum at concentrations up to 800 µg/ml [Izzo 1996].

Wound-healing properties
The effects of an Alchemilla fluid extract (3% in glycerol) on cell growth were investigated in Chang Liver and Madin Darby Bovine Kidney (MDBK) epithelial cell lines and in rat aortic myofibroblast cultures. Cell numbers increased with 0.1 - 1% of the extract, attaining 21.3 ± 2.1%, 15.5 ± 2.25% and 10.6 ± 0.6% in MDBK, myofibroblast and Chang liver cells, respectively ($p<0.005$). No morphological changes or cytotoxicity were noted [Shrivastava 2007].

In vivo experiments

Angioprotective effects
An Alchemilla extract rich in flavone monoglycosides was subjected to the capillary permeability test in male rats using the Evans Blue diffusion chamber. At doses of 30 and 60 mg/kg body weight (i.p.), angioprotection significantly ($p<0.001$) increased by 28.0 and 30.1%, respectively. Another fraction consisting of flavone diglycosides showed a non-significant increase [Jonadet 1986].

Haemorheological effects
In 16 spontaneously hypertensive male rats, and 8 female Wistar rats, with arterial hypertension and increased blood viscosity, the effects of an Alchemilla extract (standardised on flavonoids, not further specified) on the structure and function of erythrocytes were examined. The extract was administered intragastrically at a daily dose of 300 mg/kg body weight for 10 days. It had a positive effect on erythrocyte surface morphology as well as on the lipid composition of erythrocyte membranes. Furthermore, the administration of the extract improved erythrocyte deformability [Plotnikov 2006].

Wound-healing properties
The effect of an Alchemilla fluid extract (3% in glycerol) on cell growth was investigated using dorsal skin lesions (8 mm diameter) in adult male rats. The lesions treated topically with

the extract significantly decreased in size by 10.0 ± 0.7% ($p<0.005$) after 2 days of treatment. On day 3 of treatment, the diameter of the lesions was significantly reduced by 15.9 ± 1.1% in glycerine-treated rats compared with distilled water ($p<0.005$), whereas the diameter in verum-treated rats was further reduced by 23.2 ± 1.4% ($p<0.005$) [Shrivastava 2007].

Other effects
The effects of Alchemilla dried leaves and decoction on glucose homoeostasis were evaluated in normal and streptozotocin diabetic mice. The plant material was supplied ad libitum in the diet (6.25% body weight) and additionally as a decoction (1 g/400 ml water) instead of drinking water. After 12 days of administration the animals were challenged with streptozotocin (200 mg/kg b.w. i.p.). Alchemilla did not prevent the development of hyperphagia, polydipsia, body weight loss, hyperglycaemia and hypoinsulinaemia [Swanston-Flatt 1990].

Eight-day-old chicks received i.p. injections of a hydroalcoholic Alchemilla extract (not further specified) 30 minutes before being tested in the presence of two other chicks, or in isolation, for a 3-minute observation period. Sedation, separation-distress and stress-induced analgesia were not changed significantly after application of the extract [Sufka 2001].

The effect of a polyphenol-containing fraction (not further specified) from Alchemilla on the morphofunctional state of the thyroid was examined in rats. The animals were fed with 10 mg/kg b.w. of this extract by gastric tube and then were exposed to cold air (minus 10°C). Enhanced synthesis and an increase in peripheral de-iodated thyroid hormones were observed in both the treatment and the control group. After a subsequent "readaptation phase" at room temperature, the control group showed a compensatory reduction of thyroid function whereas in the treatment group hormone synthesis was stimulated [Borodin 1999].

Clinical data

An open-label study involving 48 otherwise healthy male and female patients (4-44 years of age) examined the effects of a gel containing an Alchemilla fluid extract (3% in glycerol) on common minor oral ulcers. The gel was applied topically three times daily. In 60.4 % of the patients discomfort was relieved and complete healing was achieved within 2 days. In 75 % of the patients, healing was achieved within 3 days, as compared to 15 % without treatment and 40 % with usual treatment (not further specified) [Shrivastava 2006].

Pharmacokinetic properties
No data available.

Preclinical safety data
In the Ames test with *Salmonella typhimurium* strains TA 98 and TA 100, an Alchemilla tincture (1:5, ethanol 70%, species not defined) showed no mutagenicity without activation and weak mutagenicity at a concentration of 40 µl/plate after activation with S9 mix [Schimmer 1988, 1994]. The latter effect is supposed to be caused by quercetin and does not seem to be clinically relevant as Alchemilla preparations have only a low quercetin content [Schimmer 1988].

Hydroethanolic extracts (DER 1:5) from *A. mollis* and *A. alpina* (50% ethanol), and *A. vulgaris* (70% ethanol), were tested for their antimutagenic potency against 2-nitrofluorene as a standard mutagen. The experiments were performed with *Salmonella typhimurium* strains TA 98 and TA 100 without exogenous metabolic system. At doses of 80 and 160 µl/plate all preparations inhibited the induced mutagenicity. The extracts and tincture did not show any significant mutagenicity within the dose range tested [Schimmer 1995].

Clinical safety data
A gel containing an Alchemilla fluid extract (3% in glycerol) used topically on the oral mucosa in an open-label study in 48 patients (4-44 years of age) was well tolerated [Srivastava 2006].

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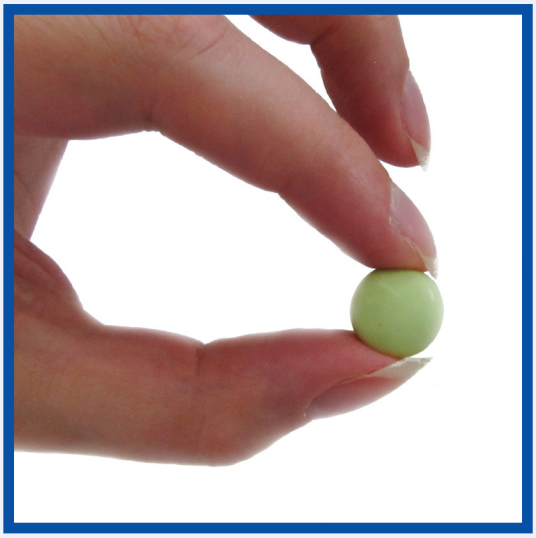

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European Scientific Cooperative On Phytotherapy

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MONOGRAPHS

***The* Scientific Foundation for**
Herbal Medicinal Products

ALLII SATIVI BULBUS
Garlic

2019

E/S/C/O/P
European Scientific Cooperative
On Phytotherapy

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Plant illustrated on the cover: *Allium sativum*

FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

ALLII SATIVI BULBUS

2019

Garlic

DEFINITION

Garlic powder consists of the bulb of *Allium sativum* L., with the outer corneous layer removed, cut, freeze-dried or dried at a temperature not exceeding 65°C and powdered. It contains not less than 0.45 per cent of allicin ($C_6H_{10}OS_2$; M_r 162.3), calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Garlic Powder].

CONSTITUENTS

Carefully dried, powdered material contains about 1 per cent of alliin [(+)-S-allyl-L-cysteine sulphoxide] as the main sulphur-containing amino acid. In the presence of the enzyme alliinase, alliin will be converted to allicin (1 mg of alliin is equivalent to 0.45 mg of allicin).

In turn, allicin is the precursor of various transformation products, including ajoenes, vinyldithiines, oligosulphides and polysulphides, depending on the conditions applied.

Material derived from garlic by steam distillation or extraction in an oily medium contains various allicin transformation products.

Other characteristic, genuine constituents are (+)-S-methyl-L-cysteine sulphoxide, gamma-L-glutamyl peptides, S-allyl-cysteine, ubiquitous amino acids, steroids, triterpenoid saponins, flavonoids, lectins and adenosine [Ackermann 2001; Benavides 2007; Blaschek 2016; Block 1985, Block 1992; Bradley 2016; Koch 1996; Lawson 1993; Sticher 1991; Wang 2015; Xiong 2015].

CLINICAL PARTICULARS

Therapeutic indications

Prophylaxis of atherosclerosis [Bordia 1989; Harenberg 1988; Kiesewetter 1991, 1993a,b; Koch 1996; Koscielny 1999; Orekhov 1995, 1996, 1997; Reuter 1991].

Treatment of elevated blood lipid levels insufficiently controlled by diet [Adler 1997; Auer 1990; Brewitt 1991; Beck 1993; De Santos 1993, 1995; Harenberg 1988; Fatima 2014; Kiesewetter 1993b; Jain 1993; Mader 1990; Orekhov 1996; Reinhard 2009; Ried 2013; Rinneberg 1989; Vorberg 1990; Schilcher 2016; Sobenin 2009b; Walper 1994; Zeng 2012].

Supportive treatment of hypertension [Ashraf 2013; Auer 1990; De Santos 1993, 1995; Holzgartner 1992; Kandziora 1988; Kiesewetter 1993b; Ried 2016; Sobenin 2009a; Vorberg 1990; Wang 2015; Xiong 2015].

Upper respiratory tract infections and catarrhal conditions [Allium BHP 1983; Andrianova 2003; Block 1992; Bradley 1992; Josling 2001; Lissiman 2009, 2014; Rafinsky 1974; Tsai 1985].

Posology and method of administration

Dosage

Prophylaxis of atherosclerosis or treatment of elevated blood lipid levels
Adults: The equivalent of 6-10 mg of alliin (approx. 3-5 mg of allicin) daily, typically contained in one clove of garlic or in 0.5-1.0 g of dried garlic powder [Adler 1997; Auer 1990; Beck 1993; Bordia 1989; Brewitt 1991; De Santos 1993, 1995; Fatima 2014; Harenberg 1988; Holzgartner 1992; Jain 1993; Kandziora 1988; Kiesewetter 1991, 1993a,b; Koch 1996; Koscielny 1999; Mader 1990; Orekhov 1995, 1996, 1997; Reuter 1991; Ried 2013; Rinneberg 1989; Schilcher 2016; Sobenin 2009b; Vorberg 1990; Walper 1994; Zeng 2012].

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Supportive treatment of hypertension
Adults: The equivalent of 6-10 mg of alliin (approx. 3-5 mg of allicin) daily, typically contained in one clove of garlic or in 0.5-1.0 g of dried garlic powder [Ashraf 2013; Auer 1990; De Santos 1993, 1995; Holzgartner 1992; Kandziora 1988; Kiesewetter 1993b; Schilcher 2016; Sobenin 2009a; Vorberg 1990].

Upper respiratory tract infections
Adults: 2-4 g of dried bulb or 2-4 ml of tincture (1:5, 45% ethanol), three times daily or 180 mg garlic powder per day [Allium BHP 1983; Josling 2001].

Method of administration
For oral administration.

Duration of administration
Long-term treatment is generally advised in the prevention of atherosclerosis and prophylaxis or treatment of peripheral arterial vascular diseases [Kiesewetter 1993b; Koscielny 1999].

Contra-Indications
None known.

Special warnings and special precautions for use
Caution is advised with regard to surgical operations due to some case reports with prolonged or increased bleeding [Burnham 1995; Carden 2002; German 1995; Izzo 2007; Kilic 2014; Shakeel 2010; Woodbury 2016].

Interaction with other medicaments and other forms of interaction
An increased International Normalized Ratio (INR) has been observed in 2 patients on warfarin and in one patient on fluindione who had used garlic products [Sunter 1991; Pathak 2003]. The results of studies with saquinavir and ritonavir in healthy volunteers remain unclear [Piscitelli 2002; Gallicano 2003].

Pregnancy and lactation
There are no objections for use during pregnancy and lactation, because neither long term nutritional experience nor any other important circumstances give reason for concern [Ziaei 2001]. From a controlled trial it is known that major sulphur-containing volatiles from garlic are transmitted to human milk leading to improved drinking habits of the babies [Mennella 1991].

Effects on ability to drive and use machines
None known.

Undesirable effects
In rare cases gastro-intestinal irritation or allergic reactions [Izzo 2007; Mane 2013; Schilcher 2016; Siegers 1989; Vovolis 2010].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties
Garlic exerts antiatherogenic, lipid-lowering, antihypertensive, antiaggregatory, endothelium-relaxing, vasodilatory and antioxidant effects. The effects are primarily due to allicin and its transformation products [Benavides 2007; Bradley 2016; Koch 1996; Ried 2016; Schilcher 2016; Xiong 2015].

In vitro experiments

Antiatherogenic and lipid-lowering effects
Inhibitory effects of extracts and isolated fractions from garlic on cholesterol biosynthesis were observed in chicken hepatocytes and monkey livers. At equivalent doses, both the drug and its lipophilic and hydrophilic fractions caused 50-75% inhibition of cholesterol biosynthesis [Quereshi 1983].

Inhibition of cholesterol biosynthesis by allicin and ajoene was evaluated in cultivated rat hepatocytes and the human liver cell line HepG2. In rat hepatocytes, ajoene inhibited sterol biosynthesis with an IC_{50} of 15 µM; alliin was almost ineffective. In HepG2 cells both allicin and ajoene inhibited sterol biosynthesis with IC_{50} values of 7 and 9 µM, respectively. The inhibition was exerted at the level of HMG-CoA-reductase [Gebhardt 1994].

In a study in primary rat hepatocytes, the water-soluble organosulphur compounds S-allyl cysteine (SAC), S-ethyl cysteine (SEC) and S-propyl cysteine (SPC) inhibited [2-^{14}C]acetate incorporation into cholesterol in a concentration-dependent manner, achieving 42 to 55% maximal inhibition. Other sulphur compounds were less potent. Alliin, S-allyl-N-acetyl cysteine, S-allylsulphonyl alanine and S-methyl cysteine had no effect. Of the lipid-soluble compounds, diallyl disulphide (DADS), diallyl trisulphide (DATS) and dipropyl disulphide (DPDS) reduced cholesterol synthesis by 10 to 25% at low concentrations (≤0.5 mmol/L) and completely inhibited synthesis at high concentrations (≥1.0 mmol/L) [Liu 2000].

Standardized garlic powder (1.3% alliin) inhibited cholesterol biosynthesis in rat hepatocytes with an IC_{50} of 90 µg/ml [Gebhardt 1991, 1993]. In HepG2 cells cholesterol biosynthesis was inhibited by the garlic powder with an IC_{50} of 35 µg/ml [Gebhardt 1993].

The mechanism of action is believed to be an interaction at the molecular level with the phosphorylation cascade of hydroxymethylglutaryl-CoA reductase. Organosulphur compounds containing an allyl disulphide or allyl sulphydryl group are capable of interacting with the thiol group of cysteine and thus forming cysteine derivatives. The antiatherogenic effects of these organosulphur compounds can be attributed to reactions that inhibit HMG-CoA reductase and other lipogenic enzymes [Gebhardt 1994; Mathew 2004; Singh 2006].

A concentration-dependent inhibition of cholesterol synthesis (^{3}H-acetate incorporation in Hep-G2) was exerted by allyl mercaptan, a metabolite of organosulphur compounds. At concentrations between 5 and 100 µg/mL, cholesterol synthesis was inhibited by 20 to 80% in cells (approximately 50% inhibition with 25 µg/mL) and cholesterol secretion into the medium was decreased by 20 to 50% compared with control ($p<0.05$). In cells incubated with 1 mM of oleic acid, allyl mercaptan decreased cholesterol synthesis compared to control ($19.5\pm1.2 \times 10^3$ dots per minute (dpm)/mg protein/4 h vs. $30.0\pm2.6 \times 10^3$ dpm/mg protein/4 h; $p<0.05$) [Xu 1999].

Allicin inhibits the incorporation of ^{14}C-acetate into non-saponifiable neutral lipids at concentrations as low as 10 µM and can be regarded as the most active of the sulphur-containing metabolic products of alliin [Gebhardt 1991].

Inhibition of cholesterol biosynthesis by up to 87% was also observed with petroleum ether, methanol and water extracts of garlic, and a garlic-preparation containing S-allylcysteine [Yeh 1994].

During a 24-hour incubation period garlic powder significantly reduced the level of cholesteryl esters by 26% and free cholesterol by 32% in cells of atherosclerotic plaques of human aorta ($p<0.05$) and inhibited their proliferative activity by 55% at 1 mg/ml [Orekhov 1995].

The antiatherogenic effect of egg-yolk-enriched garlic powder was determined on LDL oxidation and oxidant stress-induced cell injury models. The powder inhibited copper-induced LDL-oxidation in a dose-dependent manner. Pretreatment with the garlic powder significantly suppressed the production of peroxides in HL60 cells and protected endothelial cells from hydrogen peroxide-induced cell injury [Yamaji 2004].

Effects on vascular resistance, fibrinolysis and platelet aggregation
In isolated arterial strips from the dog, garlic at 0.2-200 g powder/litre and ajoene at 10^{-6} to 10^{-3} M hyperpolarised membrane potential by 9.3 mV and 4.2 mV, and reduced muscle tone by up to 0.33 g and 0.16 g, respectively [Siegel 1991,1992].

Similarly, in human coronary artery smooth muscle garlic at concentrations of 0.0002-0.2 g/litre caused dose-dependent hyperpolarisation (2.7 mV at maximum) and relaxation (20% at maximum); half-maximal effects were obtained at 1.15 mg/litre. Allicin and ajoene produced hyperpolarisation of up to 5.1 mV and 4.4 mV, and diminished vascular tone by 24% and 11%, respectively, with half-maximal effects at 6.2×10^{-9} M and 9.9×10^{-9} M [Siegel 1998].

In isolated rat aorta freeze-dried garlic powder dose-dependently inhibited noradrenaline-induced contractions. The EC_{50} values were 5.28 mg/ml in aortas with endothelia and 5.81 mg/ml in aortas without endothelia [Öztürk 1994].

A two-fold activation of endogenous nitric oxide synthesis was observed in isolated thrombocytes obtained from human volunteers after ingestion of a single dose of 4 g of fresh garlic [Das 1995; Sooranna 1996].

Garlic preparations and organosulphur compounds from garlic inhibited collagen-stimulated platelet aggregation in platelet-rich plasma with the following IC_{50} values (µg/ml): an aqueous garlic extract 460, total polar constituents 190, alliin 205, allicin 14.0, total thiosulphinates 13.2, adenosine 0.11, guanosine > 20. In whole blood the strongest effects were exhibited by allicin (IC_{50}:14.0 µg/ml) and adenosine (IC_{50}: 0.11 µg/ml) [Lawson 1992].

Diallyldisulphide (40-200 µg/mL) and diallyltrisulphide (5-100 µg/mL) dose-dependently inhibited platelet aggregation and thromboxane B2 secretion induced by arachidonic acid, adrenaline, collagen and calcium ionophore A23187 [Bordia 1998].

Antioxidant effects
Allicin inhibited lipid peroxidation induced by an ascorbic acid/Fe^{2+} system at a concentration of 0.18 mM [Rekka 1994].

The antioxidant effect of an aqueous garlic extract obtained from 1 mg of garlic powder was comparable to that of 30 nmol of ascorbic acid or 3.6 nmol of α-tocopherol in a chemiluminescence assay [Lewin 1994; Popov 1994]. Radical scavenging activity of preparations containing S-allylcysteine has also been demonstrated in both chemiluminescence and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays [Imai 1994].

Garlic powder strongly inhibited superoxide production in phorbol ester-activated granulocytes with an IC_{50} of 390 µg/ml, whereas an alliin-enriched but alliinase-inactivated garlic extract did not inhibit superoxide production at concentrations up to 1 g/ml [Siegers 1999].

An aqueous solution of garlic powder (1.3% alliin) reduced radicals generated by the Fenton reaction and liberated by cigarette smoke [Török 1994].

Trichloromethyl and trichloromethylperoxyl free radicals were scavenged by the garlic sulphur components diallyl sulphide, diallyl disulphide and allyl mercaptan. Diallyl disulphide also inhibited CCl_4-promoted liver microsomal lipid peroxidation, and diallyl sulphide was able to react with free radicals formed by UVC-activation of hydrogen peroxide or terbutyl hydroperoxide but not with those produced during UVC activation of terbutyl peroxide. However, all garlic components tested absorbed energy from UVC and became partially destroyed in the process. Allyl mercaptan was able to destroy 4-hydroxynonenal, a key reactive aldehyde produced during lipid peroxidation. Allyl mercaptan and diallyl disulphide were also able to prevent UVC and CCl_4-promoted oxidation of albumin *in vitro* [Fanelli 1998].

Allicin reversed the effect of H_2O_2 on HUVEC death. The activation of PARP-cleavage and Bax expression, and the reduction of pro-caspase-3 levels, were inhibited by allicin [Chen 2014].

Other effects
In the ischaemia-reperfusion model, garlic juice (0.01 mg/mL added to the perfusion solution) significantly ($p<0.05$) increased coronary flow before ischaemia in isolated rat heart. Compared to control, the released lactate dehydrogenase at the first minute, the recovery of rate pressure product, and the left ventricular-developed pressure after 45 minutes, were significantly ($p<0.05$) better in the garlic group [Shackebaei 2010].

An aqueous extract from fresh garlic (100-500 µg/ml) increased the proliferation and improved the morphological characteristics of cultured human fibroblasts (more thin, long and spindle cells instead of large, flat cells) [Svendson 1994].

A similar extract non-competitively and irreversibly inhibited cyclooxygenase activity in rabbit platelets and in lung and vascular aortic tissues with IC_{50} values of 0.35, 1.10 and 0.9 mg, respectively; boiled garlic had only modest effects [Ali 1995].

Anti-aggregatory [Mayeux 1988; Lawson 1992], antibacterial [Sharma 1977; Adetumbi 1983; Gupta 2010], antimycotic [Lutomski 1991; Li 2014], antiviral [Tsai 1985], neuroprotective [Koh 2005; Ishige 2007], anti-parasitic [Kinuthia 2014], and anti-tumour effects [Wargovich 1991; Dorant 1993; Lan 2013; Bagul 2015] of garlic have also been demonstrated.

In vivo experiments

Lipid-lowering and antiatherogenic effects, hypoglycaemic activity
Garlic powder (0.6% allicin) fed to rats for 11 days at 0.25, 0.5 or 1 g/kg b.w. protected against isoprenaline-induced myocardial damage, as shown by histological examination and biochemical analysis [Ciplea 1988].

In the isolated, perfused heart (Langendorff model) from rats which had been given 1% of garlic powder (1.3% alliin) in their diet for 10 weeks, the size of the ischaemic zone (32% vs 41% of total heart tissue; $p<0.05$), incidence of ventricular tachycardia (0% vs 35%; $p<0.05$) and incidence of ventricular fibrillation (50% vs 90%; $p<0.05$) were significantly reduced in comparison with untreated controls [Jacob 1991; Isensee 1993].

A petroleum-ether extract of garlic (corresponding to dried garlic at 1 g/kg b.w.), fed to rats for 5 days in combination with an atherogenic diet, significantly prevented elevation of serum cholesterol and triglyceride levels (92.4 and 93 mg/dl), respectively, compared to 320 and 370 mg/dl in control animals; $p<0.001$), and reduced the incidence and grade of atherosclerotic lesions ($p = 0.0027$) compared to animals fed on the atherogenic diet alone [Lata 1991].

Rabbits were given cholesterol in their diet at 0.5 g/kg b.w. daily for 2 months, then fed for 3 months on a normal diet with or without the addition of fresh garlic at 1 g/kg daily. Compared to rabbits receiving a normal diet over the 3-month period, those given garlic had reduced plaque areas with half as many lesions [Mand 1985].

After de-endothelialisation of the carotid artery, rats were given a diet containing 2% of cholesterol with or without the addition of 5% of garlic powder (1.3% alliin). After 4 weeks, insertion of a balloon catheter revealed only modest inhibition of the growth of neointima in the rats given garlic compared to those given cholesterol alone. However, the garlic inhibited elevation of serum cholesterol by 42% ($p<0.05$) and protected enzymes of the glutathione system, which were strongly impaired under hypercholesterolaemia [Heinle 1994].

In Wistar rats with induced hypercholesterolaemia, and their 1-day-old offspring, pathological changes to myocardial muscle and atherosclerosis of dorsal aorta were observed, affecting the progress of gestation and development of both morphological and skeletal abnormalities. Oral administration of 100 mg fresh garlic/kg b.w. for three weeks prior to onset of gestation as well as throughout the gestation period led to amelioration of histological heart and vessel abnormalities, serum total cholesterol, triglycerides, LDL and creatine kinase activity of both mother and their offspring. Moreover, as compared to those effects seen in animals with experimental hypercholesterolemia, the supplementation reduced the increased incidence of abortions, reduced the decrease in total number of offspring and reduced the marked depletion of body weight; additionally, congenital abnormalities induced by hypercholesterolaemia were not seen in the verum group [El-Sayyad 2010].

In guinea pigs fed a diet enriched with 2.5 and 5% garlic powder for 21 days, significant decreases were found in serum triglycerides (15 and 29%; $p<0.02$), total cholesterol (73 and 78%; $p<0.001$), VLDL (65 and 70%; $p<0.001$) and LDL (69 and 70%; $p<0.001$) when compared to control. HDL remained unchanged [Pourkabir 2010].

The effects of a single dose of an ethanolic garlic extract (250, 300 and 350 mg/kg b.w.) were compared to metformin in normal and alloxan-induced diabetic rabbits. In non-diabetic rabbits a maximum hypoglycaemic response was observed with the highest dose of garlic extract (350 mg/kg b.w.) 4 h after administration, while in diabetic rabbits, blood glucose levels (270.3 ± 0.8 mg/dl) were significantly ($p<0.05$) lowered as compared to control (303.8 ± 1.8 mg/dl). Triglycerides (44.0 ± 0.9 mg/dl) and cholesterol (32.8 ± 0.7 mg/dl) also significantly ($p<0.05$) decreased in non-diabetic rabbits [Sher 2012].

Administration of garlic juice by gavage to STZ-induced diabetic male Wistar rats (1 ml/100 g b.w. per day), resulted in a significant increase in body weight ($p<0.05$), together with a decrease in food intake ($p<0.0001$), and a decrease in glucose-, cholesterol- and TG-levels ($p<0.0001$) [Masjedi 2010].

In alloxan-induced diabetic rabbits, oral administration of a 1% aqueous garlic extract (300 ml three times per day) for 15 days reduced blood glucose (by 51%), cholesterol (35%) and urea (46%) [Rind 2013].

A study investigated the effects of garlic oil and gliclazide on blood glucose levels in alloxan-induced diabetic rats. Garlic oil (1 ml/kg b.w.) or a vehicle were orally administered to groups of rats for 14 days, this was followed by i.p. administration of alloxan for 2 days to induce diabetes, in all but a control group. On day 17, the diabetic rats were given single oral doses of either vehicle, gliclazide (2 ml/kg b.w.), garlic oil (1 ml/kg b.w.) or garlic oil and gliclazide. The gliclazide and combination groups demonstrated a significantly ($p<0.01$) greater mean percentage blood glucose reduction (over a 12 hour period) than the diabetic control group, while the combination had a greater effect than gliclazide alone [Nandyala 2013].

Antihypertensive effects

Garlic powder given for several weeks at 1 g/kg b.w. to spontaneously hypertensive juvenile rats, starting in their fifth week of life, inhibited the development of hypertension and reduced myocardial hypertrophy. In spontaneously hypertensive adult rats (6-7 month of age), garlic lowered blood pressure and myocardial hypertrophy ($p<0.01$) [Jacob 1993].

Garlic powder (1.3% alliin) added at 0.5% to the diet of hypertensive rats significantly ($p<0.02$) prolonged their life span by 22 days (453.2±16.2 days) compared to a control group of of hypertensive rats given the normal diet (434.5±23.5 days). During the course of the study systolic blood pressure was significantly lower in the garlic group ($p<0.01$) [Brändle 1997].

An aqueous garlic extract administered orally to hypertensive two-kidney-one-clip Goldblatt model rats as a single dose at 50 mg/kg b.w. or in repeated daily doses of 50 mg/kg for 2 weeks exerted a significant antihypertensive effect ($p<0.05$) [Al-Qattan 1999].

Other cardiovascular effects

The oral pretreatment of female Wistar albino rats with fresh garlic (125, 250 or 500 mg/kg b.w.) for 30 days, given alone or in combination with 30 mg/kg captopril during the last seven days, showed cardioprotective effects in a modified Langendorff heart model and after isoproterenol-induced myocardial damage [Asdaq 2008a; 2010a]. Given alone, or in combination with 10 mg/kg hydrochlorothiazide, fresh garlic revealed cardioprotective effects against myocardial damage induced by ischaemia-reperfusion and isoproterenol [Asdaq 2009b]. The protective effects on the myocardium after ischaemia-reperfusion injury were also demonstrated histologically and by analysis of heart tissue [Asdaq 2008a]. However, in all studies the highest dose of 500 mg/kg b.w. of fresh garlic did not show cardioprotective effects [Asdaq 2008ab, 2009ab, 2010ab].

In male wistar rats given a high-fat diet for 12 weeks prior to treatment, a garlic extract (1% allicin; 250 or 500 mg/kg b.w. for 28 days) significantly ($p<0.05$) decreased the diet-induced increases in plasma insulin, total cholesterol and oxidative stress levels, and restored heart rate variability, cardiac function and cardiac mitochondrial function [Supakul 2014].

Clotting time was delayed in rats treated orally for 11 days with an ethanolic garlic extract (10 mg/kg b.w./d), and significantly ($p<0.05$) delayed with a combination of the garlic extract at the same dose for 10 days and a single oral dose of warfarin (3 mg/kg b.w.) on day 11, when compared to untreated controls and those given a single dose of warfarin alone. Clot retraction was also shown to be poor in rats given the combination treatment [Musubika 2015].

Antioxidant effects

Pretreatment of female Wistar albino rats with an oral dose of 250 mg/kg b.w. of fresh garlic for 30 days, either alone or combined with 30 mg/kg captopril or 10 mg/kg propranolol during the last seven days of treatment, led to an increase in activities of antioxidant enzymes such as SOD and catalase ($p<0.05$), and retained the activities of lactate dehydrogenase and creatine phosphokinase isoenzyme [Asdaq 2008b, 2010a].

Oral administration of fresh garlic at 125, 250 or 500 mg/kg b.w., given alone for 30 days or in combination with 10 mg/kg b.w. propranolol for the last 7 days of treatment, augmented endogenous antioxidant synthesis at the two lower doses ($p<0.05$), whereas at the highest dose a reduction in the synthesis of SOD and catalase was observed ($p<0.05$) [Asdaq 2010a].

In a study in male Albino rats, administration of fresh garlic (250 mg/kg b.w. p.o.) for 20 days significantly ($p<0.05$) reduced nickel- and chromium-induced increases in the lipid peroxidase (LPO) level and signficantly ($p<0.05$) improved SOD activity [Das 2009].

Administration of garlic oil (10 mg/kg i.p.; n=10) for 8 weeks was compared with a saline control (i.p.; n=10) in alloxan-induced diabetic rats. The garlic oil retarded lipid peroxidation of cellular membranes induced by oxidative stress associated with diabetes. Compared to the saline control, garlic oil significantly ($p<0.05$) decreased the levels of LPO in plasma, erythrocyte lysate and liver tissue homogenate, whereas total thiols were significantly ($p<0.05$) elevated in the plasma and erythrocyte lysate. SOD and GST activity were significantly ($p<0.05$) elevated in erythrocyte lysate and liver homogenate [Saad Abdultawab 2013].

Garlic oil (5 ml/kg b.w.), administered orally 30 minutes before each daily dose of cyclosporine A (25 mg/kg b.w. p.o.) for 3 weeks, attenuated cyclosporine-induced nephrotoxicity in rats. The significant ($p<0.05$) increases in renal dysfunction parameters and levels of serum NO and plasma MDA caused by cyclosporine A were significantly ($p<0.05$) reduced by the co-administration with garlic oil [Abdelsattar 2013].

Garlic has been shown to prevent or reduce biochemical and histological effects of methotrexate (MTX)-induced nephrotoxicity in rats. An aqueous extract (100 g crushed garlic in 100 ml water; squeezed and filtered) administered orally (1 ml/100 g b.w.) for 7 days before and after a single MTX (20 mg/kg b.w.) i.p. injection resulted in decreased urea and creatinine levels, almost normalized change of electrolytes (sodium, potassium, phosphorus, calcium), and increased concentrations of renal antioxidant stress markers such as GSH and catalase as compared to a decrease with MTX alone. Decreased oxidative stress was shown via reduction of malondialdehyde adenosine deaminase and NO levels. MTX-induced alterations in renal morphology (glomerular/tubular) were prevented by garlic [Ahmed 2015].

A study investigated the ameliorative effects of garlic on gentamicin-induced nephrotoxicity in rats. Serum creatinine and blood urea nitrogen levels were significantly increased ($p<0.05$) by the administration of gentamicin (100 mg/kg bw i.p.) for 10 days. When this was followed by 10 days treatment with garlic juice (20 mg/kg b.w. i.p.), these raised levels of serum creatinine and blood urea nitrogen were significantly ($p<0.05$) reduced [Nasri 2014].

In a study investigating cisplatin-induced nephrotoxicity in rats, oral administration of an ethanolic garlic extract (150 or 300 mg/kg b.w.) for 4 days, following a single injection of cisplatin (5 mg/kg b.w.), significantly ($p<0.001$) reduced the cisplatin-induced biochemical changes (increased urea, uric acid, creatinine, blood urea nitrogen) and dose-dependently ($p<0.01$ to $p<0.001$) reduced the cisplatin-induced increase in kidney weight [Anusuya 2013].

Other effects

Post-ischaemic neuroprotective effects of allicin were seen in a rat middle cerebral artery occlusion (MCAO) model. Treatment with allicin (50 mg/kg b.w. i.p.) at 3 and 6 hours, but not 9 hours, after ischaemia induction significantly ($p<0.05$) reduced brain infarct volume, attenuated cerebral oedema and decreased the neurological deficit score when compared to vehicle only treated rats [Lin 2015].

In other studies, antiviral [Nagai 1973; Sohn 2009], antihepatotoxic [Hikino 1986; Nakagawa 1989], antidiabetic [Thomson 2007; Shariatzadeh 2008; Das 2009; Thomson 2013], anti-inflammatory [Sohn 2009; Sahbaz 2014], anti-parasitic [Khalil 2015; Kinuthia 2014], and tumour growth-inhibiting [Wargovich 1991; Dorant 1993] effects of garlic have also been demonstrated.

Pharmacological studies in humans

Rheological and antiatherosclerotic effects

In a randomized, double-blind, placebo-controlled, cross-over study, the influence on cutaneous microcirculation of a single oral dose of 900 mg of garlic powder (1.3% alliin) was evaluated in 10 healthy volunteers. After 5 hours, a significant increase of 55% was observed in erythrocyte velocity in skin capillaries ($p<0.01$) resulting from vasodilatation of precapillary arterioles, which increased in diameter by 8.6% ($p<0.01$) [Jung 1991].

In a 2-week, randomized, double-blind, placebo-controlled, cross-over study involving 10 healthy volunteers, daily ingestion of 600 mg of a standardized garlic powder preparation (1.3% alliin) significantly ($p<0.05$) lowered susceptibility to lipoprotein oxidation by 34% [Phelps 1993].

In an open study involving 101 healthy volunteers (50 – 80 years of age), the effects of 300-900 mg of standardized garlic powder (1.3% alliin) daily on elastic properties of the aorta were investigated for at least 2 years. Compared to the results from an untreated control group, garlic intake attenuated age-related increases in aortic stiffness: significant differences were observed in pulse wave velocity (8.3 vs. 9.8 m/s, $p<0.0001$) and pressure-standardized elastic vascular resistance (0.63 vs. 0.9 $m^2/s^2 \times$ mm Hg, $p<0.0001$); correlation with age was significantly different ($p<0.0001$) for pulse wave velocity (garlic, $r = 0.44$; control, $r = 0.52$) and systolic blood pressure (garlic, $r = 0.48$; control, $r = 0.54$) [Breithaupt-Grögler 1997].

In an open study 8 healthy volunteers took 1 clove of garlic (about 3 g) daily for 16 weeks. Serum thromboxane B2 was significantly ($p<0.001$) reduced from 243 to 24 ng/ml [Ali 1995].

Healthy volunteers ($n = 30$) took 10 g of fresh garlic daily for 2 months. Clotting time increased from 4.15 to 5.02 min ($p<0.001$) and fibrinolytic activity (euglobulin clot lysis time) decreased from 4.17 to 3.26 hours ($p<0.001$). There were no changes in the control group [Gadkari 1991].

In a randomized, double-blind, placebo-controlled, cross-over study, the effect of a single dose of a dried ethyl acetate extract of garlic (55 mg pure garlic oil derived from 9.9 g fresh garlic) on *ex vivo* induced platelet aggregation was investigated in 14 healthy volunteers. In blood collected 4 hours after administration there was a significant ($p<0.05$) reduction of adrenaline-induced platelet aggregation by 12%, but no effect on platelet aggregation induced by collagen or ADP [Wojcikowski 2007].

Incubation of cultured smooth muscle cells for 24 hours with serum from patients with coronary atherosclerosis increased total cell cholesterol by 46.6%. However, incubation with serum taken from the patients 2 hours after ingestion of 300 mg of standardized garlic powder (1.3% alliin) reduced total cell cholesterol to 19.3% [Orekhov 1995].

Lipid-lowering effects
In a randomized, double-blind, placebo-controlled study, significant reductions of up to 30% in fasting and postprandial plasma triglycerides were observed in 24 volunteers after 6 weeks of daily treatment with 900 mg of garlic powder ($p<0.05$ for intergroup differences in fasting plasma triglycerides) [Rotzsch 1992].

In a randomized, double-blind, placebo-controlled study, the effects of taking 600 mg of standardized garlic powder (1.3% alliin) daily were evaluated in 68 volunteers (initial total cholesterol: verum group 222 mg/dl, placebo group 217 mg/dl). After 15 weeks total cholesterol and triglycerides showed a tendency to decrease in the verum group [Saradeth 1994].

In an open study 8 healthy volunteers (initial total cholesterol, 6.1 mmol/L) took 1 clove of garlic (about 3 g) daily for 16 weeks. Total cholesterol decreased by 21% ($p<0.02$) [Ali 1995]. In another open study, total cholesterol decreased by 15.5% ($p<0.001$) in 30 healthy volunteers who took 10 g of garlic daily for 2 months; no change was observed in an untreated control group [Gadkari 1991].

In a 12-week randomized, double-blind, placebo-controlled trial, 62 healthy volunteers received 920 mg garlic per day (corresponding to 10.8 mg alliin) or placebo. No significant differences in serum lipids (total cholesterol, LDL, HDL) were found. There was a decrease (12 %; p= 0.07) in triacylglycerol concentration [Turner 2004].

In a double-blind, randomized, placebo-controlled, cross-over study in 100 healthy volunteers, 500 mg of garlic (details and daily dosage not given) administered for up to 9 months showed no effect on blood lipid levels [Tanamai 2004].

In a randomized, placebo-controlled study involving healthy volunteers, administration of garlic oil (8.2 mg allyl sulphide daily; n=19) or garlic powder (7.8 mg allicin daily; n=20) for 11 weeks did not lead to changes in plasma lipid parameters when compared to placebo (n=21). A gender-specific analysis revealed that garlic oil exerted significant increases in HDL-cholesterol ($p<0.004$) and reductions in the ratio of total cholesterol:HDL-cholesterol ($p<0.003$) in women [Zhang 2001].

Clinical studies

Antihypertensive effects

Systematic reviews/meta-analyses
In an early meta-analysis of 8 studies, the effect of garlic on blood pressure was evaluated. The overall pooled mean difference in the absolute change from baseline to final measurement of systolic blood pressure was greater (7.7 mmHg) in subjects who were treated with garlic than those treated with placebo; the effect on diastolic blood pressure (5 mmHg) was smaller. The results suggested that garlic powder may have some clinical benefit in mild hypertension [Silagy 1994a].

A systematic review of 45 randomized trials assessing the effects of garlic on several cardiovascular-related factors revealed that inconsistent effects on blood pressure were reported in the studies. The authors noted that conclusions regarding clinical significance were limited by the low quality and short duration of many trials and by the unpredictable release and inadequate definition of active constituents in the study preparations [Ackermann 2001].

A meta-analysis of 10 randomized, controlled trials studying the effect of garlic on blood pressure in both normotensive and hypertensive subjects, suggested that garlic is associated with blood pressure reductions only in patients with an elevated systolic blood pressure [Reinhart 2008].

A systematic review suggested that the contradictory results described in previous meta-analyses assessing the effect of garlic on blood pressure can be attributed to methodological deficiencies. The methodological quality of the selected studies (32 in all, 13 evaluated in previous meta-analyses) was considered to be poor. Only four trials had adequate allocation concealment, no trial reported an intention-to-treat analysis, and the evaluators were blinded in only three of the trials. Futhermore, half of the studies did not report any information regarding the details of blood pressure measurement. All trials fulfilling a predefined cut-off point for quality criteria (methodological and blood pressure measurement) were conducted in normotensive subjects and none of these found garlic to lower blood pressure. For these reasons, the authors conclude that the effect of garlic on blood pressure cannot be ascertained [Simons 2009].

A meta-analysis of 17 randomized controlled trials showed that garlic was superior to controls (placebo in most trials) in reducing blood pressure (BP), especially in hypertensive patients. Pooled analysis showed that garlic intake caused a 3.75-mm Hg reduction (95% CI, -5.04 to -2.45, $p<0.001$) in systolic BP and a 3.39-mm Hg reduction (95% CI, -4.14 to -2.65, $p<0.001$) in diastolic BP compared with controls. Meta-analysis of subgroups showed a significant reduction in systolic BP in hypertensive (-4.4 mm Hg; 95% CI, -7.37 to -1.42, p=0.004) but not normotensive patients. Significant diastolic BP reduction (-2.68 mm Hg, 95% CI, -4.93 to -0.42, p=0.02) was shown in hypertensive patients after sensitivity analysis [Wang 2015].

In a systematic review and meta-analysis of seven randomized, placebo-controlled trials comparing garlic with placebo in hypertensive patients, garlic had a significant lowering effect on both systolic BP (weighted mean difference (WMD): -6.71 mmHg; 95% CI: -12.44 to -0.99; p=0.02) and diastolic BP (WMD: -4.79 mmHg; 95% CI: -6.60 to -2.99; $p<0.00001$) [Xiong 2015].

A meta-analysis of 20 randomized, controlled trials, including 970 participants, found a mean decrease in systolic blood pressure (SBP) of 5.1 ± 2.2 mm Hg ($p<0.001$) and in diastolic blood pressure (DBP) of 2.5 ± 1.6 mm Hg ($p<0.002$) when compared with placebo. A subgroup analysis of hypertensive subjects (SBP/DBP ≥140/90 mm Hg at baseline) revealed a greater significant reduction in SBP of 8.7 ± 2.2 mm Hg ($p<0.001$) and in DBP of 6.1 ± 1.3 mm Hg ($p<0.001$) [Ried 2016].

Clinical trials
In a randomized, double-blind, placebo-controlled study, 52 patients with hypercholesterolaemia and mild systolic hypertension were treated with 900 mg of standardized garlic powder (1.3% alliin) daily for 6 months. Systolic and diastolic blood pressure (initial values: 145 and 90 mmHg) were reduced by 17% ($p<0.001$) and 10% ($p<0.01$), respectively, in the verum group, but remained unchanged in the placebo group ($p<0.001$ for intergroup comparison) [De Santos 1993].

In a randomized study the effects of daily treatment with 600 mg of standardized garlic powder (1.3% alliin, equivalent to 0.6% allicin) were compared to those of 1.98 mg of garlic oil in 80 patients with mild hypercholesterolaemia and mild hypertension. By the end of 4 months of treatment, initial systolic and diastolic blood pressures of 151 and 96 mmHg had decreased by 19 and 17% respectively (both $p<0.001$) in the garlic powder group, whereas no effects were observed in

the garlic oil group (p<0.001 for intergroup comparison) [De Santos 1995].

In a randomized, double-blind, placebo-controlled study the effects of 600 mg of standardized garlic powder (1.3% alliin) daily for 12 weeks were evaluated in 47 patients with mild hypertension. In the verum group, initial supine and standing diastolic blood pressures of 102 and 101 mmHg decreased by 13% and 11% respectively (p<0.05), while in the placebo group initial values of 97 and 94 mmHg decreased by 4% and 0.5% respectively (not significant). Initial systolic blood pressures of 171 and 161 mmHg in the verum and placebo groups respectively decreased by 12% (p<0.05) and 6% (not significant). Intergroup comparisons were not reported [Auer 1990].

In a 12-week randomized, reference-controlled study involving 98 normotensive patients with primary hyperlipoproteinaemia, the effects of daily administration of 900 mg of garlic powder (1.3% alliin) or 600 mg of bezafibrate were compared. Systolic blood pressures of 143.4 mmHg in the garlic group and 140.6 mmHg in the bezafibrate group were reduced by 5.5% and 2%, respectively (p<0.05 in favour of garlic). Initial diastolic blood pressures of 82.8 and 82.4 mmHg, respectively decreased by 5% in both groups [Holzgartner 1992].

In a randomized, double-blind, placebo-controlled study 40 patients with moderate hypertension (systolic blood pressure, 178 mmHg; diastolic, 100 mmHg) undergoing concomitant diuretic treatment with triamterene and hydrochlorothiazide received 600 mg of standardized garlic powder (1.3% alliin) or placebo daily. After 12 weeks systolic blood pressure had decreased by 9% in the verum group compared to 3% in the placebo group (p<0.001), while diastolic pressure had decreased by 15% in the verum group compared to 9% in the placebo group (p<0.001) [Kandziora 1988].

In a randomized, double-blind study 40 patients with hypercholesterolaemia and slightly elevated blood pressures received 900 mg of standardized garlic powder (1.3% alliin) or placebo daily. After 16 weeks, supine systolic and diastolic blood pressures were significantly lowered in the verum group (p<0.001 and p<0.05, respectively) compared to the placebo group [Vorberg 1990].

In a randomized, double-blind study 80 normotensive patients with peripheral arterial occlusive disease received 800 mg of standardized garlic powder (1.3% alliin) or placebo daily. After 12 weeks of therapy the initial diastolic blood pressure of 85 mmHg in the verum group had decreased by 3.5% (p<0.04), while that in the placebo group (initially 83 mmHg) remained unchanged [Kiesewetter 1993b].

In a randomized, double-blind study 42 patients with mild hypercholesterolaemia were treated with 900 mg of standardized garlic powder (1.3% alliin) or placebo daily for 12 weeks. At the end of the study systolic and diastolic blood pressures remained unchanged [Jain 1993].

In an open study, 20 patients with mild essential hypertension received two 250 mg garlic oil capsules (2.5% w/v, not clearly specified) daily for 8 weeks. A significant (p<0.05) decline in mean systolic (148±12 to 140±16 mmHg) and diastolic (94±15 to 85±23 mmHg) blood pressure was observed compared to baseline. Furthermore, significant (p<0.05) reductions in 8-hydroxy-2´-deoxyguanosine and 8-iso-prostaglandin F2α (biomarkers of oxidative stress), plasma oxidized LDL, NO levels and lipid peroxidation were observed, as well as a significant (p<0.05) increase in vitamin A, C and E levels and total antioxidant status, when compared to baseline levels [Dhawan 2004, 2005].

An acute dose study involving 75 patients with hypertension showed no significant effects on blood pressure in any of the three patient groups: placebo; 1 clove of garlic (slightly chewed and swallowed), or 12 tablets of garlic (slightly chewed and swallowed; 2.4 mg allicin) for up to an hour after consumption [Capraz 2007].

In an open study involving 50 type 2 diabetic patients with hyperlipidaemia (total cholesterol ≥220 mg/dl), administration of 900 mg garlic powder daily for 6 weeks resulted in a reduction of systolic blood pressure (p<0.03) [Parastouei 2006].

In an open study, 70 hypertensive patients received an oily garlic preparation (1.6 g garlic macerate 1:1; 1.6 mg allicin derivatives) in addition to standard antihypertensive treatment for 30 days. There were no significant changes in systolic and diastolic blood pressure. A gender-specific analysis revealed a trend for better effects in women as compared to men [Duda 2008].

A placebo-controlled study involving 100 women in their third trimester of pregnancy was conducted to evaluate whether garlic (800 mg powdered garlic with 1 mg allicin daily for 8 weeks) could prevent pre-eclampsia. No difference between the garlic and control groups in the relative risk of gestational hypertension or pre-eclampsia was observed [Ziaei 2001].

In a double-blind, placebo-controlled trial, patients with mild or moderate hypertension (systolic blood pressure 150-160 mmHg, diastolic blood pressure 90-115 mmHg) received 600 mg garlic powder daily (n=30) or placebo (n=20) for 8 weeks after an 8 week placebo-run-in phase. As compared to the baseline values (after the placebo run-in-phase), garlic significantly reduced systolic and diastolic blood pressures by 7.0 mmHg and 3.8 mmHg respectively (p<0.001). There was also a significant (p<0.001) difference compared to the placebo group [Sobenin 2009a].

In a single-blind, placebo controlled, multicentre study, patients with stage 1 essential hypertension (30 per group) received garlic tablets (not further specified) for a period of 24 weeks at daily doses of 300, 600, 900, 1200 or 1500 mg, atenolol (100 mg) or placebo. Garlic demonstrated dose- and duration-dependent significant (p<0.05) reductions in systolic and diastolic blood pressure when compared to placebo; the activity was comparable with atenolol at doses of 600 mg and above at 24 weeks [Ashraf 2013].

Rheological and antiatherosclerotic effects

Systematic reviews/meta-analyses

A systematic review of 45 randomized trials summarized the effects of garlic on several cardiovascular-related factors. The relevant trials reported modest but significant reductions in platelet aggregation with garlic treatment (without additional anti-platelet medication) compared with placebo. The authors noted that conclusions regarding clinical significance were limited by the low quality and short duration of many trials and by the unpredictable release and inadequate definition of active constituents in the study preparations [Ackermann 2001].

Clinical trials

In a randomized, double-blind study, 60 young patients (average age 24 years) with constantly elevated spontaneous platelet aggregation and at increased risk of ischaemic attack were treated with 800 mg of standardized garlic powder (1.3% alliin) or placebo daily for 4 weeks. In the garlic group the ratio of

circulating platelet aggregation decreased by 10.3% (p<0.01; placebo group unchanged) and spontaneous platelet aggregation decreased by 56.3% (p<0.01; placebo group unchanged) [Kiesewetter 1991, 1993a].

In a double-blind study 80 patients with peripheral arterial occlusive disease received 800 mg of standardized garlic powder (1.3% alliin) or placebo daily for 12 weeks. In the garlic group plasma viscosity decreased by 3.6% (p<0.0013) and spontaneous platelet aggregation dropped by 59% (p<0.01) while these parameters were unchanged in the placebo group [Kiesewetter 1993b].

In a randomized, double-blind study, 23 patients with coronary arterial disease received 900 mg of standardized garlic powder (1.3% alliin) or placebo daily for 3 weeks. The cholesterol content per mg of cell protein decreased from 40 to 28 µg, the LDL oxidation lag-time was prolonged from 133 to 176 min and the LDL-sialic acid content increased from 22 to 33 nmol/mg lipoprotein. In the control group these parameters remained unchanged [Orekhov 1996].

In an open study 20 patients with hypercholesterolaemia were treated daily for 4 weeks with 600 mg of dried garlic. Fibrinogen decreased by 10% (p<0.01) and fibrinopeptide A by 45% (p<0.01); streptokinase-activated plasminogen increased by 15% (p<0.01) and fibrinopeptide Bβ 15-42 by about 42% (p<0.01) [Harenberg 1988].

Lipid-lowering effects

Systematic reviews/meta-analyses

The effect of garlic on total serum cholesterol has been assessed in 9 meta-analyses [Warshafsky 1993; Silagy 1994b; Neil 1996; Stevinson 2000; Ackermann 2001; Reinhart 2009; Khoo 2009; Zeng 2012; Ried 2013]. In most of them it was concluded that the available data showed garlic to be superior to placebo in reducing total cholesterol; however, only a modest effect was indicated from one meta-analysis [Stevinson 2000] and no benefit on serum cholesterol was found in one meta-analysis [Khoo 2009].

A systematic review of 45 randomized trials summarized the effects of garlic on several cardiovascular risk-related factors. When compared with placebo, garlic preparations led to small reductions in the total cholesterol level at 1 month (range of average pooled reductions, 0.03-0.45 mmol/l [1.2-17.3 mg/dL]) and at 3 months (range of average pooled reductions 0.32-0.66 mmol/l [12.4-25.4 mg/dL]), but not at 6 months. Changes in the levels of LDL and triglycerides paralleled those of total cholesterol, while there were no significant changes in HDL levels observed. The authors noted that conclusions regarding clinical significance were limited by the low quality and short duration of many trials and by the unpredictable release and inadequate definition of active constituents in the study preparations [Ackermann 2001].

A meta-analysis of 13 trials (11-24 weeks in duration) including 1056 healthy or hypercholesterolaemic subjects showed that administration of garlic did not have any significant effects on lipid profile compared with placebo [Khoo 2009].

A meta-analysis of 29 trials studying garlic preparations revealed a significant reduction of total cholesterol (by 0.19 mmol/l (95% CI -0.33, -0.06); = 7.35 mg/dl) and triglycerides (by 0.11 mmol/l (95% CI -0.19, -0.03); = 9.7 mg/dl) as compared to placebo, but exhibited no significant effects on LDL or HDL [Reinhart 2009].

A meta-analysis of 26 randomized, double-blind, placebo-controlled trials concluded that garlic was superior to placebo in reducing serum total cholesterol (TC) and triglyceride levels. Compared with placebo, TC levels were significantly (p=0.001) reduced by 0.28 mmol/L (95% CI, -0.45, -0.11) and triglyceride level significantly (p<0.001) reduced by 0.13 mmol/L (95% CI, -0.20, -0.06) in the garlic group. The effects of garlic were more pronounced with long-term intervention and in subjects with higher baseline cholesterol levels. Garlic powder was more effective in reducing serum cholesterol levels, while garlic oil was more effective in lowering serum triglyceride levels. However, garlic did not influence other lipid parameters, including LDL, HDL, apolipoprotein B and TC/HDL ratio [Zeng 2012].

A meta-analysis of 39 trials investigating the effect of garlic preparations on serum lipids revealed that garlic reduced total serum cholesterol compared with placebo by 15.25 mg/dL (95% CI, -20.72, -9.78; p<0.0001). The effect was stronger in individuals with elevated total cholesterol levels (>200 mg/dL) when garlic was used for more than 2 months (-17.20 mg/dL; 95% CI, -23.10, -11.30; p<0.0001). Similar results were also seen for LDL, while HDL levels improved only slightly, and triglycerides were not influenced significantly [Ried 2013].

Comparative studies

The lipid-lowering effect of 900 mg of garlic powder (standardized to 1.3% alliin) daily (G) was compared to that of 600 mg of bezafibrate daily (B) in 98 patients with hyperlipoproteinaemia. After 12 weeks of treatment, both groups showed significant reductions in total cholesterol (G 25%, B 27%; p<0.001) and LDL (G 32%, B 33%; p<0.001) as well as an increase in HDL (G 51%, B 58%; p<0.001). No significant differences were found between the two groups [Holzgartner 1992].

In a randomized study the effects of daily treatment with 600 mg of standardized garlic powder (1.3% alliin) or 1.98 mg of garlic oil were compared in 80 patients with mild hypercholesterolaemia. After 4 months of treatment total cholesterol had decreased by 11% in the garlic powder group (p<0.001) and by 3% in the garlic oil group. LDL was reduced by 16% in the garlic powder group (p<0.001) and by 1% in the garlic oil group (intergroup comparison: total cholesterol, not significant; LDL, p<0.001) [De Santos 1995].

In a randomized study involving 20 hyperlipidaemic patients (serum cholesterol and triglyceride levels > 200 and 280 mg/dl respectively), the effects of garlic powder compared with clofibrate were investigated. Each patient took garlic at a dose of 900 mg (equivalent to 2.7 g of fresh garlic and 11.7 mg alliin) or 100 mg clofibrate daily for 8 weeks. After 4 and 8 weeks, total cholesterol was reduced with garlic by 9.8 and 17.5% and with clofibrate by 3.0 and 7.2%, respectively. Triglyceride levels were reduced by 34.8 and 43.7% with garlic and by 21.3 and 25.5% with clofibrate. Garlic demonstrated a significantly (p<0.01) greater effect than the clofibrate [Nouri 2008].

Placebo-controlled studies

A placebo-controlled, randomized study evaluated the effects of fresh or powdered garlic on plasma lipid concentrations in adults with moderate hypercholesterolaemia (LDL 3.36-4.91 mmol/L). The daily dosages of 4 g fresh garlic (n=49), 1.4 g powdered garlic (n=47) or placebo (n=48) were administered 6 days a week for 6 months. There were no statistically significant effects on the plasma lipids [Gardner 2007].

In a double-blind, randomized, placebo-controlled trial, the effects of a daily dose of 2.1 g garlic powder on biomarkers for inflammation, endothelial function and lipid metabolism were studied in 90 overweight subjects with cardiovascular risk factors (BMI >24.5 kg/m², >10 cigarettes/d, 40-75 years of age), in comparison to atorvastatin. The subjects were randomly

assigned to 3 parallel treatment groups: garlic powder (2.1 g/d), atorvastatin (40 mg/d), or placebo. In contrast to atorvastatin the garlic preparation had no effect on the variables as compared to placebo [Van 2006].

In a randomized, double-blind, placebo-controlled study the effect of standardized garlic powder tablets (1.3% alliin) was investigated in 261 patients with hyperlipidaemia (initial cholesterol: verum 266, placebo 262 mg/dl). The patients received 800 mg of garlic powder or placebo daily for 16 weeks. In the garlic group mean serum total cholesterol levels dropped by 12% ($p<0.001$) and triglyceride values by 17% ($p<0.001$); both results were significant ($p<0.001$) compared to placebo treatment. A subgroup analysis revealed better results in patients with increased initial lipid levels [Mader 1990].

In a randomized, double-blind, placebo-controlled study, 42 patients with mild hypercholesterolaemia (serum total cholesterol: verum 262, placebo 276 mg/dl) were treated with 900 mg of standardized garlic powder (1.3% alliin) or placebo daily for 12 weeks. In the garlic group serum total cholesterol decreased by 6% ($p<0.01$ vs. baseline and placebo) compared to 1% in the placebo group and LDL by 11% ($p<0.05$ vs. baseline and placebo) compared to 3% in the placebo group [Jain 1993].

In a randomized, double-blind, placebo-controlled study, 46 patients with mild hypercholesterolaemia (6.5 mmol/dl) were assigned to one of four daily treatments: group I, 900 mg of standardized garlic powder (1.3% alliin); group II, 12 g of fish oil; group III, 12 g of fish oil + 900 mg of standardized garlic powder; group IV, placebo. After 3 weeks of treatment the changes in serum total cholesterol and LDL, respectively, were: group I, -11.5% and -14.2%; group II, 0% and + 8.8%; group III, -12.2% and -9.5%; group IV, +4.5% and -1.4%. The differences in groups I and III were significant ($p<0.01$) compared to those in groups II and IV [Adler 1997].

In a randomized, double-blind study 52 patients with hypercholesterolaemia were treated with 900 mg of standardized garlic powder (1.3% alliin) or placebo daily for 6 months. Baseline serum total cholesterol levels were 6.92 mmol/litre in the garlic group and 7.05 mmol/litre in the placebo group. Serum total cholesterol and LDL, respectively, decreased by 9% ($p<0.01$ vs. baseline, $p<0.05$ vs. placebo) and 10% ($p<0.05$ vs. baseline) in the verum group and by 4% and 6% in the placebo group [De Santos 1993].

In a randomized, double-blind study 40 patients with moderate hypertension (blood pressure 178/100 mmHg) and hypercholesterolaemia (280 mg/dl) received 600 mg of standardized garlic powder (1.3% alliin) or placebo daily. After 12 weeks serum total cholesterol had decreased by 10% ($p<0.05$) and triglycerides by 8% ($p<0.05$); no changes were observed in the placebo group [Kandziora 1988].

In a randomized, double-blind study, 40 patients with hypercholesterolaemia received 900 mg of standardized garlic powder (1.3% alliin) or placebo daily. After 16 weeks of treatment, serum total cholesterol had decreased by 21% in the verum group and 3% in the placebo group (intergroup difference $p<0.001$). Serum triglycerides decreased by 24% in the verum group and 3% in the placebo group ($p<0.05$) [Vorberg 1990].

In a randomized, double-blind study 23 patients with coronary artery disease (determined by selective coronary angiography) received 900 mg of standardized garlic powder (1.3% alliin) or placebo daily. Initial serum total cholesterol levels were 218 mg/dl in the verum group and 219 mg/dl in the placebo group. After 3 weeks of treatment HDL had increased by 16% in the verum group ($p<0.05$); total cholesterol and triglycerides remained unchanged [Orekhov 1996].

In a randomized, double-blind study, 80 patients with peripheral arterial occlusive disease (determined by angiography) were assigned to treatment with 800 mg of standardized garlic powder (1.3% alliin) or placebo daily for 12 weeks. Initial serum total cholesterol levels of 266.5 and 264.4 mg/dl in the verum and placebo groups, respectively, decreased by 12% and 4% (intergroup comparison $p<0.011$) [Kiesewetter 1993b].

In a randomized, double-blind study the effects of 600 mg of standardized garlic powder (1.3% alliin) or placebo daily for 12 weeks were compared in 47 patients with mild hypertension. The baseline serum total cholesterol level of 268 mg/dl decreased by 14% in the garlic group ($p<0.05$ vs. baseline) and 6% in the placebo group (not significant). Serum triglycerides decreased by 18% in the garlic group ($p<0.05$) and 6% in the placebo group (not significant) [Auer 1990].

In a single-blind, placebo-controlled trial, administration of garlic tablets (not further specified) at 900 mg per day for 3 months to hyperlipidaemic patients (n=55) significantly ($p<0.001$) reduced total cholesterol by 9.37%, triglycerides by 1.30%, LDL by 17.59% and increased HDL-cholesterol by 5.54%, as compared to baseline values. The placebo hyperlipidaemic patient group (n=51) did not demonstrate significant changes in any of the tested parameters [Fatima 2014].

In a randomized, double-blind, placebo-controlled study 51 patients with coronary heart disease were treated with 300 mg time-released garlic powder tablets or placebo daily for 12 months. There were significant decreases in total cholesterol (-12.4 %; $p=0.004$) and LDL (-16.3%; $p=0.001$) as compared to baseline. The cholesterol lowering effects were significantly ($p=0.038$) greater for garlic than placebo. The most significant effects were observed in men [Sobenin 2009b].

Placebo-controlled studies in combination with defined diet
In a single-blind, randomized, placebo-controlled study the effects of garlic were studied in hyperlipidaemic patients (total cholesterol ≥200 mg/dl and/or LDL ≥100 mg/dl). Patients were given a garlic powder tablet (400 mg garlic,1 mg allicin; n=50) or placebo (n=50) twice daily for 6 weeks. All patients were put on the NCEP step II diet (not more than 7% of calories from saturated fat, not more than 30% of calories from total fat and less than 200 mg of cholesterol per day). Garlic had significant ($p<0.001$) effects on total cholesterol (12.1% reduction), LDL (17.3% reduction) and HDL (15.7 % increase), but no significant effect on triglycerides [Kojuri 2007].

After 8 weeks on an NCEP Step I diet (8-10% of calories from saturated fat, not more than 30% of calories from total fat and less than 300 mg of cholesterol per day), the effects of 900 mg of standardized garlic powder (1.3% alliin) or placebo daily for 12 weeks were compared in a randomized, double-blind study in 50 patients with hyperlipidaemia (initial serum total cholesterol: garlic group 274 mg/dl, placebo group 250 mg/dl). The diet was maintained during the course of study. There were no significant changes to lipid levels in either group and no intergroup differences were observed for serum total cholesterol or LDL [Isaacson 1998].

Over 1200 patients with hypercholesterolaemia embarked on an NCEP Step I diet. After 6 weeks, 115 patients who had not responded to the diet (serum total cholesterol 6.0-8.0 mmol/dL) maintained the diet and were enrolled in a randomized, double-blind study, assigned to 900 mg of standardized garlic powder (1.3% alliin) or placebo daily for 6 months. The baseline

serum total cholesterol levels were 6.96 and 6.99 mmol/litre in the garlic and placebo groups respectively. At the end of this study there were no intra- nor inter-group differences in lipid parameters [Neil 1996].

In a randomized, double-blind, crossover study (2 × 12 weeks, with a wash-out period of 4 weeks) 30 patients with hypercholesterolaemia (serum total cholesterol 6.72 mmol/litre) were treated with 900 mg of standardized garlic powder (1.3% alliin) or placebo daily. All patients maintained an NCEP Step I diet during the study. No effects on serum lipids were observed [Simons 1995].

After following an NCEP Step II diet for 6 months, 30 children with primary familial hypercholesterolaemia participated in a randomized, double-blind study and were assigned to 900 mg of standardized garlic powder (1.3% alliin) or placebo daily for 8 weeks. Baseline serum total cholesterol levels were 6.66 and 7.06 mmol/litre in the garlic and placebo groups respectively. No effects on serum lipid levels were observed except for a 10% increase in apolipoprotein A-I in the garlic group ($p = 0.03$) [McCrindle 1998].

In a randomized, double-blind study, 35 renal transplant patients with serum total cholesterol levels of 290 mg/dl followed an NCEP Step I diet and were also treated with 1.36 g of an undefined garlic preparation (equivalent to 4 mg of allicin daily) or placebo daily for 12 weeks. After 6 and 12 weeks of treatment serum total cholesterol decreased by 5% in the garlic group ($p<0.05$) while serum levels in the placebo group remained unchanged. Serum triglycerides increased by 7.5% (not significant) and HDL decreased by 5% (not significant) in the garlic group, while in the placebo group these parameters changed by +17.3% ($p<0.05$) and -6% ($p<0.05$) respectively [Lash 1998].

After following an NCEP Step I diet for at least 2 weeks (and maintaining it during the course of study), 50 patients with moderate hypercholesterolaemia (LDL subclass pattern A or B; mean serum LDL 166 mg/dl) participated in a randomized, double-blind study, taking 900 mg of standardized garlic powder (1.3% alliin) or placebo daily for 3 months. At the end of the study no significant changes were observed in lipid parameters [Superko 2000].

In a 16 week double-blind, placebo-controlled study in 33 patients with primary hypercholesterolaemia (LDL 130-190 mg/dl after at least 4 weeks of generalized dietary modification), the effects of garlic powder (22.4 mg alliin corresponding to approx. 10 mg allicin daily) on lipid profile and various mental parameters (depression, impulsiveness, hostility and temperament) were compared with placebo. Both groups received individual dietary counseling (NCEP Step I diet). No significant effects on lipid and mental parameters were observed within or between the groups [Peleg 2003].

In a randomized, double-blind, placebo-controlled trial 136 hypercholesterolaemic patients (cholesterol concentrations ≥ 5.2 mmol/L resp. >200 mg/dl) received garlic (333 mg garlic preparation equivalent to 7 g fresh garlic, standardized to 5.6 mg allicin; n=70) or placebo (n=66) for 12 weeks. All patients were given advice to follow NCEP Step I diet for 4 weeks prior to randomization and during the study. There were no statistically significant changes in serum lipid parameters or in plasma glucose, liver or renal functions [Satitvipawee 2003].

Open studies

Patients with hyperlipoproteinaemia type IV (n = 5) and hypercholesterolaemia (n = 15) were treated daily for 4 weeks with 600 mg of garlic powder. The initial mean serum total cholesterol of 278 mg/dl decreased by 7.2% ($p<0.01$) [Harenberg 1988].

Patients with hyperlipidaemia (n = 44; serum total cholesterol 298 mg/dl, triglycerides > 200 mg/dl) were treated with 1.2 g of standardized garlic powder (1.3% alliin) for 18 weeks. Serum total cholesterol decreased by 11.3% ($p<0.001$), LDL by 15.7% ($p = 0.003$) and triglycerides by 13%; HDL increased by 19.9% [Rinneberg 1989].

Patients with hyperlipidaemia (n=1024; initial serum total cholesterol 297 mg/dl) took 600-900 mg of standardized garlic powder (corresponding to 3-6 mg of allicin) for 16 weeks. Serum total cholesterol decreased by 15.8%, LDL by 13.3% and triglycerides by 13.7%; HDL increased by 14.3% [Brewitt 1991].

Patients with hyperlipidaemia (n = 1873; serum total cholesterol > 200 mg/dl or elevated triglycerides) were treated with 900 mg of standardized garlic powder (1.3% alliin) for 16 weeks. Initial average serum total cholesterol of 290 mg/dl decreased by 16.4% ($p<0.0001$), LDL by 15.1% ($p<0.0001$) and triglycerides by 21% ($p<0.0001$); HDL increased by 9.4% ($p<0.0001$) [Beck 1993].

Patients with hypercholesterolaemia (n=917; serum total cholesterol 262 mg/dl), following a cholesterol and fat-reduced diet, were treated with 600 mg of standardized garlic powder (1.3% alliin) daily for 6-8 weeks. Serum total cholesterol decreased by 2.3% ($p<0.01$) [Walper 1994].

In type 2 diabetic patients with hyperlipidaemia (n=50; total cholesterol ≥ 220 mg/dL), the administration of 900 mg garlic powder daily for 6 weeks resulted in a significant reduction of total cholesterol ($p<0.01$) and LDL ($p<0.001$), and a significant increase in HDL ($p<0.02$). There were no significant effects on diastolic blood pressure, fasting blood sugar, serum triglycerides and HbA1c [Parastouei 2006].

Hypertensive patients (n=70) received 6 capsules of an oily garlic preparation daily, in addition to standard antihypertensive medications, for 30 days. Each capsule contained 270 mg garlic macerate in rapeseed oil (1:1) with 0.27 mg of allicin derivatives standardized for allicin. Total cholesterol and LDL were significantly ($p<0.05$) reduced, while HDL increased nonsignificantly. There was also a slight increase of 7 to 10% in the proportion of patients with normal total serum cholesterol levels at the end of the study [Duda 2008].

Prophylaxis of atherosclerosis

Patients homogenous in all demographic data except age (verum > placebo, $p<0.001$), with advanced atherosclerotic plaques (measured by ultrasound in the carotid bifurcation and/or femoral arteries) and at least one risk factor (high systolic blood pressure, hypercholesterolaemia, diabetes mellitus, smoking), participated in a randomized, double-blind, placebo-controlled study. They were assigned to treatment with 900 mg of standardized garlic powder (1.3% alliin; n = 140) or placebo (n = 140) daily for 48 months; 152 patients (61 in the garlic group and 91 in the placebo group) completed the study; 38% of patients in the verum group dropped out because of annoyance with odour. In the remaining patients plaque volume decreased by 2.6% in the verum group and increased by 15.6% in the placebo group. The strongest effects (in both directions) were observed in women. There was a strong correlation ($p<0.0001$) of inhibition of the increase in plaque volume with increasing age [Koscielny 1999].

In a 3-year randomized study, 432 patients with a history of myocardial infarction were assigned to an oily extract of garlic (n = 222) or placebo (n = 210) daily, in addition to other necessary

medication. Significant reductions in the non-fatal reinfarction rate (44%; p<0.001) and mortality (31%; p<0.01) were observed in patients taking the oily extract of garlic compared to those taking placebo [Bordia 1989].

Respiratory Tract Infections / Common cold
A systematic review revealed insufficient evidence for the use of garlic in preventing or treating the common cold. Of the eight studies identified, only one (Josling 2001) met the inclusion criteria and suggested that garlic may prevent the common cold [Lissiman 2014].

In a randomized, double-blind, placebo-controlled study, a daily dose of 180 mg of stabilized allicin or placebo were given to 146 volunteers for 12 weeks. There were significantly (p<0.001) fewer occurrences of common cold in the garlic group (24) than in the placebo group (65). Symptoms lasted significantly (p<0.001) longer in the placebo group (5.01 days) compared to the garlic group (1.52 days) and there were fewer days of illness in the garlic group (n=111) compared with the placebo group (n=366) [Josling 2001].

In a randomized, double-blind, placebo-controlled, 5-month trial, garlic tablets (300 mg/day; not further specified; n=42) reduced acute respiratory disease morbidity 1.7-fold in children aged 10 to 12 years compared to placebo (n=41) [Andrianova 2003].

Other clinical effects
Other clinical studies of garlic preparations have reported antimycotic and antibacterial effects, such as in women with bacterial vaginosis [Ledezma 2000; Sabitha 2005; Mohammadzadeh 2014].

In a randomized, placebo-controlled study involving 41 patients suffering from hepatopulmonary syndrome, the effect of garlic oil capsules (n=21) was compared to placebo (n=20) over a period of 9 to 18 months. Each garlic capsule contained 250 mg oil and the mean daily garlic dose was 1.55±0.29 g. After 9 months there was a significant (p<0.001) increase in baseline arterial oxygen levels of 24.66% in the garlic group compared to only 7.37% in the placebo group (p=0.02), and a significant (p<0.001) decrease in the alveolar-arterial oxygen gradient in the garlic group (28.35%) compared to placebo (10.73%; p=0.12). The arterial oxygen level and alveolar-arterial oxygen level were also significantly (p<0.001) higher and lower, respectively, in the garlic group compared to the placebo group. Two of 21 patients in the garlic group and 7 of 20 patients in the placebo group died during the follow-up period of up to 18 months [De 2010].

In a randomized, double-blind, placebo-controlled trial, 40 women with metabolic syndrome according to NCEP ATPIII (Adult Treatment Panel III) criteria, and 10 healthy female controls, were treated with 1.8 g garlic tablets per day or placebo for 6 weeks. Compared to placebo, the garlic treatment resulted in a significantly lower weight (p=0.04) and waist circumference (p<0.001) in the women with metabolic syndrome, while no effect was seen in healthy women [Sharifi 2010].

In an open, two phase study involving 30 patients with type-II diabetes, both dry powdered garlic and a dried aqueous extract (dried filtrate of an aqueous macerate), at a dose of 45 mg/kg/d orally for 14 days, significantly (p<0.05) decreased blood glucose levels in patients taking no other medications as well as in patients taking oral hypoglycaemics but with an inadequate control of blood glucose. Lower doses (20 and 30 mg/kg/d) had no significant effect. Glycosuria, found only in the patients not also taking oral hypoglycaemics, was reversed by both garlic preparations at the 45 mg/kg/d dose. There was no significant activity found in a healthy control group (n=15) for either garlic preparation [Waheed 2014].

Various epidemiologic studies have found a negative correlation between the intake or use of garlic and the incidence of cancer [Gao 1999; Munoz 2001; Setiawan 2005; Galeone 2006; Galeone 2007; Zhou 2013; Galeone et al. 2015; Kodali 2015]. A systematic review found 4 randomized controlled trials on the effectiveness of garlic extract for reducing cancer risk. The analyzed studies were of mixed quality, and the findings from these trials were not uniformly positive [Ernst 2012].

In a randomized, placebo-controlled, double-blind trial, the preventive effect of garlic against nosocomial infections was studied in 94 patients in intensive care units. A powdered and dissolved garlic tablet (400 mg/day) or placebo were administered by gavage through nasogastric intubation for 6 days. There were no cases of venous catheter infection in the garlic group and the frequency of positive venous catheter tip cultures was significantly (p=0.03) higher in the placebo group than in the garlic group. There was no significant difference in urinary catheter cultures between the groups [Madineh 2017].

Pharmacokinetic properties
The absorption and excretion of orally administered radio-actively-labelled 35S-alliin, allicin and vinyldithiine were studied in rats. Alliin was absorbed and eliminated much faster than the other compounds. After administration of ^{35}S-alliin at 8 mg/kg b.w. maximum blood concentration was reached after 10 minutes (allicin 30-60 minutes, vinyldithiine 120 minutes). The excretion of alliin equivalents was mainly renal [Lachmann 1991].

The metabolism of allicin was analyzed in perfusion experiments using isolated rat liver. Allicin could be identified after passage through the liver only after administration of high doses (1.2 mg allicin/minute). After administration of 400 µg allicin/minute 95% was metabolized after the first hepatic passage; in this case allicin metabolites such as diallyl disulphide and allyl mercaptan were detected in the liver and gall bladder [Egen-Schwind 1991].

S-allylcysteine orally administered to rats, mice and dogs was rapidly and easily absorbed, and distributed mainly in plasma, liver and kidney; the bioavailability was 98.2% in rats, 103.0% in mice and 87.2% in dogs. It was mainly excreted in the urine as the N-acetyl metabolite in rats, while mice excreted both unchanged and N-acetyl forms [Nagae 1994].

Interactions in animals
Pharmacokinetic interactions between orally administered metformin (320 mg/kg b.w.) and garlic (500 mg/kg b.w.) were studied in rats. Compared to metformin alone, administration of the garlic with metformin for 8 days produced a significant (p-value not given) increase in the C_{max} and AUC_{0-12h}, as well as a slightly higher $t_{1/2}$, of metformin. Furthermore, blood glucose was decreased more with the combination than with metformin alone [Chourey 2011].

Oral pre-treatment of rats with a garlic homogenate (250 mg/kg b.w.) for 30 days enhanced the bioavailabilty of a single oral dose of propranolol (10 mg/kg b.w.). Significant increases in the half-life (p<0.001) and C_{max} (p<0.05) were demonstrated, as well as a significant (p<0.001) reduction in clearance, when compared to propranolol given alone [Asdaq 2011].

Interactions in humans
An increased INR in 2 patients on warfarin was attributed to garlic products as there were no other changes of medication

or habits (no further data) [Sunter 1991]. A decreased INR was reported in a patient on fluindione with concurrent use of garlic (600 mg, no further details) for 12 days [Pathak 2003].

A preparation of garlic (equivalent to approx. 8 g/day; not further defined) was given to 10 healthy volunteers on study days 5-25. Saquinavir was administered on days 1-4, 22-25 and 36-39, and blood samples were obtained on study days 4, 25 and 39. At day 25 as compared to day 4, the mean area under the curve (AUC) of saquinavir was reduced by 51%, the trough levels by 49% and the mean maximum concentrations (C_{max}) by 54%. After a 10-day washout period from garlic (day 39), the AUC, trough, and C_{max} values returned to 60-70% of their values at baseline. However, the decrease was only seen in 6 of the patients; in 3 of the patients an increase was observed. A control group as well as information on nutrition which could influence the bioavailability of saquinavir are missing [Piscitelli 2002].

A garlic preparation (equivalent to 1 g fresh garlic daily), administered for 4 days to 10 volunteers, decreased the $AUC_{0-\infty}$ of a single dose of 400 mg ritonavir by 17% and peak plasma concentration by 1%. The authors concluded that the results should not be extrapolated to steady-state conditions [Gallicano 2003].

Another study in healthy volunteers revealed no significant effects on pharmacokinetic parameters of probe substrates of the CYP2D6 (dextromethorphan) or CYP3A4 (alprazolam) pathway of metabolism after administration of 1800 mg of a garlic extract (1800 µg "potential" allicin) twice daily for 14 days [Markowitz 2003].

The effect of dried garlic powder on blood pressure was studied in a 12-week randomized, double-blind, placebo-controlled trial in 62 healthy volunteers assigned to garlic (920 mg daily, corresponding to 10.8 mg alliin) or placebo. No significant differences in blood pressure were measured between the groups [Turner 2004].

In an open study, 70 hypertensive patients received an oily garlic preparation (1.6 g garlic macerate 1:1; 1.6 mg allicin derivatives) in addition to standard antihypertensive pharmacotherapy for 30 days. A significantly lower level of lipid peroxidation products reacting with thiobarbituric acid (from 4.9±0.9 to 2.8±0.9 µmol/l, $p<0.05$), as compared to the initial values, was measured in the blood [Duda 2008].

In an open pilot study involving 13 elderly healthy adults, daily consumption of garlic (0.1 g/kg b.w.) for one month resulted in significantly lower plasma and erythrocyte malondialdehyde levels (from 381.7±39.8 to 352.2±38.4 nmol/ml, $p<0.05$) and increased activities of the antioxidant enzymes glutathione peroxidase (from 8.34±1.07 to 9.32±0.78 IU/ml) and SOD (from 1,668±495 to 2,065±298 IU/ml, $p<0.05$) as compared to the initial values [Avci 2008].

Preclinical safety data

Acute toxicity
The LD_{50} of an aqueous extract of garlic applied s.c. to rabbits (3 per group) was 3034 mg/kg b.w. and the maximum tolerated dose was 2200 mg/kg b.w. At doses of 3200 and 4200 mg/kg b.w. the extract induced behavioural signs such as loss of appetite, depression, partial paralysis and death (2 of 3 animals at 3200 mg/kg b.w.; 3 of 3 animals at 4200 mg/kg b.w.) [Mikail 2010].

Other investigators, using various non-standardized garlic extracts, reported the lowest oral LD_{50} value in rats and mice as 30 ml/kg for a 14.5% ethanolic extract from fresh bulbs [Nagakawa 1984].

The LD_{50} of an aqueous suspension of powdered garlic administered by oral gavage to mice was determined to be 25 g/kg b.w. [Alqasoumi 2012].

Repeated dose toxicity
Fresh garlic juice orally administered to rats at 5 ml/kg b.w. daily for 3 weeks resulted in reduced weight gain, gastric mucosal damage and in some cases death [Nagakawa 1980].

Daily administration of a raw garlic extract to rats at 200 mg/kg b.w. for 4 weeks resulted in weight loss and a drop in serum protein levels [Shashikanth 1986].

An aqueous extract administered by gavage to rats at doses of 1000, 3000 and 5000 mg/kg b.w. for 14 days did not induce mortality. The increased levels of liver enzymes and bilirubin, and reduced levels of urea and creatinine, were non-significant compared to control. Whereas the low and high doses demonstrated a significant ($p<0.005$) increase in white blood cells. Neither gross pathology nor histopathological analysis revealed any adverse effects [Asiedu-Gyekye 2014].

An aqueous suspension of powdered garlic administered by oral gavage to mice for 90 days, at doses of 37.5 and 75 mg/kg b.w., induced mild to moderate autonomic symptoms such as changes of respiration rate, heart rate and hypothermia, in addition to causing itching, alopecia, agressive behavior and excitation [Alqasoumi 2012].

Mutagenicity
The genotoxicity of orally administered garlic was assessed using the micronucleus test in mice; compared to controls no significant changes were found in the bone marrow cells [Abraham 1984]. No mutagenicity was evident in the Ames test [Yoshida 1984].

In the micronucleus test an aqueous suspension of powdered garlic administered to mice by oral gavage at doses of 250 and 500 mg/kg b.w. did not reveal any significant activity as compared to the control [Alqasoumi 2012].

Clinical safety data
Systematic reviews and meta-analyses have concluded that garlic preparations were well tolerated in all trials with minimal side effects [Ried 2013] and that no serious adverse events were reported in any of the included trials [Xiong 2015].

In a 5-month, open study involving 172 children with acute respiratory disease, there was no difference in the prevalence of gastrointestinal side effects between the groups receiving garlic tablets (not further specified), benzimidazole or placebo [Andrianova 2003].

In published studies involving consumption of up to 1.2 g of garlic powder daily, garlic odour was the most common side effect, with an incidence of up to 50%. However, this effect is not considered to be adverse. Gastrointestinal discomfort was a common adverse effect reported and there have been rare cases of allergic reactions [Hughes 2002; Pires 2002; Izzo 2007; Polat 2007].

In healthy volunteers 10 g of raw garlic consumed daily for 2 months induced no adverse events [Gadkari 1991].

Daily administration of high doses of garlic oil (approx. 120 mg, equivalent to 60 g/day fresh garlic) over a period of 3 months did not result in any toxic effects or adverse events [Bordia 1978].

In 4 cases reduced platelet aggregation and prolonged bleeding time have been reported after intake of raw garlic or garlic powder [Rose et al. 1990; Burnham 1995; German 1995; Carden 2002; Woodbury & Sniecinski 2016], and in one further case also in combination with acetyl salicylic acid and milk thistle tablets (no further details) [Shakeel 2010].

In an 8 week, randomized, single-blind, placebo-controlled study involving 100 women in the third trimester of pregnancy, given either garlic (n=50; 800 mg garlic powder per day, 2 mg allicin) or placebo (n=50), no serious side effects were reported. A slight feeling of nausea was reported in 8 of the garlic group patients and 2 of the placebo patients. An "odour due to garlic" was reported by 34% of the garlic group and 4% of the placebo group [Ziaei 2001].

In a case report, consumption of approx. 6 stir-fried garlic cloves three times per week was associated with HIV virologic failure during treatment with boosted atazanavir [Duncan 2013].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Online Series, 2019
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHAMOMILLAE ROMANAE FLOS	Roman Chamomile Flower	Online Series, 2019
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Online Series, 2018

JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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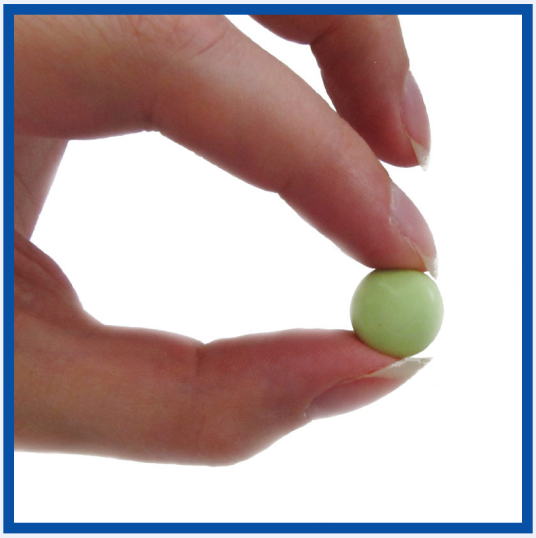
Barbados Aloes

2014



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Herbal Medicinal Products

ALOE BARBADENSIS
Barbados Aloes

2014

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

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Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

ALOE BARBADENSIS

2014

Barbados Aloes

DEFINITION

Barbados aloes[1] consists of the concentrated and dried juice of the leaves of *Aloe barbadensis* Miller. It contains not less than 28.0 per cent of hydroxyanthracene derivatives, expressed as barbaloin ($C_{21}H_{22}O_9$; M_r 418.4) and calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [EP].

CONSTITUENTS

The main active constituents are 25-40% of barbaloin, which is a mixture of aloin A (10*S*) and aloin B (10*R*), and their respective 6-O-*p*-coumaroyl esters; 3-4% of 7-hydroxyaloins A and B and their 6-O-*p*-coumaroyl esters (characteristic for Barbados aloes); and 8-O-methyl-7-hydroxyaloins A and B and their 6-O-cinnamoyl esters; all these compounds are aloe-emodin anthrone C-glycosides. Small amounts of the aglycones aloe-emodin and chrysophanol are also present. Other constituents include 5-methylchromone glycosides, mainly the 8-glucosyl derivative aloeresin B with smaller amounts of its coumaroyl and cinnamoyl esters [Sigler 2007; Hiller 2009; Sticher 2010].

CLINICAL PARTICULARS

Therapeutic indications
For short term use in cases of occasional constipation [Schilcher 1979; Sigler 2007; Hiller 2009].

Posology and method of administration

Dosage
The correct individual dosage is the smallest required to produce a comfortable soft-formed motion.

Adults and children over 12 years: preparations equivalent to 10-30 mg of hydroxyanthracene derivatives, calculated as barbaloin, to be taken once daily at night [Sigler 2007; Hiller 2009].

Elderly: dose as for adults.

Not recommended for use in children under 12 years of age.

The pharmaceutical form must allow lower dosages.

Method of administration
For oral administration.

Duration of administration
Stimulant laxatives should not be used for periods of more than 2 weeks without medical advice. If symptoms persist after intake of the preparation medical advice should be sought [Tack 2011].

Contra-indications
Known hypersensitivity; intestinal obstruction and stenosis, atony, inflammatory bowel diseases (e.g. Crohn's disease, ulcerative colitis), appendicitis; abdominal pain of unknown origin; severe dehydration states with water and electrolyte depletion [Schilcher 1979; Reynolds 1996; Hiller 2009; Pasricha 2011].

Special warnings and special precautions for use
As for all laxatives, aloes should not be given when any undiagnosed acute or persistent abdominal symptoms are present.

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[1]Synonym: Aloe vera

Chronic use of anthraquinone laxatives may cause pigmentation of the colon (pseudomelanosis coli) which is harmless and reversible after drug discontinuation. Abuse, with diarrhoea and consequent fluid and electrolyte losses, may cause disturbance of the water and electrolyte balance (mainly hypokalaemia). A symptom of hypokalaemia is an atonic colon with impaired function (aggravation of constipation). In severe cases hypokalaemia can result in cardiac and neuromuscular dysfunction, especially if cardiac glycosides, diuretics or corticosteroids are taken. Chronic use may result in albuminuria and haematuria.

Long-term use of stimulant laxatives is not recommended. If laxatives are needed every day the cause of the constipation should be investigated. Use of stimulant laxatives for more than 2 weeks should be avoided and requires medical supervision. In chronic constipation, stimulant laxatives are not an acceptable alternative to a change in dietary habits, adequate fluid intake, physical activities and training for normal bowel evacuation [Schilcher 1979; Müller-Lissner 1993, 2005; Reynolds 1996; Nusko 2000; Morales 2009; Sticher 2010; Keller 2011; Pasricha 2011].

Anthraquinone laxatives enhance circulation in the pelvic region and thus are not recommended for patients suffering from haemorrhoids [Frasch 2007; Sigler 2007; Hiller 2009; Sticher 2010].

Not recommended for children under 12 years.

Interaction with other medicaments and other forms of interaction
Hypokalaemia (resulting from long term laxative use) potentiates the action of cardiac glycosides and interacts with antiarrhythmic drugs and with drugs which induce reversion to sinus rhythm (e.g. quinidine). Concomitant use with other drugs inducing hypokalaemia (e.g. thiazide diuretics, adrenocorticosteroids and liquorice root) may aggravate electrolyte imbalance [Sigler 2007].

Pregnancy and lactation

Pregnancy
Not to be taken by pregnant women as there is a greater risk of abortion due to increased circulation in the pelvic region and nervous stimulation of uterine muscles [Frasch 2007; Sigler 2007; Hiller 2009].

Lactation
Not to be taken during breastfeeding as small amounts of active metabolites (rhein) may appear in breast milk [Faber 1988; Sigler 2007; Hiller 2009].

Effects on ability to drive and use machines
None.

Undesirable effects
Abdominal spasms and pain, in particular in patients with irritable colon; yellowish-brown or red (pH dependent) discolouration of urine by metabolites, which is not clinically significant [Ewe 1986; Tedesco 1986; Reynolds 1996; Pasricha 2011; Tack 2011].

Overdose
The major symptoms are griping and severe diarrhoea with consequent losses of fluid and electrolytes, which should be replaced [Pasricha 2011]. Treatment should be supportive with generous amounts of fluid. Electrolytes, especially potassium, should be monitored; this is particularly important in the elderly and the young.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

1,8-dihydroxyanthracene derivatives possess a laxative effect [Fairbairn 1965, 1970]. Aloins A and B and 7-hydroxyaloins A and B are precursors which are not absorbed in the upper gut. Studies in conventional and germ-free animals have established that glycosidases from the intestinal flora are responsible for the breakdown of glycosides. The ability to metabolize various anthranoids varies greatly across species depending upon the composition of intestinal flora [Dreessen 1988; de Witte 1993; Sigler 2007]. In humans, anthranoid glycosides ingested orally pass into the colon unmodified. Human intestinal flora is able to break down O-glycosides easily, but only to some extent the C-glycosides of most anthranoids [Hattori 1988; Che 1991]. The main active metabolite is aloe-emodin-9-anthrone, which acts specifically on the colon [Ishii 1990].

There are different mechanisms of action:

(i) reduced resorption of sodium and water and an influence on the motility of the large intestine (inhibition of the Na^+/K^+ pump and of Cl^- channels at the colonic membrane) resulting in accelerated colonic transit [Ishii 1990; Hönig 1992; Rauwald 1992; Wanitschke 2003; Hiller 2009], and

(ii) an influence on secretion processes (stimulation of mucus and chloride secretion) resulting in enhanced fluid secretion [Ishii 1990, 1994a, 1994b; Wanitschke 2003].

(iii) direct and indirect stimulation of colonic neurons (indirect stimulation by prostaglandins) resulting in enhanced motility [Capasso 1983; Ishii 1990; Wintola 2010; Teuscher 2012].

Due to the time taken for transport to the colon and metabolism into the active compounds, defecation may take place after a delay of 6-12 hours [Sigler 2007; Müller-Lissner 2009; Pasricha 2011].

Anti-tumour activity
Isolated aloe-emodin specifically inhibited the growth of neuroectodermal tumour cell lines at ED_{50} of 1-13 µg *in vitro*. However, no effect was shown on other tumour cell lines or by aloe-emodin glycosides, which was explained by a unique drug uptake of aloe-emodin in neuroectodermal tumour cells. These results were confirmed by further experiments in rats [Pecere 2000].

In two human hepatic cancer cell lines, Hep G2 and Hep 3B, aloe-emodin inhibited cell proliferation by different mechanisms [Kuo 2002]. Aloe-emodin was also shown to inhibit the growth of Merkel carcinoma cells [Wassermann 2002]. In isolated rat hepatocytes, a cytoprotective effect of aloes extract against 1,4-naphthoquinone-induced toxicity was demonstrated [Norikura 2002].

Pharmacokinetic properties

Aloins A and B as well as hydroxyaloins A and B are C-glycosides which pass directly into the large intestine without breakdown in the stomach or the jejunum. At their site of action, the colon and rectum, they are metabolized by bacterial enzymes into the active anthrone compounds. It has been shown that *Eubacterium* sp. BAR isolated from human faeces is capable of transforming aloin A into the active metabolite aloe-emodin-9-anthrone. This bacterial strain is necessary to cause aloin A-induced diarrhoea in the rat [Hattori 1988; Schulz 1993; Morales 2009; Sticher 2010].

In a human pharmacokinetic study performed with Cape aloes (equivalent to 16.4 mg of hydroxyanthracene derivatives) administered orally for 7 days, aloe-emodin was detected as a metabolite in the plasma only sporadically and with maximum concentrations of less than 2 ng/ml. In the same study rhein was detected in the plasma in concentrations ranging from 6-28 ng/ml (median c_{max} 13.5 ng/ml at median t_{max} 16 h) after single dose administration. In 7-day administration there was no evidence of accumulation of rhein [Schulz 1993].

Using an Ussing-type chamber with rat colonic mucosa as test system, isolated aloe emodin anthrone significantly increased the permeation of the poorly permeable compound 5(6)-carboxyfluorescein [Kai 2002]. Isolated rhein and danthrone enhanced permeation of furosemide in a Caco-2 cell monolayer test system [Laitinen 2007].

In vitro studies on various membrane models confirmed membrane-related effects of barbaloin. Measurements of the phospholipid/water partition coefficient of barbaloin, and investigations on its influence on membrane thermotropic behaviour, illustrated barbaloin's high affinity for phospholipid membranes. Barbaloin induced leakage of intraliposomal carboxyfluorescein, and the antimicrobial effect of barbaloin-containing liposomes were traced back to interaction of this anthraquinone [Alves 2004].

In vitro absorption studies demonstrated that 5-14 % of aloin, aloe-emodin and aloesin were absorbed directly from the gut [Park 2009].

From another study it was concluded that free anthranoids absorbed systemically in humans are partly excreted in the urine as rhein or as conjugates [Vyth 1979].

Systemic metabolism of free anthranoids depends upon their ring constituents [de Witte 1988; Sendelbach 1989]. In the case of aloe-emodin, it has been shown in animal experiments that at least 20-25% of an oral dose will be absorbed. The bioavailability of aloe-emodin is much lower than the absorption, because it is quickly oxidized to rhein and an unknown metabolite, or conjugated. Maximum plasma values of aloe-emodin were reached 1.5-3 hours after administration [Lang 1993]. Elimination of anthranoid metabolites occurs mainly via faeces, but also renally as glucuronides and sulphates [Teuscher 2012].

Preclinical safety data
No specific toxicity was observed when aloes extract (up to 50 mg/kg daily for 12 weeks) and aloin (100 mg/kg daily for 20 weeks) were administered orally to mice [Siegers 1986,1993].

Aloes fermentation products, which contain higher amounts of aloin A and B and less glucans compared to unfermentated aloe preparations, were administered orally to rats at doses of 1, 2 or 5 g/kg for single-dose toxicity tests, and at 0.5, 1 or 2 g/kg for repeated-dose toxicity tests. Treatment, even at high dose levels, did not affect function of the autonomic nervous system, general behavior, reaction, vigilance, mortality, body weight, feed and water intake, nor haematological and histopathological parameters [Cho 2011].

There was no evidence of any embryolethal, teratogenic or foetotoxic effects in rats after oral treatment with aloes extract (up to 1000 mg/kg b.w./day) or aloin (up to 200 mg/kg b.w./day) [Bangel 1975].

Results from *in vitro* (gene mutation and chromosome aberration) tests and *in vivo* (micronucleus test in mice bone marrow cells) genotoxicity studies performed with various aloes preparations and isolated constituents, as well as human and animal pharmacokinetic data, indicate no genotoxic risk [Brown 1979, 1980; Westendorf 1988, 1990; Wölfle 1990; CCR 1992a, 1992b, 1992c; Heidemann 1993].

In a 2-year study, male and female F344/N rats were exposed to 280, 830 or 2500 ppm of emodin in the diet, corresponding to an average daily dose of emodin of 110, 320 or 1000 mg/kg b.w. in male rats and 120, 370 or 1100 mg/kg in female rats. No evidence of carcinogenic activity of emodin was observed in male rats. A marginal increase in the incidence of Zymbal's gland carcinoma occurred in female rats treated with the high dosage but was interpreted as questionable [NIH 1999].

In a further 2-year study, on $B6C3F_1$ mice, males were exposed to 160, 312 or 625 ppm of emodin (corresponding to an average daily dose of 15, 35 or 70 mg/kg b.w.) and females to 312, 625 or 1250 ppm of emodin (corresponding to an average daily dose of 30, 60 or 120 mg/kg). There was no evidence of carcinogenic activity in female mice. A low incidence of renal tubule neoplasms in exposed males was not considered relevant [NIH 1999].

Aloin fed to mice in the diet at a level of 100 mg/kg/day for 20 weeks did not promote dimethylhydrazine-induced colorectal tumours [Siegers 1993].

The data from *in vivo* studies concerning the carcinogenic risk of aloe in rats are supported by studies on herbal stimulant laxatives containing senna extract, with similar anthroquinone constituents. Senna preparations showed no carcinogenic or genotoxic effect, and from studies in rats no connection to tumours occurred [Lydén-Sokolowski 1993; Morales 2009].

Clinical safety data
In a case control study with retrospective and prospective evaluation, no causal relationship between anthranoid laxative use and colorectal cancer could be detected [Loew 1994a, 1994b].

In another prospective case control study involving 202 patients with colorectal carcinomas, 114 patients with adenomas and 238 patients in the control group, an association between the use of anthranoid laxatives and the development of colorectal adenoma or carcinoma could not be shown [Nusko 2000].

A longer-term use of laxatives has been recommended in chronic constipation [Andresen 2013].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Second Edition, 2003
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BALLOTAE NIGRAE HERBA	Black Horehound	Supplement 2009
BETULAE FOLIUM	Birch Leaf	Second Edition, 2003
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Second Edition, 2003
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
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ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Second Edition, 2003
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Second Edition, 2003
FUMARIAE HERBA	Fumitory	Supplement 2009
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Supplement 2009
GRINDELIAE HERBA	Grindelia	Supplement 2009
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
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HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Second Edition, 2003
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
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LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Second Edition, 2003
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003

LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Supplement 2009
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
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MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Second Edition, 2003
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Second Edition, 2003
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Second Edition, 2003
RATANHIAE RADIX	Rhatany Root	Supplement 2009
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SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
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SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
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TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Second Edition, 2003
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VIOLAE HERBA CUM FLORE	Wild Pansy	Supplement 2009
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The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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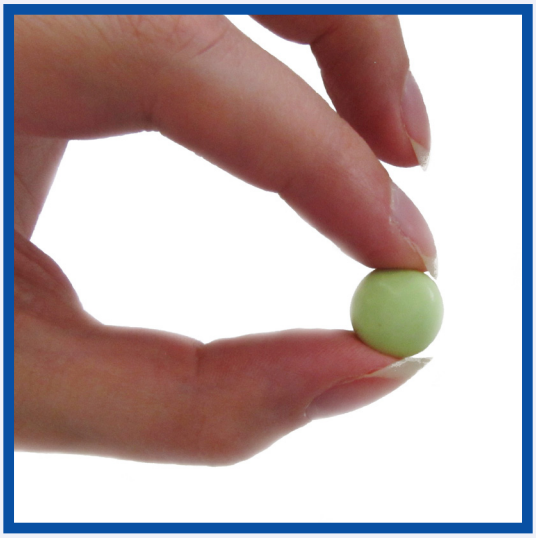

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

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Abbreviations
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Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

ALOE CAPENSIS

2014

Cape Aloes

DEFINITION

Cape aloes consists of the concentrated and dried juice of the leaves of various species of *Aloe*, mainly *Aloe ferox* Miller and its hybrids. It contains not less than 18.0 per cent of hydroxyanthracene derivatives, expressed as barbaloin ($C_{21}H_{22}O_9$; M_r 418.4) and calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [EP].

CONSTITUENTS

The main active constituents are 13-27% barbaloin, which is a mixture of aloin A (10*S*) and aloin B (10*R*), the aloe-emodin anthrone C-glycoside 5-hydroxyaloin A (characteristic for Cape aloes) and the anthrone C- and O-glycosides aloinosides A and B [Rauwald 1993a, 1993b]. Other constituents include 2-acetonyl-5-methylchromones (also known as aloeresins), small quantities of 1.8-dihydroxyanthraquinones (e.g. aloe-emodin), cinnamic acid and 1-methyl-tetralin derivatives [Sigler 2007; Hiller 2009; Sticher 2010; Chen 2012].

CLINICAL PARTICULARS

Therapeutic indications
For short term use in cases of occasional constipation [Schilcher 1979; Sigler 2007; Hiller 2009].

Posology and method of administration

Dosage

The correct individual dosage is the smallest required to produce a comfortable soft-formed motion.

Adults and children over 12 years: preparations equivalent to 10-30 mg of hydroxyanthracene derivatives, calculated as barbaloin, to be taken once daily at night [Sigler 2007; Hiller 2009].

Elderly: dose as for adults.

Not recommended for use in children under 12 years of age.

The pharmaceutical form must allow lower dosages.

Method of administration
For oral administration.

Duration of administration
Stimulant laxatives should not be used for periods of more than 2 weeks without medical advice. If symptoms persist after intake of the preparation medical advice should be sought [Tack 2011].

Contra-indications
Known hypersensitivity; intestinal obstruction and stenosis, atony, inflammatory bowel diseases (e.g. Crohn's disease, ulcerative colitis), appendicitis; abdominal pain of unknown origin; severe dehydration states with water and electrolyte depletion [Schilcher 1979; Reynolds 1996; Hiller 2009; Pasricha 2011].

Special warnings and special precautions for use
As for all laxatives, aloes should not be given when any undiagnosed acute or persistent abdominal symptoms are present.

E/S/C/O/P
MONOGRAPHS

Chronic use of anthraquinone laxatives may cause pigmentation of the colon (pseudomelanosis coli) which is harmless and reversible after drug discontinuation. Abuse, with diarrhoea and consequent fluid and electrolyte losses, may cause disturbance of the water and electrolyte balance (mainly hypokalaemia). A symptom of hypokalaemia is an atonic colon with impaired function (aggravation of constipation). In severe cases hypokalaemia can result in cardiac and neuromuscular dysfunction, especially if cardiac glycosides, diuretics or corticosteroids are taken. Chronic use may result in albuminuria and haematuria.

Long-term use of stimulant laxatives is not recommended. If laxatives are needed every day the cause of the constipation should be investigated. Use of stimulant laxatives for more than 2 weeks should be avoided and requires medical supervision. In chronic constipation, stimulant laxatives are not an acceptable alternative to a change in dietary habits, adequate fluid intake, physical activities and training for normal bowel evacuation [Schilcher 1979; Müller-Lissner 1993, 2005; Reynolds 1996; Nusko 2000; Morales 2009; Sticher 2010; Keller 2011; Pasricha 2011].

Anthraquinone laxatives enhance circulation in the pelvic region and thus are not recommended for patients suffering from haemorrhoids [Frasch 2007; Sigler 2007; Hiller 2009; Sticher 2010].

Not recommended for children under 12 years.

Interaction with other medicaments and other forms of interaction
Hypokalaemia (resulting from long term laxative use) potentiates the action of cardiac glycosides and interacts with antiarrhythmic drugs and with drugs which induce reversion to sinus rhythm (e.g. quinidine). Concomitant use with other drugs inducing hypokalaemia (e.g. thiazide diuretics, adrenocorticosteroids and liquorice root) may aggravate electrolyte imbalance [Sigler 2007].

Pregnancy and lactation

Pregnancy
Not to be taken by pregnant women as there is a greater risk of abortion due to increased circulation in the pelvic region and nervous stimulation of uterine muscles [Frasch 2007; Sigler 2007; Hiller 2009].

Lactation
Not to be taken during breastfeeding as small amounts of active metabolites (rhein) may appear in breast milk [Faber 1988; Sigler 2007; Hiller 2009].

Effects on ability to drive and use machines
None.

Undesirable effects
Abdominal spasms and pain, in particular in patients with irritable colon; yellowish-brown or red (pH dependent) discolouration of urine by metabolites, which is not clinically significant [Ewe 1986; Tedesco 1986; Reynolds 1996; Pasricha 2011; Tack 2011].

Overdose
The major symptoms are griping and severe diarrhoea with consequent losses of fluid and electrolytes, which should be replaced [Pasricha 2011]. Treatment should be supportive with generous amounts of fluid. Electrolytes, especially potassium, should be monitored; this is particularly important in the elderly and the young.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties
1,8-dihydroxyanthracene derivatives possess a laxative effect [Fairbairn 1965, 1970]. Aloins A and B, 5-hydroxyaloin A and the aloinosides A and B are precursors which are not absorbed in the upper gut. Studies in conventional and germ-free animals have established that glycosidases from the intestinal flora are responsible for the breakdown of glycosides. The ability to metabolize various anthranoids varies greatly across species depending upon the composition of intestinal flora [Dreessen 1988; de Witte 1993; Sigler 2007]. In humans, anthranoid glycosides ingested orally pass into the colon unmodified. Human intestinal flora is able to break down O-glycosides easily but only to some extent C-glycosides of most anthranoids [Hattori 1988; Che 1991]. The main active metabolite is aloe-emodin-9-anthrone, which acts specifically on the colon [Ishii 1990].

There are different mechanisms of action:

(i) reduced resorption of sodium and water and an influence on the motility of the large intestine (inhibition of the Na^+/K^+ pump and of Cl^- channels at the colonic membrane) resulting in accelerated colonic transit [Ishii 1990; Hönig 1992; Rauwald 1992; Wanitschke 2003; Hiller 2009], and

(ii) an influence on secretion processes (stimulation of mucus and chloride secretion) resulting in enhanced fluid secretion [Ishii 1990, 1994a, 1994b; Wanitschke 2003].

(iii) direct and indirect stimulation of colonic neurons (indirect stimulation by prostaglandins) resulting in enhanced motility [Capasso 1983; Ishii 1990; Wintola 2010; Teuscher 2012].

Due to the time taken for transport to the colon and metabolism into the active compounds defecation may take place after a delay of 6-12 hours [Sigler 2007; Müller-Lissner 2009; Pasricha 2011].

Anti-tumour activity
Isolated aloe-emodin specifically inhibited the growth of neuroectodermal tumor cell lines at ED_{50} of 1-13 µg in vitro. However, no effect could be shown on other tumor cell lines or by aloe-emodin glycosides, which was explained by a unique drug uptake of aloe emodin in neuroectodermal tumour cells. These results were confirmed by further experiments in rats [Pecere 2000].

In two human hepatic cancer cell lines, Hep G2 and Hep 3B, aloe-emodin inhibited cell proliferation by different mechanisms [Kuo 2002]. Aloe-emodin was also shown to inhibit the growth of Merkel carcinoma cells [Wassermann 2002]. In isolated rat hepatocytes, a cytoprotective effect of aloes extract against 1.4-naphthoquinone-induced toxicity was demonstrated [Norikura 2002; Chen 2012].

An ethanolic fraction of Cape aloes was reported to have anti-tumour activity *in vivo* against Sarcoma 180 and Ehrlich ascites cancer cells [Soeda 1969]. After a screening of 31different plants, aloes extract showed strong growth inhibiting effects on Ehrlich ascites tumor cells (EATC). From subsequent *in vitro* studies with Cape aloes dichloromethane extract it was concluded that constituents of aloes contribute synergistically to this effect. Aloe-emodin has no growth-inhibiting effect on EATC but enhanced the effect of the other constituents of the extract [Kametani 2007].

Pharmacokinetic properties
Aloins A and B as well as hydroxyaloins A and B are C-glycosides which pass directly into the large intestine without breakdown

in the stomach or the jejunum. At their site of action, the colon and rectum, they are metabolized by bacterial enzymes into the active anthrone compounds. It has been shown that *Eubacterium* sp. BAR isolated from human faeces is capable of transforming aloin A into the active metabolite aloe-emodin-9-anthrone. This bacterial strain is necessary to cause aloin A-induced diarrhoea in the rat [Hattori 1988; Schulz 1993; Morales 2009; Sticher 2010].

In a human pharmacokinetic study performed with Cape aloes (equivalent to 16.4 mg of hydroxyanthracene derivatives) administered orally for 7 days, aloe-emodin was detected as a metabolite in the plasma only sporadically and with maximum concentrations of less than 2 ng/ml. In the same study rhein was detected in the plasma in concentrations ranging from 6-28 ng/ml (median c_{max} 13.5 ng/ml at median t_{max} 16 h) after single dose administration. In 7-day administration there was no evidence of accumulation of rhein [Schulz 1993].

Using an Ussing-type chamber with rat colonic mucosa as the test system, isolated aloe-emodin anthrone significantly increased the permeation of poorly the permeable compound 5(6)-carboxyfluorescein [Kai 2002]. Isolated rhein and danthrone enhanced permeation of furosemide in a Caco-2 cell monolayer test system [Laitinen 2007].

In vitro studies on various membrane models confirmed membrane-related effects of barbaloin. Measurements of the phospholipid/water partition coefficient of barbaloin, and investigations on its influence on membrane thermotropic behaviour, illustrated barbaloin's high affinity for phospholipid membranes. Barbaloin-induced leakage of intraliposomal carboxyfluorescein and the antimicrobial effect of barbaloin-containing liposomes were traced back to the membrane interaction of this anthraquinone [Alves 2004].

In vitro absorption studies demonstrated that 5-14 % of aloin, aloe-emodin and aloesin were absorbed directly from the gut [Park 2009].

From another study it was concluded that free anthranoids absorbed systemically in humans are partly excreted in the urine as rhein or as conjugates [Vyth 1979].

Systemic metabolism of free anthranoids depends upon their ring constituents [de Witte 1988; Sendelbach 1989]. In the case of aloe-emodin, it has been shown in animal experiments that at least 20-25% of an oral dose will be absorbed. The bioavailability of aloe-emodin is much lower than the absorption, because it is quickly oxidized to rhein and an unknown metabolite, or conjugated. Maximum plasma values of aloe-emodin were reached 1.5-3 hours after administration [Lang 1993]. Elimination of anthranoid metabolites occurs mainly via faeces, but also renally as glucuronides and sulphates [Teuscher 2012].

Preclinical safety data
No specific toxicity was observed when aloes extract (up to 50 mg/kg daily for 12 weeks) and aloin A (up to 60 mg/kg daily for 20 weeks) were administered orally to mice [Siegers 1986, 1993].

Aloes fermentation products, which contain higher amounts of aloin A and B and less glucans compared to unfermentated aloe preparations, were administered orally to rats at doses of 1, 2 or 5 g/kg for single-dose toxicity tests, and at 0.5, 1 or 2 g/kg for repeated-dose toxicity tests. Treatment, even at high dose levels, did not affect function of the autonomic nervous system, general behavior, reaction, vigilance, mortality, body weight, feed and water intake, nor in haematological and histopathological parameters [Cho 2011].

There was no evidence of any embryolethal, teratogenic or foetotoxic effects in rats after oral treatment with aloes extract (up to 1000 mg/kg b.w./day) or aloin A (up to 200 mg/kg b.w./day) [Bangel 1975].

Results from *in vitro* (gene mutation and chromosome aberration) tests and *in vivo* (micronucleus test in mice bone marrow cells) genotoxicity studies as well as human and animal pharmacokinetic data indicate no genotoxic risk from Cape aloes [Brown 1979, 1980; Morimoto 1982; Bootman 1987a, 1987b; Marquardt 1987; Westendorf 1988, 1990, 1993; Wölfle 1990; CCR 1992a, 1992b, 1992c; Heidemann 1993; Lang 1993].

In a 2-year study, male and female F344/N rats were exposed to 280, 830 or 2500 ppm of emodin in the diet, corresponding to an average daily dose of emodin of 110, 320 or 1000 mg/kg b.w. in male rats and 120, 370 or 1100 mg/kg in female rats. No evidence of carcinogenic activity of emodin was observed in male rats. A marginal increase in the incidence of Zymbal's gland carcinoma occurred in female rats treated with the high dosage but was interpreted as questionable [NIH 1999].

In a further 2-year study, on $B6C3F_1$ mice, males were exposed to 160, 312 or 625 ppm of emodin (corresponding to an average daily dose of 15, 35 or 70 mg/kg b.w.) and females to 312, 625 or 1250 ppm of emodin (corresponding to an average daily dose of 30, 60 or 120 mg/kg). There was no evidence of carcinogenic activity in female mice. A low incidence of renal tubule neoplasms in exposed males was not considered relevant [NIH 1999].

Aloin fed to mice in the diet at a level of 100 mg/kg/day for 20 weeks did not promote dimethylhydrazine-induced colorectal tumours [Siegers 1993].

The data from *in vivo* studies concerning the carcinogenic risk of aloe in rats can be supported by studies on herbal stimulant laxatives containing senna extract, another anthraquinone containing plant with similar constituents. Senna preparations showed no carcinogenic or genotoxic effect, and from studies in rats no connection to tumours could be identified [Lydén-Sokolowski 1993; Morales 2009].

Clinical safety data
In a case control study with retrospective and prospective evaluation, no causal relationship between anthranoid laxative use and colorectal cancer could be detected [Loew 1994a, 1994b].

In another prospective case control study involving 202 patients with colorectal carcinomas, 114 patients with adenomas and 238 patients in the control group, no association between the use of anthanoid laxatives and the development of colorectal adenoma or carcinoma was shown [Nusko 2000].

A longer-term use of laxatives has been recommended in chronic constipation [Andresen 2013].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Second Edition, 2003
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
BALLOTAE NIGRAE HERBA	Black Horehound	Supplement 2009
BETULAE FOLIUM	Birch Leaf	Second Edition, 2003
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Second Edition, 2003
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Supplement 2009
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Second Edition, 2003
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Second Edition, 2003
FUMARIAE HERBA	Fumitory	Supplement 2009
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Supplement 2009
GRINDELIAE HERBA	Grindelia	Supplement 2009
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Second Edition, 2003
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Second Edition, 2003
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003

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MALVAE FLOS	Mallow Flower	Supplement 2009
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
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MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
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PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Second Edition, 2003
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
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SAMBUCI FLOS	Elder flower	Online Series, 2013
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SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
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SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Second Edition, 2003
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VIOLAE HERBA CUM FLORE	Wild Pansy	Supplement 2009
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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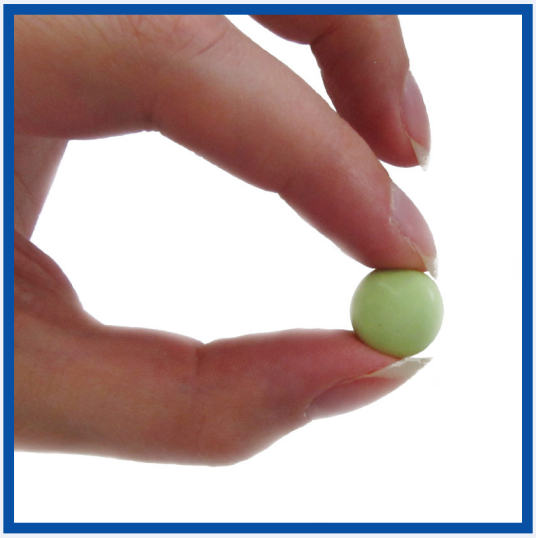
Althaeae radix
Marshmallow Root

2019



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Marshmallow Root

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

Edited by Simon Mills and Roberta Hutchins
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Plant illustrated on the cover: *Althaea officinalis*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

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Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
CCl_4	carbon tetrachloride
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase

INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

ALTHAEAE RADIX

2019

Marshmallow Root

DEFINITION

Marshmallow root consists of the peeled or unpeeled, whole or cut, dried root of *Althaea officinalis* L. It has a swelling index of minimum 10, determined on the powdered herbal drug.

The material complies with the monograph of the European Pharmacopoeia [Marshmallow Root].

CONSTITUENTS

Characteristic constituents of the dried root are mucilage polysaccharides (from 5% up to 20% in late autumn and winter, in biennial roots), consisting of rhamnogalacturonans (≤30%), arabinans, glucans and arabinogalactans [Franz 1966; Karawya 1971; Tomoda 1977; Tomoda 1980; Capek 1983; Akhtardzhiev 1984; Rosík 1984; Capek 1984; Shimizu 1985; Capek 1987; Capek 1988; Evans 1996; Nosalova 2005; Blaschek 2014]. The mucilage can withstand temperatures of 40-60°C [Franz 1990].

Other constituents include flavone glycosides (ca. 0.2% as aglycones) [Gudej 1990; Shah 2011], mainly isoscutellarein 4′-methyl ether 8-glucoside-2″-sulphate, 4 sulfated hypolaetin-glucosides, phenolic acids, the coumarin scopoletin, fatty acids: linolenic acid (omega 3) and hexadecanoic acid [Gudej 1991; Ninov 1992; Valiei 2011; Sendker 2017], starch, pectin [Evans 1996; Blaschek 2014] and tannins [Bieloszabska 1966].

CLINICAL PARTICULARS

Therapeutic indications

a) Dry cough; irritation of the oral or pharyngeal mucosa [Braun 1987; Weiss 1991; Bradley 1992; Bone 1993; Barnes 2002; Sweetman 2002; Schulz 2004; Fasse 2005; Rouhi 2007; Wichtl 2009; Blaschek 2014].

b) For symptomatic relief of mild gastrointestinal discomfort and irritation of the gastric mucosa [Villar 1984; Schilcher 2003; Bäumler 2007; Blaschek 2014].

In these indications, efficacy is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

a) Internal use for dry cough and irritation of the oral mucosa.

Adult single and daily dose:
- 0.5-3 g herbal substance as an aqueous cold macerate* (several times daily up to an equivalent of 15 g of the drug).
- 10 ml of syrup (DER 1:20), repeated up to 6 times daily [Bradley 1992; Schilcher 2003; Schulz 2004; Wichtl 2009; Blaschek 2014].

Daily dose for children, between 6 and 12 years of age, for dry cough:
- 0.5-1.5 g as a macerate*, up to 3 times daily.
- 5-10 ml of syrup (DER 1:20), up to 4-6 times daily. [Weiss 1999; Schilcher 2003; Fasse 2005].

Daily dose for children, between 3 and 6 years of age, for dry cough:
- 0.5-1 g as a macerate*, up to 3 times daily.
- 2.5-5 ml of syrup (DER 1:20), up to 4-6 times daily. [Weiss 1999; Schilcher 2003; Fasse 2005].

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b) Internal use for gastrointestinal irritation.

Adult single and daily dose:
- 3-5 g herbal substance, as an aqueous cold macerate*, up to 3 times daily
  [Bradley 1992; Schilcher 2003; Wichtl 2009].

* To make a macerate pour 150 ml of water (max. temp. 40°C) over one dose of comminuted marshmallow root. Steep for 30 min., stirring frequently. The filtered macerate should be used immediately after preparation.

Method of administration
For oral administration.

Duration of administration
If symptoms persist or worsen, medical advice should be sought.

Contraindications
None known.

Special warnings and special precautions for use
For dry cough: the use in children under 3 years of age is not recommended [Fasse 2005].

Interaction with other medicinal products and other forms of interaction
The absorption of other drugs taken simultaneously may be retarded [Barnes 2002; Blaschek 2014]. As a precaution, marshmallow root should be taken ½ to 1 hour before or 2 hours after intake of other medicinal products.

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy and lactation, without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No case of overdose reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties
The mucilage from marshmallow root covers the mucosa, especially of the mouth and pharynx, and the gastric mucosa, protecting them from local irritation [Meyer 1956; Müller-Limmroth 1980; Braun 1987; Franz 1989; Weiss 1999; Schulz 2004; Wichtl 2009; Shah 2011; Blaschek 2014].

In vitro experiments

Antimicrobial activity
A dry methanol extract (15 g extracted, filtered and evaporated) demonstrated significant antibacterial activity against periodontal pathogenic bacteria resident in the oral cavity (*Porphyromonas gingivalis*, *Prevotella* spp., *Actinomyces* spp.). The MIC for 9/12 strains was ≤3125 mg/L [Iauk 2003].

Antimicrobial activity against *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Staphylococcus aureus* has been documented for chloroform and methanol extracts of marshmallow root [Recio 1989].

Good antimicrobial activity was reported for hexane extracts containing mainly fatty acids and hexadecanoic acid methylester. The strongest antibacterial effects, by zone of inhibition (mm), were demonstrated for *Bacillus subtilis* (18.9 mm), *Staphylococcus aureus* (16.8 mm), *S. epidermidis* (17.3 mm) and *Escherichia coli* (16.0 mm). Antifungal effects were observed against *Candida albicans* (14.7 mm) and *Saccharomyces cerevisiae* (14.7 mm) [Valiei 2011].

Protection of mucilage
Mucociliary transport in isolated, ciliated epithelium of the frog oesophagus was inhibited up to 17% by 200 µl of a cold, 30-minute macerate of marshmallow root (6.4 g/140 ml) [Müller-Limmroth 1980].

In a study of the bioadhesive effects of purified polysaccharides (>95%) on isolated porcine buccal membranes, polysaccharides from marshmallow root showed dose dependent moderate adhesion to epithelial tissue [Schmidgall 2000].

A dried cold aqueous extract (100 g powdered root yielding 9.9 g (w/w) dry extract), at 10 µg/ml, stimulated cell viability (marginally) and proliferation (significantly, $p<0.01$) of human epithelial cells. Isolated polysaccharides, at 10 µg/ml, significantly ($p<0.01$) increased cell viability but not proliferation. Primary dermal human fibroblasts were not stimulated by the extract or the polysaccharides. Fluorescence-labelled-polysaccharides were detected inside epithelial cells with detectable changes in cell physiology. The fibroblasts did not demonstrate any internalisation, but were found to be covered with a bioadhesive layer of polysaccharides [Deters 2010].

A 50% methanolic extract, depleted of high molecular weight material by alcohol precipitation, yielded 8.1% plant material (relative to the dried drug) in a raw extract. This low molecular weight raw extract contained, among others, flavonoid glycosides, four hypolaetin glycosides and coumarins. The raw extract inhibited human hyaluronidase-1 activity (expressed on *E. coli*) and hyaluronidase mRNA expression in keratinocytes (representing skin and mucosal epithelial cells) at 125 and 250 µg/ml [Sendker 2017].

The bioadhesive properties of 2 mucopolysaccharide-containing aqueous marshmallow root extracts, added to 2 commercial cough syrups (syrup 1: 2.5 g extract (DER: 7-9:1) in 100 ml syrup; syrup 2: 35.6 g extract (DER: 1:19.5-23.5) in 100 g syrup), were investigated in isolated porcine buccal mucosa (attached to glass plates) kept humid by artificial saliva, which was also used as a control solution. The positive adherence of these 2 syrups (coloured with eosin) to porcine mucosa was demonstrated by measuring a reduction in flow-velocity (6% flow for syrup 1; 25% flow for syrup 2) compared to the control solution which did not demonstrate any adhesive properties (100% flow). A subsequent experiment, using the same 2 marshmallow extract-containing syrups and an identical mucosal test system, measured inhibition of caffeine transport through the mucosa (a protective property). Both syrups inhibited caffeine transport: syrup 1 by 30% and syrup 2 by 10%; compared to 100% transport of caffeine alone. A control marshmallow extract (DER: 7-9:1) showed only slight inhibition, indicating that the galenic composition of the syrup supports the inhibitory activity of the marshmallow polysaccharides [Appel 2018].

Immunological activity and effects on melanocytes and keratinocytes
An acidic polysaccharide isolated from marshmallow root, althaea-mucilage O, exhibited weak anti-complement activity (alternative route) in normal human serum at concentrations of 100-1000 µg/ml [Yamada 1985].

A filtered extract (dissolved in 45% 1,3-butylene glycol) was found to inhibit intracellular calcium mobilization in normal human melanocytes (involved in pigmentation) and to strongly inhibit endothelin-1 induced proliferation of melanocytes. The extract also reduced the secretion of endothelin-1 in normal human keratinocytes. The authors suggest that because of these effects, the extract may be a useful ingredient in a whitening agent [Kobayashi 2002].

Pronounced antioxidant activity (tests: ABTS+ radical cation scavenging assay; hypochlorous acid scavenging assay; trolox as positive control) of ethanol/water extracts (50:50 and 70:30 V/V) of marshmallow root (100 mg/ml) was found to correlate well with the phenolic and flavonoid content of the extracts [Benbassat 2013].

In vivo experiments
Mucilaginous herbs like marshmallow root may inhibit coughing by forming a protective coating on the mucosal lining of the respiratory tract, shielding it from irritants.

Antitussive effects
Extracts from marshmallow root and isolated mucilage polysaccharides were administered orally to cats at doses of 50 or 100 mg/kg b.w. in order to investigate their antitussive effects in comparison with controls. Both the extract and isolated polysaccharides, as well as syrupus Althaeae (1000 mg/kg), significantly diminished the intensity and amount of coughing induced by mechanical irritation [Nosalova 1992a, 1992b, 1993].

Cats (with experimentally induced cough reflex, induced by mechanical stimulation of the airway mucosa) were treated orally with either a syrup (1 g/kg b.w.), or an aqueous extract (1 g/kg), or root mucilage (prepared as a crude mixture of polysaccharides obtained by precipitation of the aqueous extract with ethanol and subsequent dialysis of the precipitate; 100 mg/kg), or with isolated rhamnogalacturonan (50 mg/kg). These were compared to commonly used cough suppressants, both a narcotic (codeine at 10 mg/kg) and a non-narcotic drug (dropropizine at 100 mg/kg). The marshmallow-derived polysaccharides exhibited impressive antitussive activity after 30 min., 1, 2 and up to 5 hours, for various cough-related parameters. Rhamnogalacturonan was 2.5 times more active than the other marshmallow preparations [Nosalova 2005].

Antitussive activity of the marshmallow polysaccharide, rhamnogalacturonan, was shown to be dose dependent (25 mg/kg and 50 mg/kg b.w.), when given orally to unsensitized guinea pigs, with experimental citric acid aerosol-induced airways inflammation. The highest oral rhamnogalacturonan dose with strong cough reflex suppressant activity was comparable to the effect of codeine at 10 mg/kg b.w. This dose suppressed the cough reflex significantly ($p \leq 0.01$) up to 5 hours after application [Sutovska 2011].

Anti-inflammatory effects
An ointment containing an aqueous marshmallow root extract (20%), applied topically to the external ear of rabbits, reduced irritation induced by ultraviolet irradiation or tetrahydrofurfuryl alcohol; the anti-inflammatory effect was less than that of an ointment containing dexamethasone (0.05%). An ointment containing both active ingredients at these levels had an anti-inflammatory effect superior to that of the individual active ingredients [Beaune 1966].

On the other hand, a dry 80%-ethanolic extract, administered orally at 100 mg/kg b.w., did not inhibit carrageenan-induced rat paw oedema [Mascolo 1987].

Hypolaetin 8-glucoside has been shown to possess anti-inflammatory activity [Alcaraz 1989; Villar 1987; Villar 1984]. When administered i.p. at 90 mg/kg b.w., it dose-dependently inhibited carrageenan-induced rat paw oedema by 74% after 3 hours ($p<0.01$) compared to 49% inhibition by phenylbutazone at the same dose. The anti-inflammatory effect of hypolaetin 8-glucoside declined more rapidly than that of phenylbutazone, but gastric erosions were only seen after phenylbutazone [Villar 1984]. Hypolaetin 8-glucoside also showed gastric anti-ulcer activity in rats [Alcaraz 1988] and was more potent than troxerutin in inhibiting histamine-induced capillary permeability in rats [Villar 1987].

Hypoglycaemic activity
Mucilage polysaccharides isolated from marshmallow root and administered intraperitoneally to mice at doses of 10, 30 and 100 mg/kg reduced plasma glucose levels respectively to 74%, 81% and 65% of the control level after 7 hours, demonstrating significant hypoglycaemic activity [Tomoda 1987].

Phagocytic activity, immunemodulatory effect
Isolated mucilage polysaccharides from marshmallow root, administered intraperitoneally to mice at 10 mg/kg, produced a 2.2-fold increase in phagocytic activity of macrophages in the carbon-clearance test [Wagner 1985; Hänsel 2014].

Clinical studies
In a randomized, double-blind, placebo-controlled clinical study 63 adults suffering from dry cough, associated with angiotensin-converting enzyme inhibitors, were assessed using a cough score of 0 to 4 (0 = no cough, 1 = tickling in throat; 2 = mild cough; 3 = moderate tolerable cough; 4 = severe persistent cough). The patients were randomized to receive 20 drops 3 times per day of either a marshmallow root preparation (not further defined) or a placebo for 4 weeks. After 4 weeks the severity of cough in the marshmallow group was significantly ($p<0.05$) reduced. Eight patients in the marshmallow group showed almost complete abolition of cough, as opposed to 1 patient in the placebo group. Three patients were excluded from the study because of noncompliance with the test drug [Rouhi 2007].

In a postmarkeing surveillance study, 313 children in 3 groups (0-3 y, n=100; 3-6y, n=115; 6-12y, n=98) were treated 4-6 times daily with 2.5, 5 and 10 ml (according to their age group) of mashmallow root syrup (Phytohustil®; DER: 1:20). Duration of treatment was three days for ¾ of the patients and was continued longer for the remaining ¼ (2.2% were treated for < 3 days). Three children were excluded from efficacy evaluation due to disallowed concomitant medication. The following cough symptoms were evaluated: cough intensity, cough frequency and extent of coughing periods per day. Coughing intensity and frequency as well as cough-dependent symptoms were strongly reduced after three days. Two adverse events occurred in the 0-3 age group which were not attributed to the drug. Tolerability of marshmallow root was reported as very good [Fasse 2005].

Pharmacokinetic properties
No data available.

Preclinical safety data
No data available.

Clinical safety data
A total of 373 adults and children with cough were treated with various doses of marshmallow root in 2 clinical studies. In thirty patients treated with 3 x 40 mg/d marshmallow root for 4 weeks, adverse events were not reported [Rouhi 2007]. In the study with 313 children that received a marshmallow root

containing syrup (Phytohustil®, DER 1:20), for 3 days or more, one adverse event (development of obstructive bronchitis) and one serious adverse event (development of bronchopneumonia resulting in hospitalisation) occurred in the 0-3 years age group, but these were not attributed directly to the drug. The tolerability of marshmallow root was reported as very good [Fasse 2005].

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E/S/C/O/P
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PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2019
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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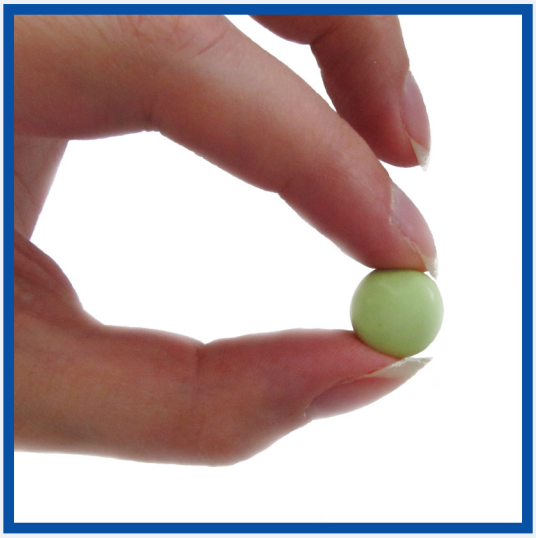
The Scientific Foundation for Herbal Medicinal Products

Angelicae radix
Angelica Root

2021



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MONOGRAPHS

***The* Scientific Foundation for**
Herbal Medicinal Products

ANGELICAE RADIX
Angelica Root

2021

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

Edited by Roberta Hutchins and Simon Mills
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Plant illustrated on the cover: *Angelica archangelica*

FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

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Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in the production of ESCOP monographs and other activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

ANGELICAE RADIX

2021

Angelica Root

DEFINITION

Angelica root consists of the whole or cut, carefully dried rhizome and root of *Angelica archangelica* L. (syn. *A. officinalis* Hoffm.). It contains not less than 2.0 mL/kg of essential oil, calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Angelica root].

CONSTITUENTS

The main characteristic constituents are:

Essential oil (0.2-1%) of variable composition, consisting mainly of monoterpenes, such as α-pinene, δ-3-carene, β-phellandrene, sabinene, limonene, *p*-cymene and myrcene, together with small amounts of sesquiterpenes, such as α-copaene, germacrene D, β-bisabolene and caryophyllenes and macrocyclic lactones [Taskinen 1975; Kerrola 1994; Lawrence 1989; Lawrence 1996; Chalchat 1997; Wedge 2009].

Coumarins, principally the prenylcoumarins, osthenol (0.04%) and osthole. Furanocoumarins including auraptene, bergapten, angelicin, imperatorin, isoimperatorin, oxypeucedanin, oxypeucedanin hydrate, 7-isopentenyl-oxycoumarin, phellopterin, ostruthol, byakangelicin angelate, xanthotoxin, umbelliprenin, psoralen and isopimpinellin, and the dihydrofuranocoumarins archangelicin and 2′-angeloyl-3′-isovaleryl vaginate [Genius 1981; Härmälä 1992a,b; Roos 1997; Eeva 2004; Taddeo 2017].

Other constituents include phenolic acids (chlorogenic and caffeic acids, 4′-geranyloxyferulic acid), sitosteryl esters, angelic acid and various fatty acids, tannins and starch [Baerheim Svendsen 1951; Eichstedt 1963; Czygan 1998; Taddeo 2017].

CLINICAL PARTICULARS

Therapeutic indications

Dyspeptic complaints such as mild gastrointestinal spasms, sluggish digestion, flatulence and feeling of fullness, loss of appetite, bronchitis [Ożarowski 1987; Bruneton 1995; Vieweger 1998; Chevallier 2001; Sarker 2004; Bradley 2006, Stahl-Biskup 2016].

Efficacy in these indications is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Adult daily dose: 3-6 g of the drug, or as an infusion; liquid extract 1:1 in 25% ethanol, 1.5-6 ml; tincture 1:5 in 50% ethanol, 1.5-6 ml, preferably divided into three doses [Todd 1967; Angelica root 1983; Bradley 2006; Stahl-Biskup 2016].

Elderly: dose as for adults.

Children over 4 years, average daily dose: 4-10 years of age, 2-3 g; *10-16 years of age*, 3-4 g [Dorsch 2002].

Method of administration
For oral administration.

Duration of administration
No restriction.

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Contra-indications
None known.

Special warnings and special precautions for use
Care should be taken concerning prolonged exposure to sunlight while taking angelica root preparations since skin photosensitization due to the presence of furocoumarins was deduced from preclinical studies with the essential oil [Søborg 1996;Tisserand 2014].

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy or lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Spasmolytic activity
Significant spasmolytic activity was exerted by a 50% methanolic extract on isolated guinea pig ileum ($p<0.05$ to $p<0.01$). Spontaneous contractions of the circular smooth muscle were inhibited with an IC_{50} of 265 µg/mL compared to 3.94 µg/mL for papaverine. Acetylcholine- and barium chloride-induced contractions of the longitudinal smooth muscle were reduced with IC_{50} values of 242 and 146 µg/mL, respectively, compared to 4.82 and 4.90 µg/mL for papaverine [Izzo 1996].

The essential oil inhibited contractions of isolated guinea pig tracheal and ileal smooth muscle with IC_{50} values of 2.5 µg/mL and 10 µg/mL respectively [Reiter 1985].

Angelicin at 20 µg/mL produced marked relaxation of isolated rabbit duodenum and 50% inhibition of acetylcholine-, histamine- and barium chloride-induced contractions of isolated guinea pig ileum [Chandhoke 1975].

Calcium-blocking activity
Depolarization-induced uptake of $^{45}Ca^{2+}$ ions into cultured rat pituitary GH_4C_1 cells was inhibited by extracts of angelica root [Härmälä 1992b]. The effect was attributed to coumarins and furanocoumarins; osthole, imperatorin, isoimperatorin and phellopterin gave IC_{50} values in the range of 4-12 µg/mL [Härmälä 1992a]. A methanolic extract (not further specified) and archangelicin had IC_{50} values of 3.9 µg/mL and 1.2 µg/mL respectively, compared to verapamil (IC_{50} 2.0 µg/mL) [Härmälä 1991, 1992a, 1992b, Vuorela 1997].

Anti-inflammatory activity
A dry extract (24:1, petroleum ether-dichloromethane 1:1) at 50 µg/mL inhibited microsomal COX-1 from sheep seminal vesicles by 55% and 5-LOX in porcine leucocytes by 85%. In similar assays, coumarins showed no inhibitory activity on COX-1; however, osthole and oxypeucedanin hydrate isovalerate concentration-dependently inhibited 5-LOX with IC_{50} values of 36.2 and 127.5 µM, compared to 36.3% inhibition by nifedipine at 100 µM [Roos 1997].

An essential oil (α-pinene 21.3%, β-3-carene 16.5%, limonene 16.4%) significantly ($p<0.05$) reduced IL-6 release in LPS-stimulated human umbilical vein endothelial cells at 73 µg/mL [Fraternale 2018].

Antimicrobial activity
An essential oil (α-pinene 28.7%, β-3-carene 14.2%) exhibited MIC values of 14.2 µL/mL and 28.4 µL/mL against *S. aureus* and *E. coli* respectively, while the MBC values were 56.8 µL/mL and 113.6 µL/mL respectively. Gentamicin as a positive control showed MIC/MBC values of 0.25/0.75 µg/mL for *S. aureus* and 0.5/0.1 µg/mL for *E. coli* respectively [Aćimović 2017].

Antiviral activity
A dichloromethane extract (12:1) and isolated compounds such as imperatorin, phellopterin, isoimperatorin and bergapterin, as well as the mixture of imperatorin and phellopterin, were investigated in the virus titre reduction assay against *Herpes simplex* virus-1 (HSV-1) and Coxsackievirus B3 (CVB3). The extract, imperatorin, phellopterin and the mixture reduced the HSV-1 titre (expressed as $TCID_{50}$/mL) significantly ($p<0.05$) while the activity on CVB3 replication was not significant [Rajtar 2017].

Cytotoxic activity
Various furanocoumarins inhibited cell proliferation of HeLa S3 cells at 25 µg/mL; the order of potency was osthole > isopimpinellin > bergapten > xanthotoxin > imperatorin. Xanthotoxin and imperatorin also inhibited the proliferation of lymphocytes *in vitro*, and xanthotoxin inhibited the growth of tumours induced in mice (Ehrlich carcinoma) and rats (sarcoma 45) [Gawron 1987].

A 65% ethanolic extract (not further specified) was cytotoxic to human breast cancer cells MCF-7 and mouse mammary carcinoma cells 4T1 at 250 µg/mL and inhibited 50% of cell viability ($p<0.001$). No toxicity was observed in normal CCD1072Sk fibroblasts [Oliveira 2019].

An essential oil (α-pinene 21.3%, β-3-carene 16.5%) at more than 200 µg/mL induced apoptosis and to a lesser extent necrosis in U937 lymphoma cells after 4 hours of treatment ($p<0.05$). With 150 µg/mL only approximately 50% apoptosis and no necrosis were observed [Fraternale 2018].

Other effects
A hexane extract inhibited butyrylcholinesterase (BChE) with an IC_{50} of 16 ±5 µg/mL. Imperatorin showed IC_{50} values of 14.4±3.2 µg/mL (BChE) and 156±15 µg/mL (acetylcholinesterase, AChE). The IC_{50} for heraclenol-2'-O-angelate was 7.5±1.8 µg/mL for BChE as compared to physostigmine (1.73±0.4 µg/mL for BChE and 0.21±0.1µg/mL for AChE) and to galanthamine (8.3±2.6 µg/mL for BChE and 0.37±1.1 µg/mL for AChE) [Wszelaki 2011].

Chloroform and methanolic extracts (not further specified), the combination of these extracts, as well as osthole, were tested for their ability to inhibit ketohexokinase isoform C (KHKC) activity. The chloroform extract and the combination showed KHKC inhibition with IC_{50} values of 57.3 and 30.3 µg/mL respectively. The IC_{50} value for osthole was 0.7 µg/mL [Le 2016].

An aqueous methanolic extract (not further specified) inhibited urease with an IC_{50} of 64.03 µg/mL [Biglar 2014].

Phellopterin strongly inhibited the binding of [^{3}H]diazepam to CNS benzodiazepine receptors in isolated rat cortical membrane with an IC_{50} of 0.36 µM as compared to diazepam (IC_{50}: 0.018 µM). The selectivity of the benzodiazepine receptor for phellopterin was much greater than for various structurally similar furanocoumarins [Bergendorff 1997].

In vivo experiments

Gastro-intestinal effects
An ethanolic liquid extract (osthenol 53 µg/mL), administered orally to rats at 2.5-10 mL/kg b.w. one hour before the oral administration of indomethacin (10 mg/kg b.w.), reduced the ulcer index by 35-45%. This was associated with reduced acidity of the gastric juice, increased mucin secretion and prostaglandin E_2 release, and a decrease in leukotrienes (all $p<0.05$ compared to indomethacin). The anti-ulcerogenic effect of the extract was confirmed histologically [Khayyal 2001].

Hepatoprotective effects
Mice with ethanol-induced hepatotoxicity were treated orally with a dry aqueous extract at 10, 25 and 50 mg/kg three times per week for 4 weeks. The extract significantly ($p<0.05$) reduced both serum GOT and GTP levels and significantly ($p<0.05$) and dose-dependently inhibited malondialdehyde formation in mouse liver homogenates [Yeh 2003].

Anti-inflammatory and analgesic effects
Osthole inhibited carrageenan-induced rat paw oedema by 61.8% at 500 mg/kg b.w. p.o. and by 65.3% at 50 mg/kg i.p. ($p<0.01$), compared to 47.2% for indomethacin (10 mg/kg b.w. i.p.). Osthole inhibited acetic acid-induced writhing in mice by 53.9% at 250 mg/kg b.w. p.o. and by 61.2% at 50 mg/kg b.w. s.c. ($p<0.01$) compared to 69.7% for acetylsalicylic acid at 100 mg/kg b.w. s.c. [Kosuge 1985].

Sedative activity
Angelicin exhibited potent sedative, anticonvulsant and central muscle relaxant activity in mice, rats and rabbits. When administered p.o. to rats at 40-300 mg/kg or i.p. at 20-80 mg/kg b.w. it caused marked sedation, flaccidity, inhibition of spontaneous motor activity (77% at 80 mg/kg i.p.) and hypothermia. The ED_{50} for most of the parameters in rats was 30-40 mg/kg b.w. p.o. and 6-15 mg/kg b.w. i.p. [Chandhoke 1975].

Anxiolytic effects
A 95% methanolic extract (not further specified) was evaluated in albino rats using the elevated plus maze test. The rats were treated with diazepam (1 mg/kg b.w.) or doses of 200 mg/kg and 400 mg/kg b.w. of the extract. At the higher dose, the extract significantly ($p<0.05$) increased the number of entries and the duration of time spent in the open arms while both parameters decreased in the closed arms [Kumar 2012].

In the elevated plus-maze test, light and dark-box and whole board-box, the anxiety-like behaviour of albino rats was investigated using diazepam (1 mg/kg b.w.), imperatorin (5 mg/kg b.w.), isoimperatorin (5 mg/kg b.w.) or vehicle only. One hour after administration all substances showed significant activity ($p<0.05$, $p<0.01$) on the anxiety-like behaviour [Kumar 2013].

Anticonvulsant effects
An essential oil (not further specified) was evaluated against electrically- and chemically-induced seizures as well as in the rota-test. In maximal electroshock-induced seizures in mice, 30 min after i.p. administration of either vehicle (10 mL/kg b.w.), the essential oil (50, 100, 200, 400, 500 mg/kg b.w.) or phenytoin sodium (25 mg/kg b.w.), only the oil at 500 mg/kg b.w. and phenytoin resulted in a 100% protection against the tonic hind limb extensor phase. In pentylenetetrazol-induced seizures, the highest dose of the essential oil resulted in significant reduction of mortality, postponed the onset of general clonus ($p<0.05$) and decreased the duration of clonus compared to vehicle. In the rota-test, the essential oil at a dose of 500 mg/kg b.w. had a significant ($p<0.05$) influence on the motor function [Pathak 2010].

Imperatorin, osthole and valproate (positive control) were tested in maximal electroshock (MES)-induced seizures and chimney tests in mice. The animals were treated (i.p.) at 15, 30, 60 and 120 min before the test. The ED_{50} values for imperatorin were in the range of 185-290 mg/kg b.w., for osthole 266-639 mg/kg b.w. and for valproate 189-255 mg/kg b.w., depending on the timing of pre-treatment. In the chimney test, acute adverse effects on motor coordination were seen with TD_{50} values of 329-433 mg/kg b.w. for imperatorin, 531-648 mg/kg b.w. for osthole and 363-512 mg/kg b.w. for valproate. Imperatorin exhibited greater anticonvulsant activity than osthole while osthole demonstrated less acute neurotoxic effects than imperatorin, both in relation to valproate [Luszczki 2009].

Antitumour activity
A 65% ethanolic extract (not further specified) significantly ($p<0.05$) reduced tumour growth and tumour weight when administered by gavage at a dose of 500 mg/kg b.w. to mice inoculated with 4T1 cells as compared to the untreated group [Oliveira 2019].

Clinical studies
None published.

Pharmacokinetic properties
No data available.

Preclinical safety data
Angelica root is accepted as a food flavouring by the Council of Europe [Angelica archangelica 2000] and is generally recognized as safe (GRAS) in the USA [Anonymus 2018].

Angelica root oil has been assessed as non-toxic (i.e. safe for oral use at therapeutic dose levels), very mildly irritant and non-sensitizing, but phototoxic if applied in concentrations greater than 0.78% to areas of skin exposed to sunshine [Tisserand 2014].

Acute toxicity
The oral LD_{50} of angelica root oil was determined as 2.2 g/kg b.w. in mice and 11.2 g/kg b.w. in rats [Opdyke 1975].

An extract (not further specified) administered orally to rats at a dose of 3200 mg/kg b.w. was found to be safe [Kumar 2012].

Oral doses of 20 mg/kg b.w. imperatorin as well as isoimperatorin were determined as safe in rats [Kumar 2013]. The oral LD_{50} of angelicin in rats was found to be 321.6 mg/kg b.w. [Chandhoke 1975].

Subchronic toxicity
When angelica root oil was administered to rats for 8 weeks, the tolerated dose was concluded to be 1.5 g/kg b.w. [Opdyke 1975].

Mutagenicity
A tincture and a liquid extract showed weak mutagenic potential in the Ames test using *Salmonella typhimurium* strains TA 98 and TA 100 [Göggelmann 1986].

In the micronucleus test in murine bone marrow cells, aqueous and alcoholic extracts exhibited antimutagenic activity against triethylenethiophosphoramide (thio-TEPA)-induced

mutagenicity. The reduction of micronucleus frequencies was more marked when the extracts were injected 2 hours before thio-TEPA treatment. No genotoxic effects of the extracts were observed at concentrations of 50-100 mg/kg [Salikhova 1993, 1995].

Clinical safety data
No clinical data available for mono-preparations.

Data from clinical studies and post-marketing surveillance studies involving a total of over 5000 patients, as well as from adverse event reporting systems, showed that angelica root was safe when used as a herbal combination in which 10% by volume is angelica root tincture (1:3, ethanol 30% V/V) [Saller 2002].

Data from randomized, controlled studies (413 patients), prospective non-interventional studies (5,795 patients) and more than 40,000 children in retrospective database surveillances, showed that angelica root was safe when used as a herbal combination in which 10% by volume is angelica root extract (1-2.5-3.5, ethanol 30% V/V) [Ottillinger 2013].

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MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
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ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Online Series, 2021
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Online Series, 2019
ARCTII RADIX	Burdock Root	Online Series, 2016
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BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Online Series, 2019
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
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CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
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CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
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ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Online Series, 2021
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Online Series, 2021
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FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
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FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
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GENTIANAE RADIX	Gentian Root	Online Series, 2014
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HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
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LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Online Series, 2020
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
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PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Online Series, 2020
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Online Series, 2020
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
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ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
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SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
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SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
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TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
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URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Online Series, 2020
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
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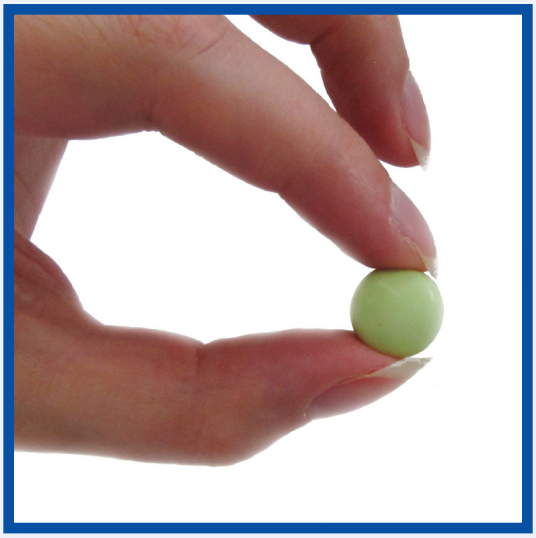

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

ANISI FRUCTUS

2014

Aniseed

DEFINITION

Aniseed consists of the whole dry cremocarp of *Pimpinella anisum* L. It contains not less than 20 ml/kg of essential oil.

The material complies with the monograph of the European Pharmacopoeia [Aniseed].

Note: In The European Pharmacopoeia (Eds 1,2 and 3), the monograph for Anise Oil permits oils obtained by steam distillation from the fruits of *Pimpinella anisum* L. (aniseed) or *Illicium verum* L. (star anise). *Trans*-anethole is the predominant component of both oils, but the other components are not identical. The term 'anise oil' is therefore avoided in the following text and the terms 'essential oil' or 'oil' refer specifically to the essential oil from aniseed.

CONSTITUENTS

The essential oil (2-6%) contains predominantly *trans*-anethole (80-95%) with smaller amounts of estragole, *cis*-anethole, anisaldehyde and pseudoisoeugenyl-2-methylbutyrate [Blaschek 2007; Kubeczka 1976; Kubeczka 1978; Schultze 1987]; sesquiterpene and monoterpene hydrocarbons are also present [Kubeczka 1976; Scultze1987; Orav 2008].

Other constituents include flavonol glycosides [El-Moghazi 1979; Kunzemann 1977], phenolic acids [Schulz 1980; Baerheim-Svendsen 1951; El-Wakeil 1986], phenolic and hydroxyalkylglucosides [Dirks 1984; Fujimatu 2003] furanocoumarins [Ceska 1987; Kartnig 1969], hydroxycoumarins [Blaschek 2007] and fatty oil [Kartnig 1969].

CLINICAL PARTICULARS

Therapeutic indications
Dyspeptic complaints and mild spasmodic gastro-intestinal complaints, such as bloating, flatulence [Blaschek 2007; Czygan 2002; Weiss 1997; Pimpinella 1983; Sweetman 2011; Czygan 1992; Hänsel 1999].

In mild inflammation of the upper respiratory tract, as expectorant [Blaschek 2007; Czygan 2002; Weiss 1997; Pimpinella 1983; Sweetman 2011; Czygan 1992; Hänsel 1999].

In these indications the effects are plausible on the basis of human experience and longstanding use.

Posology and method of administration

Dosage

Adult average daily dose: 3 g of crushed fruits as an infusion or similar preparation [Czygan 2002; Pimpinella 1983].

Children, average daily dose: *0-1 year of age*, 0.5 g of crushed fruits as an infusion; *1-4 years of age*, 1 g; *4-10 years of age*, 2 g; *10-16 years of age*, the adult dose [Dorsch 2002].

Method of administration
For oral administration.

Duration of use
No restriction.

If symptoms persist, consult your doctor.

Contra-indications
Persons with known sensitivity to anethole should avoid aniseed.

E/S/C/O/P MONOGRAPHS

Special warnings and special precautions for use
Persons with known sensitivity to anethole should avoid aniseed [Newall 2002; Andersen 1978; Franks 1998]. The sensitizing potential of aniseed is considered low [Hausen 1988].

Interaction with other medicaments and other forms of interaction
None known. Experimental data on weak enzyme induction in rodents cannot be directly extrapolated to man [Marcus 1982].

Pregnancy and lactation
Aniseed may be used during pregnancy and lactation at the recommended dosage, as aqueous infusions only.

Preparations containing the essential oil [Tisserand 1995] or alcoholic extracts should not be used during pregnancy and lactation. Mild oestrogenic activity and antifertility effects of the essential oil and anethole (the major constituent of the essential oil) have been demonstrated *in vitro* and in rats [Dhar 1995].

Effects on ability to drive and use machines
None known.

Undesirable effects
Rare cases of contact dermatitis caused by anethole-containing toothpastes and cosmetic creams [Barnes 2007; Andersen 1978], occupational rhinoconjunctivitis and food allergy caused by working in a bakery making biscuits with aniseed [Garcia-Gonzales 2002] and repeated episodes of tongue angioedema in a patient after drinking aniseed liqueur [Garcia 2007].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties
The medicinal use of aniseed is largely due to secretolytic, antispasmodic and secretomotor effects of its essential oil [Blaschek 2007].

In vitro experiments

Antimicrobial effects
An acetone extract of aniseed inhibited the growth of a range of bacteria including *Escherichia coli* and *Staphylococcus aureus*, and also exhibited antifungal activity against *Candida albicans* and other organisms [Gülcin 2003; Maruzella 1959].

Essential oil of aniseed inhibited the growth of *Escherichia coli* (MIC: 0.5% V/V), *Staphylococcus aureus* (MIC: 0.25%), *Salmonella typhimurium* (MIC: 2.0%) and *Candida albicans* (MIC: 0.5%) using the agar dilution method [Hammer 1999]. Antimicrobial activity of the oil has also been demonstrated in other studies [Ramadan 1972; Shukla 1987; Ibrahim 1991].

A methanolic dry extract of aniseed reduced the resistance of *Pseudomonas aeruginosa* to certain antibiotics when used in combination with the individual antibiotic (both the extract and antibiotic being at concentrations which did not inhibit growth when used alone; antibiotic concentrations were half their minimum inhibitory concentrations). The aniseed extract was particularly effective in combination with chloramphenicol, gentamicin, cephalexin, tetracycline or nalidixic acid against the standard strain of *P. aeruginosa*, causing almost complete inhibition of growth; it was less effective against a particularly resistant strain of *P. aeruginosa*, but inhibited growth by 74% in combination with tetracycline [Aburjai 2001].

The essential oil (0.0125-0.1%) concentration-dependently inhibited the growth of the fungi *Aspergillus flavus, A. parasiticus, A. ochraceus* and *Fusarium moniliforme*. The formation of mycotoxins (aflatoxins, ochratoxin A and fumonisin) in wheat infected with the fungi were also inhibited [Soliman 2002].

Secretolytic and expectorant effects
A modest increase in mucociliary transport velocity of isolated ciliated epithelium from the frog oesophagus was observed 90 seconds after application of 200 µl of an infusion from aniseed (4.6 g per 100 mL of water) [Müller-Limroth 1980].

Spasmolytic effects
The essential oil at 200 mg/litre produced complete relaxation of carbachol-induced contractions of isolated tracheal smooth muscle from the guinea pig. In contrast, the oil increased the contraction force in electrically-stimulated guinea pig ileal smooth muscle with an EC_{50} of 6-7 mg/litre (a positive inotropic effect) [Reiter 1985].

The essential oil (0.02 mL), an aqueous extract (0.6 mL, equivalent to 1.5 g of aniseed) and an ethanolic extract (0.1 mL, equivalent to 0.25 g of aniseed) exhibited relaxant effects on methacholine-induced contractions of guinea pig tracheal chains (prepared from rings of isolated tracheal smooth muscle). The bronchodilatory effects were significant ($p<0.05$, $p<0.005$, $p<0.001$ respectively) compared to those of controls (0.6 mL of saline for the essential oil and aqueous extract; 0.1 mL of ethanol for the ethanolic extract) [Boscabady 2001].

Local anaesthetic activity
Trans-anethole concentration-dependently reduced electrically-evoked contractions of rat phrenic nerve-hemidiaphragm, by 10.3% at 10^{-3} µg/mL, by 43.9% at 10^{-2} µg/mL, by 79.7% at 10^{-1} µg/mL and by 100% at 1 µg/mL [Ghelardini 2001].

Tumour-inhibiting activity
Anethole at a concentration below 1 mM has been shown to be a potent inhibitor of tumour necrosis factor (TNF)-induced cellular responses, such as activation of nuclear factor-kappa B (NF-κB) and other transcription factors, and also to block TNF-induced activation of the apoptotic pathway. This might explain the role of anethole in suppression of inflammation and carcinogenesis [Chainy 2000].

Oestrogenic activity
Dry extract of aniseed with hot water (DER 10:1) exhibited selective oestrogen receptor modulator (SERM)-like properties in various *in vitro* assays. At a concentration of 25 µg/mL, the extract caused a significant ($p<0.05$) increase in alkaline phosphatase activity. At 50 µg/mL it significantly ($p<0.05$) increased the formation of mineralized nodules [Kassi 2004].

The essential oil, and the pure isolated compound *trans*-anethol, exhibited extremely weak oestrogenic effect using the yeast oestrogen screen (YES) assay (yeast cells expressing the human oestrogen receptor) with an EC_{50} of 600 µg/mL for the essential oil and 625 µg/mL for *trans*-anethol. Relative potency compared to 17β-estradiol was 9.47×10^{-8} for the essential oil and 8.6×10^{-8} for trans-anethol [Tabanca 2004].

Using the same model, the potency of 17β-estradiol was found to be at least 10^5-10^6 times that of anethole, as well as for the other essential oil constituents [Howes 2002].

Antioxidant activity
The essential oil in concentrations of 2µM and 5µM inhibited the copper-catalyzed oxidation of human LDL by 40% and 71% respectively [Teissedre 2000]. An aqueous dry extract of

aniseed exhibited antioxidant activity in six different *in vitro* assays [Hinneburg 2006].

In vivo experiments

Secretolytic and expectorant effects
An emulsion of 2 drops of the essential oil, administered intragastrically to cats, caused hypersecretion of mucus in the air passages and stimulated ciliary removal of mucus (which had been inhibited by opium alkaloids) [VanDongen 1953].
A solution of the essential oil in 12% ethanol, administered intragastrically to anaesthetised guinea pigs at 50 mg/kg b.w., induced a 3- to 6-fold increase in respiratory tract fluid during the first 2 hours after administration; even 10 mg/kg caused a 2-fold increase [Boyd 1946]. A similar experiment in anaesthetised rats, dosed orally with the oil at 0.0015 mL/kg, resulted in a 28% increase of respiratory tract fluid without influencing chloride concentration or density [Boyd 1954]. Administration of the oil vapour to anaesthetised rabbits by inhalation (in steam) dose-dependently increased the volume of respiratory secretion by 19-82%; doses added to the vaporizer were 0.7-6.5 g/kg b.w. (the amount to which the animals were exposed being considerably less). However, at the highest dose level there were signs of tissue damage and a mortality rate of 20% [Boyd 1968].

Sedative effect
The pentobarbital-induced sleeping time of mice was prolonged by 93.5% ($p<0.01$) after simultaneous intraperitoneal administration of essential oil at 50 mg/kg b.w.; *trans*-anethole gave similar results [Marcus 1982].

Anticonvulsant effect
The anticonvulsant effect of intraperitoneally administered aniseed oil in mice with seizures induced by pentylenetetrazole (PTZ) and maximal electroshock (MES) was investigated. Pretreatment with the oil significantly ($p< 0.001$) suppressed PTZ-induced hind limb tonic extensions (HLTE) and mortality with an ED_{50} value of 0.52 mL/kg b.w., and MES-induced HLTE and mortality with an ED_{50} value of 0.20 mL/kg b.w. ($p<0.001$). The essential oil dose-dependently increased the dose of i.v. administered PTZ needed to induce seizures in the mice ($p<0.01$ for 1 mL oil/kg b.w.) [Pourgholami 1999].

Oestrogenic activity
Trans-anethole administered orally to immature female rats at 80 mg/kg b.w. for 3 days significantly increased uterine weight, to 2 g/kg compared to 0.5 g/kg in controls and 3 g/kg in animals given oestradiol valerate subcutaneously at 0.1 µg/rat/day ($p<0.001$). The results confirmed that *trans*-anethole had oestrogenic activity; other experiments showed that it has no anti-oestrogenic, progestational, anti-progestational, androgenic or anti-androgenic activity [Dhar 1995].

Local anaesthetic activity
In the rabbit conjunctival reflex test, solutions of *trans*-anethole administered into the conjunctival sac concentration-dependently increased the number of stimuli required to evoke the conjunctival reflex ($p<0.01$): 9.7 stimuli at 10 µg/mL, 31.8 stimuli at 30 µg/mL and 65.2 stimuli at 100 µg/mL, compared to about 3 stimuli for vehicle controls. The effect was comparable to that of procaine [Ghelardini 2001].

Anti-tumour activity
In Swiss albino mice with Ehrlich ascites tumour (EAT) in the paw, anethole administered orally at 500 or 1000 mg/kg on alternate days for 60 days significantly and dose-dependently reduced tumour weight ($p<0.05$ at 500 mg/kg, $p<0.01$ at 1000 mg/kg), tumour volume ($p<0.01$ at 500 mg/kg, $p<0.001$ at 1000 mg/kg) and body weight ($p<0.05$ to 0.01) compared to EAT-bearing controls. Mean survival time increased from 54.6 days to 62.2 days (500 mg/kg) and 71.2 days (1000 mg/kg). Histopathological changes were comparable to those after treatment with cyclophosphamide (a standard cytotoxic drug). These and other results demonstrated the anticarcinogenic, cytotoxic and non-clastogenic nature of anethole [Al-Harbi 1995].

Anti-ulcer activity
An aqueous suspension of aniseed, administered to rats intragastrically at a dose equivalent to 250 and 500 mg crude drug/kg b.w., significantly ($p<0.001$) prevented gastric mucosal lesions induced by 80% ethanol, 0.2M NaOH or 25%NaCl [Al Mofleh 2007].

Enzyme induction
Subcutaneous administration of the essential oil to partially (two-thirds) hepatectomized rats at 100 mg/animal/day for 7 days stimulated liver regeneration ($p<0.01$) [Gershbein 1977].

Experiments in which rats were injected intraperitoneally with a mixture of *trans*-anethole (100 mg/kg b.w.) and [14C] parathion (1.5 mg/kg) showed no significant effect of *trans*-anethole on metabolism and excretion of the insecticide. However, when rats were fed a diet containing 1% of *trans*-anethole for 7 days and subsequently cell fractions from the livers of these rats were incubated for 2 hours with [14C] parathion, significantly less unchanged parathion (1.6%) was recovered compared to controls (12.5%). The data were interpreted as suggesting that feeding *trans*-anethole to rats for 7 days induced the synthesis of parathion-degrading liver enzymes [Marcus 1982].

Anti-diuretic activity
Aniseed oil, added to drinking water at a 0.05% concentration, exerted a significant ($p<0.027$) anti-diuretic effect in rats, as expressed by the urine to water intake ratio [Kreydiyyeh 2003].

Pharmacokinetic properties

Pharmacokinetics in animals
No data available for aniseed.

In mice and rats *trans*-anethole is reported to be metabolized by O-demethylation and by oxidative transformation of the C3-side chain. After low doses (0.05 and 5 mg/kg b.w.) O-demethylation occurs predominantly, whereas higher doses (up to 1500 mg/kg b.w.) give rise to higher yields of oxygenated metabolites [Sangster 1984a; Sangster 1984b].

Pharmacokinetics in humans
No data available for aniseed.

After oral administration of radioactively-labelled *trans*-anethole (as the *methoxy*-^{14}C compound) to 5 healthy volunteers at dose levels of 1, 50 and 250 mg on separate occasions, it was rapidly absorbed. 54-69% of the dose (detected as ^{14}C) was eliminated in the urine and 13-17% in exhaled carbon dioxide; none was detected in the faeces. The bulk of elimination occurred within 8 hours and, irrespective of the dose level, the principal metabolite (more than 90% of urinary ^{14}C) was 4-methoxyhippuric acid [Caldwell 1988]. An earlier study with 2 healthy subjects taking 1 mg of *trans*-anethole gave similar results [Sangster 1987].

Preclinical safety data
Most toxicity studies relevant to aniseed have been conducted on *trans*-anethole, the major constituent of the essential oil.

Acute toxicity
Oral LD_{50} values per kg b.w. have been determined for the

essential oil as 2.7 g in rats [Sangster 1987] and for *trans*-anethole as 1.82-5.0 g in mice, 2.1-3.2 g in rats and 2.16 g in guinea pigs [Lin 1991].

Intraperitoneal LD_{50} value for the essential oil from aniseed was determined as 0.93 (1.11-0.79) mL/kg bw. for mice [Pourgholami 1999].

Intraperitoneal LD_{50} values for *trans*-anethole have been determined as 0.65-1.41 g/kg in mice and 0.9-2.67 g/kg in rats [Lin 1991].

Repeated-dose toxicity
No data available for aniseed.

In 90-day experiments in rats, 0.1% of *trans*-anethole in their diet caused no toxic effects. However, dose-related oedema of the liver was reported at higher levels of 0.3%, 1% and 3%, which have no therapeutic value [Lin 1991]. Male rats receiving 0.25% of anethole in their diet for 1 year showed no toxic effects, while others receiving 1% for 15 weeks had slight oedematous changes in liver cells [Hagan 1967]. Rats given *trans*-anethole as 0.2, 0.5, 1 or 2% of their diet for 12-22 months showed no effects at any level on clinical chemistry, haematology, histopathology or mortality. Slower weight gain and reduced fat storage were noted at the 1% and 2% levels [Lin 1991, LeBourhis 1973].

Reproductive toxicity
Trans-anethole exerted dose-dependent anti-implantation activity after oral administration to adult female rats on days 1-10 of pregnancy. Compared to control animals (all of which delivered normal offspring on completion of term), *trans*-anethole administered at 50, 70 and 80 mg/kg b.w. inhibited implantation by 33%, 66% and 100% respectively. Further experiments at the 80 mg/kg dose level showed that in rats given *trans*-anethole only on days 1-2 of pregnancy normal implantation and delivery occurred; in those given *trans*-anethole only on days 3-5 implantation was completely inhibited; and in those given *trans*-anethole only on days 6-10 three out of five rats failed to deliver at term. No gross malformations of offspring were observed in any of the groups. The results demonstrated that *trans*-anethole has antifertility activity. From comparison with the days 1-2 group (lack of antizygotic activity), the lower level of delivery in the days 6-10 group was interpreted as a sign of early abortifacient activity [Dhar 1995].

Mutagenicity
A dry ethanolic aniseed extract was mutagenic at high concentrations (5 mg/plate) to streptomycin-dependent strains of *Salmonella typhimurium* TA 98 [Shashikanth 1986]. An ethanolic aniseed extract gave negative results at the maximum non-toxic concentration of 0.1 mg/mL in chromosomal aberration tests using a Chinese hamster 1984 cell line [Ishidate].

The essential oil and *trans*-anethole were mutagenic at 2 mg/plate in the Ames test using *Salmonella typhimurium* strains TA 98 and TA 100, and mutagenicity was potentiated by S13 activation [Marcus 1982]. In another study, *trans*-anethole was mutagenic to *Salmonella typhimurium* TA 100 in the Ames test with S9 activation, doses of 30-120 µg/plate showing a dose-dependent increase in revertants, which did not exceed twice the number of the control [Sekizawa 1982]. Other investigations with metabolic activation have confirmed that *trans*-anethole is weakly mutagenic [Lin 1991].

Estragole, a minor constituent of anise oil, has also shown mutagenic potential in various Ames tests, demonstrating the need for carcinogenicity studies [DeVincenzi 2000; European commission 2001].

Anti-genotoxic activity of trans-anethole
In the mouse bone marrow micronucleus test, oral pre-treatment of mice with trans-anethole at 40-400 mg/kg b.w., 2 and 20 hours before intraperitoneal injection of genotoxins, led to moderate, dose-dependent protective effects against known genotoxins such as cyclophosphamide, procarbazine, N-methyl-N'-nitrosoguanidine, urethane and ethyl methane sulfonate ($p<0.05$ to $p<0.01$ at various dose levels). No significant increase in genotoxicity was observed when *trans*-anethole (40-400 mg/kg b.w.) was administered alone [Abraham 2001].

Carcinogenicity
A 1-year experiment in which mice received *trans*-anethole in their diet gave no evidence of carcinogenic potential: low levels of DNA adducts were observed in liver tissue [Lin 1991]. In another chronic study of *trans*-anethole in mice there were no histological differences between treated and control animals [Miller 1983]. In a study in rats, the highest dose feeding group (1% *trans*-anethole for 117 weeks) presented with hyperplastic and partially neoplastic changes in the livers of female (but not male) rats; a review of these results confirmed that the observed neoplastic changes in the liver were not due to a direct genotoxic effect induced by *trans*-anethole [Lin 1991].

Genotoxicity studies performed with aniseed extracts, the essential oil, *trans*-anethole and estragole do not provide adequate data to fully evaluate the carcinogenic risk. However, the carcinogenic risk from aniseed in man is assumed to be low [Aniseed].

Clinical safety data
A severe case of poisoning with anise oil after intake of 10-15 ml oil is reported. The symptoms were convulsions, unconsciousness and vomiting [Bang 2008].

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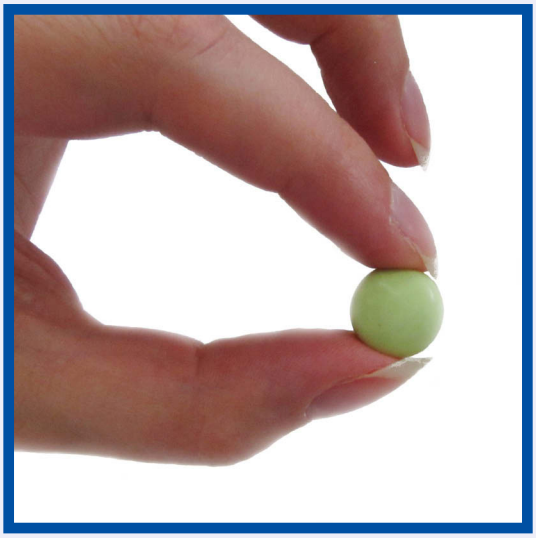

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Plant illustrated on the cover: *Arctium lappa*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
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Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

ARCTII RADIX

2016

Burdock root

DEFINITION

Burdock root consists of dried, whole or cut roots of *Arctium lappa* L. (= *A. major* Gaertn.), *A. minus* (Hill) Bernh., *A. tomentosum* Mill. and hybrids or mixtures thereof. The root is collected in the autumn of the first year or in the spring of the second year. The material complies with DAC 2014 or with the French Pharmacopoeia [Ph. Fr, 1989].

CONSTITUENTS

The main characteristic constituents are:

- Carbohydrates (ca. 70%) containing up to 45% of inulin [Wichtl 2009; Yotchkova 1990] in *A. lappa,* up to 27% in *A. minus* and up to 19% in *A. tomentosum* [De Smet 1993], a xyloglucane [Kato 1993]; 6.8% starch [Pandey 2007] and other fructans [Olennikov 2011], fructooligosaccharides with degrees of polymerization between 3 and 9 [Li 2013]. A fructan composed of fructose and glucose in the ratio of 13.0:1.0 [Liu 2014].

- Essential oil (0.06 to 0.2%) which contains mainly aplotaxene, dehydrocostuslactone, 11,13-dihydrodehydrocostuslactone and costic acid. Other components are aliphatic hydrocarbons, aliphatic and aromatic aldehydes, carbonic acids, pyrazines and sesquiterpenes [Washino 1985; Yotchkova 1990; Blaschek 2006].

- Polyacetylenic compounds (0.001 to 0.002%), the main one is trideca-1,11-diene-3,5,7,9-tetrayne, sulfur-containing acetylenic compounds (arctic acid, arctinone-a and -b, arctinol-a and -b, arctinal) [Washino 1986; Yotchkova 1990] and lappaphen-a and -b [Washino 1987].

- Phenolic acids (1.9 to 3.65%) [Wichtl 2003]: caffeic acid [Maruta 1995, Ferracane 2010, Liu 2012], hydroxycinnamoyl quinic acids [Lin 2008] such as chlorogenic acid [Pandey 2004 and 2007] and derivatives [Ferracane 2010, Jaiswal 2011; Haghi 2013] as well as tannins [Pandey 2007].

- Other constituents include triterpenes [Washino 1985, Yotchkova 1990], unsaturated sterols [Yotchkova 1990], arctigenin [De Souza Predes 2011, 2014] and arctiin [Ferracane 2010, Liu 2012] as well as b-asparagin [Boev 2005].

CLINICAL PARTICULARS

Therapeutic indications

Internal use
Seborrhoeic skin, eczema, furuncles, acne, psoriasis [Rolet and Bouret 1928; Planchon 1946; Fournier and Leclerc 1947; Paris and Moyse 1971; Valnet 1976; BHMA 1983; Van Hellemont and Delfosse 1986; Dorvault 1987; Rombi 1991; Bradley 1992; Cazin and Cazin 1997; Barnes 2007].

As an adjuvant in minor urinary tract complaints, by increasing the amount of urine to achieve flushing of the urinary tract [Rolet and Bouret 1928; Planchon 1946; Fournier and Leclerc 1947; Paris and Moyse 1971; Valnet 1976; BHMA 1976, 1983; Van Hellemont and Delfosse 1986; Dorvault 1987; Rombi 1991; Cazin and Cazin 1997; Barnes 2007].

External use
Seborrhoeic skin, eczema, furuncles, acne [Fournier et Leclerc 1947; Garnier 1961; Leclerc 1976; Paris et Moyse 1971; Valnet 1976; Van Hellemont and Delfosse 1986; Rombi 1991; Bradley 1992].

Efficacy in these indications is plausible on the basis of long-standing use.

E/S/C/O/P MONOGRAPHS

Posology and method of administration

Dosage

Internal use
Adult dose
At three times daily: 2 – 6 g as an infusion; 2 to 8 mL as a liquid extract (1:1, 25% ethanol); 8-12 mL as a tincture (1:10, 45% ethanol) [BHMA 1983, Bradley 1992, Barnes 2007].
As a decoction at 500 mL (1:20) per day [Barnes 2007].

External use:
Adults
Fresh pulp of root [Fournier et Leclerc 1947; Garnier 1961; Paris et Moyse 1971 ; Leclerc 1976].
Decoction (60 g/litre) [Garnier 1961; Leclerc 1976].

Method of administration
For oral administration and topical application.

Duration of use
If symptoms persist or worsen, medical advice should be sought.

Contra-indications
Hypersensitivity to plants of the Asteraceae family [Hausen 1997].

Special warnings and precautions for use
Burdock root should not be used in patients with oedema due to impaired heart and kidney function [Wichtl 2009].

Interaction with other medicinal products and other forms of interaction
None reported.

Pregnancy and lactation
The product should not be used during pregnancy and lactation [Barnes 2007].

Effects on ability to drive and use machines
None known.

Undesirable effects
Hypersensitivity can occur with topical application [Yotchkova 1990; Rodriguez 1995] or by oral route [Sasaki 2003].

Overdose
No case of overdose reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antibacterial activity
A dry ethanolic extract (1:5, 70%) demonstrated activity against *Bacillus subtilis* and several Gram negative bacteria (*E. coli*, *Shigella flexneri*, *Shigella sonnei*) but no activity against *Staphylococcus aureus* at 400 µg/disc [Moskalenko 1986].

Ethanolic extracts (70%; not further specified) demonstrated greater antimicrobial activity than dry water extracts (not further specified). MIC values for the ethanolic extracts were found to be 31.25 mg/mL for *Bacillus subtilis* and *Staphylococcus aureus*, 62.50 mg/mL for *Proteus vulgaris* and 250 mg/mL for *E. coli* [Mi 2010].

An extract (not further specified) at a concentration of 0.01% significantly inhibited the chemotaxis effect of *Propionibacterium acnes* on polymorphonuclear leucocytes ($p<0.05$) and exerted antibacterial activity against two strains of *P. acnes* with MICs of 1400 µg/mL, whereas the MICs for erythromycin were 300 and 0.5 µg/ml [Nam 2003].

A 25% ethanolic dry extract of *Arctium minus* exhibited greater than 60% inhibition of *S. aureus* at 40 µg/mL and more than 50% inhibition of *B. subtilis* at 200 µg/mL[Watkins 2012].

Antiviral properties
Epstein Barr virus activation induced by 40 ng 12-*O*-hexadecanoylphorbol-13-acetate and 88 µg of n-butyric acid was moderately inhibited at 50-69% by 40 µg of the ethylacetate soluble part of burdock root but not by 200 µg of a methanolic extract or 40 µg of a water soluble part (29% or less) [Koshimizu 1988].

When added to the medium at the time of viral infection and immediately after the viral infection, arctiin showed antiviral activity against influenza A virus (H1N1) with an IC_{50} of 24 and 22 µM respectively [Hayashi 2010].

Antioxidant activity
Ethanolic, aqueous and chloroform extracts showed activity in the DPPH radical scavenging test [Duh 1998, Dos Santos 2008, Chen 2009, Predes 2011, da Silva 2013].

Aqueous dry extracts showed antioxidant and radical scavenging effects in different test systems such as inhibition of linoleic acid peroxidation, inhibition of malondialdehyde formation, anti-$FeCl_2$ ascorbic acid-stimulated lipid peroxidation in rat liver homogenate, scavenging of superoxide, hydrogen peroxide and hydroxyl radical scavenging [Duh 1998].

A methanolic dry extract (10% m/V, DER not specified) showed significant antioxidant activity on liposome, deoxyribose, protein and LDL oxidation ($p<0.05$). In 3T3 cells, at 200 µg/mL, the extract enhanced GSH levels and increased significantly the activities of glutathione peroxidase, glutathione reductase, glutathione-*S*-transferase and catalase ($p< 0.05$). In RAW 264.7 cells stimulated with LPS, the extract scavenged NO in a dose-dependent manner and reduced COX-2 and iNOs protein expressions at 500 µg/mL [Wang 2006].

Elastase activity was inhibited by 50.9% by 10 mg/mL of an aqueous extract (no further details, approximately 0.1 mg/mL total phenolic content expressed as gallic acid). The antioxidant activity of the extract was equivalent to 4.73 µM Trolox. The extract did not show SOD mimetic activity and did not inhibit collagenase [Thring 2009].

A strong correlation ($r^2 = 0.98$) was shown between total phenolic contents of different burdock extracts (45.8% in seeds, 15.3% in leaves and 2.87% in roots) and the total antioxidant activity (0.069, 0.029 and 0.0016 mmol of Trolox/100 g dry weight) [Ferracane 2010].

The polysaccharide ALP1 isolated from the roots showed moderate ABTS radical scavenging activity, strong hydroxyl radical scavenging activity and strong ferrous ion chelating activity [Liu 2014].

Antiinflammatory effects and antiallergic properties
Diarctigenin inhibited the production of NO, prostaglandin E2, tumour necrosis factor-α, and interleukins (IL)-1β and IL-6 with IC_{50} values of 6 to 12 µM in zymosan- or lipopolysaccharide-(LPS) activated macrophages. It attenuated zymosan induced mRNA synthesis of inducible NO synthase (iNOS) and also

inhibited promotor activities of iNOS and cytokine genes in the cells. Diarctigenin inhibited the transcriptional activity and DNA binding ability of NF-κB in zymosan-activated macrophages but did not affect the degradation and phosphorylation of inhibitory κB (IκB) proteins. Moreover, it suppressed expression vector NF-κBp65-elicited NF-κB activation and also iNOS promotor activity,indicating that the compound could directly target an NF-κB activating signal cascade downstream of IκB degradation and inhibit NF-κB-regulated iNOS expression. Diarctigenin also inhibited the *in vitro* DNA binding ability of NF-κB but did not affect the nuclear import of NF-κB p65 in the cells [Kim 2008].

The butanolic fraction of an ethanolic (30%) extract significantly suppressed the dinitrophenyl-bovine serum albumin (DNP-BSA) induced β-hexominidase secretion in IgE-sensitized RBL-2H3 cells in a dose-dependent manner at 1 ($p<0.01$), 10 ($p<0.01$) and 100 μg/mL ($p< 0.05$) compared to DNP-BSA alone. In concanavalin A-treated cells, the extract significantly increased murine splenocyte proliferation at 10 and 100 μg/mL ($p<0.05$) and significantly decreased mRNA expression of IL-4 and IL-5 at 100 μg/mL and their secretion compared with concanavalin A-stimulated splenocytes ($p<0.01$). The same dose of the extract attenuated the NF-κB activation and also the phosphorylation of mitogen-activated protein kinases such as p38, c-Jun N-terminal kinase (JNK) and extracellular-signal-regulated kinases (ERK) [Sohn 2011].

Effects on glucose

Incubation of L6 myotubes with 1 μM arctigenin resulted in a 1.49-fold increase in glucose uptake, which is comparable with the effect of 100 nM insulin. Arctigenin (0.1, 0.3, 1 and 3 μM) increased basal glucose uptake in a dose- and time-dependent manner ($p<0.01$). Arctigenin (1, 3, 10 and 30 μM) dose dependently increased ($p<0.01$) AMPK and acetyl co-enzyme A synthetase (ACC) phosphorylation in L6 myotubes. Significant increases of AMPK and ACC phosphorylation were observed within 15 min after arctigenin treatment (3 μM) and were maintained for up to 120 min. Incubation of EDL or soleus muscles from C57BL/6J mice with 3 μM arctigenin resulted in an increase of glucose uptake by 31.7% ($p=0.1$) and 18% ($p<0.05$), or 42% ($p<0.05$) and 20.6% ($p<0.01$) under basal and insulin-stimulated conditions, respectively. Arctigenin (1, 3 and 10 μM) also dose-dependently increased AMPK and ACC phosphorylation in isolated EDL and soleus muscles. In primary rat hepatocytes incubation with arctigenin at 2.5, 5, 10, 20 or 40 μM for 1h increased AMPK and ACC phosphorylation in a dose-dependent manner. Glucagon induced hepatic gluconeogenesis (10 nM for 20h) was significantly suppressed by arctigenin treatment in a dose-dependent manner (10, 20 and 40 μM); arctigenin (5, 10, 20 and 40 μM) also significantly decreased the insulin stimulated synthesis (10nM) of fatty acids and sterols. The suppression of gluconeogenesis and lipid synthesis by a treatment of 20 μM arctigenin for 20 h was completely blocked by compound C pretreatment (20 μM) [Huang 2012].

At a concentration of 100 μg/mL an ethanolic extract containing approximately 15% chlorogenic acid induced a reduction in insulin release in INS-1cells in the presence of 5.6 mM of glucose ($p<0.05$) and a significant increase in glucose uptake on L6 muscular cells ($p<0.05$) in the presence of insulin (100 nM). This effect was not observed in the absence of insulin in the medium. At concentrations of 50 and 100 μg/mL, the extract showed a significant reduction in glucagon (100 nM)-induced glucose production ($p<0.01$) [Tousch 2011].

Antimutagenic / Antiproliferative effects

In *Salmonella* strain TA 98, the supernatant of a burdock root juice inhibited the mutagenicity induced by 4-nitro-1,2-diaminobenzene and 2-nitro-1,2–diaminobenzene without metabolic activation, and by ethidium bromide, 2-amino-anthracene, 3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole, 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole with metabolic activation at 10.4 and 0.2 μg/plate, respectively. It did not inhibit mutagenicity induced by 2-(2-furyl)-3-(nitro-furyl)acrylamide (AF-2) in TA 100 strain [Morita 1984].

In human breast cancer MCF-7 cells, a metabolite of arctiin, (2*R*,3*R*)-2-(3′-hydroxybenzyl)-3-(3″,4″-dihydroxybenzyl) butyrolactone, showed significant antiproliferative activity at 10 μM ($p<0.001$) compared to control. In another experiment, this compound at 10 μM inhibited the oestradiol-mediated proliferative effect [Xie 2003].

The number of human prostate cancer PC-3 cells treated with arctiin, at doses up to 100 μM for 36, 42 and 48h in a serum-containing condition, decreased in a concentration- and time-dependent manner. According to microscopic examination, arctiin induced cell detachment from culture plates in a concentration-dependent manner. This effect was inhibited by cycloheximidine, showing that protein synthesis was required. Cells treated with 30 μM of arctiin in serum-containing medium for 24h significantly ($p<0.001$) increased MUC-1 expression (protein inhibiting cell-cell interactions) on the cell surface by up-regulating the mRNA [Huang 2004].

b-Asparagine isolated from burdock roots at a concentration of 0.4. and 4 mM induced apoptosis in Ehrlich's carcinoma tumour cells (2.5×10^5 cells/mL) by 5.4% and 87% respectively. b-Asparagine (0.075 to 75 mM) suppressed phytohaemagglutinin-induced proliferative activity of lymphocytes. Synthetic L-asparagine did not exhibit cytostatic and apoptosis-inducing activity [Boev 2005].

Arctiin inhibited the growth of human immortalized keratinocyte HaCaT cells in a dose-dependent manner. Treatment with 100 μM of arctiin for 48 h significantly ($p<0.05$) increased the percentage in the G1 phase from 38.2 to 48.8%. Arctiin had no effect or slightly affected the expression of cyclin-dependent kinase (CDK) inhibitor proteins (p21, p27, p57, p15, p18 and p19), whereas the expression of the cyclin D1 (promotor of the G1-to-S phase transition of the cell cycle) was strongly decreased in a time- and dose-dependent manner by phosphorylating the tumour-suppressor retinoblastoma protein. The down-regulation of cyclin D1 was demonstrated in human lung, colorectal, cervical, breast, melanoma, renal and prostate tumour cell lines [Matzuzaki 2008].

Two dichloromethane extracts (yield 0.12 and 0.10%) showed moderate antiproliferative activity against K562 (leukaemia) cell lines with tumour growth inhibition (TGI) of 17.06 and 3.62 μg/mL, respectively, as well as against 786-0 (renal) cell lines with TGI 155.79 and 60.32 μg/mL compared to doxorubicin as a positive control with TGI 0.03 and 0.20 μg/mL, respectively [Predes 2011].

Arctigenin extracted from seeds showed ED_{50} values of 4.5, 5.4 and 11 μg/mL for Hep G2 (human liver carcinoma), A459 (lung cancer) and Kato III cells (stomach cancer) respectively, whereas ED_{50} values for normal cells were more than 100μg/mL. Incubated with arctigenin (50 μg/mL) for 4, 8, 24 and 48 h, A459 cell numbers slightly decreased ($p<0.01$) with the treatment time compared to control (PBS) whereas the number of lung normal diploid fibroblast (WI-38) cells did not change with or without treatment with arctigenin. After incubation with 50 μg/mL of arctigenin for 4h, caspase-3 activity is significantly increased in A459 cells ($p<0.01$) in a time-dependent manner and slightly increased in normal WI-38cells. It was shown that activation of caspase-3 activity by arctigenin was correlated

with the decrease in the viability of both cancer and normal cells ($p<0.01$) [Suzanti 2012].

An ethanolic extract containing 23.5 ± 1.8 mg/g total polyphenols expressed as gallic acid showed antileukaemic properties against J-45.01 human T cell leukaemia cell line with an IC_{50} of 0.35 ± 0.03 mg/mL [Wegiera 2012].

Other effects
Arctigenin (isolated from a CH_2Cl_2 fraction of a methanolic extract of *T. nucifera*) at 0.1-1 µM inhibited LPS-inducible phosphorylation of MAP kinases ERK1/2, p38 kinase and JNK1/2 in Raw 264.7 cells after 1 h of contact. Arctigenin inhibited the activity of MKK1 with an IC_{50} of 1 nM. Arctigenin (0.01-1µM) inhibited LPS-inducible Activator Protein-1 (AP1) DNA binding and it was shown that arctigenin significantly inhibited AP-1-mediated reporter gene expression at 0.01 µM and 0.1 µM ($p<0.05$) and 1 µM ($p<0.01$) compared to LPS alone. After 24h of incubation, arctigenin at 1µM significantly ($p<0.01$) inhibited by 30% the production of TNF alpha in LPS-treated macrophages compared to LPS alone [Cho 2004].

In myoblasts isolated from 1 to 3-day-old newborn ICR mice limbs, arctiin at 2.5 µg/mL significantly ($p<0.01$) inhibited intracellular cAMP phosphodiesterase and significantly ($p<0.05$) increased intracellular cAMP and the amount of cell total protein [Gu 2008].

Burdock inulin (1% w/V) significantly stimulated the anaerobic growth of *Bifidobacterium adolescentis* as compared to control ($p<0.05$) [Li 2008].

After incubation for 2h with 125 to 1000 ppm of an aqueous extract (12.8 mg/g of polyphenols expressed as gallic acid), the viability of RGM1 cells treated with 250 ppm indomethacin increased significantly ($p<0.05$) in a dose-dependent manner similar to sucralfate [Chen 2009].

Arctigenin, isolated from roots at 6.25, 12.5 and 25 µM, inhibited T lymphocytes activated by anti-CD3/CD28 Ab ($p=0.0374$) and suppressed IL-2 ($p=0.0278$) and IFN-gamma ($p=0.0642$) production in a concentration-dependent manner. After 3 days of incubation with arctigenin, the ratio of IL-2 to GAPDH mRNAs in the activated T lymphocytes were significantly decreased by the three concentrations ($p=0.01$) and the ratio of IFN-gamma to GAPDH mRNAs was significantly decreased by 25 µM arctigenin ($p<0.01$) compared to cells treated with DMSO and anti-CD3/CD28. In Jurkat cells, arctigenin significantly decreased NF-AT-mediated reporter gene expression in a dose-dependent manner ($p=0.0418$) [Tsai 2011].

In the MTT assay, arctiin significantly increased viability of retinal microvascular endothelial cells in a dose-dependent manner when compared to control ($p<0.01$) [Lu 2012].

The *n*-hexane fraction of an ethanolic extract (containing *a*-linoleic acid, methyl *a*-linolenate and methyl oleate) reduced HepG2 cellular lipid synthesis and increased phosphorylation of acetyl-coenzyme A carboxylases on serine 79. It decreased fatty acid synthase protein level in a time and dose-dependent manner in HepG2 cells and stimulated AMPK phosphorylation in a time- and dose-dependent manner through the LKB1 pathway [Kuo 2012].

Arctigenin depolarized mitochondrial membrane potential in a dose-dependent manner but with a mild effect (max effect lower than 30%) and dose-dependently increased the AMP/ATP ratio in L6 myotubes. Arctigenin produced a dose dependent inhibition of oxygen consumption with complex I-linked substrate ($p<0.01$), but not with complex II-linked respiration. Arctigenin (3 µM) significantly increased lactate production in L6 myotubes after 1 and 4h ($p<0.01$) [Huang 2012].

Ex vivo experiment
Goto-Kakizaki rats (a diabetic animal model) in non-fasting conditions were given a decoction *ad libitum* (125 g/L; mean consumption 42.3 mL/day) for 4 weeks. At 4 weeks, rat liver mitochondria were isolated and respiratory parameters evaluated. Compared to control, decreases in state 3 (50% inhibition; $p<0.001$) and carbonyl cyanide p-trifluoro-methoxy-phenylhydrazone (about 30% inhibition; $p<0.01$) respirations were observed. The respiratory control ratio was reduced by approximately 40% ($p<0.05$) [Ferreira 2010].

In vivo experiments

Antioxidant activity
Doses of 100 to 400 mg/kg b.w. of the polysaccharide ALP1 isolated from the roots significantly ($p<0.01$) enhanced anti-oxidant enzyme activities and total antioxidant capacity and decreased the levels of malondialdehyde (MDA) in both the serum and liver of aging mice [Liu 2014].

Antiviral properties
Ten BALB/C mice, intranasally infected with influenza A virus (H1N1), received arctiin (5 mg/day) or oseltamivir (0.2 mg/day) orally, twice a day, for 7 days. Half of the mice were also injected s.c. with 0.5 mg of 5-fluorouracil (5-FU), every other day from 7 days before virus inoculation to 13 days afterwards. At day 3, arctiin alone significantly ($p<0.01$) reduced the virus titre in the lungs and in bronchoalveolar lavage fluids compared to control, whereas no reduction was observed in mice treated with arctiin and 5-FU. By day 7, arctiin had reduced the virus production in the 5-FU group, but to a lower extent than oseltamivir. There was no significant difference in antibody response to influenza A virus in any of the groups at day 3, but a marked increase of sera antibody titre in mice which did not receive 5-FU was observed at day 7. Arctiin produced a higher antibody titre than oseltamivir or control in 5-FU mice at day 7. In another experiment, 18 mice infected with influenza A virus received orally arctiin (1 mg/day) or oseltamivir (0.02, 0.05 or 0.2 mg/day) alone or in combination, twice a day for 3 days after virus inoculation. In the bronchoalveolar lavage fluids, the combination of arctiin and oseltamivir significantly reduced the virus titres to 21% ($p<0.001$), 29% ($p<0.01$) and 60% ($p<0.01$) compared to oseltamivir alone. In the lungs, the combination with the lower doses of oseltamivir decreased the virus titres to 61% ($p<0.001$) and 64% ($p<0.01$), compared to oseltamivir alone [Hayashi 2010].

Antimutagenic / Antitumour effects
In mice, s.c. administration of a fresh burdock root methanolic extract inhibited the growth of Ehrlich ascites carcinoma with a test to control ratio of 0.6 and 0.8 at dose levels of 340 and 160 mg/kg b.w. respectively, 24 h after transplantation. An aqueous fraction of an ethanolic extract (310 mg/kg b.w.) and a dichloromethane extract (310 mg/kg b.w.) gave a test to control ratio of 0.5 and 0.57 respectively, 48h after transplantation. The methanolic extract caused haemorrhagic necrosis and liquefaction of the established solid Yoshima sarcoma at 400 mg/kg b.w. [Dombradi 1966].

Female Sprague-Dawley rats were administered intragastric doses of 2-amino-1-methyl-6-phenylimidazol[4,5-*b*]pyridine (PhIP) at 100 mg/kg b.w. once a week for 8 weeks in order to induce breast cancer. Three days later the rats were divided into three groups, receiving either a basal diet or a diet containing 0.2 or 0.02% arctiin for 40 weeks. A further three

groups without PhIP initiation were given the same test diets. Carcinoma multiplicity was significantly ($p<0.05$) decreased in arctiin treated rats compared to the PhIP/basal diet control. The number of colon aberrant crypt foci also significantly ($p<0.05$) decreased in both arctiin groups while the incidence and multiplicity of pancreatic lesions was not significantly different from the PhIP/basal diet group. Two weeks after a single injection of diethylnitrosamine (DEN) at 200 mg/kg b.w., male F344 rats received a diet containing either 0.03% 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) alone, MeIQx with 5% arctiin, 0.1% arctiin or basal diet for 6 weeks. The average number and area of preneoplastic glutathione S-transferase placental positive foci significantly increased in the hepatocarcinogen MeIQx alone group (21.1 ± 9.6 and 1.7 ± 1.0 respectively) and MeIQx associated with 0.5% arctiin (28.0 ± 5.6 and 2.6 ± 0.6 respectively) compared to basal diet control values (6.3 ± 1.4 and 0.5 ± 0.2 respectively). In contrast, treatment with 0.1% arctiin alone after DEN administration did not affect foci development [Hirose 2000].

Gastrointestinal effects
In female Wistar rats, oral doses of 10, 30 and 100 mg/kg b.w. of a chloroform extract (DER = 104:1) significantly ($p<0.05$) reduced the acute gastric lesions induced by an oral dose of 80% ethanol by 61%, 70% and 76% respectively, compared to 75% by omeprazole (40 mg/kg b.w.). The same extract administered orally at 100 mg/kg b.w. daily for 7 days reduced the chronic gastric ulcerations induced by acetic acid by 52% while omeprazole (20 mg/kg b.w.) reduced them by 71% ($p<0.05$). After pylorus ligatures, intraduodenal administration of 100, 300 and 600 mg/kg b.w. of the extract reduced the total acidity of gastric secretion by 22, 22 and 33% respectively, whereas i.p. administration inhibited total acidity by 50, 60 and 67% respectively. *In vitro*, the extract inhibited H^+, K^+-ATPase activity with an IC_{50} of 53 µg/mL and a fraction (*n*-hexane/ethyl acetate 3:1 *v/v*) of this extract containing unsaturated fatty acids and esters (30 and 100 µg/mL) by 48% and 89% respectively. In female Swiss mice treated orally with 30, 100 and 300 mg/kg b.w., the gastric emptying and the intestinal transit were not altered [Dos Santos 2008].

In Swiss mice, an aqueous dry extract (1:10 m/V) orally administered at 500 mg/kg for 24 days had no effect on abdominal lesions caused by the parasite *Angiostrongylus costaricensis* [Fante 2008].

In female Kunming mice receiving a diet containing 5% of burdock inulin for 14 days, the proliferation of caecal Bifidobacteria and Lactobacilli was significantly improved compared to control group ($p<0.05$). Enterobacteria and Enterococci counts were not significantly affected [Li 2008].

Male Wistar rats were orally treated with 260, 1300 and 2600 mg/kg b.w. of an aqueous extract (12.8 mg/g of polyphenols expressed as gallic acid) before administration of a dose of 10 mL/kg b.w. of 70% ethanol. In the verum groups the gastric mucosal lesions were 16.67, 57.22 and 48.72 mm^2 respectively, whereas without burdock treatment, the lesion area was 132.95 mm^2 ($p<0.05$) [Chen 2009].

In female Wistar rats, oral administration of an ethanolic extract (1, 3, 10 and 30 mg/kg b.w., twice daily for 7 days) significantly ($p<0.001$) reduced acetic acid-induced gastric ulceration by 29.2%, 41.4%, 59.3% and 38.5% respectively, when compared to control; whereas a dose of 100 mg/kg b.w. did not reduce gastric lesions. At 10 mg/kg, the extract did not increase the gastric mucus content but did induce cell proliferation in the stomach according to proliferating cell nuclear antigen expression. Compared to control, the 10 mg/kg extract also significantly ameliorated acetic acid-induced ulcer-associated increases in SOD activity ($p<0.01$), lipid hydro-peroxide levels ($p<0.05$), myeloperoxidase activity ($p<0.01$) and microvascular permeability ($p<0.01$), and the reduction in glutathione ($p<0.001$). Intraduodenal administration of doses of 10 and 30 mg/kg b.w., immediately after pyloric ligation, reduced the gastric volume from 6.0 ± 0.5 mL (control group) to 3.0 ± 0.1 mL and 3.4 ± 0.5 mL respectively, and acidity of gastric secretion by 80% and 72% respectively when compared to control ($p<0.001$). At 100 mg/kg, the extract did not affect gastric acid secretion [da Silva 2013].

Diuretic activity
In male albino rats, an oral dose of 175 mg/kg b.w. of a 50% ethanolic extract induced diuretic activity after 5h compared to urea (75 mg/100 g) [Sharma 1978].

An infusion (3 g/L) ingested *ad libitum* by 12 female Wistar rats for 12 days showed beneficial effects on urolithiasis by increasing urinary pH [Grases 1994].

Hepatoprotective effects
After i.p. injection of carbon tetrachloride in rats, an aqueous extract (DER 4:1) dose-dependently decreased serum GOT and GPT levels when administered i.p. at 100 ($p<0.05$), 300 ($p<0.01$) and 1000 mg/kg b.w. ($p<0.01$), compared to control. Doses of 300 and 1000 mg/kg showed greater activity than silymarin at 25 mg/kg. The liver of animals treated with the extract exhibited a marked reduction in necrosis [Lin 1996].

In male ICR mice an aqueous dry extract, orally administered at a dose of 300 mg/kg b.w., significantly suppressed the SGOT and SGPT elevation induced by 32 µL/kg of carbon tetrachloride, i.p. ($p<0.001$) or 600 mg/kg of paracetamol, i.p. ($p<0.05$). The extract significantly reversed the decrease of glutathione concentration ($p<0.05$) and of cytochrome P450 activity ($p<0.001$) in both groups whereas it decreased significantly the liver malondialdehyde content ($p<0.01$ and $p<0.001$ respectively). After treatment with the extract, lesions induced by carbon tetrachloride and paracetamol were reduced and hepatocytes were regenerated [Lin 2000].

Groups of 10 Wistar rats were treated as follows: one group was fed a liquid ethanolic diet (4 g absolute ethanol / 80 mL liquid ethanol) for 28 days and another received the same treatment and an i.p. injection of carbon tetrachloride (0.5 mL/kg b.w.) on the 21st day. Both groups received orally 300 mg/kg of an aqueous extract of burdock root, 3 times per day at day 22. In a second experiment, the pre-treatments with ethanol and ethanol / carbon tetrachloride were the same but the extract was administered 3 times daily from day 22 to day 28. No significant differences were observed between liver weights for all groups. The extract significantly improved serum levels of transaminases (SGOT and SGPT), triglycerides hepatic glutathione, cytochrome P450, cytochrome b5, NAPH cytochrome c reductase and MDA levels which were increased by ethanol and ethanol plus carbon tetrachloride pre-treatments. The extract restored the histopathological lesions induced by these substances [Lin 2002].

An extract (1:5; ethanol 70 %) was tested for liver protective effects against experimentally-induced cadmium toxicity in male Wistar rats. The extract was administered by gavage at a daily dose of 300 mg/kg b.w. for 7 or 56 days, either alone or after a single i.p. injection of cadmium chloride, saline solution was used as a control. At days 7 and 56, compared to control, plasma GOT was significantly higher in rats administered cadmium, but not in those co-administered the extract. Plasma GPT was significantly lower than control in rats given the extract, with

or without cadmium, at day 7 but not day 56. Liver GOT was significantly higher in rats given cadmium, with or without the extract, at day 7, but only in the group given both at day 56. No alterations in plasma levels of creatinine, total bilirubin and total protein were observed at days 7 or 56 [De Souza Predes 2014].

Effects on glucose
In non-fasting conditions, a decoction given *ad libitum* (125 g/L; mean consumption of 42.3 mL/day) for 4 weeks induced a significant ($p<0.05$) decrease in glucose levels in Goto-Kakizaki rats. No difference in glucose levels was observed under fasting conditions (16-18 h) and 2 h after i.p. glucose loading (1.8 g/kg b.w.) as compared to control [Ferreira 2010].

Wistar rats received 15 mg of an ethanolic extract by gavage or i.p. daily for 4 days. On the fourth day a glucose tolerance test was performed by oral (OGTT, 3 g/kg) or i.p. (IPGTT, 1 g/kg) glucose administration. After oral administration of the extract a rapid reduction in hyperglycaemia associated with a significant transient increase in plasma insulin was observed ($p<0.05$), whereas after i.p administration the extract did not modify glucose tolerance [Tousch 2011].

In C57BL/J6 mice, pre-treatment with 300 mg/kg b.w. arctigenin by gavage decreased blood glucose levels. The phosphorylation of AMPK and ACC liver isolated from arctigenin–treated mice was significantly increased by 36% and by 74% ($p<0.01$) respectively [Huang 2012].

Oral administration of an ethanolic extract (400 mg/kg) daily for 14 days to streptozotocin-induced diabetic rats significantly ($p<0.01$) decreased blood glucose (31.5%), body weight, serum triglycerides, total cholesterol, LDL levels, serum urea, creatinine and MDA levels in liver and kidney tissues at the end of the experiment compared to diabetic control. Serum insulin, muscle glycogen content and HDL level were significantly increased ($p<0.01$) as was liver glycogen content ($p<0.05$) [Cao 2012].

Streptozotocin-induced diabetic rats received arctiin at 40 or 60 mg/kg b.w. i.p.daily for 8 weeks. These treatments significantly decreased the levels of 24h-urinary albumin ($p<0.01$), prevented glomerulosclerosis and effectively restored glomerular filtration barrier damage ($p<0.01$) compared to non-treated diabetic rats; up-regulating the expression of nephrine and podocine and down-regulating heparanase [Ma 2012].

Three groups (n = 20) of male Sprague-Dawley rats with streptozotocin-induced diabetic retinopathy received daily oral doses of arctiin of 30, 90 and 270 mg/kg b.w. for 16 weeks. The serum glucose level was significantly decreased by the highest dose of arctiin from weeks 8 to 16 when compared to week 0 ($p<0.05$) whereas the lower doses demonstrated no effect at the end of the experiment. The glycated haemoglobin (HBA1c) level was significantly decreased at week 8 with the dose of 270 mg/kg of arctiin and at week 16 with the two other doses ($p<0.05$). In retinal tissue, the inner plexiform layer or the rod layer were restored, and angiogenesis was decreased in a dose-dependent manner [Lu 2012].

Three groups (n=10) of male Sprague-Dawley rats with cationic bovine serum albumin (cBSA)-induced glomerulonephritis received oral doses of arctiin at 30, 60 and 120 mg/kg b.w. per day for three weeks (starting one week after initial cBSA injection). Levels of urinary protein had decreased at weeks 2, 3 and 4 at the doses of 60 and 120 mg/kg ($p<0.05$ or $p<0.01$) but only at week 4 at 30 mg/kg, compared to control. When compared to control, all three arctiin dosages at week four demonstrated a dose-dependent signfificant ($p<0.05$ or $p<0.01$) decrease in serum blood urea nitrogen, creatinine, IL-6 and TNF-α levels, as well as significantly ($p<0.01$) decreasing the level of malondialdehyde and NF-κB DNA binding activity, and increasing super oxide dismutase activity. The parameters of renal lesion such as hypercellularity, infiltration of polymorphonuclear leukocyte, fibrinoid necrosis, focal and segmental proliferation and interstitial infiltration were ameliorated in arctiin-treated animals compared to control group [Wu 2009].

A single oral administration of arctigenin (200 mg/kg b.w.) to ob/ob mice significantly decreased blood glucose levels ($p<0.01$) by 34.2% after 2h compared to vehicle only control. Chronic oral administration of the same dose for 23 days significantly decreased random and fasting blood glucose levels with an average reduction rate over that period of 32.2% ($p<0.05$) and 35.2% ($p<0.05$ and $p<0.01$) compared to control. Chronic i.p. administration of arctigenin (25 mg/kg, twice daily) to ob/ob mice for 22 days significantly increased ($p<0.05$) insulin-stimulated AKT phosphorylation in gastrocnemius muscle and liver, but not in perirenal fat. The treatment had no effect on GLUT4 levels in gastrocnemius muscle and had no significant effect on hepatic Pepck expression, but did significantly decrease G6pase mRNA expression ($p<0.05$) [Huang 2012].

Clinical Studies
No published clinical data currently available.

Pharmacokinetic properties

Pharmacokinetics in vitro
Arctiin was stable after incubation for up to 24h with gastric juice (pH 1.2-1.5) of male Wistar rats at 37°C. After incubation for 24h with 5 mL of the suspension of intestinal flora obtained from gut, at 37°C, arctiin (500 µg) was transformed into arctigenin (55.4% of arctiin) after 1h and further metabolised into 2-(3'',4''-dihydroxybenzyl)-3-(3',4'-dimethoxybenzyl)-butyrolactone after 3h (90.9%) [Nose 1992].

A weak inhibition of cytochrome CYP3A4 (<13.5%), CYP19 (<28.5%) and CYP2C19 (<25%) was observed with a 55% ethanolic extract of burdock root (DER 2.6-4.6:1) [Scott 2006].

Metabolism
After incubation with human faecal inoculum, arctigenin was metabolized to enterolactone, 3'-demethyl-4'-dehydroxy-arctigenin and 3'demethylarctigenin, an isomer of matairesinol [Heinonen 2001].

Six metabolites were identified after anaerobic incubation of arctiin with a mixture of human faecal bacteria: (-)-arctigenin, (2*R*,3*R*)-2-(3',4'-dihydroxy-benzyl)-3-(3'',4''-dimethoxybenzyl) butyrolactone, (2*R*,3*R*)-2-(3'-hydroxybenzyl)-3-(3'',4''-dimethoxybenzyl)-butyrolactone, (2*R*,3*R*)-2-(3'-hydroxybenzyl)-3-(3''-hydroxy-4''-methoxybenzyl)butyrolactone, (2*R*,3*R*)-2-(3'-hydroxybenzyl)-3-(3'',4''-dihydroxybenzyl)butyrolactone and (-)-enterolactone [Xie 2003].

Pharmacokinetics in vivo
After i.v. injection of 0.3 mg/kg b.w of arctigenin to Wistar rats, the C_{max}, half life and AUC were 323 ± 65.2 ng/mL, 0.830 ± 0.166 and 81.0 ± 22.1 hng/mL, respectively [Zou 2013].

After oral administration of 30, 50 and 70 mg/kg b.w of arctigenin to Sprague-Dawley rats, the AUC values in plasma were 86.4 ± 11.4, 206.2 ± 57.3, and 261.7 ± 61.4 mg/mL, respectively; arctigenin was undetected after 4h. T_{max} was 90 min for the 3 doses [He 2013].

Metabolism
In BALB/c mice, arctiin was immediately hydrolysed to arcti-

genin after oral administration (5.0 mg/mL in 1% ethanol). The metabolite was present in the blood for over 12 h [Hayashi 2010].

After oral administration of arctigenin to Sprague Dawley rats, three metabolites were identified in plasma: arctigenic acid, 4-*O*-demethyl-arctigenin and arctigenin-4-*O*′-glucuronide [Gao 2013].

Distribution
In adult Sprague Dawley rats, the AUC of arctiin was not proportional to the orally administered dosage (30 to 70 mg/kg b.w.). Half-life, elimination rate constant, absorption rate constant, peak concentration, apparent volume, total body clearance and area under curve values of arctiin were significantly different in female compared to male rats ($p<0.05$) [Fan 2011].

The terminal half-life of arctiin and arctigenin was not found to be dose proportional in adult Sprague Dawley rats orally adminstered 25, 50 and 100 mg/kg of arctiin. Thirty minutes after administration the highest arctiin concentration was found in the spleen, followed by the liver, heart, small intestine, stomach, lungs and kidneys. The highest arctigenin concentration was found in the stomach and the small intestine, the lowest in the liver. Three hours after administration the tissue concentrations of the two compounds decreased markedly in all tissues, but especially in the spleen [He 2012].

After oral administration to Sprague Dawley rats, the highest content of arctigenin was found in the spleen: two times greater than in liver and several times more than other organs [He 2013].

Excretion
Following an oral daily dose of 120 mg/kg b.w. of arctiin for 20 days, three metabolites were identified in the faeces of Sprague Dawley rats: (-)-enterolactone (M1), (-)-arctigenin (M2) and [(2*R*,3*R*)-2-(3′-hydroxybenzyl)-3-(3′′,4′′-dimethoxybenzyl)-butyrolactone] (M3). After a dose of 30 mg/kg b.w. of arctiin by oral gavage, the total cumulative excretion rates of arctiin, M1, M2 and M3 were 19.84%, 0.35%, 0.28% and 0.02% in urine and 1.80%, 35.80%, 0.30% and 0.48% of intragastric dose in faeces, respectively. Excretion rate of M1 reached 35.80% in faeces, whereas the excretion rates of the two other metabolites were lower in urine and faeces. The cumulative excretion rate of arctiin and its metabolites reached a steady state from 76 to 96 h [Wang 2013].

Preclinical safety data

Acute toxicity
The i.p. LD_{50} of a 50% ethanolic extract was 700 mg/kg b.w. in albino rats [Sharma 1978].

In male Wistar rats, the oral LD_{50} of an aqueous extract (100 g, DER = 4:1) was higher than 2 g/kg b.w. [Lin 2002].

After 72h, mortality and toxic reactions were not observed in Sprague Dawley rats after oral administration of dosages up to 3 g/kg b.w. of an ethanolic dry extract 70% V/V (1:10 w/v) [Cao 2012].

Neither mortality, significant difference in body weight development nor signs of toxicity were observed in female Wistar rats after oral administration of up to 100 mg/kg b.w. of an ethanolic extract, twice daily for 7 days [da Silva 2013].

Repeated dose and chronic toxicity studies
Inbred strain ACI rats were fed with a basal diet mixed with 33% dried and powdered burdock root for 120 days and then returned to the basal diet for 120 days. At the end of the experiment, no tumours were detected [Hirono 1977].

In female Sprague Dawley rats receiving either 0.2% or 0.02% arctiin included in their diet for 48 weeks, no mammary tumours or pancreatic lesions were found, but a significant decrease in body weight gain was observed (448 ± 88 g, $p<0.05$ and 416 ± 26 g, $p<0.01$, respectively) [Hirose 2000].

No intestinal discomfort such as eructation or bloating was observed in female Kunming mice receiving a diet containing 5% of burdock inulin for 14 days [Li 2008].

Body weights of male Sprague Dawley rats fed with 500 and 1000 mg/kg b.w. of burdock root for 4 weeks significantly ($p<0.05$) decreased as compared to control, whereas 100 mg/kg of burdock root had no effect [Kuo 2012].

Chronic oral administration of arctigenin (200 mg/kg b.w.) for 23 days did not induce changes in body weight, but did cause a significant reduction of subcutaneous ($p<0.05$; 21)) but not perirenal or mesenteric fat weight, and significantly decreased cholesterol levels ($p<0.05$) [Huang 2012].

Lappaol F administered at 5 or 10 mg/kg b.w. for 15 days was well tolerated in mice, without significant toxicity [Sun 2013].

Reproductive toxicity
An aqueous extract did not affect fertility of female mice after s.c. administration twice a day for five days [Matsui 1967].

Clinical safety data

A 20% aqueous solution of a propylene glycol extract of burdock root (no further details), applied to the skin of volunteers according to the Jadssohn block test, did not induce skin sensitisation or skin and eye irritation [Yotchokova 1990].

Three cases of contact dermatitis caused by burdock root plasters applied for anti-inflammatory purposes have been reported [Rodriguez 1995].

In a 53-year-old Japanese man with multiple prior episodes of urticaria following ingestion of boiled burdock, an anaphylactic reaction was observed after further ingestion of boiled burdock in a meal. Immediate type allergic reaction to boiled and raw burdock was confirmed after medical tests [Sasaki 2003].

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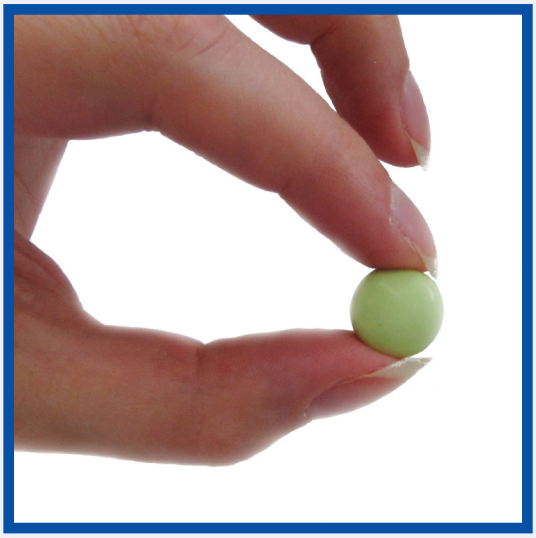

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FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in the production of ESCOP monographs and other activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

ARNICAE FLOS

2019

Arnica flower

DEFINITION

Arnica flower consists of the whole or partially broken, dried flower-heads of *Arnica montana* L. It contains not less than 0.4 per cent m/m of total sesquiterpene lactones expressed as dihydrohelenalin tiglate, calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Arnica flower].

Fresh material may also be used, provided that when dried it complies with the monograph of the European Pharmacopoeia.

CONSTITUENTS

Sesquiterpene lactones of the pseudoguaianolide type, 0.2-0.8%; principally helenalin and 11α,13-dihydrohelenalin and their esters with acetic, isobutyric, methacrylic, tiglic and other carboxylic acids. Other constituents include diterpenes, arnidiol (a triterpene), flavonols, flavonol glycosides and -glucuronides, pyrrolizidine alkaloids (tussilagine and isotussilagine), polyacetylenes, caffeic acid and derivatives, coumarins, fatty acids and carotenoids [Santer 1962; Güntzel 1967; Schulte 1969; Kating 1970; Vanhaelen 1973; List 1974; Willuhn 1983, 1994; Merfort 1984, 1985, 1986, 1987, 1988a,b, 1992a,b,c; Schmidt 1992; Paßreiter 1992a, b; Kolodziej 1993, Pietta 1994; Kos 2005; Teuscher 2016].

CLINICAL PARTICULARS

Therapeutic indications

External use
Treatment of bruises, sprains and inflammation caused by insect bites; gingivitis and aphthous ulcers; symptomatic treatment of rheumatic complaints and muscular pain [Hänsel 1991; Van Hellemont 1988; Hänsel 1991; Merfort 1992b; British Pharmacopoeia 1996; Knuesel 2002; Alonso 2002; Widrig 2007; Mendes 2008; Ross 2008; Pumpa 2014; Teuscher 2016; Simsek 2016; van Exsel 2016].

Posology and method of administration

Dosage

External use
Ointments, creams, gels or compresses made with 5-50% V/V tinctures or 5-25% V/V fluid extracts; diluted tincture (1:3 to 1:10), diluted fluid extracts or a decoction of 2.0 g of dried arnica flower in 100 ml of water [Merfort 1992b; Knuesel 2002; Widrig 2007; Mendes 2008].

Method of administration
Topically as diluted tincture, ointment, cream, gel or compress [Merfort 1992a,b; Knuesel 2002; Widrig 2007; Mendes 2008].

Duration of use
If symptoms persist or worsen, medical advice should be sought.

Contraindications
Allergy to Arnica or other members of the Compositae [Eberhartinger 1984; Hausen 1985; Machet 1993; Hörmann 1995].

Special warnings and precautions for use
For external use only.

Interaction with other medicaments and other forms of interaction
None reported.

E/S/C/O/P MONOGRAPHS

Pregnancy and lactation
No restriction for external use; no harmful effects have been reported.

Effects on ability to drive and use machines
None known.

Undesirable effects
Skin irritation has been reported. Contact dermatitis from arnica may occur in susceptible individuals [Herrmann 1978; Hausen 1978, 1979, 1980, 1985, 1997; Eberhartinger 1984; Machet 1993; Hörmann 1995; Reynolds 1996; Spettoli 1998; Widrig 2007; Jocher 2009; Roth 2012].

Overdose
Not applicable to external use.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Anti-inflammatory activity
The effect of three tinctures (fresh flowerheads of Central European chemotype, 1:20, 70%v/v; dried flowerheads of Central European chemotype, not further specified; dried flowerheads of Spanish chemotype, not further specified) and a gel (50 g tincture of fresh flowerheads of Central European chemotype, 1:20, 70% v/v in 100 g gel) on IL-1β-induced MMP1 and MMP13 gene expression in primary bovine and human articular chondrocytes was investigated. The ability to inhibit DNA binding of the transcription factors AP-1 and NF-κB was also studied. The increased expression levels of MMP1 and MMP13 in IL-1β stimulated chondrocytes were concentration-dependently reduced following pre-treatment with arnica. All preparations inhibited AP-1 and NF-κB DNA binding in both cell lines in a concentration-dependent manner [Jäger 2009].

The production of cytokines was measured in H_2O_2-inflamed mouse fibroblast cells (NCTC clone L929), with and without pre-treatment with extracts enriched in polysaccharides (0.36% m/m) or polyphenols (18.44% m/m). The secretion of IL-8 and TNF-α was significantly ($p<0.05$) lower in cells pre-treated with the extracts [Gaspar 2014].

An extract (not further specified, yield 3.8%) showed inhibition of 5-LOX (IC_{50}=9 ng/mL) and COX-1 (IC_{50}= >50µg/mL) [Chagas-Paula 2015].

In mouse and rat liver homogenates and human polymorphonuclear neutrophils, helenalin and 2,3-dihydrohelenalin significantly suppressed various parameters of inflammation; at 5×10^{-5} M they inhibited the chemotactic migration of human polymorphonuclear neutrophils by 100% and 20% respectively [Hall 1980]. Unsaturated structures such as α-methylene-γ-lactone and/or α,β-unsubstituted cyclopentenone moieties (helenalin has both) seem to play a role in the anti-inflammatory activity. The compounds exerted their activities at multiple receptor sites, e.g. by suppression of the synthesis of prostaglandins at 10^{-3} M in a prostaglandin synthetase assay and uncoupling of oxidative phosphorylation of human polymorphonuclear neutrophils at 5×10^{-4} M [Hall 1979, 1980].

Helenalin and (to a much lesser degree) 11α,13-dihydrohelenalin inhibited NF-κB activation in response to four different stimuli in T-cells, B-cells and epithelial cells. Inhibition was seen at the µM level and was selective. The activities of transcription factors Oct-1, TBP, Sp1 and STAT5 were not affected [Lyss 1997]. Inhibition of the activation of transcription factor NF-AT has also been demonstrated [Klaas 2002].

The electrophoretic mobility shift assay was performed with isolated sesquiterpene lactones (helenalinisobutyrate 39.89 mg/100 ml; 11α,l3-dihydrohelenalinmethacrylate 69.93 mg/100 ml; 11α,13 dihydrohelenalinisobutyrate 69.93 mg/100 ml) at doses of 20µL and 50µL. An activation of NF-κB DNA binding in dendritic cells was observed at low concentrations and an inhibition at high concentrations [Lass 2008].

Activated Jurkat CD4+ T cells were exposed for 24h to various concentrations of helenalin (0.5 µM, 0.75 µM, 1 µM and 2 µM). Helenalin significantly ($p<0.05$) reduced proliferation in a dose-dependent manner by triggering the mitochondrial pathway of apoptosis. Activated CD4+ T cells which survived exposure to helenalin exhibited a cell cycle arrest in the G2/M phase [Berges 2009].

The interactions of helenalin with the NF-κB protein p65 (RelA), with recombinant IκB kinases (IKKs) α and β, and with glutathione, were characterized with a surface plasmon resonance-based method. Helenalin interacted with the NF-κB protein RelA, but not with IKKα or IKKβ or with glutathione. The influence of the binding between helenalin and RelA on the binding of RelA to double-stranded DNA carrying NF-κB binding sequences was analysed. At physiological pH 7.4, helenalin inhibited the binding of RelA to the DNA NF-κB binding sites with an IC_{50} of 5.0 ± 2.8 µM, while at pH 8.0 the IC_{50} value was approximately 3-fold higher [Büchele 2010].

Antimicrobial activity
Using isolated constituents such as helenalin and 11,13-dihydrohelenalin acetate, antimicrobial activity against the Gram-positive bacteria *Arthrobacter citreus*, *Bacillus brevis*, *Bacillus subtilis*, *Corynebacterium insidiosum*, *Micrococcus roseus*, *Mycobacterium phlei*, *Sarcina lutea* and *Staphylococcus aureus* and Gram-negative *Proteus vulgaris*, was demonstrated [Lee 1977; Willuhn 1982; Merfort 1992a]. Antifungal activity of helenalin and derivatives against *Botrytis cinerea* has also been demonstrated [Willuhn 1982].

Some sesquiterpene lactones including helenalin showed activity against *Trypanosoma brucei rhodesiense* and *T. cruzi* in rat skeletal myoblasts (L-6 cells) infected with trypomastigotes. Helenalin was most effective against *T. brucei* (IC_{50}=0.051µM) and *T. cruzi* (IC_{50}=0.695 µM) and showed less cytotoxicity against L-6 cells (LC_{50}=0.992µM) [Schmidt 2002].

Mammary epithelial T-cells were incubated with 10 µM helenalin, then stimulated with TNF-α and finally infected with *Staphylococcus aureus*. A significant ($p<0.05$) reduction of the multiplication of *S. aureus* in comparison with the control group was shown after 6 and 9 hours. The same effect was observed under non-inflammatory conditions [Boulanger 2007].

Antioxidant effects
An extract (not further specified, yield 10.9%) was tested for its ability to inhibit the lipid peroxidation induced by the Fe^{3+}/ascorbate system in a young rat brain cytosolic fraction and on DPPH, H_2O_2, superoxide and NO radicals. The radical-scavenging activity of the extract was dose-dependent and significant ($p<0.05$) [Sharma 2016].

The effect of concurrent treatment (10-100 mg/L) and pre-treatment (24h, 10-100 mg/L) with an extract (not further specified) on H_2O_2-induced (50µM) oxidative stress in NCTC cells was studied. Co-treatment did not increase cell viability

or decrease LDH secretion as compared to controls. In contrast, pre-treatment showed a significant ($p<0.01$) increase in cell viability and a decrease in LDH secretion. In the co treated cells a significant ($p<0.01$) difference in cell cycle distribution was observed as compared to the untreated controls, pre-treatment showed no significant difference [Craciunescu 2012].

Immunotoxic effects
Treatment of THP-1 cells positive for intracellular ROS with helenalin resulted in a significant ($p<0.01$) increase of positive cells from 6.6% to 26.5% (IC_{10}=0.52µM) and 55.7% (IC_{50}=1.16µM) respectively. In Jurkat cells, a significant increase of intracellular ROS was not observed. The treatment of THP-1 cells with subtoxic concentrations of helenalin (IC_{10} =0.52µM) lead to a significant ($p<0.05$) influence on protein folding, the intrinsic apoptosis pathway, and carbohydrate metabolism shifting the cells to anaerobic glycolysis. Human peripheral blood mononuclear cells showed a decreased secretion of various cytokines after helenalin (IC_{10} =0.52µM) treatment [Zwicker 2017].

Cytotoxic effects
Cell viability of NCTC mouse fibroblasts was significantly ($p<0.01$) decreased by an ethanolic extract (70% v/v; yield 18.4%) at concentrations above 100 mg/L (300 to 1000 mg/L) in comparison to control. Whereas at concentrations of 10 mg/L and 100 mg/L, cell viability was more than 80% compared to control [Craciunescu 2012].

The cytotoxicity of a multi-step extract (containing 26% carbohydrates, 21.3% phenolics, 12% uronic acids), at concentrations of between 1.9 and 2000 µg/mL, was tested in human A549 cells and mouse L929 fibroblasts. The extract showed no cytotoxic effects at concentrations up to 500 µg/mL. Concentrations of 1000 µg/mL and 2000 µg/mL resulted in a lower cell proliferation rate compared to control [Šutovská 2014].

An extract (not further specified) did not have a significant influence on the cell viability of blood platelets at a concentration range between 5 µg/mL and 100 µg/mL [Rywaniak 2015].

Helenalin and 11α,13-dihydrohelenalin were cytotoxic against GLC_4 (a human small cell bronchial carcinoma cell line) and COLO 320 (a human colorectal cancer line). IC_{50} values for helenalin were 0.44 µM against GLC_4 and 1.0 µM against COLO 320; corresponding values for 11α,13-dihydrohelenalin were 66.1 and 64.7 µM, compared to 4.6 and 8.8 µM for the reference compound cisplatin. Most flavonoids in arnica flower had low cytotoxic activity at 200 µM [Woerdenbag 1994; Anon 2001]. Isolated flavonoids from arnica flower, applied to human bronchial carcinoma cell lines, effectively reduced the cytotoxic activity of helenalin [Woerdenbag 1995]. Inhibition of cellular respiration of Ehrlich ascites cells was reported [Hall 1978]. Helenalin and 11,13-dihydrohelenalin, as well as an unspecified extract, inhibited platelet activation [Weil 1988; Schröder 1990].

The effects of 2β-ethoxy-6-O-acetyl-2,3-dihydrohelenalin on IL-8 were studied in HeLa229 cells, with parthenolide as positive control. The compound suppressed IL-8 production at low concentrations (IC_{50}=4.61µM) [Kos 2005].

A β-cyclodextrin-helenalin complex was studied in T47D breast cancer cells using the MTT assay. After 24, 48 and 72 hours, a dose- and time-dependent cytotoxicity with IC_{50} values of 2.1 nM, 1.64 nM and 1.4 nM was observed. The inhibitory effect of the complex on telomerase gene expression in a T47D cell line was investigated by qRT-PCR, which showed that the complex dose-dependently decreased hTERT gene expression in treated cells compared to control [Samaneh 2013].

In THP-1 and Jurkat cells, as well as in PBMC, a dose-dependent cytotoxicity of helenalin was demonstrated with IC_{50} values of 1.1 µM, 1.2 µM and 0.2 µM respectively [Zwicker 2017].

Anti-platelet effect
Pre-incubation with an extract rich in phenolic acids (not further specified), at concentrations of 7.5 µg/mL and 15 µg/mL, significantly ($p<0.05$) reduced ADP-induced aggregation in whole blood and platelet-rich plasma, decreased the platelets' reactivity index in whole blood and showed antioxidant capacity [Rywaniak 2015].

Other effects
Aqueous extracts (2 mg/ml of dried plant extract redissolved after lyophilisation) had an antihistamine effect on smooth muscle preparations [Brunelin-Geray 1969].

An ethanolic extract (25:1) and eight isolated sesquiterpene lactones were evaluated for heat shock protein 70 (HSP-70)-inducing activity, cytotoxicity, melanin production, and production and expression of tyrosinase in B16 cells. The extract was active in inducing HSP70, with low cytotoxicity. Within 9 h, a significant ($p<0.05$) up-regulation of the expression of HSP70 by helenalin 2-methylbutyrate (AM-2) and the extract was shown. An upward band-shift of heat shock factor-1 was observed in cells treated with AM-2 or the extract. Pre-treatment of the cells with AM-2 or the extract significantly ($p<0.05$) supressed the increase of melanin caused by 3-isobutyl-1-methylxanthine and lead to a decrease of tyrosinase [Usui 2015].

Ex vivo experiments
Airway smooth muscle reactivity was examined using tracheal smooth muscle strips from guinea pigs treated with a multi-step extract containing 26% carbohydrates and 21.3% phenolics (100 mg/kg), salbutamol (10 mg/kg i.p.; positive control group) or water (2 mL/kg; negative control group). The extract concentration-dependently significantly ($p<0.01$ to $p<0.05$) attenuated acetylcholine- and histamine-induced contractions in comparison to the negative control. Furthermore, relaxation of isolated tracheal smooth muscle strips after treatment with the extract was similar to the positive control [Šutovská 2014].

In vivo experiments

Anti-inflammatory effects
Rats with ulcerative mouth lesions induced by 40% sodium hydroxide were treated topically with either a tincture (30% concentration, starting material not quantified; n=25) or a saline control (n=25). The arnica treated group exhibited faster re-epithelialization than the control group, and the number of blood vessels was higher in the treatment group on days 2, 7, 14, 21 and 42 (significance data not provided). The number of lymphocytes in the arnica-treated rats was significantly ($p<0.05$) lower on day 42 than day 2 [Sawada 2016].

In rats with collagen-induced arthritis, oral treatment with a methanolic extract (yield 10.85%) at 75 mg/kg b.w. resulted in a significant ($p<0.001$) reduction in the severity of arthritis and a significantly ($p<0.0001$) attenuated arthritic index as compared to untreated arthritic rats. Behavioural tests such as the open field test showed significantly ($p<0.0001$) improved locomotor and exploratory behaviour and a significantly ($p<0.0001$) increased motility score in comparison with the untreated arthritic rats. Pro-inflammatory cytokines were significantly ($p<0.0001$) downregulated to the baseline of healthy controls in comparison to the untreated arthritic rats [Sharma 2016].

Intraperitoneal pre-treatment with helenalin at 2.5 mg/kg b.w. significantly ($p<0.001$) inhibited carrageenan-induced rat paw

oedema [Hall 1979]. In mice, helenalin (20 mg/kg b.w. i.p.) significantly ($p<0.001$) inhibited the acetic acid-induced writhing reflex by 93% [Hall 1979].

Intraperitoneal treatment of rats with chronic adjuvant arthritis with helenalin at 2.5 mg/kg/day for 3 weeks confirmed that sesquiterpene lactones are the constituents mainly responsible for anti-inflammatory activity [Hall 1980]. 11α,13-Dihydrohelenalin acetate and methacrylate applied topically at 1 µmol/cm^2 inhibited croton oil-induced mouse ear oedema by 54 and 77% respectively ($p<0.001$), compared to 44% inhibition by indometacin at 0.2 µmol/cm^2 [Klaas 2002].

At a dose of 1 µmol/cm^2, 2β-ethoxy-6-O-acetyl-2,3-dihydrohelenalin induced a 42% inhibition of croton oil-induced mouse ear oedema [Kos 2005].

Tumour-inhibiting activity
Sesquiterpene lactones from arnica flower inhibited the growth of Ehrlich ascites in mice and Walker 256 carcinosarcoma in rats [Hall 1978].

Antimicrobial activity
The effects of helenalin on *Staphylococcus aureus* infection were investigated in the mammary glands of lactating mice. Helenalin (20 mg/kg i.p.) was administered at both 9 and 3 hours prior to infection of the mammary glands with *S. aureus*. Examination after 12 hours showed that helenalin reduced bacterial proliferation and significantly ($p<0.05$) reduced leukocyte infiltration in the mammary glands when compared to control (DMSO) [Boulanger 2007].

Bronchodilatory effects
The bronchodilatory and antitussive effects of a multi-step extract containing 26% carbohydrates and 21.3% phenolics were tested in guinea pigs exposed to a citric acid aerosol. The animals were divided into six groups, three test groups received either 50 mg/kg, 75 mg/kg or 100 mg/kg p.o. of the extract, positive control groups received either codeine (10mg/kg p.o.) or salbutamol (10mg/kg i.p.) and the negative control group received water (2 mL/kg). All extract doses produced a significant cough-suppressive effect after 120 minutes ($p<0.01$) and 300 minutes (50 and 75 mg/kg $p<0.01$; 100mg/kg $p<0.05$). This antitussive effect was not dose-dependent and was lower than that of the positive control codeine ($p<0.001$ at 120 mins, $p<0.01$ at 300 mins). However citric-acid induced bronchial smooth muscle reactivity was significantly ($p<0.05$ to $p<0.01$) reduced by the extract compared to negative control (water) in a dose-dependent manner; while at 300 minutes the extract demonstrated significantly greater activity (75 mg/kg $p<0.01$; 100 mg/kg $p<0.001$) than the positive control group (salbutamol) [Šutovská 2014].

Pharmacological studies in humans
In a randomized, double-blind, placebo-controlled trial, the effectiveness of an arnica gel on pain, indicators of inflammation, muscle damage and improvement of performance was tested in 20 well-trained sportmen who undertook an exercise protocol designed to induce delayed onset muscle soreness (DOMS) and muscle damage. Each 1 g of gel contained arnica tincture equivalent to 10 mg of dry flower. The arnica gel or an identical placebo gel were applied (2.5 g to an approximate diameter of 2 cm) immediately after completing the exercise and every 4 waking hours for 4 days. The outcome was measured with a VAS, a Somedic algometer, an isokinetic dynamometer, as well as by determination of IL-1β, IL-6, TNF-α, C-reactive protein, myoglobin and creatinine kinase. Application of the arnica gel led to significantly ($p=0.045$) less pain in the quadriceps, 72 hours after the exercise, when compared to the placebo gel. Muscle tenderness was significantly ($p<0.05$) lower in the arnica group when compared to placebo, at 72 hours post-exercise in the quadriceps muscle, and at various time points in the gastrocnemius muscle (immediately after, and 4, 24 and 72 hours after). There were no significant differences between the groups in any of the inflammatory or muscle damage markers [Pumpa 2014].

Clinical studies

Varicosis
In a randomized, double-blind, placebo-controlled study, patients with chronic venous insufficiency received basic hydrotherapy treatment in addition to topical application to the lower legs and feet with either an extract ointment (n = 39) or a placebo ointment (n = 39). After 3 weeks treatment, from objective plethysmographic measurements and subjective assessments (feelings of tension and swelling in the legs, pain in the legs), improvements were observed in both groups but the degree of improvement was greater after verum treatment. A further study in 100 patients (verum 50, placebo 50), under identical conditions except using an extract gel, showed comparable improvements but no difference between groups [Brock 2000].

Osteoarthritis
A six week, open, multicentre trial investigated the efficacy of a gel containing 50% tincture (1:20; 50% ethanol m/m) in 79 patients (ITT population) with mild to moderate osteoarthritis of the knee. Fifty four patients completed the study (PP population). The gel was applied twice daily as a thin layer on the affected knee. The patients assessed effectiveness with the WOMAC Score and a five-point-scale for subjective assessment. After 3 weeks the WOMAC Score was significantly ($p<0.0001$) reduced by 6.0 points in the ITT population and 7.4 points in the PP population; after 6 weeks it was significantly ($p<0.0001$) lowered by around 8.5 points (ITT) and 10.5 points (PP). Efficacy of the gel was stated as being "very good" or "good" in 52% (ITT) and 63% (PP), and as having "no effect" in 13% (ITT) and 11% (PP) [Knuesel 2002].

A randomized, double blind study in 198 patients with hand osteoarthritis (ITT), of which 174 completed the study (PP), investigated the effect of topical treatment with the same gel in comparison to ibuprofen gel (5%), applied 3 times daily for 3 weeks. Reduction of pain was recorded on VAS (100 mm) and functional capacity of the hand was validated with the Hand Algofunctional Index (HAI). There was almost no difference between the Arnica and the ibuprofen gel from the 1st to the 21st day in the VAS (-26.6 points to -23.9 points) as well as in the HAI (-4.3 points to -4.6 points). "Very good" or "good" efficacy was stated by 64% of the arnica group and by 58.8% of the ibuprofen group, while global efficacy evaluation by physicians was "very good" or "good" in 56.5% (ibuprofen) and 64.0% (arnica) [Widrig 2007].

A randomized, double-blind, controlled, multi-centre study compared the efficacy of an arnica gel (50 g fresh herbal tincture (1:20) / 100 g gel) with an ibuprofen (5%) gel for osteoarthritis of the hands in 204 patients. The gels (4 cm strip) were applied 3 times daily for 3 weeks. The effects of the arnica gel were comparable to the ibuprofen gel, regarding hand functional capacity, pain intensity, number of painful joints, duration and severity of morning stiffness and paracetamol consumption, in a clinically relevant manner [Ross 2008].

Bruises and ecchymosis
A randomized, double-blind, placebo-controlled trial investigated the efficacy of an ointment (30% of a tincture,

1:10) used after bilateral upper blepharoplasty in 116 patients. The patients applied the ointment or placebo twice daily on one periorbital area, the untreated contralateral side served as a control. The primary outcome measure was the appearance of the periorbital areas based on light photography, as assessed by a medical and nonmedical panel after 3 days, 7 days and 6 weeks. The secondary outcome measures were the degrees of ecchymosis, erythema and swelling, assessed by light photography, pain and patient satisfaction using a VAS. No significant ($p>0.05$) differences between verum and placebo were observed at any of the time points [van Exsel 2016].

In a randomized, double-blind, placebo-controlled study, the effect of a gel (not further specified) on the bruises of nineteen patients with facial telangiectasia was tested both pre- and post-treatment with a 585 nm pulsed dye laser. The pre-treatment group (n=9) applied the gel to one side of the face and vehicle to the other side, twice a day for two weeks prior to laser treatment; the post-treatment group (n=10) used the same application for 2 weeks after the laser treatment. On days 0, 3, 7, 10, 14 and 17 bruising was assessed using a VAS by the patient and the physician. No significant differences between the mean scores of verum and vehicle were seen in either group [Alonso 2002].

A randomized, controlled, prospective clinical trial in patients who had undergone an open technique rhinoplasty, compared the effect of topical treatment with a cream (not further specified; n=36) applied four times a day for 10 days, with no post-operative treatment (n=36), on the regression of postoperative peri-orbital oedema and ecchymosis. According to a 4 point scale (ranging from 0 to 4), on postoperative days 1, 5 and 7, ecchymosis was significantly ($p<0.005$) less after verum, and regression of the oedema was significantly ($p<0.005$) faster than in the control group [Simsek 2016].

Lesions of the oral mucosa
Male and female individuals (n=21) with recurrent ulcers of the oral mucosa (more than four episodes per year) received an ointment containing 30% of a tincture, the control group (n=10) received placebo. The ointment was applied three times a day after oral hygiene on the ulcer. 52% of the patients of the verum group perceived a faster ulcer lesion healing process in the oral mucosa, and 45% reported an analgesic effect, as compared to placebo [Mendes 2008].

Pharmacokinetic properties
No data available.

Preclinical safety data
The safety of arnica flower has been assessed in a review. In general, extracts have been found to have low toxicity in acute tests in mice, rats and rabbits. They were not irritating, sensitizing or phototoxic to mouse or guinea pig skin, and did not produce significant ocular irritation [Anon 2001].

The safety profile of arnica flower has also been reviewed with respect to sesquiterpene lactones, flavonoids and pyrrolizidine alkaloids [Merfort 1992a; Hausen 1997]. The pyrrolizidine alkaloids tussilagine and isotussilagine [Paßreiter 1992a, 1992b] are considered non-toxic because they lack the key structure, a 1,2-unsaturated necine group, considered to be responsible for the toxicity of certain pyrrolizidine alkaloids [Merfort 1992a; Paßreiter 1992b].

Acute toxicity
The oral LD_{50} of an extract (not further specified) in rats has been reported as > 5g/kg. In mice, oral and intraperitoneal values of an extract have been reported as 123 mg/kg and 31 mg/kg respectively [Anon 2001].

LD_{50} values of helenalin were determined as 150 mg/kg b.w. in mice, 125 mg/kg in rats, 90 mg/kg in rabbits and 85 mg/kg in hamsters, and estimated as 100-125 mg/kg in sheep [Witzel 1976].

Mutagenicity
In the Ames mutagenicity test, a hydroethanolic extract showed a weak mutagenic potential in *Salmonella typhimurium* strain TA98 with or without S9 activation and in strain TA100 with activation; the effects were possibly due to the flavonoids present in the extract [Göggelmann 1986]. Helenalin showed no mutagenic potential in the Ames test using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537, but was relatively toxic to the test organisms [MacGregor 1977].

Genotoxicity
Helenalin (dose not further specified) was tested for genotoxicity using six strains of *Bacillus subtilis* (rec A8, rec E4, mc-1, Exc-strain, hcr-9 and strain fh 2006-7). It produced lethal DNA damage in strains mc-1 and rec E4 in the spot assays. Exposure of the fractional survival of *B. subtilis* strains to increasing concentrations of helenalin (1-1000 µg) demonstrated a significant ($p<0.05$) decrease in the survival of the repair-deficient strains [Jones 1981].

Chronic toxicity
The effect of a hydroethanolic extract (25 mg/kg/day p.o.; not further specified) on male and female albino rabbits for three months was investigated. In the male test group, all measured blood parameters were significantly ($p<0.05$) elevated, whereas they were significantly ($p<0.05$) lowered in females, except platelet count which was elevated. Creatinine and uric acid levels were significantly ($p<0.05$) raised in female animals as compared to males. Cardiac enzymes were significantly ($p<0.05$) elevated in males and significantly ($p<0.05$) lowered in females. Males showed significantly ($p<0.05$) decreased lipid profile and liver enzyme parameters, whereas females exhibited significantly ($p<0.05$) raised lipid profile and liver enzymes levels. Urine volume and specific gravity were significantly ($p<0.05$) lowered in both male and female animals. Blood cells were observed in urine of female animals. No significant ($p>0.05$) toxic effects were observed on the stomach, kidney and liver tissues of male rabbits. In contrast, areas of myocytolysis in the right ventricular wall and inter-ventricular septum were observed in the heart tissues [Farah-Saeed 2016].

Allergenic activity
Tinctures from fresh flowers of Spanish (SP; 0.7 mg/mL 11α,13-dihydrohelenalin esters) or Central European (CE; 0.83 mg/mL helenalin esters) chemotypes and several isolated sesquiterpene lactones were investigated for their allergenic potential in the mouse ear swelling test. On day 0, mice were sensitized epicutaneously with the application to shaved abdominal skin of either 100 µl of 2,4,6- trinitrochlorobenzene (TCNB; 7% in acetone), undiluted SP or CE tinctures or the isolated sesquiterpene lactones 11α,13-dihydrohelenalinisobutyrate or 11α,13-dihydrohelenalinmethacrylate (2.12 and 21.1 mM; from SP) or helenalinisobutyrate (1.2 and 12 mM; from CE) in ethanol; while controls received acetone or ethanol. The mice were then challenged on day 5 with application to both ears of either 20µL of TNCB (1%), 20 µL of the tinctures or the sesquiterpene lactones. Ear thickness was measured prior to challenge and 24 hours later. TNCB induced a strong ear swelling, whereas neither the tinctures nor the sesquiterpene lactones induced ear swelling. Pre-treatment with the tinctures significantly ($p<0.05$) decreased the ear swelling response to TNCB in TNCB-sensitized mice. Sensitization was also induced with 50 µL of the undiluted tinctures on 3 consecutive days in CD4-depleted mice (treated i.p. with anti-CD4 monoclonal

antibody on the 3 days prior to sensitization and 4 days later). The ear challenge in this case was performed on days 5 and 6 with 25 µL tincture. Here the CE but not the SP tincture, induced contact hypersensitivity [Lass 2008].

Clinical safety data

Allergenic potential
The allergic potential of a standard arnica patch test (extract) compared with 6 different arnica preparations (two extracts, tincture, gel, ointment, oil) containing various quantities of sesquiterpene lactones, a standard sesquiterpene lactone mix, 3 isolated sesquiterpene lactones and vehicles-only (gel and ointment base) was investigated in eight patients with a history of a positive standard arnica patch test within the previous 2 years. One patient showed positive reactions to each of the arnica preparations, while three patients showed positive patch tests to 1, 2 or 3 of the 6 test preparations and one patient showed no reaction to the test preparations. None of the patients that tested positive to the standard Arnica patch test displayed positive reactions to the individual isolated sesquiterpene lactones [Jocher 2009].

In a six week, open, multicentre trial patients (n=79) applied a gel (50% tincture, 1:20; ethanol 50% m/m) twice a day for 6 weeks. Six patients experienced mild or moderate local reactions at the treatment site, which consisted of red spots and itching, a localized rash, dry skin, pruritus or petechiae that disappeared after treatment with corticosteroid ointment [Knuesel 2002].

A randomized, double blind study involving 198 patients investigated the effect of a gel (50% tincture, 1:20; ethanol 50% m/m) for topical treatment of hand osteoarthritis in comparison to ibuprofen gel (5%) for 3 weeks. Treatment-related adverse events on the skin were recorded in 6.1% and 4.8% of patients on ibuprofen and arnica respectively [Widrig 2007].

Application of an ointment (30% tincture, 1:10) twice daily for six weeks to one periorbital area after bilateral upper blepharoplasty in 136 patients, produced no adverse drug reactions [van Exsel 2016].

In a randomized, double-blind, placebo-controlled study, patients with facial telangiectasia (n=19) applied a gel (not further specified) twice daily for two weeks before or after laser treatment on the bruises. No side effects from verum or vehicle gel were reported [Alonso 2002].

In a randomized, double-blind, placebo-controlled trial, topical application of a gel by 10 well-trained sportsmen for 4 days after intense excessive exercise did not show any adverse effects [Pumpa 2014].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Online Series, 2019
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Online Series, 2019
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Online Series, 2019
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHAMOMILLAE ROMANAE FLOS	Roman Chamomile Flower	Online Series, 2019
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HELICHRYSI FLOS	Sandy Everlastin Flower	Online Series, 2019
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013

HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

E/S/C/O/P Monographs

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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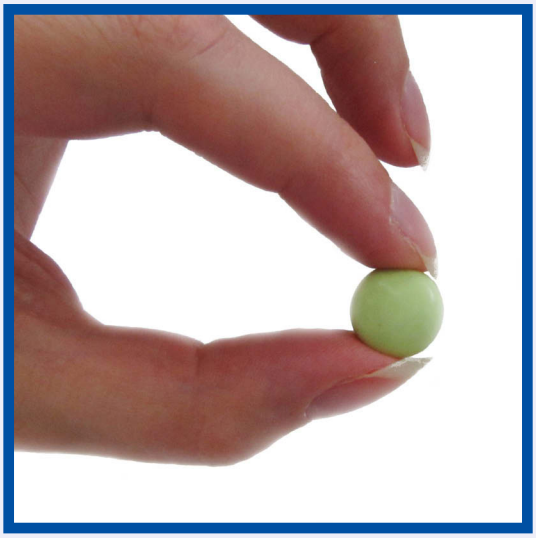
The Scientific Foundation for Herbal Medicinal Products

Ballotae nigrae herba

Black Horehound

2015



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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

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Plant illustrated on the cover: *Ballota nigra*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

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The monograph text
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Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

BALLOTAE NIGRAE HERBA 2015

Black Horehound

DEFINITION

Black horehound consists of the dried flowering tops of *Ballota nigra* L. It contains not less than 1.5 per cent of total *ortho*-dihydroxycinnamic acid derivatives, expressed as acteoside ($C_{29}H_{36}O_{15}$; M_r 624.6) and calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Black horehound].

CONSTITUENTS

The main characteristic constituents are: phenylpropanoids (ca. 5.5%) including the *ortho*-dihydroxycinnamic acid glycosides acteoside (verbascoside), forsythoside B, arenarioside [Seidel 1996a], ballotetroside [Seidel 1997] and martynoside [Tóth 2007], and non-glycosidic (+)-*E*-caffeoyl-L-malic acid [Didry 1999]; flavonoids, mainly derivatives of luteolin and apigenin, e.g. luteolin 7-lactate and 7-glucosyl-lactate [Bertand 2000], apigenin 7-*O*-glucoside and apigenin 6,8-*C*-diglucoside (vicenin 2) [Darbour 1986]; and labdane diterpene lactones [Savona 1976; Savona 1977a; Savona 1977b; Seidel 1996b].

CLINICAL PARTICULARS

Therapeutic indications
Tenseness, restlessness and irritability with difficulty in falling asleep [Huriez 1991; Hänsel 1992].

Black horehound has also been documented for the relief of mild spasmodic gastric complaints [Hänsel 1992].

Posology and method of administration

Dosage

Adult single dose: 1.5-5 g of the drug (e.g. as a tea infusion) or equivalent preparations prepared with water or ethanol (maximum 45% V/V) [Huriez 1991; Ballota; Tisanes].

Elderly: as for adults.

Children from 3 to 12 years under medical supervision only: proportion of adult dose according to body weight, as non-alcoholic preparations.

Method of administration
For oral administration.

Duration of administration
No restriction. If symptoms persist or worsen, medical advice should be sought.

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
Due to the sedative effects of black horehound, the ability to drive or use machines might be affected.

E/S/C/O/P MONOGRAPHS

Undesirable effects
None reported.

Overdose
No case of overdose reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Receptor-binding activity
The competitive receptor binding activity of four phenylpropanoid glycosides, acteoside, forsythoside B, arenarioside and ballotetroside, and the non-glycosidic phenylpropanoid caffeoyl-L-malic acid, has been studied. Apart from ballotetroside, they showed affinity for benzodiazepine, dopaminergic and morphinic receptors isolated from rat brain with IC_{50} values between 0.4 mg/ml and 4.8 mg/ml [Daels-Rakotoarison 2000].

Antioxidant effects
A 75%-ethanolic extract from *Ballota nigra* subsp. *anatolica* inhibited the formation of superoxide anion with an IC_{50} of 0.74 mg/ml [Citoglu 2004].

An aqueous dry extract from black horehound showed a concentration-dependent activity in the DDPH assay (IC_{25} = 4.81 µg/ml), concentration-dependent superoxide radical scavenging activity using the xanthine/xanthine oxidase system (IC_{25} = 14.6 µg/ml) and NO radical scavenging activity (IC_{25} = 122 µg/ml) [Vrchovská, 2007].

The antioxidant activity of five phenylpropanoids (acteoside, forsythoside B, arenarioside, ballotetroside, and caffeoyl-L-malic acid) was investigated against the reactive oxygen species (ROS) superoxide anion, hydrogen peroxide, hypochlorous acid and hydroxyl radical. These phenylpropanoids exhibited ability to scavenge ROS with IC_{50} values comparable to that of *N*-acetylcysteine. Furthermore, they significantly and dose-dependently inhibited the release of ROS from isolated and stimulated polymorphonuclear neutrophils from human blood ($p<0.05$), with activity in the order acteoside > forsythoside B > caffeoyl-L-malic acid > arenarioside > ballotetroside. Proteine kinase C or phospholipase C pathways are involved in the mechanism of action [Daels-Rakotoarison 2000].

Five phenylpropanoids from black horehound were evaluated for their ability to inhibit Cu^{2+}-induced peroxidation of human LDL. They strongly inhibited peroxidation of LDL in a dose-dependent manner with ED_{50} values of 1 µM for acteoside and forsythoside B, 1.8 µM for arenarioside, 7.5 µM for ballotetroside and 9.5 µM for caffeoyl-L-malic acid, compared to 2.3 µM for quercetin. The capacity of phenylpropanoids to inhibit Cu^{2+}-induced LDL oxidation is linked to their free radical scavenging properties [Seidel 2000].

Antimicrobial activity
An aqueous dry extract from black horehound dose-dependently inhibited the formation of biofilm of MRSA with an IC_{50} of 8 µg/ml [Quave 2008].

Acteoside, forsythoside B and arenarioside, but not ballotetroside or caffeoyl-L-malic acid, exhibited moderate antibacterial activity against *Staphylococcus aureus* (Gram-positive) and *Proteus mirabilis* (Gram-negative) at a concentration of 128 µg/ml [Didry 1999].

In vivo experiments

Sedative and antidepressive effects
The psychotropic effects of a black horehound aqueous dry extract, intraperitoneally administered to rodents, were evaluated from a range of tests. A dose of 200 mg/kg b.w. significantly reduced mobility and, to a lesser extent, curiosity in mice; produced a significant tranquillizing effect in mice; significantly reduced anxiety but not locomotor activity in rats; and significantly prolonged barbiturate-induced sleeping time in rats. No muscle relaxant effects were evident in mice from doses of 200 or 400 mg/kg [Mongold 1991].

After i.p. administration to mice at 0.5 ml per animal a 10% aqueous extract from black horehound had a marked sedative effect on the central nervous system, reducing spontaneous motor activity by 60% after 1 hour and 65% after 3 hours [Rácz-Kotilla 1980].

After i.p. administration to rats an aqueous dry extract from black horehound showed significant antidepressant activity in the forced swimming test. Mean durations of immobility were 570 seconds for untreated control animals, 496 seconds after the extract at 240 mg/kg ($p<0.001$) and 379 seconds after amitriptyline at 5 mg/kg i.p. ($p<0.001$). This extract did not exert significant anxiolytic activity in the elevated plus-maze test [Vural 1996].

Hypoglycaemic effects
Oral administration of a 70%-ethanolic dry extract from black horehound to rats at 400 mg/kg b.w. daily for 7 days caused significant decreases in blood glucose and total serum cholesterol (both $p<0.01$). In the glucose tolerance test in rats, a single oral dose of the same extract at 400 mg/kg significantly reduced blood glucose levels ($p<0.001$) and increased serum insulin levels ($p<0.01$) within 15 minutes [Nusier 2007a].

In a further experiment with the same extract, a single oral dose at 400 mg/kg significantly reduced plasma glucose levels in healthy normoglycaemic rats by 32% and in alloxan-induced diabetic rats by 22% (both $p<0.001$) after 6 hours. At this dose level no significant changes were observed in spontaneous motor activity of the animals, nor noticeable changes in behaviour and intake of food and water [Nusier 2007b].

Clinical studies
In an open study, 28 patients with general anxiety disorder, diagnosed in accordance with the DSM (at least 6 of the 18 criteria comprising the DSM III R classification) and involving depression and sleep disorders, were treated daily for 90 days with 3 × 5 ml of a black horehound liquid preparation (14% ethanol, corresponding to 3 × 0.5 g of black horehound). Based on clinical examination and DSM III R assessment, responder rates after 60 and 90 days were 65 and 73% of patients respectively. Patients with sleep disorders showed particularly marked improvement. Out of 10 patients taking benzodiazepines prior to the study, 3 discontinued and 4 reduced their dose by half [Huriez 1991].

Pharmacokinetic properties
No data available.

Preclinical safety data

Acute toxicity
After administration of a single large dose of a black horehound aqueous dry extract to mice at 2 g/kg b.w. no mortality occurred and no signs of toxicity were evident over a period of 15 days or from post-mortem examination [Mongold 1991].

Clinical safety data

In an open clinical study, no major adverse effects were reported after treatment of 28 patients with 3 × 5 ml of a black horehound liquid preparation (14% ethanol, corresponding to 3 × 0.5 g black horehound) for 90 days. Feelings of fatigue, which diminished as the study progressed, and nausea, which was alleviated by taking the black horehound preparation after meals, were reported [Huriez 1991].

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MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
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ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
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ALOE CAPENSIS	Cape Aloes	Online Series, 2014
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COLAE SEMEN	Cola	Online Series, 2014
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FUMARIAE HERBA	Fumitory	Supplement 2009
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The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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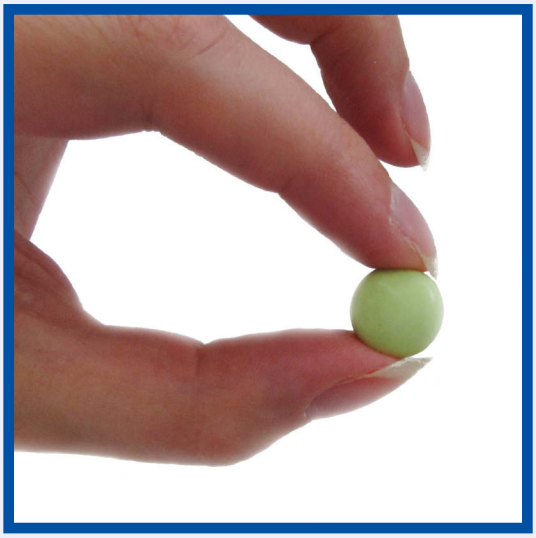

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

BETULAE FOLIUM

2015

Birch Leaf

DEFINITION

Birch leaf consists of the whole or fragmented dried leaves of *Betula pendula* Roth and/or *Betula pubescens* Ehrh. as well as hybrids of both species. It contains not less than 1.5 per cent of flavonoids, calculated as hyperoside ($C_{21}H_{20}O_{12}$; M_r 464.4) with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Birch Leaf].

Fresh material may also be used, provided that when dried it complies with the monograph of the European Pharmacopoeia.

CONSTITUENTS

1-3% of flavonol glycosides, principally hyperoside and other quercetin glycosides together with glycosides of myricetin and kaempferol [Tissut 1972; Pawlowska 1982; Pokhilo 1983; Dallenbach-Tölke 1986; 1987a; 1987b; Keinänen 1996; Ossipov 1996; Hänsel 2010, Raal 2015]; other phenolic compounds including 3,4′-dihydroxypropiophenone 3-glucoside (ca. 0.8% in *B. pendula*, 0.08% in *B. pubescens*) and chlorogenic acid (ca. 2% in *B. pubescens*, 0.02-0.1% in *B. pendula*) [Keinänen 1996; Ossipov 1996]; triterpene alcohols of dammarane type and their malonyl esters [Pokhilo 1983, 1986, 1988; Baranov 1983; Rickling 1992, 1993; Hilpisch 1997].

Other constituents include monoterpene glucosides [Tschesche 1977], a sesquiterpene oxide [Pokhilo 1984], roseoside [Tschesche 1976], tannins, traces of essential oil [Hänsel 2010, Orav 2011] and approx. 4% of minerals, particularly potassium [Szentmihályi 1998, Hänsel 2010].

Fresh leaves contain up to 0.5% of ascorbic acid [Jones 1984].

CLINICAL PARTICULARS

Therapeutic indications
Irrigation of the urinary tract, especially in cases of inflammation and renal gravel, and as an adjuvant in the treatment of bacterial infections of the urinary tract [Schilcher 1984, 1987; Hänsel 2010].

Posology and method of administration

Dosage
2-3 g of dried leaf as an infusion, three to four [Gorecki 2013] times daily; equivalent preparations [Schilcher 1987; Schilcher 1990].

Method of administration
For oral administration.

Duration of administration
No restriction. If symptoms persist or worsen, medical advice should be sought.

Contra-indications
None known.

Special warnings and special precautions for use
Oedema due to impaired heart and kidney function [Schilcher 1987].

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

E/S/C/O/P
MONOGRAPHS

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Diuretic activity
Various flavonoids were investigated for their inhibitory activity on specific neuropeptide hydrolases which regulate the formation of urine through excretion of sodium ions [Borman 2000]. The results led to the conclusion that certain flavonoids, especially quercetin, and other phenolic compounds present in birch may contribute to accelerated formation of urine [Melzig 2000].

Anti-inflammatory effect
An aqueous extract (0.63 mg/mL) was tested for its anti-proliferative effect on human peripheral blood mononuclear cells, activated with anti-CD3/CD28 monoclonal antibodies. In the highest tested concentration (160 µg/mL) the optical density (OD) was reduced from 0.54 ±0.1 in untreated cells to 0.18 ±0.03. Methotrexate as a positive control reduced the OD to 0.25 ±0.09 at a concentration of 1 mg/mL. The extract had no effect on the proliferation of resting cells [Gründemann 2011].

Antioxidant effect
A dry extract (ethanol 70%; mass/volume ratio 1:10) was tested for its Trolox Equivalent (TE) antioxidative capacity. The constituents hyperoside and chlorogenic acid showed the strongest activity with 28.9 µmol TE/g and 22.8 µmol TE/g respectively [Raudone 2014].

In vivo experiments

Diuretic effect
After oral administration of an infusion of birch leaf to rabbits, urine volume increased by 30% and chloride excretion by 48% [Vollmer 1937]; in mice, urine volume increased by 42% and chloride excretion by 128% [Vollmer 1937]. Young birch leaves administered orally to mice and rats did not produce these effects, in fact an anti-diuretic action was apparent [Elbanowska 1966].

Oral administration of powdered birch leaf to dogs at 240 mg/kg b.w. increased urine volume by 13.8% after 2 hours; a flavonoid fraction at 14 mg/kg increased urine volume by 2.8% [Borkowski 1960].

Further studies in rats showed increased excretion of urine after oral administration of aqueous and alcoholic extracts rich in flavonoids (48, 76 and 148 mg/100 mL). The aquaretic effect correlated with the amount of flavonoids, but no saluretic effect could be demonstrated [Schilcher 1987; Schilcher 1988; Schilcher 1989].

However, diuretic effects do not appear to be due entirely to flavonoids since lesser effects were achieved with isolated flavonoid fractions [Schilcher 1984; Schilcher 1990; Borkowski 1960; Schilcher 1988; Schilcher 1989; Lübben 1982].

Various extracts from birch leaf were administered orally to rats: an extract prepared with ethanol 70% (43 mg flavonoids/kg b.w.), a fraction obtained from this extract with butanol (192 mg flavonoids/kg b.w.), a butanolic fraction low in flavonoids (14 mg flavonoids/kg b.w.) and the aqueous residue from the separation process (0.7 mg flavonoids/kg b.w.). No increase in diuresis or saluresis could be demonstrated for any of these preparations [Rickling 1992].

It has been suggested that the potassium content of birch leaf may contribute to the diuretic effect [Schilcher 1987; Schilcher 1990; Schilcher 1988; Schilcher 1989]. High potassium-sodium ratios were determined in dried leaf (189:1) and in a 1% decoction (168:1) [Szentmihályi 1998].

Gastroprotective effect
The gastroprotective effects of a dried methanolic (70%; not further specified) extract were investigated in rats with ethanol-induced (90%) gastric ulcers. Pre-treatment with doses of 100, 200 and 400 mg/kg b.w. demonstrated dose-dependent reductions in the lesions. Pre-treatment at the highest dose significantly reduced alcohol-induced lesions by 71.4% ($p<0.01$) compared to the positive control misoprostol at 0.50 mg kg^{-1} (85.7%) [Germano 2013].

Clinical studies
In a field study, 1066 patients received a dry aqueous extract of birch leaf (4-8:1) at various daily doses (from 180 mg to 1080 mg or more daily) for irrigation of the urinary tract. In most cases (63%) the treatment period was 2-4 weeks. The patients could be classified into four groups: 73.8% suffered from urinary tract infections, cystitis or other inflammatory complaints, 14.2% from irritable bladder, 9.3% from stones and 2.7% from miscellaneous complaints. In the first group 56% of patients also received antibiotic therapy. After treatment the symptoms disappeared in 78% of patients in the first group, 65% in the second group and 65% in the third group. Symptoms disappeared in 80% of patients treated with, and in 75% of those going without, antibiotics. Both physicians and patients considered efficacy to be very good (39% and 48% respectively) or good (52% and 44% respectively) [Müller 1999].

In a randomized, double-blind, placebo-controlled pilot study, 15 patients with infections of the lower urinary tract were treated with 4 cups of birch leaf tea or placebo tea daily for 20 days. Microbial counts in the urine of the birch leaf tea group decreased by 39% compared to 18% in the control group. At the end of the study, 3 out of 7 patients in the verum group and 1 out of 6 in the placebo group no longer suffered from a urinary tract infection [Engesser 1998].

Pharmacokinetic properties
No data available.

Preclinical safety data
An extract of birch leaf gave a very weak mutagenic response in the Ames test [Göggelmann 1986]; no other studies have been performed to confirm this.

Clinical safety data
In an open post-marketing study, mild adverse effects were reported in only 8 out of 1066 patients who received a dry aqueous extract of birch leaf (4-8:1) at daily doses of up to 1080 mg for 2-4 weeks [Müller 1999].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Supplement 2009
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Second Edition, 2003
FUMARIAE HERBA	Fumitory	Supplement 2009
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Supplement 2009
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Second Edition, 2003
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Second Edition, 2003

LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Supplement 2009
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Second Edition, 2003
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Second Edition, 2003
RATANHIAE RADIX	Rhatany Root	Supplement 2009
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Second Edition, 2003
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Second Edition, 2003
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Second Edition, 2003
SALICIS CORTEX	Willow Bark	Second Edition, 2003
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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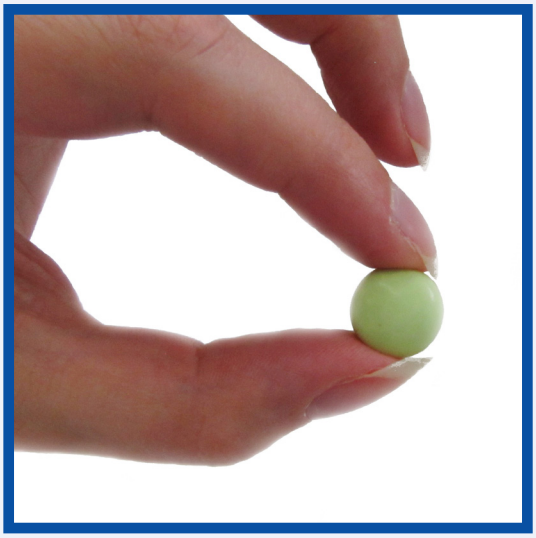
The Scientific Foundation for Herbal Medicinal Products

Calendulae flos
Calendula flower

2019



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MONOGRAPHS

***The* Scientific Foundation for**
Herbal Medicinal Products

CALENDULAE FLOS
Calendula flower

2019

E/S/C/O/P
European Scientific Cooperative
On Phytotherapy

ESCOP Monographs were first published in loose-leaf form progressively
from 1996 to 1999 as Fascicules 1-6, each of 10 monographs

Second Edition, completely revised and expanded

Second Edition, Supplement 2009

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Calendulae flos - Calendula flower

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

Edited by Roberta Hutchins and Simon Mills
Cover photographs by Roberta Hutchins (*Calendula officinalis*) and Martin Willoughby
Cover and text design by Martin Willoughby
Typeset in Optima by Roberta Hutchins

Plant illustrated on the cover: *Calendula officinalis*

FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in the production of ESCOP monographs and other activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

CALENDULAE FLOS

2019

Calendula flower

DEFINITION

Calendula flower consists of the whole or cut, dried, fully opened flowers, which have been detached from the receptacle, of the cultivated, double-flowered varieties of *Calendula officinalis* L. It contains not less than 0.4 per cent of flavonoids, calculated as hyperoside ($C_{21}H_{20}O_{12}$, Mr 464.4) with reference to the dried drug [Calendula flower, Ph. Eur.].

The material complies with the monograph of the European Pharmacopoeia [Calendula flower, Ph. Eur.].

Fresh material may also be used, provided that when dried it complies with the monograph of the European Pharmacopoeia [Calendula flower, Ph. Eur.].

CONSTITUENTS

Triterpene saponins (2-10%), mainly oleanolic acid glycosides; free and esterified triterpene alcohols, especially faradiol 3-mono- and diesters, carotenoids (up to 3%), flavonoids (0.3-0.8%) based on quercetin and isorhamnetin, polysaccharides e.g. of the rhamnogalacturonan I type, sterols, sesquiterpenoids, phenolcarbonic acids, fatty acids, tocopherols and essential oil (0.2-0.3%) with α-cadinol and δ-cadinen as major components [Scheffer 1979, Komissarenko 1988, Varljen 1989, Vidal-Ollivier 1989a, Vidal-Ollivier 1989b, Isaac 1992, Della Loggia 1994, Akihisa 1996, Gracza1987, Zitterl-Eglseer 1997, Marukami 2001, Mukhtar 2004, Kishimoto 2005, Naved 2005, Petrovic 2011, Korzh 2012, Ollenikov 2013, Ollenikov 2014, D´Ambrosio 2015, Isaac 2017, Mohamed 2015, Caamal-Herrera 2016, Khidoyatova 2016, Lichius 2016, Miguel 2016, Nicolaus 2016].

CLINICAL PARTICULARS

Therapeutic indications
Treatment of minor inflammations of the skin and mucosa; as an aid to the healing of minor wounds [Scheffer 1979, Lichius 2016, Willuhn 1992, Duran 2005, Amoian 2010].

Posology and method of administration

Dosage

External use
Infusion for topical application: 1-2 g of dried flower per 150 mL of water.
Fluid extract 1:1-2.2 in 40-50% ethanol or tincture 1:5 in 70-90% ethanol [Isaac 2015]. For the treatment of wounds the tincture is applied undiluted; for compresses the tincture is usually diluted at least 1:3 with freshly boiled water [Willuhn 1992, Van Hellemont 1988].
Semi-solid preparations containing 2-10% of fluid extract 1:1 [Willuhn 1992, Isaac 2017, Farmacopoea Italiana].

Method of administration
For topical application.

Duration of administration
No restriction.

Contra-indications
Known sensitivity to members of the Asteraceae (Compositae) family.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

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Pregnancy and lactation
No data available.

However, there are no objections to external use during pregnancy and lactation.

Effects on ability to drive and use machines
Not relevant.

Undesirable effects
Rare cases of skin sensitisation have been shown [Reider 2001, Mailhol 2009], whereas no allergic reactions were observed in 16 Asteraceae-sensitive persons in a patch test with a 10% petroleum ether extract [Paulsen 2001].

Overdose
Not applicable.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Wound-healing effects
In a scratch assay with Swiss 3T3 albino mouse fibroblasts a hexane (DER 7.7:1) and an ethanolic (DER 5.2:1) extract at 10 µg/mL significantly ($p<0.05$) enhanced cell numbers by 64.35% and 70.53%, respectively, mainly by stimulating migration. Faradiol myristate and palmitate were shown to contribute partially to the effect [Fronza 2009].

The proliferation of endothelial progenitor cells (EPC) was significantly stimulated by an extract (prepared in phosphate buffered saline) at concentrations of 50, 500 and 1000 ng/mL ($p<0.005$, 0.05 and 0.05, respectively). The expression of molecules involved in EPC chemotaxis and adhesion (VCAM-1, VE-cadherin, ICAM-1, PECAM-1, P-selectin, Tie-2, CXCR4) and cell migration ability in a scratch assay were significantly increased with 1 µg/mL ($p<0.05$ for VCAM-1, $p<0.005$ for all other assays) [Iordache 2011].

A CO_2 extract (18.2% faradiol esters and 0.97% carotenes) at 5 µg/mL and 20 µg/mL led to significant re-epithelialisation after 48 h incubation in a scratch assay using human conjunctival WKD cells ($p<0.05$ and 0.01 respectively) [Arana 2015].

Proliferation of Vero cells was increased by a methanolic and a 20% methanolic extract (1 µg to 100 µg/mL); the effect of apolar extracts was less. Cell migration was stimulated by the extracts (100 µg/mL) in the order hexane > 20% methanol > dichloromethane; the methanolic extract was inactive [Un 2015].

A tincture (41% ethanol) at dilutions of 1:50 and 1:100 significantly increased proliferation of human dermal and human lung fibroblasts ($p<0.05$ and 0.01) via a phosphatidylinositol-3-kinase-dependent pathway. The growth of HeLa cells remained unchanged. Cell migration of both fibroblast cell lines was stimulated significantly ($p<0.05$) after 24 hours [Dinda 2015].

The proliferation of human dermal fibroblasts was significantly and dose-dependently increased by a 50% ethanolic extract (yield 32%) and a water fraction thereof, with the strongest effect of both at 100 µg/mL ($p<0.001$). At 100 µg/mL both extracts enhanced cell migration in a scratch assay ($p<0.01$ and 0.05, respectively). Expression of CTGF and α-SMA (markers of increased fibroblast activity and wound contraction) was induced by the extract ($p<0.01$) [Dinda 2016].

Cultivation of non-malignant human CCD16 fibroblasts with a decoction (not further specified) for 24 and 48 hours resulted in a gradual but not significant increase of their numbers, as compared to control, and in beneficial morphological changes of the cell structure [Wyganowska-Swiatkowska 2016].

Three extracts (n-hexane, DER 11:1; ethanol, 8.3:1; water, 10:1) were investigated to analyse molecular effects in the inflammatory phase of wound healing. In human immortalized keratinocytes, the n-hexane and the ethanolic extract at 50 µg/mL activated NF-κB and IL-8 at the transcriptional and the protein level. A dose-dependent inhibition of collagenase activity was observed for the ethanolic (62.5 to 500 µg/mL) and aqueous extracts (62.5 to 2500 µg/mL), significant at all tested doses ($p<0.001$). The content of soluble collagen in the supernatant of human primary fibroblasts was increased by the ethanolic extract at 50 µg/mL [Nicolaus 2017].

Immunomodulatory effects
A dry 70% ethanolic extract caused an inhibitory effect in the mitogen-induced lymphocyte proliferation assay. In the mixed lymphocyte reaction the extract showed stimulatory effects at 0.1-10 µg/mL, followed by inhibition at higher concentrations [Amirghofran 2000].

A dose-dependent (125-500 µg/mL) increase in the proliferation of peripheral blood lymphocytes was observed after treatment with an aqueous dry extract from Laser-irradiated Calendula flower. Cell cycle analysis revealed a re-entering of the lymphocytes into S phase [Jiménez-Medina 2006].

Isolated polysaccharides from calendula flower were found to stimulate phagocytosis of human granulocytes [Varljen 1989].

Antiviral and antimicrobial effects
A 70% alcoholic tincture had high virucidal activity against influenza viruses and a marked ability to suppress the growth of herpes simplex virus [Bogdanova 1970].

A dry extract (dichloromethane-methanol 1:1) exhibited anti-HIV activity in an MTT (dimethylthiazole-diphenyltetrazolium)-based assay. In the presence of 10-30 µg/mL of the extract, 90% inhibition of HIV-1 replication in acutely infected lymphocytic Molt-4 cells was observed. At concentrations of 500 µg/mL the extract suppressed cell fusion and protected uninfected Molt-4 cells against subsequent HIV-induced cytolysis caused by co-cultivation with persistently infected U-937/HIV-1 cells for up to 24 hours. The extract (50, 100 and 200 µg/mL) also caused marked dose- and time-dependent inhibition of HIV-1 reverse transcriptase activity by up to 85% in a cell-free system [Kalvatchev 1997].

Extracts from the flowers had trichomonacidal activity, which has been attributed to oxygenated terpenes [Gracza 1987].

Suture material after extraction of the unerupted maxilliary third molars was investigated for different microorganisms. After left molar extraction the patients (n=12) did not use any mouthwash, whereas after extraction of the right molar 15 days later patients used a mouthwash with 1% of a tincture or a 0.12% solution of chlorhexidine digluconate. The suture material was removed after 7 days and extracted. Dilution series of the extacts were grown on 5 different media. The mean number of colony forming units of aerobic, facultative anaerobic microorganisms and *Candida* ssp. as compared to control was reduced by the calendula mouthwash but to a lesser extent than by chlorhexidine [Faria 2011].

In the disc diffusion assay, a methanolic and an ethanolic

extract at 300 µg/disc showed antifungal activity against a panel of different fungi, comparable to the effect of 30 µg/disc of fluconazole as positive control. Growth inhibition of numerous Gram-positive and Gram-negative bacteria by the extracts was less than that found for ciprofloxacin (4.5 mg/disc) [Efstratiou 2012].

A significant inhibition of *Staphylococcus epidermidis*, *S. aureus* (both $p<0.001$) and *Propionibacterium acnes* ($p<0.01$) was seen after treatment with a methanolic extract (not further specified) when compared to control [Nand 2012].

An 80% methanolic extract inhibited *Leishmania major* promastigotes (IC_{50} = 108.2 µg/mL). The infection rate of macrophages with amastigotes after incubation with the extract and the parasite survival rate were significantly ($p<0.001$) lower as compared to untreated control [Nikmehr 2014].

Combination of cefotaxime with a methanolic extract led to a pronounced reduction of MICs due to synergistic or additive effects in 10 multiresistent *Klebsiella pneumoniae* isolates co-producing extended spectrum beta lactamase and AmpC beta lactamase. The extract and cefotaxime alone were active at much higher concentrations only [Shah 2015].

In a similar approach, the essential oil (15 µL/disc) exhibited antifungal effects against numerous *Candida* species which were more pronounced than that of 20 µg/disc of nystatin [Gazim 2008].

Oleanolic acid from calendula flower showed marked antibacterial activity against several bacteria, of which the Gram-positive were more susceptible than the Gram-negative [Szakiel 2008].

Antioxidant and radical scavenging effects
Extracts of differing polarities exhibited antioxidative effects on liposomal lipid peroxidation induced by Fe^{2+}/ascorbic acid. Marked activity was observed with ether, butanol and aqueous extracts. In combination with pro-oxidants, different antioxidative effects were obtained: with pyralene the butanol and aqueous extracts were most effective; with carbon tetrachloride the chloroform and ethyl acetate extracts showed the strongest activity. Combination of fullerenol with each extract resulted in a further decrease in lipid peroxidation [Popovic 1999, Popovic 2000].

DPPH and ABTS radicals were scavenged by an ethanolic extract with IC_{50} values of 100 and 6.5 µg/mL. In several other radical scavenging or antioxidant assays the IC_{50} values were much higher [Preethi 2006].

Antioxidant activity was shown for an extract prepared with propylene glycol/water (85:15). Respiratory burst in human polymorphonuclear leukocytes after incubation with L-arginine followed by stimulation with N-formyl-methionyl-leucyl-phenylalanine was significantly reduced at extract concentrations from 0.05 µg/mL to 1.6 µg/mL ($p<0.01$) and after phorbol-12-myristate-13-acetate (PMA) from 0.10 µg/mL to 1.6 µg/mL ($p<0.05$ to $p<0.01$) [Braga 2009].

A 50% ethanolic extract (yield 15.7%) resulted in IC_{50} values of 97.1 µg/mL, 350.0 µg/mL and 4.4 µg/mL in the DPPH, the lipid peroxidation and the xanthine/luminol/XOD assay, respectively [Fonseca 2010].

A methanolic and a 20% methanolic extract at 100 µg/mL scavenged DPPH radicals (inhibition 93.6 and 89.8% respectively). These extracts, as well as a dichloromethane and a hexane extract, protected Vero cells against H_2O_2-induced oxidative stress ($p<0.001$) [Un 2015].

Pretreatment of HaCat human keratinocytes with two polar calendula extracts (not further specified) resulted in protection of H_2O_2-induced oxidative stress, which was most pronounced after 48 hours of pretreatment [Alnuqaydan 2015].

A methanolic extract (DER 5:1; 0.1, 0.2, 0.3 and 0.4 mg) dose-dependently protected rat brain mitochondria from oxidative damage induced by ascorbate as shown by reduced chemoluminescence and the composition of polyunsaturated fatty acids [Zeinsteger 2018].

A butanolic fraction of a 50% ethanolic extract inhibited the lipid peroxidation induced by Fe^{2+}/ascorbate in liver microsomes with an IC_{50} of 150 µg/mL [Cordova 2015].

Arnidiol-3-O-myristate from Calendula flower significantly reduced ROS generation induced by H_2O_2 at all the tested concentrations (0.01, 0.1 and 1 µg/mL), while ψ-taraxasterol-3-O-myristate resulted in a significant effect only at the lowest concentrations (0.01 µg/mL). In transepithelial resistance measurements neither compound altered the permeability of a Caco-2 cell layer. Under inflammatory stimulation with H_2O_2, or interferon-γ + TNF-α modulated oxidative stress and inflammatory conditions, a significant ($p<0.05$ to <0.0001) increase of the trans-epithelial resistance at 0.01 and 0.1 µg/mL was observed [Dall´Acqua 2015].

Rat brain microsomes incubated with a methanolic extract (DER 4.8:1) were protected against lipid peroxidation induced by Fe^{2+}/ascorbate [Palacios 2016].

Anti-inflammatory effects
A defatted, 70% ethanolic extract (DER 14:1) inhibited COX-2 with an IC_{50} value of 0.1 µg/mL, but remained without effect on 5-LOX [Chagas-Paula 2015].

In AGS cells, after stimulation with TNF-α, a significant inhibition of the NF-κB driven transcription by a CH_2Cl_2 extract was shown (5, 10 and 20 µg/mL; $p<0.05$, <0.001 and <0.001 respectively) [Colombo 2015].

Three isorhamnetin glycosides from calendula flower, at concentrations of 1.5×10^{-5} M, showed inhibitory effects on arachidonate 12-lipoxygenase from rat lung cytosol [Bezákova 1996].

Effects on buccal membranes
In a test system based on porcine buccal membranes, strong concentration-dependent adhesive processes were observed with a low-viscosity, polysaccharide-enriched extract (98% carbohydrates) of calendula flower [Schmidgall 2000].

Cytotoxic and anti-genotoxic effects
An ethyl acetate extract showed a proliferative effect on human skin fibroblast between 25 and 75 µg/mL; higher concentrations were cytotoxic. For a heptane and a methanol extract, proliferation was only seen at 25 µg/mL; all higher doses were cytotoxic. In human breast cancer cells T47D, the ethyl acetate extract resulted in proliferation at concentrations up to 175 µg/mL and the heptane extract up to 50 µg/mL. These two extracts at higher concentrations and the methanol extract from 25 µg/mL were cytotoxic [Matysik 2005].

An aqueous extract from Laser-irradiated Calendula flower suppressed proliferation of B9 murine fibrosarcoma cells with an IC_{50} of 60 µg/mL. In several other cancer cell lines the extract

at 250 µg/mL caused inhibition of between 72 and 100% by caspase-3 induction of apoptosis and accumulation in G_0/G_1 phase due to the downregulation of several cyclins and cyclin-dependent kinases [Jiménez-Medina 2006].

No cytotoxcicity was observed with a 50% ethanolic extract (yield 15.7%) for L929 and HepG2 cells up to 15 mg/mL [Fonseca 2010].

Human acute T leukaemia cells (J-45.01) were inhibited by a 70% methanolic dry extract with an IC_{50} of 330 µg/mL [Wegiera 2012].

An infusion (2 g drug in 100 mL water) was slightly cytotoxic for human melanoma Fem-x cells, HeLA cells and human chronic myelogenous leukaemia cells (IC_{50} 360µg/mL, 750 µg/mL and 870 µg/mL respectively). Lower cytotoxicity was observed against other cancer cell lines, but also against human peripheral blood mononuclear cells with and without stimulation by phytohaemagglutinin [Matíc 2013].

In human conjunctival WKD cells the LD_{50} of a CO_2 extract was 245 µg/mL [Arana 2015].

A hydromethanolic (80%) extract exhibited weak cytotoxic effects on HeLa and HepG2 cells with IC_{50} values of 256 and 330 µg/mL respectively [Miguel 2016].

In rat liver cell cultures, an aqueous and a hydroethanolic extract reversed unscheduled DNA synthesis induced by the carcinogen diethylnitrosamine at around 50 ng/mL and between 0.4 to 16 ng/mL, respectively. Evaluation of the genotoxicity of the extracts without diethylnitrosamine showed a genotoxic effect at concentrations from 25 to 100 µg/mL and from 3.7 to 100 µg/mL, which was three orders of magnitude above the protective dose [Pérez-Carreón 2002].

A butanol fraction and two triterpene saponin glycosides were cytotoxic for HepG2 cells (IC_{50} = 14.3, 7.35 and 3.73 µg/mL respectively) [Mohamed 2015].

In B16F1 melanoma cells, an ethyl acetate fraction from a 50% ethanolic extract (6.5-50 µg/mL) significantly ($p<0.05$) decreased melanin production induced by α-melanocyte-stimulating hormone in a dose-dependent manner. The fraction inhibited migration of the cells via a reduction of MMP-2 activity and expression, which was caused by the inhibition of the MAPK signaling pathway [Xuan 2019].

In a panel of 60 cancer cell lines, calenduloside F-6'-O-methylether was most potent against leukaemia (MOLT-4 and RPMI-8226), colon (HCC-2998), and melanoma (LOX IMVI, SK-MEL-5, and UACC-62) cells, with GI_{50} values of 0.77-0.99 µM. Calenduloside G-6'-O-methylether exhibited GI_{50} values of less than 20 µM against all of the cell lines, except for ovarian (IGROV1: GI_{50} 20.1 µM) and renal (UO-31: 33.3 µM) cancer cells [Ukiya 2006].

In the MTT assay three sesquiterpene glycosides from a dichloromethane extract showed cytotoxicity against human epithelial gastric cells AGS with IC_{50} values between 5.4 and 6.46 µM [D´Ambrosio 2015].

Other effects
An 80% ethanolic extract (DER 5:1) showed a spasmolytic effect on spontaneous contractions in rabbit jejunum (EC_{50} 0.78 mg/mL) and in acetylcholine- and histamine-induced contractions in guinea pig ileum (80-90% inhibition at 0.3 mg/mL), mediated by calcium channel blockade [Bashir 2006].

In vivo experiments

Effects on wound healing
Dried alcoholic and aqueous extracts of calendula flower in combination with allantoin, applied topically as a 5% ointment, stimulated epithelisation in surgically-inflicted standard wounds in rats [Klouchek-Popova 1981, Klouchek-Popova 1982].

Experimental incisions in rats were infected with *Staphylococcus epidermidis*. Topical treatment with a calendula cream (not further specified) resulted in accelerated cicatrisation compared to controls. The results were confirmed by histological examination [Perri de Calvalho 1991].

In a study in rabbits, the time taken for complete cicatrisation of experimental wounds was shortened by approximately 25% after topical treatment with hydrogel or powder preparations containing 10% of an ethanolic dry extract in comparison with vehicle-only controls [Oana 1995].

Enhanced healing of experimental wounds in buffalo calves was observed after topical treatment with an ointment containing 5% of a dry extract. Epithelisation accelerated and histopathological parameters improved in comparison with wounds treated with saline only [Ansari 1997].

In incision wounds of rabbits, topical treatment with a lotion containing 5% of a tincture (0.1 mL for 7 days) resulted in a significantly higher number of fibroblasts ($p<0.05$) as compared to controls and in a higher number of fibrocytes ($p<0.05$ as compared to baseline) in the wounds [de Oliveira Pagnano 2008].

A 70% ethanolic extract (yield 1.1%) was administered orally (20 and 100 mg/kg b.w.) or topically (dosage not given) to female Wistar rats with excision wounds for 10 days. The percentage of wound closure was significantly ($p<0.01$) greater in the verum groups (91.7%, 93.9% and 86.9% respectively) compared to the control group (51.1%) on day 8. Days for re-epithelisation were 17.7 for control and 16.1 for topical application, but significantly less after oral treatment (14 days with 20 mg/kg ($p<0.05$) and 13 days with 100 mg/kg ($p<0.01$)). On days 5 and 15 a significant increase ($p<0.05$ to $p<0.001$) was observed in the hydroxyproline and hexosamine content in the granuloma tissue after oral treatment with 100 mg/kg as compared with control [Preethi 2009c].

The same extract was tested in a burn wound model in rats at doses of 20, 100 and 200 g/kg b.w. orally for 10 days. The amounts of hydroxyproline and hexosamine significantly increased with the two higher doses at day 10 ($p<0.01$). The levels of acute phase proteins (haptoglobin, orosomucoid) were reduced significantly ($p<0.05$ to $p<0.001$ at 200 mg/kg). Antioxidant parameters (GSH, MDA, SOD, catalase) in the liver and markers of hepatic function (ALP, GOT, GPT) were also improved in the animals with burns [Chandran Preethi 2008].

After topical treatment of circular wounds in rats for 4, 7 and 14 days with an ethanolic dry extract (100 µL of a 1% solution in water), a better contraction of the wounds and of collagen formation on days 4 and 7 was observed. Significantly less epidermal hyperaemia ($p<0.026$) on day 7 and epithelial hyperplasia ($p<0.046$) on day 14 were seen [Parente 2009a].

An ethanolic extract, and two apolar fractions (hexane, dichloromethane) thereof, led to significant neovascularisation in the chorioallantoic membrane model ($p<0.05$). In rats with experimental wounds treated topically with 100 µL of a 1% aqueous solution of the extract, macroscopic evaluation demonstrated a faster cessation of serous exudation and thinner

and wetter crusts as compared to control. In the wounds of the verum group, less fibrin and hyperaemia and a higher collagen quantity were observed. Immunohistochemistry revealed an increase in the number of blood vessels as shown by VEGF staining [Parente 2012].

In New Zealand white rabbits, treatment of full-thickness dorsolumbar wounds (2 x 2 cm) with a 5% aqueous extract (not further specified; daily for 28 days) resulted in significantly better scores ($p<0.05$) for epithelialisation, inflammation, fibroblasts, neovascularisation and collagen fibre density, thickness and arrangement at days 21 and 28 as compared to saline [Mahdu 2013].

In Sprague Dawley rats with incision wounds, topical treatment with a gel containing 7% of a 70% ethanolic extract for 14 days resulted in significantly ($p<0.05$) higher collagen and hydroxyproline contents in the wounds or scars after 14, 21 and 45 days, as compared to control (no treatment) or gel base [Naeini 2012]. In a biomechanical investigation in the same experimental set-up, after 21 days, treatment with the 7% gel resulted in significantly ($p<0.05$) higher ultimate tensile strength of skin samples from the edges of the wounds as compared to control. In the histopathological study, treated tissues at 14 days showed complete re-epithelialisation and thicker epithelium, and more fibroblasts and neovascularisation were seen than in the control and placebo groups. After 21 days, treated tissues were more organised with thinner epithelium and very few inflammatory cells in the lesion in comparison to control and placebo. The number of fibroblasts was reduced due to their change to mature organized fibrocytes, which were aligned along the reparative connective tissue [Shafeie 2015].

Wistar rats with partial transection of the Achilles tendon were treated with a cream containing 4% of a glycolic extract (1:1) on the wound before suture and for 5 further days topically. In the transected region the hydroxyproline concentration (as a marker for collagen concentration) and the amount of non-collagenous proteins were significantly ($p<0.05$) increased as compared to the operated control group. Other parameters characteristic for remodeling (amount of the isoforms of metalloproteinase-2 and -9 (MMPs), birefringence of tendon sections, chondroitin-6-sulfate) were not or only slightly improved with the cream [Aro 2015].

Topical treatment of excisional wounds in BALB/c mice for 6 days with 150 mg/kg of either a 50% ethanolic extract (yield 32%) or a water fraction thereof, significantly enhanced wound closure ($p<0.05$ at day 7) with complete closure at day 13 [Dinda 2016].

Photoprotective effects
The depletion of GSH in the skin of hairless HRS/J mice due to irradiation with UV-B was restored to non-irradiated control levels after oral treatment with a 50% ethanolic extract (yield 15.7%) at doses of 150 or 300 mg/kg b.w. 30 minutes and 18 hours prior to radiation ($p<0.05$). MMP-2 and MMP-9 were elicited by the extract [Fonseca 2010]. In a similar experiment, the animals received the extract at a concentration of 5% in a topical formulation 1 hour before, immediately before and 1 hour after irradiation. The treatment restored GSH levels to non-irradiated control levels ($p<0.05$). Modifications of collagen fibrils in the skin induced by irradiation were reduced [Fonseca 2011].

In Wistar rats treated topically with formulations containing 3 and 4% (w/v) of the essential oil, the photoprotection was not superior to the effect of the same formulation containing sunflower oil instead of calendula essential oil [Mishra 2012a].

Topical treatment of Wistar rats prior to UV-B radiation with creams containing 4 or 5 % of the essential oil resulted in significantly increased SOD, catalase, GSH, total protein and ascorbic acid levels ($p<0.05$ for all parameters and both dosages) in the skin as compared to irradiated controls. The elevated MDA content after irradition was significantly ($p<0.05$) reduced [Mishra 2012b].

Anti-inflammatory effects
A lyophilized extract, administered i.p., suppressed inflammatory effects and leukocyte infiltration induced by simultaneous injection of carrageenan and prostaglandin E1 into rats [Shipochliev 1981].

A dry 80% ethanolic extract, given orally 1 hour before oedema elicitation, inhibited carrageenan-induced rat paw oedema by 11% at a dose of 100 mg/kg body weight. In the same experiment indometacin at 5 mg/kg resulted in 45% inhibition of oedema [Mascolo 1987].

An aqueous dry extract (not further specified), at single doses of 50, 150 and 450 mg/kg b.w., was administered orally to Wistar rats. In paw oedema induced by carragenan and histamine, and in ear oedema induced by croton oil, the two higher doses exhibited significant inhibition ($p<0.05$). In serotonin-induced paw oedema all doses showed significant effects ($p<0.05$), but after challenge with dextran only the highest dose was significant ($p<0.05$). In cheek pouch granuloma the animals were treated with the extract for 7 days, and at 150 and 450 mg/kg significant ($p<0.05$) improvement was observed [Núnez-Figueredo 2007].

Oral pretreatment of BALB/C mice with a 70% ethanolic extract (yield 1.1%; 250 and 500 mg/kg b.w.) resulted in a significant ($p<0.001$) inhibition of dextran-induced (41.9 and 42.4% respectively) and carrageenan-induced (50.6 and 65.9% respectively) paw oedema. In the model of chronic inflammation with formalin-induced oedema the reduction was 32.9 and 62.3% respectively. The pronounced increase of inflammatory cytokines (IL-1β, IL-6, TNF-α, INF-γ and CRP) in serum after stimulation with LPS decreased significantly after pretreatment with 50, 100 or 250 mg/kg of the extract ($p<0.001$ for all doses and cytokines). COX-2 expression in the spleen was inhibited by 250 mg/kg of the extract [Preethi 2009a].

The effects of topical application of gels containing 5 or 10% of an 80% ethanolic extract on 5-fluorouracil-induced mucositis were studied in Syrian hamsters from day 12 (maximum severity of mucositis) until day 17. Weight gain of the animals was significantly higher from day 13 until the end of the study with both treatments ($p<0.04$ to $p<0.001$) as compared to control and animals treated with gel base. Microscopic and macroscopic scores of mucositis were lower in the 5% and 10% gel groups as compared to the gel base and control groups ($p<0.05$) [Tanideh 2013].

Oral treatment of male Sprague–Dawley rats with 0.5g/kg daily of a dry ethanolic extract (not further specified) for 6 weeks significantly ($p<0.05$) reduced aflatoxin-induced increases of TNF-α and IL-1β [Abdel-Aziem 2014].

The anti-inflammatory effect of a 70% ethanolic extract was studied in formalin-induced inflammation of the rat paw. A significant ($p\leq0.001$) decrease in paw thickness was observed after oral administration of 250 mg/kg [Elzorba 2016].

An ethanolic extract (8.7% yield; 400 mg/kg b.w. orally/day for 14 days) attenuated several pathological parameters of pancreatitis induced by L-arginine in Sprague-Dawley rats: the enhanced serum lipase and drastically reduced pancreatic

amylase, as well as changes in pancreatic DNA, RNA and total protein content, were significantly ameliorated ($p<0.05$). The extract protected against damage to the pancreatic architecture by markedly decreasing inflammatory cells and oedema. Markers of oxidative stress and indicators of regeneration were impoved [Kaur 2016].

A calendula extract administered orally and by enema exhibited dose-dependent beneficial activity in Sprague-Dawley rats with acetic acid-induced ulcerative colitis. Treatment for 7 days with an 80% ethanolic dry extract (18.2% yield) at doses of 1.5 or 3 g/kg b.w. p.o., or as an enema containing 10 or 20% of the extract, resulted in significantly ($p<0.05$) higher weight gain in each of the calendula treated groups, as compared to negative control (oral saline or base gel). Most of the histopathological changes of the colonic tissue, such as acute inflammation and granular atrophy, were significantly ($p<0.05$) improved in the 20% intracolonic gel and both oral calendula groups, as compared to the negative controls, with the higher oral dose exhibiting almost complete reversal. The inflammatory markers, MDA and myeloperoxidase (MPO) activity, were also significantly ($p<0.05$) lower in the treated groups than negative controls [Tanideh 2016].

In a similar study, male Wistar rats were treated orally with 100 or 200 mg/kg b.w. of a 70% ethanolic dry extract (yield 18.7%) for 7 days before intracolonic challenge with acetic acid for the induction of ulcerative colitis. Clinical and macroscopic scores including weight loss, stool consistency, bleeding etc. were significantly ($p<0.001$) less in the pretreated groups as compared to acetic acid control. Increased concentrations of MPO, colonic lipoxyperoxidase and LDH, as well as the decreased GSH level in colonic tissue, were improved significantly ($p<0.001$) by the lower dose of the extract, with a less pronounced effect shown by the higher dose [Banakar 2016].

In an experimental model of periodontitis in male Wistar rats, the alveolar bone loss by ligature of the second upper left molar was improved by an aqueous extract (not further specified; 90 mg/kg/day by gavage for eleven days). Changes in bone topography and dearrangement of collagen fibers in the periodontal ligament were impeded. Reduced gingival GSH, SOD and CAT levels, increased gingival MDA and the changes in the count of immunopositive cells for WNT 10b, β-catenin and DKK-1 were significantly (all $p<0.05$) reversed by the treatment [Lima 2017].

In a similar study with the same extract (10, 30 and 90 mg/kg/day for eleven days), the highest extract dose significantly ($p<0.05$) prevented alveolar bone loss and the reduction of bone-specific alkaline phosphatase in serum and of osteoprotegerin immuno-expression. Leukocyte infiltration, increased myeloperoxidase activity and gingival concentrations of TNF-α, IL-1b and RANKL due to ligature were decreased at the highest dose ($p<0.05$). The extract did not affect kidney and liver functions as shown by serum AST/ALT levels [Alexandre 2018].

A 70% alcoholic extract and a supercritical CO_2 extract were applied topically in the croton oil ear oedema test in mice. The hydroalcoholic extract had a mild dose-dependent effect, inhibiting oedema by 20% at a dose of 1200 µg/ear, corresponding to 4.16 mg of crude drug. The CO_2 extract produced 30% inhibition at 150 µg/ear, corresponding to 3.6 mg of the crude drug, and 71% inhibition at 1200 µg/ear, corresponding to 28.6 mg of crude drug. The activity at the higher concentration was comparable to that of indometacin at 120 µg/ear [Della Loggia 1990]. In the same model, triterpenoids were shown to be the most important anti-inflammatory principles in the CO_2 extract [Della Loggia 1994].

In the same test system in male albino Swiss mice, a faradiol monoester mixture, faradiol-3-myristic acid ester, faradiol 3-palmitic acid ester and ψ-taraxasterol isolated from calendula flower all showed significant ($p<0.05$) anti-oedematous activity compared to controls at doses of 240 and 480 µg/cm^2. Faradiol (which is not present in the free form in calendula flower) proved to be even more active than the esters with an anti-oedematous effect comparable to that of an equimolar dose of indometacin, but 3-esterification reduces the activity by more than 50% [Della Loggia 1994, Zitterl-Eglseer 1997].

Helianol and ψ-taraxasterol from calendula flower inhibited inflammation induced by 12-O-tetradecanoylphorbol-13-acetate (1 µg per ear topically) in female ICR mice. The 50% inhibitory doses were 0.1 mg/ear for helianol and 0.4 mg/ear for ψ-taraxasterol, compared to 0.3 mg/ear for indometacin [Akihisa 1996]. Several other triterpenes from calendula flower showed ID_{50} values of 0.03 to 0.2 mg/ear in the same animal model [Yasukawa 1996].

A pronounced anti-inflammatory activity was shown for several oleanane-type triterpene glycosides in TPA-induced mouse ear inflammation with ID_{50} values between 60 and 200 µg/ear [Ukiya 2006].

Topical application of faradiol in mouse ear oedema induced by croton oil resulted in an ED_{50} of 0.13 µmol/cm^2 of ear [Neukirch 2005].

Angiogenic effects
The angiogenic activity of a lyophilized aqueous infusion (1:10) was tested in the chick chorioallantoic membrane (CAM) test. The numbers of micro-vessels in tissue sections of treated CAMs were significantly higher ($p<0.0001$) than in control CAMs. All treated CAMs were positive for hyaluronan, while no hyaluronan was detected in control CAMs [Patrick 1996].

Antitumour effects
In ANDO-2 human melanoma implanted nude mice, treatment with an aqueous extract (laser-irradiated at 650 nm during extraction) at 50 mg/kg/day p.o. three times a week for 12 weeks, or 25 mg/kg i.p. twice a week for 9 weeks, resulted in similar inhibitions of tumour growth of 60%, which was also similar to taxol as positive control. The survival rate at the end of the study was 0% for saline control, 40% for taxol, 75% for the extract i.p and 60% for the extract p.o. [Jiménez-Medina 2006].

In male C57BL/6 mice injected with B16F-10 melanoma cells and simultaneously treated with an ethanolic dry extract (yield 1.1%; 250 mg/kg p.o. daily for 10 days), the number of lung tumour nodules was decreased significantly ($p<0.001$) and the life span of tumour bearing mice increased by 43.3%. The extract caused a pronounced reduction of the elevated levels of lung hydroxyproline, uronic acid, hexosamine, serum sialic acid and γ-glutamyl transpeptidase as compared to metastatic controls; the drastic increase in the level of proinflammatory cytokines (IL-1β, IL-6, TNF-α, GM-CSF) and the growth factor (VEGF) in metastasis-bearing animals was significantly ($p<0.001$) inhibited by the extract. The expression of genes involved in metastasis, including MMPs, prolyl hydroxylase and lysyl oxidase, was inhibited, while tissue inhibitors of MMPs were activated [Preethi 2010].

In male Sprague-Dawley rats, aflatoxin-induced micronucleus formation in bone marrow cells, changes in the gene expression of p53, Bax and Bcl-2 as well as in the hepatic tumour marker AFP, were significantly ($p<0.05$) improved by oral treatment with 0.5 g/kg daily of a dry ethanolic extract (not further specified) for 6 weeks. DNA fragmentation in the liver was not fully restored [Abdel-Aziem 2014].

A methanolic extract was tested in a model for experimental skin carcinogenesis in female Swiss albino mice for 32 weeks. The animals were challenged with 7,12-dimethylbenz(a) anthracene and treated twice weekly with croton oil as a tumour promoter for 16 weeks. The extract (100 µL; 10 mg/kg) was applied topically 1 hour before croton oil, and alone twice a week for a further 16 weeks. The tumour incidence was reduced by the extract; the decrease of tumours per mouse was significant ($p<0.05$). Histological changes in the skin due to the carcinogens were improved. The proliferative population in tumours was diminished by restoration of the endogenous antioxidant defense, the inhibition of NFκB, the reduction of inflammation, the enhancement of immunosurveillance of the genetically mutated cells, along with silencing of the cell cycle progression signals. The extract induced stable expression of p53 tumour suppressor protein within the tumours [Ali 2014].

Wistar rats challenged with *N*-diethylnitrosamine, followed by three doses of 2-acetylaminofluorene (2-AAF) as promotor on days 7, 8 and 9 after challenge, and partial hepatectomy on day 10, were treated orally with various doses of an enriched hydroethanolic extract (0.45% yield; ethanol concentration 0.8%) for 18 days. Histological inspection of the development of altered hepatocyte foci (AHF) revealed a protective effect of the extract as shown by the area and number of AHF starting at 0.1 mg/kg, increasing at 0.5 and reaching a maximum at 2.5 mg/kg b.w. At higher doses (10, 20 and 40 mg/kg) an increase of AHF area and numbers above the level of the control were observed. Substitution of the promotor 2-AAF by 40 mg/kg of the extract also resulted in significant promotion ($p<0.01$ for area and <0.006 for numbers) [Barajas-Farias 2006].

A triterpene-enriched fraction given orally to mice inoculated with Ehrlich mouse carcinoma prevented the development of ascites and increased survival time compared to controls [Boucaud-Maitre 1988].

Hepatoprotective effects

Administration of a fluid extract (1:1; 10 mL/kg b.w. p.o.) to male Wistar rats with CCl_4-induced hepatotoxicity resulted in a marked improvement of the increased SGPT activity but did not show an effect on the increased SGOT activity. CCl_4-induced histological changes were reduced. Modifications in the activity of several enzymes and in steatosis were ameliorated [Rusu 2005].

Oral pretreatment with an ethanolic extract (yield 1.1%; 100 and 250 mg/kg b.w. daily for 3 days) protected female Wistar rats against CCl_4-induced hepatotoxicity. The CCl_4-induced increases in serum marker enzymes of liver injury (SGOT, SGPT and ALP), total bilirubin, and MDA as a marker of lipidperoxidation in liver tissue, were significantly ($p<0.05$ to $p<0.001$) reduced by both doses of the extract [Preethi 2009b].

Antioxidant effects

Oral administration of an ethanolic extract (100 and 250 mg/mL) to female Swiss albino mice led to an inhibition (12.6 and 38.7% respectively) of superoxide radical generation during the activation of sodium caseinate-induced macrophages with phorbol-12-myristate-13-acetate. In a second experiment, mice were treated with three different doses (50, 100 and 250 mg/kg b.w.) for 30 days. Changes of the antioxidant status in the blood included an increase in catalase, significant for 50 and 100 mg/kg ($p<0.005$), a decrease in superoxide dismutase ($p<0.001$ for 50 mg/kg) and of glutathione peroxidase ($p<0.001$ for 250 mg/kg). Glutathione was significantly enhanced in all treated groups ($p<0.005$, $p<0.001$). Changes in glutathione reductase were not significant [Preethi 2006].

In male pigs, DNA damage was induced by oxidative stress due to a high intake of polyunsaturated fatty acids (30% energy from linseed oil). Oral treatment with 3 mL/day of an aqueous propylene glycol extract from fresh flowers for 14 days resulted in a significant ($p<0.05$) reduction of DNA damage in peripheral blood lymphocytes. The amount of 8-hydroxy-2′-deoxyguanosine, a biomarker of generalized oxidative DNA damage, in urine decrased [Frankič 2009].

Sprague-Dawley rats exposed to cigarette smoke daily received a dry ethanolic extract (100 mg/mL/day) or placebo orally for 23 days. Body weight increased in unexposed controls, decreased in animals exposed to smoke and remained unchanged in the extract group. Serum markers of renal function remained unchanged; the HDL-reduction and increased serum AST, LDH and creatine after smoke exposure were significantly ($p<0.05$) improved by the extract [Ozkol 2011].

The influence of an extract (appr. 35% ethanol; 100 mg/kg b.w./day p.o.) on alterations in the oxidant status generated by exposure to cigarette smoke was investigated in Sprague-Dawley rats. Parameters of the cellular antioxidant defense system such as SOD, glutathione peroxidase, glutathione reductase and GSH were investigated in lung, brain and heart. All changes due to cigarette smoke were improved by the extract [Özkol 2012].

In male Sprague–Dawley rats receiving 0.5 g/kg daily of a dry ethanolic extract (not further specified) orally for 6 weeks, the levels of the inflammatory cytokines TNF-α and IL-1β remained unchanged. Markers of oxidative stress (MDA in the liver, hepatic glutathione peroxidase, SOD) were improved as compared to untreated controls [Abdel-Aziem 2014].

The effects of a dry ethanolic and a dry aqueous extract (not further specified, 300 mg/kg b.w. p.o. 1 hour before and 24 and 48 hours after challenge with cisplatin) on cisplatin-induced nephrotoxicity in Wistar rats were studied. The extracts significantly ($p<0.05$) reduced the elevated blood creatinine levels after cisplatin. Both treatments improved the cisplatin-detoriated plasma total antioxidant status (TAS), total thiols and oxidative stress index ($p<0.05$), the ethanolic extract also improved the total oxidant status, GSH, CAT and GST ($p<0.05$). In renal tissue both extracts restored TAS and MDA levels ($p<0.05$), and the ethanolic extract restored Cat, GPx and GST activities. Histopathological findings showed less severe degenerative and necrotic changes in renal tubular epithelium after co-treatment with both extracts [Verma 2016].

Other effects

A methanolic extract (yield 35.3%) and its 1-butanol-soluble fraction were tested for their effects on serum glucose after glucose-load and on gastric emptying in mice, as well as for gastroprotective effects in rats. A significant reduction of the glucose level after challenge was only seen for the butanolic fraction (500 mg/kg p.o.; $p<0.01$). The extract (500, 1000 mg/kg) and the fraction (250, 500 mg/kg) inhibited gastric emptying in carboxymethyl cellulose sodium salt meal-loaded mice after oral application ($p<0.01$). Gastric lesions in rats after ethanol or indometacin challenges were significantly ($p<0.01$) improved by the extract (100 and 200 mg/kg) and the fraction (50 and 100 mg/kg). Some of the oleanane-type triterpene oligoglycosides showed effects as well [Yoshikawa 2001].

Oral administration of a 50% hydroethanolic extract to male Wistar rats (200 mg/kg daily for 60 days) significantly decreased the weight of the testis, epididymis, seminal vesicle and ventral prostate ($p<0.001$). Sperm motility and sperm density were reduced ($p<0.001$), resulting in 80% loss of fertility. Serum testosterone as well as total protein and sialic acid in the testis, epididymis, seminal vesicles and ventral prostate showed

significant reduction ($p<0.001$); testicular cholesterol was elevated. All measured haematological parameters remained unchanged [Kushwaha 2007].

Treatment of Swiss albino mice with an ethanolic extract (yield 1.1%; 100 and 250 mg/kg b.w.daily p.o.) for 3 days prior to and 3 days after challenge with cisplatin diminished kidney damage as shown by a significant ($p<0.001$) reduction of increased urea and creatinine at both doses. Increased lipid peroxidation was not influenced by the extract. SOD activity and glutathione content, which remained unchanged by cisplatin, were significantly elevated with the 250 mg extract ($p<0.05$ and 0.01 respectively). The decrease of catalase was abolished by both doses of the extract ($p<0.01$ and 0.001). Diminished white blood cell count was improved at the higher dose ($p<0.01$) [Preethi 2009b].

In male Swiss albino mice receiving a dry ethanolic extract (0.1, 0.3 and 1 g/kg b.w. p.o.), a dose-dependent elongation of pentobarbital-induced sleeping time, significant ($p<0.05$) at the highest dose, was determined. In the Rotarod performance test, the extract at 0.5, 1 and 2 g/kg p.o. slightly decreased endurance and increased the number of falls to a similar extent at all doses. In the open field test, entrances in the open square were diminished and time of immobilisation raised, both significant ($p<0.05$) at the two higher doses [Parente 2009b].

An 80% ethanolic extract was administered to male Wistar rats at doses of 100, 150 or 250 mg/kg b.w. (i.p.). In the tail flick test, a significant elongation of tail flick latency was observed ($p<0.05$, 0.01 and 0.05 respectively). The number of writhings induced by acetic acid was significantly reduced ($p<0.01$, 0.001 and 0.001 respectively) [Shahidi 2012].

The neuroprotective activity of a 70% methanolic extract (18.7% yield) against 3-nitropropionic acid-induced damage was studied in Wistar rats. Oral treatment with the extract at 100 or 200 mg/kg/day for 14 days significantly improved the decrease in body weight ($p<0.001$ and 0.05 respectively). Most of the changes in motor- and cognition-related behaviours as studied in various models and in the antioxidant status in the rat brain such as catalase, MDA etc. were ameliorated significantly ($p<0.05$ to $p<0.001$) and more pronounced with the lower dosage [Shivasharan 2013a].

In a similar study with the same extract (100 or 200 mg/kg p.o. for 7 days), neurotoxic effects in Wistar rats were induced by sodium glutamate. Decreased locomotor activity was enhanced by the extract ($p<0.01$ at the higher dose). The negative influence of sodium glutamate on oxidative stress parameters in the brain such as GSH, total thiols, glutathione-S-transferase, catalase, MDA and nitrite was improved [Shivasharan 2013b].

Dogs infected with sarcoptic mange were treated with the antiparasitic compound ivermectin (0.2 mg s.c.) alone, in combination with 50 mg p.o. of an 80% ethanolic extract per day (CFE), or combined with an oral dose of 70 mg *N*-acetylcysteine twice daily as a positive control (NAC). Parasitical cure rates were 25%, 75% and 87.5%, respectively, after 14 days of treatment and 75%, 100% and 100% after 28 days.The improvement of the *Sarcoptes*-induced skin lesions score was significant after 14 and 28 days in the CFE and NAC groups as compared to baseline, but only after 28 days in the ivermectin alone group ($p<0.01$). CFE and NAC at both time points were superior to ivermectin alone ($p<0.01$) with no significant differences between CFE and NAC. All tested markers of oxidative stress in peripheral blood (lipid peroxides, reduced glutathione, glutathione peroxidase, glutathione-S-transferase, SOD and catalase) that had been significantly influenced by the infection ($p<0.001$) were ameliorated similarly and significantly in the CFE and NAC groups ($p<0.001$) compared to baseline [Singh 2013].

Streptozocin-induced diabetic male Wistar rats received a dry, 80% ethanolic extract (300 mg/kg b.w. by gavage) for 13 weeks. Levels of serum glucose, urea, creatinine and malondialdehyde and the total oxidant status increased, the total anti-oxidant capacity decreased and damage in the glomerulus and mass infiltration in kidney tissue was observed in diabetic rats as compared to non-diabetic controls. The extract had no effect on blood glucose, but did decrease blood urea nitrogen, creatinine, the total oxidant status and malondialdehyde and increase total anti-oxidant capacity. It did not prevent histological signs of nephropathy in the diabetic rats [Salehi 2015].

In brewer´s yeast-induced hyperthermia in rats, a 70% ethanolic extract (250 mg/kg p.o.) led to a significant ($p\leq0.05$) reduction of the elevated temperature after 3 hours, comparable to metamizol sodium (50 mg/kg) as the positive control. Administration of the extract to mice (250 mg/kg p.o.) in the writhing test showed a 20% inhibition of acetic acid-induced writhing for 5 hours [Elzorba 2016].

Treatment with saponins from calendula flower changed the glycosylation pattern in infective larvae of *Heligmosomoides polygyrus*, an intestinal parasitic nematode, which resulted in a significantly ($p<0.001$) reduced level of infection in BALB/c mice infected with third stage larvae. In these mice the production of TNF-α in the intestine and in serum increased significantly ($p<0.05$) [Doligalska 2013].

Oleanolic acid and three fractions from calendula flower containing oleanolic acid 3-O-glucoside, other glucosides of oleanolic acid and oleanolic acid glucuronides, respectively, significantly ($p<0.001$) inhibited the development of L3 larvae of *Heligmosomoides polygyrus*. The concentration of a mixture of all oleanolic acid glycosides, at which 50% of hatched larvae failed to develop fully into infective stage L3 larvae, was 350 µg/mL of oleanolic glucuronides [Szakiel 2008].

Pharmacological studies in humans

The effects of various preparations on sodium lauryl sulfate-induced irritant contact dermatitis were tested in 20 healthy volunteers. A dyed and an undyed extract, a faradiol ester-enriched fraction, faradiol myristic acid ester and faradiol palmitic acid ester (as 5% in a cream) were applied topically 10 min after irritation on four days. All preparations resulted in significant ($p<0.05$ to $p<0.001$) improvement as evaluated by visual inspection, measurement of transepidermal water loss and of erythema as compared to untreated controls [Fuchs 2005].

In four healthy males, topical application of a 50% solution of the essential oil of calendula flower in absolute ethanol showed weak repellency against *Anopheles stephensi* with a mean protection time of 2.15 hours and an ED_{50} of 0.6034 mg/cm^2 [Tavassoli 2011].

Clinical studies

Wound healing

In a randomized, open, controlled study, the effects of three ointments were compared after topical treatment of patients with 2nd or 3rd degree burns for 17 days: a calendula flower ointment (prepared by digestion in vaseline, no further information; n = 53) or vaseline only (n = 50) or a proteolytic ointment (n = 53). The success rates were considered to be 37/53 for the calendula ointment, 27/50 for vaseline and 35/53 for the proteolytic ointment. Calendula flower ointment was marginally superior to its base, vaseline ($p = 0.05$) [Lievre 1992].

In an open, uncontrolled pilot study, 30 patients with burns or scalds (degrees 1 and 2a) were treated 3 times per day for up to 14 days with a hydrogel containing 10% of a hydroethanolic extract. The symptoms reddening, swelling, blistering, pain, soreness and heat sensitivity were scored before, during and at the end of treatment. The total score and individual scores for each symptom improved [Baranov 1999].

Patients with lower leg venous ulcers received treatment for 3 weeks with either local application of 1g/cm^2 of an ointment (7.5% of an ethanolic extract (DER 15.6:1) in propylene glycol) twice daily (n=21) or saline solution dressings as control (n=13). The reduction of the total surface of all ulcers in the verum group was 14.6%, 33.3% and 41.7% after the first, second and third week respectively. In 7 patients complete epithelialisation was observed. In the control group the reductions were 4.1%, 10% and 14.5% and complete epithelialisation occurred in four patients [Duran 2005].

After surgical removal of small pigment neoformations, 28 patients were randomly assigned to one of two groups to determine wound healing and inflammation. Wounds were treated either with iodine solution on days 0, 3, 7 and 10 (A), or on day 0 with iodine and on days 3, 7 and 10 with a hydroethanolic extract (B). Wound areas decreased from day 3 to day 15 from 0.55 to 0.30 cm^2 (42.1%) in group A and from 0.80 to 0.45 cm^2 (44.7%) in group B. Inflammation, as determined by differences in the colour of the affected area and the adjacent unaffected area, was reduced from 22.5 to 14.9 (group A) and from 31.3 to 29.9 (group B) [Raposio 2007].

In a randomized, double-blind, comparative study, the effects of an ointment containing 1.5% of an extract (not further specified; n=34) were compared to an aloe cream (containing aloe vera gel and olive oil in a base cream; n=32) in diaper dermatitis in infants (up to 3 years of age). The creams were applied 3 times a day for 10 days. Severity was determined by a 5-point scale at the beginning and on days 5 and 10 of treatment, and decreased significantly ($p<0.001$) in both groups as compared to baseline. The reduction of symptoms was significantly ($p<0.001$) higher in the group receiving the calendula ointment [Panahi 2012].

In a randomized, controlled, blinded study, primiparous women with episiotomy were treated on the episiotomy area with either 3 cm^3 calendula ointment (not further specified) every 8 hours (n=37) or with a povidone iodine solution every four hours (n=37) as control, both for five days. Healing was evaluated with the REEDA (redness, oedema, ecchymosis, drainage, approximation) scale. After 5 days a significantly ($p<0.001$) better healing was seen with the calendula ointment in the REEDA scale as compared to control [Eghdampour 2013].

During surgical extraction of a third molar, patients (n=44) received irrigation with either a dilution of a mother tincture (10% in physiological solution) or with physiological solution alone. The same agents were used for 1 week as a mouthwash after brushing the teeth. The verum resulted in significantly ($p<0.05$) better bone preservation after extraction. Patients in the verum group experienced less pain during the first 24 hours after extraction and after one week, less bleeding and needed less antibiotics [Uribe-Fentanes 2018].

Effects on radiation-induced skin damage

In a randomized, single-blind trial, 254 patients undergoing postoperative radiation therapy, following breast cancer surgery, applied either trolamine (n=128; A) or an ointment containing a lipophilic extract of calendula flower (n=126; B) to the irradiated field after each session. The occurrence of acute dermatitis of grade 2 or higher was significantly lower (41% vs. 63%; $p<0.001$) with the use of calendula compared to trolamine. The mean maximal pain evaluated on a VAS was 2.10 in group A and 1.54 in group B ($p=0.03$). Patients in group B had less frequently interrupted radiotherapy [Pommier 2004].

To reduce acute radiation skin reactions (ARSR), 411 patients with adjuvant radiotherapy (RT) for breast cancer applied a thin layer of a calendula cream (10% of a non-specified extract) or an aqueous moisturizing cream twice daily from the onset of RT until two weeks after the end of RT. In this randomized, blinded study, ARSR were evaluated by the RTOG/EORTC scale at the first and the final radiation and 5 to 17 days after the final radiation. Patients recorded pain, burning, itching, pulling and tenderness on a VAS and completed the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core-30 and the MoS sleep questionnaire. No differences in ARSR were observed between the groups [Sharp 2013].

The effect of a gel containing 2% of an extract (70% ethanol) on radiation-induced oropharyngeal mucositis was studied in a randomized, double-blind, placebo-controlled study in 40 patients with head-and-neck cancers undergoing radiotherapy. The patients applied 5 mL of the gel twice daily for at least one minute for 7 weeks from the outset of radiation. Oropharyngeal mucositis was evaluated by the oral mucositis assessment scale (OMAS). The verum significantly decreased the intensity of oropharyngeal mucositis compared to placebo at week 2 (score: 5.5 vs. 6.8, $p = 0.019$), week 3 (score: 8.25 vs. 10.95, $p < 0.0001$) and week 6 (score: 11.4 vs. 13.35, $p = 0.031$) [Babaee 2013].

In a randomized, double-blind, controlled study 51 patients with head and neck cancers undergoing radiotherapy were treated twice daily with a calendula preparation (4% calendula oil (not further specified) and 1% vitamin A in liquid petroleum jelly) or a fatty acid preparation (EFA; 5% capric acid, 1% vitamin A and 0.2% vitamin E in sunflower oil) on the complete treatment field. Radiodermatitis was evaluated every 5th radiation and developed from the 10th session with severity of grades 1 to 3 according to RTOG. In the calendula group the proportion of radiodermatitis grade 2 was significantly lower ($p=0.012$), and the survival curve remained significantly higher ($p=0.00402$), as compared to EFA [Schneider 2015].

Effects on gingivitis and periodontitis

Forty patients with established gingivitis brushed their teeth with standard toothpaste (control), or standard toothpaste with 2% of a dry ethanolic extract, three times daily for two minutes according to the Bass method in a 4 week randomized, double-blind, controlled study. Plaque index (PI), gingivitis index (GI) and bleeding on probing (BoP) were analysed at baseline and at the end of weeks 2, 3 and 4. The PI was reduced significantly after 4 weeks as compared to baseline and to control ($p<0.0001$; reduction 33.4% vs. 9.8% after 4 weeks). A significant difference between control and verum was observed after 2, 3 and 4 weeks in GI ($p<0.004$, 0.0001 and 0.0001 respectively), with a decrease of 46.3% after verum and 18.4% after control at the end of the study. A similar progress was seen in BoP ($p<0.001$, 0.0001 and 0.0001 respectively), with a decrease of 50.8% after verum and 21.8% after control after 4 weeks [Amoian 2010].

In a controlled study, 40 patients with chronic periodontitis applied a mouthwash containing a standardized tincture (not further specified) or a 0.12% chlorhexidine solution, three times per week for two weeks. Clinical attachment level, marginal bleeding, Plaque Index of O'Leary and Gingival Index were determined. The effects of both rinses were similar in most parameters, the difference was significant only on the Gingival

Index, with chlorhexidine being superior to the tincture (p = 0.02) [Vinagre 2011].

In a randomized, controlled study, 240 patients exhibiting gingivitis, with bleeding on probing and a probing depth ≤3 mm, used a mouthwash of either a mother tincture (not further specified; 2 mL diluted with 6 mL water) or 8 ml distilled water (control) twice daily for six months. All patients received a thorough scaling procedure at a 3 month visit. Plaque index, gingival index, sulcus bleeding index and oral hygiene index–simplified were all significantly (p<0.01) improved after six months in the verum group; while after three months (prior to the scaling procedure) only the latter index had not significantly improved. In the control group no significant changes in the four parameters were observed after three months. The improvement after six months was less pronounced than after verum [Khainar 2013].

Effects on diabetic ulcers
In a prospective study, 41 patients with an average glycaemic level of 153 mg/dL, and diabetic foot ulcers (DFU; size 0.5–40 cm^2) of more than 3 months duration, were treated twice daily with a spray solution containing 4% of a hydroglycolic extract (6.7% amyrin, 4.7% lupeol, 8.1% ψ-taraxasterol, 5.5% calenduladiol monoesters, 15.7% arnidiol monoesters, 35.2% faradiol monoesters and 120mg/mL total flavonoids). Size, microbial flora, odour and tissue type of DFU, exudate, retraction rate and pain on a 10-point rating scale were evaluated every second week for 30 weeks or until healing. Blood parameters and neuropathic pain were assessed at baseline and at the end of treatment. Complete wound closure after 11, 20 and 30 weeks was observed in 54%, 68% and 78% of the cases. After 30 weeks, the number of colonized wounds decreased from 29 at baseline to 5, and the number of odorous wounds decreased from 19 to 1. A significant (p=0.001) reduction in the amount of exudate, fibrin slough and necrotic tissue was observed [Buzzi 2016a].

In a randomized, controlled study, 32 type-II diabetic patients with lower limb ulcers received either no treatment, low-level laser therapy, calendula oil (5 mL/day; not further specified) or a combination of low-level laser therapy with calendula oil for 30 days. The oil did not influence the diameter of the posterior tibial artery and the Ankle-Brachial Index (parameters of peripheral circulation). In the Brief Pain Inventory Questionnaire and a VAS to quantify pain the oil produced no effect, although the total ulcer area was reduced [Carvalho 2016].

Effects in vaginal candidiasis
In an open study, 42 patients with recurrent vaginal candidiasis were treated topically with 8.6 mL of a solution prepared from 15 mL of a tincture (200 g drug in 1 L ethanol 70%) in 200 mL water, three times per week for 2 weeks. Evaluations on day 0, day 21 and day 30 included grade and persistence of leucorrhea, persistence of pruritus, a gynaecological examination and a check of vaginal exudate for *Candida* sp. All parameters were improved significantly on day 30 as compared to baseline (p=0.000, each) [Milián Vázquez 2010].

In a triple-blind, randomized study, patients with vaginal candidiasis (n=150) applied 5 g of either a cream containing 1% of an ethanolic dry extract (DER 5:1) or of a 1% clotrimazole cream every night for 7 nights. In the follow-up between day 10 and 15, significantly (p<0.001) more women in the clotrimazole group had a negative mycological test for Candidiasis as compared to the calendula group (55 vs. 34%). In contrast, the outcome in the assessment after 30 and 35 days was reversed with 54% without Candidiasis in the calendula group and 25% in the clotrimazole group (p<0.001). In individual complaints such as vaginal discharge, vulvar pruritus, irritation, dysuria, abdominal pain and dyspareunia, no significant differences between the two treatments were seen after 10 to 15 days. After 30 to 35 days vaginal discharge, vulvar pruritus and irritation were significantly (p<0.001, 0.001 and 0.003 respectively) improved in the calendula group in comparison to clotrimazole [Saffari 2017].

Other effects
Case studies reported positive healing effects after application of not further characterized topical preparations in small diabetic lesions of the limbs, in exfoliative cheilitis or after periodontal surgery [Cioinac 2016; Roveroni-Favaretto 2009; Fang Mercado 2013].

Pharmacokinetic properties
In an *in vitro* assay on porcine ears, the skin penetration and percutaneous delivery of rutin and narcissin from a 50% ethanolic extract (yield 15.7%) in three topical formulations containing different excipients was studied. The compounds were absorbed. The amounts in viable skin and in the receptor phase depended on the composition of excipients in these formulations [Fonseca 2011].

Preclinical safety data

Acute toxicity
In the brine shrimp lethality assay, an ethyl acetate fraction (containing mainly flavonoids and phenolic acids), and a butanol fraction (containing mainly saponins), from a 70% methanol extract produced LC_{50} values of 60.1 and 50.2 mg/mL respectively [Mohamed 2015].

For an aqueous extract administered to mice, the intravenous LD_{50} was determined as 375 mg/kg b.w. and the intraperitoneal LD_{100} as 580 mg/kg [Manolov 1964].

For a hydroalcoholic extract (DER 1:1, 30% ethanol) the subcutaneous LD_{50} was 45 mg in mice and the intravenous LD_{50} was 526 mg/100 g in rats [Boyadzhiev 1965].

An ethylene glycol extract (DER 2:1) was non-toxic in albino mice after subcutaneous administration of 10 mL/kg [Russo 1972].

Swiss male mice did not show any behavioural changes after single oral doses of 0.5, 1, 2 and 3 g/kg b.w. of an extract (80% methanol; DER 6.25:1) [Ali 1999].

Single oral doses of a 70% ethanolic extract up to 5 g/kg b.w. did not cause deaths or any signs of toxicity in Wistar rats and albino Swiss mice [Silva 2007].

Oral administration of a dried decoction (prepared from 1 part drug with 3 parts water) to male and female Wistar rats at a dose of 2 g/kg b.w. remained without any signs of toxicity. Over a period of 14 days normal tendency in body weight gain was observed and after this time no changes in examined tissues were seen [Lagarto 2011].

After i.p. administration, the LD_{50} of a 70% ethanolic extract in mice was determined as 2.45 mg/kg b.w. [Elzorba 2016].

A 70% ethanolic extract (18.7% yield) at oral doses of 50, 300, 1000 and 2000 mg/kg b.w. did not produce any symptoms of toxicity or mortality in male Wistar rats [Banakar 2016].

Application of the hydrodistilled essential oil (yield 1.25%) on the shaved skin of Wistar rats under occlusion at doses of 2.5, 5 and 10 mL/kg b.w. for 24 hours did not result in any

mortalities, signs of toxicity, erythema or oedema up to 48 hours [Mishra 2018].

Subchronic toxicity
Oral treatment of male Wistar rats with a 70% ethanolic extract (0.025, 0.25, 0.5 or 1 g/kg b.w.) for 30 days did not result in any deaths or clinical and behavioural signs of toxicity. Haematological and biochemical parameters remained largely unchanged. Only a slight fluctuation in erythrocyte count, mean corpuscular volume, mean corpuscular haemoglobin and differential leukocyte counts ($p<0.05$) was observed in the groups receiving the three higher doses. A dose-dependent increase in blood urea nitrogen and ALT was also seen in these groups. Of all tissues investigated, only lung weight decreased ($p<0.05$). No histological changes were seen in heart, kidney and brain. Microscopic changes in the liver (centrilobular cells with acidophilic cytoplasm and heterochromatic nuclei etc.) were only seen after the highest dose [Silva 2007]. Similar results on haematological and biochemical parameters were observed earlier in the same set-up in female Wistar rats receiving the same extract at 0.25, 0.5 or 1 g/kg b.w. for 30 days. The weight of the investigated organs including lung did not change [Silva 2005].

Various strains of mice did not show signs of toxicity after oral treatment with an aqueous extract from laser-irradiated calendula flower (11 or 55 mg/kg b.w. daily for 30 days). The LD_{50} was 500 mg/kg. Rats tolerated 11, 55 and 500 mg/kg in the same experimental setup, the observed LD_{50} was 2.75 g/kg. The surviving animals did not show signs of toxicity during a 90 day observation period following treatment [Jiménez-Medina 2006].

During treatment of male Wistar rats with an ethanolic extract (0.1, 0.3 or 1 g/kg b.w. p.o.) for 4 weeks, a pronounced increase of water consumption and urine volume was observed in weeks 3 and 4 with the lowest dose. At the end of treatment, an increase of neutrophils and a decrease of lymphocytes were seen with 1 g of the extract (both $p<0.05$). Creatinine was augmented with 0.1 and 0.3 g; glucose was reduced in the same groups. Total proteins and albumin were reduced with 0.1 g of the extract (all significant, $p<0.05$) [Parente 2009b].

Male and female Wistar rats received 50, 250 or 1000 mg/kg/day of a dried decoction (prepared from 1 part drug with 3 parts water) with drinking water for 90 days. Food consumption and body weight gain were similar to control in all groups and no behavourial signs of toxicity were detected. After 28 days a significant increase in total leukocyte counts in females ($p<0.001$) and in neutrophils in males ($p<0.05$), as well as a decrease in lymphocytes in males ($p<0.01$) were observed. After 90 days erythrocyte and leukocyte counts were increased dose-dependently ($p<0.01$) in both sexes. A raise in blood clotting time occurred only in males. Variations in ALT and AST differed in both sexes and slight histological changes in the liver were observed [Lagarto 2011].

In Wistar rats, after topical application of the essential oil (2.5, 5 and 10 mL/kg b.w. daily) for 13 weeks, no mortalities, signs of abnormality, changes in food consumption or body weight were observed. Significant ($p<0.01$) changes in haemotological parameters, including white blood cell count, haemoglobin content and % lymphocytes, were mainly seen after the highest dose. In the groups treated with 5 and/or 10 mL/kg, there were significant ($p<0.01$) changes in biochemical parameters including ALP, AST, ALT, cholesterol, albumin and total protein [Mishra 2018].

Chronic toxicity
An aqueous extract was reported to be non-toxic in chronic administration to mice [Manolov 1965].

No symptoms of toxicity were observed after oral administration of an extract (solvent unspecified) at 0.15 g/kg b.w. to hamsters over 18 months and to rats over 21 months [Avramova 1988].

Mutagenicity and Carcinogenicity
In the Ames test using *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100, a fluid extract (60% ethanol) was non-mutagenic at concentrations of 50-5000 µg/plate. With *Aspergillus nidulans* diploid strain D-30, genotoxic effects with mitotic crossing over and chromosome malsegregation were observed at higher concentrations of 0.1-1.0 mg/mL, at which a concentration-dependent increase of cytotoxicity also occurred. These findings were not confirmed *in vivo* in the mouse bone marrow micronucleus test; after oral administration of the extract at up to 1 g/kg b.w. for two days no increase in the number of micronucleated polychromated erythrocytes was observed [Ramos 1998].

In a somatic mutation and recombination test in *Drosophila melanogaster*, an infusion (20 g drug/100mL) and a dried tea concentrate (DER appr. 10:1), redissolved in water at concentrations of 20 and 40%, did not show any genotoxic effect [Graf 1994].

Carcinogenicity studies with calendula flower extract have been performed in rats over a period of 22 months and in hamsters over a period of 18 months with a daily oral dose of 0.15 g/kg body weight. The extract was not carcinogenic in either species [Avramova 1988].

Oral treatment of male Sprague-Dawley rats with 0.5g/kg of a dry ethanolic extract (not further specified) daily for 6 weeks produced no changes in DNA fragmentation or the level of the tumour marker AFP in the liver. Micronucleus formation in bone marrow cells as well as the expression of p53, Bax and Bcl-2 remained unchanged [Abdel-Aziem 2014].

Reproductive toxicity
A 70% ethanolic extract (30% yield; ≤0.4% flavonoids) was tested at oral doses of 0.25, 0.5 and 1.0 g/kg for 60 consecutive days on the reproductive function of Wistar rats. From day 53 to 60, rats were mated with untreated, fertile female rats. Reproductive parameters including testicular morphology, reproductive organ weights, fertility index and offspring viability were not affected as compared to control. In a second protocol, groups of pregnant rats were treated orally with the same doses from days 1 to 6 (preimplantation period), 7 to 14 (organogenic period) or 15 to 19 (foetal period) of pregnancy. The treatment was non-toxic in the preimplantation and organogenic periods. During the foetal period, a dose-dependent decrease of the maternal weight gain (significant at the highest dose; $p<0.05$) and of placental absolute weight, but not of placental relative weight ($p<0.05$) was observed as compared to control [Silva 2009].

Sensitizing potential
An oily extract from calendula was tolerable to mucosa in the Draize primary mucosa irritation test using the rabbit eye [Isaac 1992].

Various preclinical toxicological data, mainly from company reports, were compiled for three commercial extracts (1-5% in soybean oil; 10-25% in glycerol; unspecified amount in butylene and water) with a broad use in cosmetics [Anonymus 2001].

Clinical safety data
In a randomized study involving 156 patients with 2nd or 3rd degree burns a calendula ointment was significantly better tolerated ($p = 0.002$) than a proteolytic ointment [Lievre 1992].

No side effects or irritations were observed in 30 patients with

burns or scalds during treatment with a hydrogel containing 10% of a hydroethanolic extract 3 times daily for up to 14 days [Baranov 1999].

Topical treatment of patients with lower leg venous ulcers with a preparation containing an ethanolic extract did not result in systemic or local adverse effects [Duran 2005].

The three adverse reactions reported in 2,135 patients were skin and subcutaneous tissue symptoms, of which two occurred after application of combinations [Jeschke 2009].

No adverse effects were observed in 42 patients with recurrent vaginal candidiasis treated topically with an aqueous solution [Milián Vázquez 2010].

No adverse effects were reported in a study in 34 children with diaper dermatitis treated with an ointment containing 1.5% calendula flower extract three times daily for ten days [Panahi 2012].

In 120 patients with gingivitis using a mouthwash containing 25% of a tincture twice daily for six months, no adverse effects were observed [Khainar 2013].

In 41 patients with diabetic foot ulcers, no adverse events were observed during treatment with a spray solution containing 4% of a hydroglycolic extract [Buzzi 2016a].

In a very detailed safety assessment, it was concluded that calendula flower and its extracts are safe for use in cosmetics [Andersen 2010].

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MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Online Series, 2019
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Online Series, 2019
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Online Series, 2019
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHAMOMILLAE ROMANAE FLOS	Roman Chamomile Flower	Online Series, 2019
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Online Series, 2018

JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
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SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
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The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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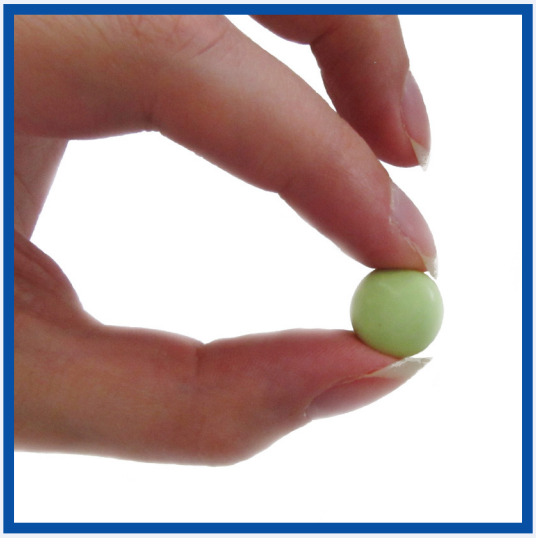

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FOREWORD

It is a great pleasure for me, on behalf of my colleagues in ESCOP, to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

CARVI AETHEROLEUM 2019

Caraway Oil

DEFINITION

Caraway oil is obtained by steam distillation from the dry fruits of *Carum carvi* L.

The material complies with the monograph of the European Pharmacopoeia [Caraway oil].

CONSTITUENTS

Mainly oxygenated monoterpenes: (S)-(+)-carvone: 50 to 65%; *trans*-dihydrocarvone: up to 2.5%; *trans*-carveol: up to 2.5%; and monoterpene hydrocarbons: (*R*)-(+)-limonene: 30 to 45%; β-myrcene: 0.1 to 1.0% [Caraway oil; Samojlik 2010; Raal 2012].

CLINICAL PARTICULARS

Therapeutic indications

Internal use
Symptomatic relief of digestive disorders such as bloating, flatulence and spasm of the gastrointestinal tract [Martindale 2014; Sticher 2015; Stahl-Biskup 2016]. Flatulent colic of infants and children [BPC 1973; Dorsch 2002; Bradley 2006, Martindale 2014].

In these indications, efficacy is plausible on the basis of human experience and long-standing use.

External use
Flatulent colic of infants [Fintelmann 2009]. In this indication, efficacy is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Internal use
Adults:
3 to 6 drops daily in divided doses [BPC 1973, Fintelmann 2009, Schilcher 2010].

Children
Infants over 6 months:
2–3 drops concentrated caraway water (0.26 - 0.38 µL of caraway oil) [BPC 1973] for use in feeding bottles (equivalent to circa 60 mg caraway fruit) per day [Bradley 2006]
Children up to 1 year: 1–2 drops daily [Dorsch 2002]
Children from 1 to 4 years: 2-4 drops daily [Dorsch 2002]
Children above 4 years: 3-6 drops daily [Dorsch 2002]

External use
Children and adolescents: An ointment (2% w/w) to be applied once daily as a thin layer on the abdominal area after bathing in the evening.
Adults: 10% in olive oil, rub 10-12 drops onto the stomach [Weiss & Fintelmann 2000].

Method of administration
For oral administration or external application to the abdomen.

Duration of use
If symptoms persist or worsen, use should be discontinued and medical advice sought.

Contra-indications
Patients with known sensitivity to Apiaceae (Umbelliferae) should not use caraway oil and its preparations.

E/S/C/O/P MONOGRAPHS

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
In accordance with general practice the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known

Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties
Caraway oil acts as a carminative, spasmolytic, choleretic and cholagogue [Georg 2003; Mickelfield 2003].

In vitro experiments

Muscle relaxant / spasmolytic activity
Caraway oil had a relaxant effect on guinea pig tracheal muscle, decreasing the force of phasic contractions by 50% at 27 mg/L, but no antispasmodic effect was observed on electrically-stimulated guinea pig ileum. Caraway oil (20 – 60 mg/L) produced a marked increase in resting force (i.e. contracture) of the ileal mesenteric plexus- longitudinal muscle preparation [Reiter 1985].

The oil administered to isolated rat uterus inhibited the tonic contraction to KCl (80 mM) and the phasic contraction to ACh (320 nM) in a concentration-dependent manner [Sadraei 2003].

(*S*)-(+)-carvone and (*R*)-(+)-limonene produced relaxation in isolated guinea-pig ileum but were less potent than (*R*)-(-)-carvone [de Sousa 2008].

Smooth muscle contractility was evaluated using longitudinal strips isolated from the gastric fundus and duodenum of mice. Under Ca^{++}-free conditions, (*S*)-(+)-carvone (600 µM) significantly ($p<0.05$) inhibited both the phasic and tonic phases of carbachol (1 µM) - induced contractions and contractions caused by electrical field stimulation, indicating muscle relaxant and antispasmodic effects. Effects were more pronounced in duodenal tissues where 100 µM concentrations were also active [Silva 2015].

Antimicrobial activity
The MIC of caraway oil against *Helicobacter pylori* was found to be 0.03 % V/V (273.1 µg/mL) with an MBC of 0.06-0.125 % V/V (546.1-1092.2 µg/mL) in comparison with amoxicillin (MIC: 0.02 µg/mL), ampicillin (MIC: 0.064 µg/mL) and levofloxacin (MIC: 0.39 µg/mL) [Weseler 2005].

The oil was found to have antibacterial and antifungal activities. Gram-positive bacteria in general were more sensitive: *Bacillus cereus* (MIC = 0.5 µL/mL; MBC = 1.0 µL/mL); *Micrococcus luteus* (MIC = 1.0 µL/mL; MBC = 1.0 µL/mL); *Staphylococcus aureus* (MIC = 0.1 µL/mL; MBC = 0.25 µL/mL), than Gram-negative bacteria: *Escherichia coli* (MIC = 2.0 µL/mL; MBC = 4.0 µL/mL); *Proteus mirabilis* (MIC = 2.0 µL/mL; MBC = 4.0 µL/mL); *Pseudomonas tolaasii* (MIC = 4.0 µL/mL; MBC = 6.0 µL/mL); *Salmonella enteritidis* (MIC = 0.25 µL/mL; MBC = 0.5 µL/mL). Evaluation of antifungal activity against a series of micromycetes, found a concentration of 0.25 – 2.5 µL/mL inhibited their growth, except for *Trichoderma viride* when 10.0 µL/mL was effective [Simic 2008].

The oil displayed a high degree of antimicrobial selectivity, inhibiting the growth of potential pathogens associated with intestinal dysbiosis at concentrations that had no effect on the beneficial bacteria examined (*Lactobacillus acidophilus*, *L. plantarum*). MIC for *Candida albicans* was 0.55% V/V and 0.275% V/V for *Clostridium difficile* [Hawrelak 2009].

The antimicrobial activity of the oil was stronger against fungi than bacteria. The following MIC values were observed: *Aspergillus niger* (0.12%); *Penicillium expansum* (0.12%); *Saccharomyces cerevisiae* (0.06%); *Candida knusei* (0.06%); *Salmonella enteritidis* (0.12%); *E. coli* (0.12%); *S. aureus* (0.12%); *Bacillus subtilis* (0.12%). Results confirmed that the oil was more effective against Gram-positive than Gram-negative bacteria [Gniewosz 2013].

The antimicrobial activity of caraway oil differs, depending on the genotypes, ranging from MIC = 0.16 mg/mL to 1.75 mg/mL. A significant negative correlation was found between MIC and carvone content and a positive correlation with limonene content [Seidler-Lozykowska 2013].

Antioxidant activity
The antioxidant activity of caraway oil was measured in the DPPH assay. The oil reduced the DPPH in a dose-dependent manner. Effects on lipid peroxidation were determined by the thiobarbituric acid assay (TBA) assay. In the Fe^{2+}/ascorbate system the oil expressed a strong and partly dose-dependent antioxidant capacity (IC_{50} of <2.5 µL/mL), with the highest inhibition (68.1%) at 5 µL/mL [Samojlik 2010].

Other effects
Foam height of simulated gastric juice was reduced by caraway oil at concentrations of 0.025 - 0.1% [Harries 1978].

De-sheathed sciatic nerves isolated from male Wistar rats were used to evaluate the effects of *R*-(-)- and *S*-(+)-carvone on inhibition of the compound action potential (CAP). Using a modified single sucrose-gap technique both enantiomers (at 10 mM) reduced peripheral nerve conduction (V_{CAP}) compared to control. *S*-(+)-Carvone produced an IC_{50} of 8.7+0.1mM. After 30 min of incubation *S*-(+)-carvone inhibited the CAP amplitude to about 25% of control reducing from 39.4+ 2.0 mV to 9.8+3.0 mV ($p<0.05$) and then reverted to 29.9+1.6 mV after 30 min nerve washing with the vehicle. Carvone may decrease nerve excitability by blocking voltage-gated sodium channels in a reversible manner. (+)-limonene (10mM) had no CAP-blocking effect [Goncalves 2010].

The effect of (+)-carvone on glutamatergic spontaneous excitatory postsynaptic currents (sEPSCs) was examined in substantia gelatinosa (SG) neurons of adult rat spinal cord slices by using the whole-cell patch-clamp technique. The stereoisomers (+)- and (-)-carvone were found to activate different types of TRP channels. The (+)-carvone (1mM) activity was inhibited by the TRPA1 antagonist HC-030031, indicating activation of TRPA1 channels, resulting in an increase in spontaneous L-glutamate release into SG neurons. The EC_{50} value for this activation was 0.72 mM [Kang 2015].

In vivo experiments

Anti-colitic activity
Caraway oil was given to groups (n=6) of male Wistar rats orally (100, 200, 400 µL/kg) or intraperitoneally (100, 400 µL/kg) 6 hours after induction of trinitrobenzene sulfonic acid (TBNS)-induced colitis and daily for 5 consecutive days. Colon tissue lesions (ulcer areas) and colitis indices were significantly reduced ($p<0.001$) in all cases in comparison to control groups, irrespective of dose and route of administration [Keshavarz 2013].

Anti-carcinogenic activity
Premalignant lesions were induced in rat colon by administration of 1,2-dimethylhydrazine (DMH; 20 mg/kg b.w. for 5 weeks). Rats given caraway oil (0.01 and 0.1% in the diet) for 16 weeks showed a suppression of DMH-induced lesions (72–87%) when compared to controls. Results indicated that long-term feeding of caraway oil altered colonic CYP1A1 and GST activities and the expression of β-catenin (a dual-function protein, which regulates the coordination of cell–cell adhesion and gene transcription) [Dadkhah 2011; Allameh 2013]. In a further experiment with DMH-treated rats given caraway oil (0.2% in the diet), a significant inhibition of colonic β-catenin was shown. A 65% inhibition ($p<0.05$) of aberrant crypt foci (ACF) formation was also reported. In addition, a significant reduction of hepatic CYP450 activity ($p<0.05$) and increase of GST ($p<0.05$) in DMH-treated rats were shown, when compared to the DMH-treated control group [Dadkhah 2014].

Effects on central nervous system
The effect of chirality on locomotor activity (LA) was evaluated in mice pre-treated with analeptics (amphetamine, methamphetamine, caffeine) and sedatives (diazepam, phenobarbital). Results were dependent on the chirality of the terpene and the circadian rhythm of mice. Inhalation of (*S*)-(+)-carvone by 'morning' mice (i.e. 'highly-active') produced a stimulating effect with phenobarbital-treatment ($p<0.05$) but a decreased LA in over-stimulated animals (with caffeine $p<0.05$) [Buchbauer 2005].

(*S*)-(+)-carvone (200 mg/kg i.p.) given to mice produced a depressant effect in the CNS and had anticonvulsant-like activity [De Sousa 2007].

Mice treated with methylphenidate (5 mg/kg i.p.) or sleep-deprived for 24 h exhibited an increase in locomotor activity. Single dose acute pre-treatment and chronic treatment once daily for 3 weeks with 100 mg/kg i.p. of (*S*)-(+)-carvone significantly ($p<0.05$) reduced methylphenidate-induced hyperlocomotion compared to a saline control group. Lithium (100 mg/kg), used as a positive control, showed significant activity with chronic treatment. Acute pre-treatment with (*S*)-(+)-carvone and lithium at the same doses significantly ($p<0.001$) blocked sleep deprivation-induced hyperlocomotion. Spontaneous locomotor activity was not significantly altered by (*S*)-(+)-carvone (50 or 100 mg/kg) or lithium (100 g/kg) in the first experiment; in the sleep deprivation experiment a non-sleep deprived control group given (*S*)-(+)-carvone (100 mg/kg) did exhibit a significant ($p<0.05$) reduction in spontaneous locomotion [Nogoceke 2016].

Effects on gastrointestinal motility
The effects of (*R*)-(-)- and (*S*)-(+)-carvone (100 mg/kg dissolved in vehicle) on gut motility were evaluated in 'awake' mice by measuring gastric dye emptying and the rate of intestinal transit. Intragastric pressure was also monitored. Gastric emptying (expressed as % of dye) was significantly lower ($p<0.05$) in mice treated with (*R*)-(-)-carvone (32.1%) and (*S*)-(+)-carvone (41.9%), when compared to vehicle (control, 59.2%), but higher than the positive control loperamide (29.1%). Both enantiomers also significantly ($p<0.05$) delayed intestinal transit when the liquid test meal was injected directly into the duodenum, with effects being comparable to that produced by the positive control (loperamide). Spontaneous and rhythmic intragastric mean amplitudes of pressure waves were significantly ($p<0.05$) decreased by (*R*)-(-)-carvone (13.2 mmHg) and (*S*)-(+)-carvone (10.7 mmHg) when compared to vehicle alone (24.6 mmHg), but higher than loperamide (6.9 mmHg) [Silva 2015].

Other effects
Male albino rats (n = 30) were divided into 3 groups: control (A), diabetic positive control (B) receiving 60 mg/kg s.c. streptozotocin (STZ) for 3 days and the experimental group (C) receiving 60 mg/kg STZ s.c. for 3 days followed by 10 mg/kg/day p.o. caraway oil. After 22 days rats in group B showed an increase in serum glucose and a decrease in glutathione peroxidase. Caraway oil reduced these changes in rats of group C. Examination of the kidneys of group C rats showed minor pathological changes compared with group B rats where kidneys showed glomerular and tubular degeneration, with haemorrhage and deformed renal tissue architecture [Abou El-Soud 2014].

To assess hepatoprotective activity against CCl_4, 2 groups of NMRI mice (n=6 each) were pre-treated daily for 5 days p.o. with saline (control) or caraway oil (0.13 g/kg). 20 h before sacrifice animals were administered i.p. a single dose of CCl_4 in olive oil (1:1; 2 mL/kg), 4 h after the last dose of caraway oil. Pre-treatment with caraway oil prevented a pro-oxidative effect in liver tissue homogenate, despite increased peroxidase activity, while xanthine oxidase activity showed a statistically significant decrease ($p<0.05$ vs control). The oil pre-treated group given CCl_4 showed significant ($p<0.05$) increases in glutathione peroxidase (oil+CCl_4: 1.76 vs CCl_4:1.08 nmol/mg of protein/min). Plasma aspartate transaminase activity (U/L) rose significantly ($p<0.05$) compared to the saline control (oil+CCL_4:173 U/L vs CCl_4:229 U/L vs control 89 U/L). In spite of the CCl_4 potential to form lipid peroxidation products (LPx), the LPx remained unchanged in the oil-treated group compared to both control and CCl_4 groups [Samojlik 2010].

Carvone or limonene, both isolated from caraway oil, given by oral administration (3 times over 6 days) to mice (20 mg/animal) significantly increased ($p<0.05$ to $p<0.005$) glutathione *S*-transferase (GST) activity in the liver, forestomach, small intestinal mucosa and colon. Limonene was less effective [Zheng 1992].

Pharmacological studies in humans

Antispasmodic effect
Healthy volunteers (n=12) took a capsule containing caraway oil (50 mg) on an empty stomach, followed 15 mins later by a non-fat test drink (400 mL apple juice + 1 mL lactulose). Sonographic measurements showed that the gall bladder emptied in response to the drink. On refilling the gall bladder showed a volume increase of 90% (from initial) compared to 40% in the controls (test drink only) indicating that caraway oil had a relaxing effect on the gall-bladder without significantly prolonging orocaecal transit time [Goerg 2003].

The effect of an intraduodenal administration of caraway oil (50 mg) on gastroduodenal motility was studied in healthy volunteers (n = 7). Results showed significant reductions in contraction amplitudes of the duodenum ($p = 0.025$) as well as reduced contraction duration and amplitudes in the gastric corpus and antrum ($p = 0.028$), indicating smooth-muscle relaxing effects of the oil [Mickelfield 2003].

Effects on the central nervous system
The effects of chirality on the human autonomic nervous system were studied in healthy volunteers (n = 20). Inhalation of *R*-(+)-limonene increased systolic blood pressure, subjective alertness and restlessness, while *S*-(+)-carvone increased levels of both systolic and diastolic blood pressure [Heuberger 2001].

Clinical studies

Irritable bowel syndrome
In a monocentric, randomized, open-label, cross-over trial, 48 patients with Irritable Bowel Syndrome (IBS) were randomly assigned to one of 6 groups. Each group received, in different orders, three interventions: (i) hot caraway oil-containing poultice (2% solution in olive oil; CarO); (ii) hot olive oil poultice (OliveH) as control and (iii) cold olive oil (OliveC). Patients applied each intervention to their abdominal area daily for 3 weeks. By means of patient questionnaires symptom severity was measured using the IBS-symptom severity scale [Francis 1997]. A significant difference (p = 0.033) was found for symptom severity in favour of CarO compared to OliveC, but not compared to OliveH [Lauche 2015].

Pharmacokinetic properties

Pharmacokinetics in vitro
A membrane diffusion model was used to study the transfer of caraway oil (0.5 g) from a buffer solution pH 1.1 (= stomach) and pH 6.5 (= intestine) to pH 7.5 (= plasma). Only very low concentrations of carvone, *d*-limonene and other components (no amounts given) were transferred through the membrane [Lado 2005].

Gastrointestinal absorption
The absorption of carvone from a caraway fruit extract (1:3, ethanol 30% V/V) was tested in everted intestinal sacs prepared from male adult rats. After 30 minutes, the uptake from an extract concentration of 11.7µg/mL was about 3 µg/cm^2 carvone [Kelber 2000].

Percutaneous absorption
Caraway oil increased permeation of salicylic acid by 48% when evaluated using goat skin [Kaza 2006].

After a single topical application to an area of the lower abdomen (376 cm^2) of healthy subjects (n=4), *S*-(+)-carvone (300 mg in 1.5 g arachis oil) was rapidly absorbed (T_{max} (min) 32.4), resulting in a C_{max} of 88 ng mL^{-1} and distribution half-life ($t_{1/2\alpha}$) of 19.4 min [Jäger 2001].

Using human abdominal skin, (*R*)-(+)-limonene (5% V/V) was found to improve the permeability of haloperidol (2.5 mg/mL) by 26.5 times ($p<0.05$) and reduce the lag time from 14.1 to 9.2 h ($p<0.05$), compared to control [Lim 2006].

A terpene-based hydroxypropyl cellulose gel drug reservoir system was used to study the transdermal permeation of ondansetron hydrochloride through prepared rat epidermis. Carvone (8% m/m) produced an optimal permeation of 87.4 mg/cm^2·h ($p<0.001$) and limonene (3% m/m) of 181.9 mg/cm^2·h ($p<0.01$). The enhancement ratio in drug permeability was 10.8 with carvone and 22.5 with limonene, when compared with 1.0, i.e. that obtained without a terpene-enhancer (control) [Krishnaiah 2008].

Biotransformation
R-(-)- and *S*-(+)-carvone are stereoselectively biotransformed by human liver microsomes to 4(*R*),6(*S*)-(-)- and 4(*R*),6(*S*)-(+)-carveol respectively and 4(*R*),6(*S*)-(-)-carveol is further glucuronidated [Jäger 2000].

Pharmacokinetics in animals

Absorption, distribution, metabolism and elimination
The absorption rate of a caraway fruit extract (1:3, ethanol 30%) determined as carvone, was carried out using the mouse everted gut sac technique. Incubation time was 30 min and extract concentrations were 10, 25, 50 and 100 µL/mL. Data indicate a fast uptake of carvone linearly correlated to the mucosal concentration [Kelber 2006].

In rabbits, (*S*)-(+)- and (*R*)-(-)-carvone is reduced to yield carveol which is converted to the glucuronic acid conjugate and excreted in the urine [Ishida 1989].

The main route of elimination of (*R*)-(+)-limonene administered orally was via the urine in animals and man, 75-95% of the administered radioactivity being excreted in the urine during 2-3 days. Faecal excretion accounted for less than 10% of the dose in animals during 2-3 days. In addition to six metabolites, namely p-mentha-1,8-dien- 10-ol (M-I), p-menth-1-ene-8,9-diol (M-II), perillic acid (M-III), perillic acid-8,9-diol (M-IV), p-mentha-1,8-dien-10-yl-beta-D-glucopyranosiduronic acid (M-V) and 8-hydroxy-p-meth-1-en-9-yl-beta-D-glucopyranosiduronic acid (M-VI) isolated from rabbit urine previously, five new metabolites have been isolated from dog and rat urine, and which were characterized as 2-hydroxy-p-menth-8-en-7-oic acid (M-VII), perillylglycine (M-VIII), perillyl-beta-D-glucopyranosiduronic acid (M-IX), p-mentha-1,8-dien-6-ol (M-X) and probably p-menth-1-ene-6,8,9-triol (M-XI). The major metabolite of (*R*)-(+)-limonene in the urine was M-IV in rat and rabbit, M-IX in hamster, M-II in dog and M-VI in guinea pig [Kodama 1976].

Pharmacokinetics in humans
Oral administration of an immediate-release preparation containing peppermint oil (180 mg) and caraway oil (100 mg) to healthy male volunteers (n=15) yielded a mean C_{max} for carvone of 12.57 ng/mL, a mean T_{max} of 1.24 h and mean $t_{1/2}$ of 2.0 h [Mascher 2001].

(S)-(+)-Carvone

Percutaneous absorption
To determine whether an enantioselective difference in the metabolism of topically applied *R*-(-)- and *S*-(+)-carvone could be observed in man, the metabolism and pharmacokinetics of *R*-(-)- and *S*-(+)-carvone was investigated in four healthy subjects. Following separate topical applications at a dose of 300 mg, *R*-(-)- and *S*-(+)-carvone were rapidly absorbed, resulting in significantly higher C_{max} levels for (*S*)-(+)-carvone (88.0 vs 23.9 ng/mL) and longer distribution half-lives ($t_{(1/2\alpha)}$; 19.4 vs 7.8 min), resulting in 3.4-fold higher areas under the blood concentration-time curves (5420 vs 1611 ng x min/mL). The biotransformation products for both enantiomers in plasma were below detection limit. Analysis of control- and beta-glucuronidase pre-treated urine samples, however, revealed a stereoselective metabolism of *R*-(-)-carvone to 4*R*,6*S*-(-)-carveol and 4*R*,6*S*-(-)-carveol glucuronide. No metabolites could be found in urine samples after (*S*)-(+)-carvone application. These data indicate that stereoselectivity in phase-I and phase-II metabolism has significant effects on *R*-(-)- and *S*-(+)-carvone pharmacokinetics and could explain the increased blood levels of (*S*)-(+)-carvone [Jäger 2001].

Metabolism
Using the Metabolism Ingestion-Correlated Amounts (MICA) approach, volunteers (n=6) receiving a controlled diet were given a single dose of (*S*)-(+)- or (*R*)-(-)- carvone (0.5 mmol; ~ 1 mg/kg b.w.) ingested as a solution in full-fat milk (500 mL). 24-hour urine samples were collected before (control) and after

ingestion of carvone. Carvonic acid, dihydrocarvonic acid, carveol, dihydrocarveol and uroterpenolone were identified as the metabolites in humans. 10-Hydroxycarvone was not detected, indicating either concentration effects or interspecies differences. No differences in metabolism between (*S*)-(+)- and (*R*)-(-)-carvone were detected [Engel 2001].

Lactation/Breast milk
Lactating women (n=18) were each given 100 mg of (*S*)-(+)-carvone mixed with lactose and talc in a capsule on 3 test days. Milk samples were collected every 2 hours for 8 hours starting at the time of ingestion. Carvone was detected in milk at all times, with the average concentrations of 1.3 µg/L at 0 hours, reaching a maximum of 7.2 µg/L at 2 hours, 5.6 µg/L at 4 hours, 4.3 µg/L at 6 hours and 2.7 µg/L at 8 hours. The average peak carvone concentration in milk was 10.5 µg/L. It is considered that lipophilic flavour compounds are transported from blood into milk via passive diffusion [Hausner 2008].

In another study, 20 mothers consumed 30 mg of (*S*)-(+)-carvone in 75 grams of hummus every third day for 28 days (10 exposures) at about 2 hours before a "usual" nursing time. Breastmilk samples were obtained 2 hours after ingestion on the first and last days of carvone intake. Carvone was detectable in the milk of 18 mothers. Average carvone concentrations in breastmilk were 2.5 µg/L and 3.8 µg/L on the first and last days of sampling, respectively. However, these values did not differ statistically and the combined average carvone concentration was 3.2 µg/L. A control group of 20 women who did not ingest (*S*)-(+)-carvone had no detectable carvone in their breastmilk [Hausner 2010].

(R)-(+)-Limonene
About 25-30% of an oral dose of (*R*)-(+)-limonene in humans was found in urine as (*R*)-(+)-limonene-8,9-diol and its glucuronide; about 7-11% was eliminated as perillic acid and its metabolites [Kodama 1976].

Healthy human volunteers (n=7) ingested 100 mg/kg limonene. Blood was drawn at 0 and 24 h for chemistry-panel analysis and at 0, 4, and 24 h for limonene-metabolite analysis.Atleast five compounds were present at 4 h that were not present at time zero. Two major peaks were identified as dihydroperillic acid and perillic acid, and two minor peaks were found to be the respective methyl esters of these acids. A third major peak was identified as limonene-1,2-diol. Limonene was a minor component. At a dose of 100 mg/kg, limonene caused no gradable toxicity [Crowell 1994].

In a single centre, open-label pharmacokinetic study, healthy male volunteers (n=24) were given a single dose of 300 mg (*R*)-(+)-limonene with 240 mL water. The maximum plasma concentration of (*R*)-(+)-limonene ranged from 40.1 to 327.4 ng/mL. T_{max} values ranged from 0.75 to 3.0 h and oral clearance ranged from 53.7 to 312.1L/h [Wang 2007].

Patients (n=32) with refractory solid tumours completed 99 courses of (*R*)-(+)-limonene 0.5 to 12 g/m^2 per day administered orally in 21-day cycles. Additional breast cancer patients (n=10) received 15 cycles of (*R*)-(+)-limonene at 8 g/m^2 per day. One partial response in a breast cancer patient on 8 g/m^2 per day was maintained for 11 months; three patients with colorectal carcinoma had prolonged stable disease. There were no responses in the phase II study. Peak plasma concentration (C_{max}) for (*R*)-(+)-limonene ranged from 10.8+6.7 to 20.5+11.2 mM. Predominant circulating metabolites were perillic acid (C_{max} 20.7+13.2 to 71+29.3 mM), dihydroperillic acid (C_{max} 16.6+7.9 to 28.1+3.1 mM), limonene-1,2-diol (C_{max} 10.1+8 to 20.7+8.6 mM), uroterpenol (C_{max} 14.3+1.5 to 45.1+1.8 mM), and an isomer of perillic acid. Both isomers of perillic acid, and cis- and trans- isomers of dihydroperillic acid were in urine hydrolysates. Intratumoural levels of (*R*)-(+)-limonene and uroterpenol exceeded the corresponding plasma levels. Other metabolites were trace constituents in tissue [Vigushin 1998].

Preclinical safety data

Allergenicity
In a 24-hour closed-patch test in humans a 4% concentration of caraway oil in petroleum jelly produced no irritation [Opdyke 1973].

The Scientific Committee on Consumer Safety [SCCS] on fragrance allergens in cosmetic products has listed carvone among the established contact allergens (skin sensitisers) in humans [EFSA 2014].

Although the non-oxidized *R*-(+)-limonene itself is not allergenic, it easily forms allergenic autoxidation products. A limonene hydroperoxide fraction proved to be the most important allergen of the oxidation mixture, showing positive reactions in around 60% of the limonene-allergic patients. Testing limonene oxide and carvone separately resulted in very few positive reactions. In Europe, *R*-(+)-limonene containing oxidation products are classified as a skin sensitizer in animals and humans [Matura 2002; 2003; Kim 2013].

Acute toxicity
The oral LD_{50} for caraway oil in rats is 3.5 mL/kg b.w. corresponding to 3720 mg/kg b.w. [EMEA 1998] and 3500 mg/kg; LD_{50} for rabbit skin 1780 mg/kg [Lewis 1996].

LD_{50} value for (*S*)-(+)-carvone in mice was 484.2 mg/kg [De Sousa 2007].

The EFSA Scientific Committee established an Accepted Daily Intake (ADI) for (*S*)-(+)-carvone of 0.6 mg/kg b.w./day, based on the $BMDL_{10}$ of 60 mg/kg b.w./day for an increase in relative liver weight in the rat, 90-day studies and an uncertainty factor of 100 [EFSA 2014].

The oral LD_{50} for *R*-(+)-limonene in male and female mice is reported to be 5.6 and 6.6 g/kg b.w. respectively, and 4.4 and 5.1 g/kg b.w. in male and female rats respectively [IARC 1999].

Administration to rats and mice of *R*-(+)-limonene at doses ranging from 413 - 6,600 mg/kg/day for 5 days/week for 3 weeks, resulted in no signs of compound-related toxicity at doses <1,650 mg/kg daily [NTP 1990b].

The dose-response for *R*-(+)-limonene has shown NOEL of 3.6 mg/kg/day in the development of hyaline-droplet nephropathy in male rats and no development of kidney tumours are expected to occur in male rats below this NOEL. Neither female rats nor mice developed tumours or hyaline-droplet nephropathy, which is associated with elevated alpha2u-globulin as found in male rats. *R*-(+)-limonene is stated as not posing a risk to humans for the formation of renal neoplasms [Whysner 1996].

Subchronic and chronic toxicity
A study in rats demonstrated that 0.1% carvone in their diet for 28 weeks and 0.25% for one year had no effects, while 1% of (*S*)-(+)-carvone in the diet for 16 weeks caused growth retardation and testicular atrophy [EMEA 1998; Hagan 1967].

Based on short-term and long-term toxicity studies in rodents, including a NOEL of 93 mg/kg b.w./day in rats, the World Health Organization established the ADI for (*S*)-(+)-carvone of 0-1 mg/kg b.w. per day [WHO 1999].

For (*S*)-(+)-carvone a daily intake of 1 mg/kg b.w. was considered harmless. For a 60 kg person this corresponds to a dose of 60 mg, approx. equivalent to 120 mg caraway oil (corresponding to approx. 6 drops) [EMEA 1998].

Mutagenicity and carcinogenicity
(*S*)-(+)-Carvone was not mutagenic in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537, with and without metabolic activation [EMA 1998].

(*S*)-(+)-Carvone was reported to be non-carcinogenic in a 103-week study in mice dosed with oral doses of 375 and 750 mg/kg b.w., 5 days per week [NTP 1990a; EMA 1998; EFSA 2009].

R-(+)-Limonene was not mutagenic in four strains of *S. typhimurium* (TA98, TA100, TA1535, or TA1537), did not significantly increase the number of trifluorothymidine (Tft)-resistant cells in the mouse L5178Y/TK&plusmn assay and did not induce chromosomal aberrations or sister chromatid exchanges (SCEs) in cultured CHO cells [NTP 1990b].

R-(+)-Limonene was administered by gavage to F344/N male rats for 5 days per week for 103 weeks at doses of 0, 75 or 150 mg/kg, and female F344/N rats at doses of 0, 300 or 600 mg/kg. There was no evidence of carcinogenic activity of *R*-(+)-limonene for the female rats that received 300 or 600 mg/kg. Evidence of carcinogenic activity was found only in male F344/N rats as shown by increased incidences of tubular cell hyperplasia, adenomas and adenocarcinomas of the kidney [NTP 1990b]. Similarly there was no evidence of carcinogenic activity of limonene for male B6C3F1 mice that received 250 or 500 mg/kg or female B6C3F1 mice that received 500 or 1,000 mg/kg [NTP 1990b]. Renal toxicity of limonene results from the accumulation in male rat kidney proximal tubule lysosomes of alpha-2u-globulin, a protein synthesized exclusively by adult male rats [Whysner 1996].

Male Big Blue ™ rats were given limonene (525 mg/kg) as part (1%) of their diet for 10 consecutive days. The rat carcinogen 4-aminophenyl was used as positive control. Limonene failed to increase the mutant frequency in the rat liver or kidney, results consistent with a non-genotoxic mechanism of carcinogenic action [Turner 2001].

R-(+)-Limonene has demonstrated no gradable toxicity in humans after single (100 mg/kg) [Crowell 1994] and repeated dosing (0.5-12 g/m^2/day for 21 days) with one person receiving 15 g/day for 11 months [Vigushin 1998; Sun 2007].

Clinical safety data

Safety of a standardized preparation of enteric coated capsules containing 90 mg peppermint oil and 50 mg caraway oil per capsule was studied in a double-blind, placebo-controlled multicentre trial in patients with non-ulcer dyspepsia. The test preparation was well tolerated, even over a 4-week period [May 1996; Madish 1999].

A second double-blind, randomized, parallel-group evaluation of the fixed peppermint oil/caraway oil combination vs placebo was carried out in patients suffering from functional dyspepsia (n=96). Patients received one capsule twice daily of the combination or placebo for 28 days. No side effects were reported by patients receiving the verum [May 2000].

In further randomized, placebo-controlled, double-blind clinical trials with the same combination (2 x 1 capsule daily), the test preparation did not show any adverse events [Holtmann 2001; Holtmann 2003].

R-(+)-Limonene has been found to be safe, when 100 mg/kg (equivalent to about 7 g for an average adult male) was ingested. Only mild eruction for 1-4 h, mild satiety for 10 h and mild fatigue for 4 h were reported [Crowell 1994].

In a dose-escalation study in patients (n=32) with refractory solid tumours, *R*-(+)-limonene (0.5 – 12 g/m^2/day) was given orally for 21 days. The maximum tolerated oral dose was 8 g/m^2/day (15 g/day). Nausea, vomiting and diarrhoea were observed and effects were dose-dependent. *R*-(+)-Limonene was considered to have low toxicity after single and repeated doses for up to one year [Vigushin 1998].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2019
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003

LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2019
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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The second edition of ESCOP Monographs, published as a hardback book in 2003 with a Supplement in 2009, has been widely acclaimed for its authoritative information on the therapeutic uses of herbal medicines. Monographs covering a total of 107 herbal substances include extensive summaries of pharmacological, clinical and toxicological data, and copious references to scientific literature form an important part of each text.

Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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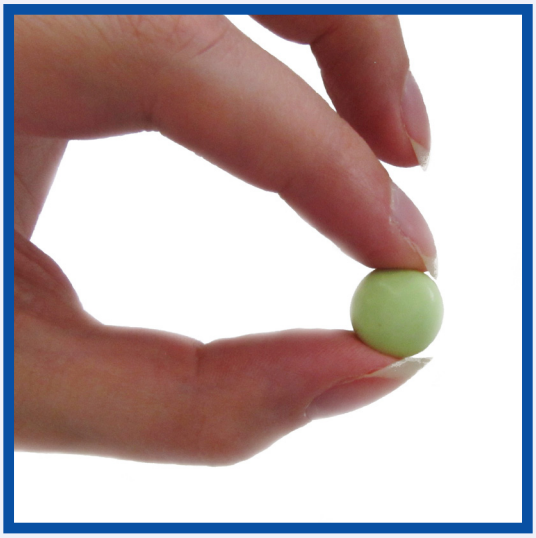
The Scientific Foundation for Herbal Medicinal Products

Caryophylli aetheroleum
Clove Oil

2014



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Herbal Medicinal Products

CARYOPHYLLI AETHEROLEUM
Clove oil

2014

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Caryophylli aetheroleum - Clove oil

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

Edited by Simon Mills and Roberta Hutchins
Cover photographs by Simon Mills (*Syzygium aromaticum*)
Cover and text design by Martin Willoughby
Typeset in Optima by Roberta Hutchins

Plant illustrated on the cover: *Syzygium aromaticum*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
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Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

CARYOPHYLLI AETHEROLEUM 2014

Clove oil

DEFINITION

Clove oil is obtained by steam distillation of the dried flower buds of *Syzygium aromaticum* (L.) Merrill et L.M. Perry (*Eugenia caryophyllata* (C. Spreng., Bull. et Harr.). The material complies with the monograph of the European Pharmacopoeia [Clove oil].

CONSTITUENTS

The main characteristic constituents of the essential oil are β-caryophyllene (5 - 14 %) and phenylpropanoids eugenol (75 - 88 %) and acetyleugenol (4 – 15 % of the oil); minor constituents, less than 1%, are 2-heptanone, ethyl hexanoate, humulenol; sesquiterpenes, α-humulene, α-humulene epoxide, β-humulene, β-caryophyllene oxide, α-cubebene, α-copaene, α-cadinene, δ-cadinene, α-ylangene, calacorene and calamenene [Clove oil; Iwamuro 1983; Gopalakrishnan 1984,1985; Zheng 1992; WHO 2002; Chaieb 2007; Blaschek 2008].

CLINICAL PARTICULARS

Therapeutic indications
In dental healthcare, for short term use, as a local anaesthetic [Leung 1980; Reynolds 1989], as a disinfectant mouthwash [Wichtl 2002; Bradley 2006; Blaschek 2008]. In these treatments the efficacy is plausible on the basis of human experience and longstanding use.

Symptomatic treatment in chronic anal fissure, after diagnosis by a physician [Elwakeel 2007].

Posology and method of administration

Dosage

Adult daily dose
As a mouthwash with 1-3% diluted clove oil, up to three times daily [Tisserand 1995; Wichtl 2002; Bradley 2006; Lis-Balchin 2006; Blaschek 2008].
For anorectal use: clove oil in a 1% cream, applied three times daily [Elwakeel, 2007].

Method of administration
Oromucosal or anorectal applications with liquid or semisolid preparations of clove oil [Markowitz 1992; Wichtl 2002; Elwakeel 2007; Blaschek 2008].

Duration of use
No restrictions. If symptoms persist or worsen medical advice should be sought.

Contraindications
Topical use of clove oil is contraindicated in case of hypersensitivity to clove oil, as well as hypersensitivity to Peru balsam due to possible cross reactivity. Of 78 patients with allergy to Peru balsam, 36 showed a positive reaction to clove powder [Niinimäki 1984]. Four out of 4 patients sensitized to Peru balsam reacted positive to a 1% hexane extract of clove in petrolatum [Bouhlal 1989].

Special warnings and special precautions for use
None required.

Interaction with other medicinal products and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

E/S/C/O/P MONOGRAPHS

Effects on ability to drive and use machines
None known.

Undesirable effects
Very rare cases of contact dermatitis and food allergy have been reported for clove oil or eugenol.

Investigations on contact allergy from essential oils were conducted by the Information Network of Departments of Dermatology (IVDK). In patch test results (from the years 2000 to 2008) of 15,682 patients with dermatitis (from 84,716 consulting), there were 637 cases positive for any oil: of these, 1.5% were positive for clove oil [Uter 2010].

In a worldwide, multi-centre investigation of allergy to cosmetics and toiletries, 43 (19%) of 218 subjects with proven contact dermatitis due to fragrance materials tested positive for clove oil (10% solution) in a new patch test [Larsen 2002].

In 589 cases of food allergy, none were allergic to clove [Moneret-Vautrin 2002].

45 patients occupied in stomatological services (dentists, dental surgeons, nurses and dental technicians), presenting with contact dermatitis of various unknown causes, were exposed to 17 potential allergens, including eugenol. Eugenol (1% solution) tested positive in 9% of the cases (3 dentists and 1 dental surgeon) [Berova 1990].

Allergic contact dermatitis was documented during a 5-year investigation. Only 1 of 1000 patients, working with food and diagnosed to have hand dermatitis, was found to be allergic to clove and simultaneously to carrot and allspice [Kanerva 1996]. Occupational allergic contact dermatitis from eugenol, oil of cinnamon and oil of cloves was reported in a physiotherapist [Sanchez-Perez 1999].

A survey of consumer patch-tests to investigate sensitization demonstrated a very low sensitization potential for eugenol. In 11,632 patch tests only 2 positive eugenol tests were detected at a concentration of 0.05 % (induced hypersensitivity) and at 0.09 % (pre-existing sensitization) [Rothenstein 1983].

Overdose, cutaneous and oral
Permanent local anaesthesia and anhidrosis was reported 11 months after an unknown amount of clove oil was spilled on the face of a patient with toothache. The symptoms were considered to be a neurotoxic effect after cutaneous use [Isaacs 1983].

Toxic effects were reported after oral overdoses of clove oil. Two children, that ingested overdoses of clove oil of 1 teaspoon (7 mo. old, corresponding to approx. 0.5 g/kg body weight of eugenol) and 10 ml (2 yr. old), presented with CNS depression and liver failure, respectively; both recovered [Lane 1991; Brown 1992]. After drinking 5-10 ml clove oil a young child presented with coma and acute liver damage, but recovered [Hartnoll 1993]. Fulminant hepatic failure was reported for a 3-month old patient after ingesting less than 8 ml clove oil, and for a 15 month-old boy after ingesting 10-20 ml clove oil. Both were successfully treated with intravenous N-acetylcysteine, according to a standard protocol used for paracetamol (acetaminophen) poisoning [Eisen 2004; Janes 2005].

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In view of the vast amount of scientific literature on clove oil and eugenol, the selection of literature cited in the following sections is aimed solely at bringing together relevant examples of the possible physiological roles of clove oil and its major constituents eugenol and β-caryophyllene.

Several monographs and reviews have described pharmacological and other properties of clove oil and/or eugenol, its isolated major constituent [Deininger 1991; Markowitz 1992; Tisserand 1995; Lis-Balchin 2006; Chaieb 2007; Edris 2007; Blaschek 2008].

In vitro experiments

Antibacterial activities (Table 1)
Reports on multiple antimicrobial activities of clove oil and other essential oils have been reviewed [Kalemba 2003; Ceylan 2004; Baser 2009]. The antibacterial activity of clove oil compares in magnitude with oils from spices with the highest antibacterial properties, such as the oils of thyme, cinnamon, oregano and rosemary. In direct comparison, clove oil exhibited slightly greater antibacterial activity than that of rosemary oil. For example, *Escherichia coli* was inhibited at 0.125 %V/V (MIC) by clove oil, as compared to 0.250 %V/V (MIC) by rosemary oil [Ceylan 2004; Prabuseenivasan 2006; Fabio 2007; Fu 2007].

Mechanism of antibacterial action
Eugenol at 1% V/V (MIC of 0.0125%) increased permeability of the bacterial cell membrane of *Salmonella typhi* and caused disruption of the cytoplasmic membrane [Devi 2010]. Clove oil had an antibacterial effect on *Propionibacterium acnes* (MBC/MIC 0.31 mg/mL). Loss of the bacterial membrane integrity, i.e. ruptured cell walls and membranes, resulted at 0.031-0.62 mg/mL clove oil, measured by flow cytometry [Fu 2009].

Antibiotic–resistant bacteria in hospital isolates
Clove oil was shown to be effective against hospital-acquired isolates of Methicillin-resistant *Staphylococcus aureus* (MRSA) and was proposed, together with other essential oils, for antiseptic topical treatment [Warnke 2009]. Inhibition zones of 26 multi-resistant *Staphylococcus epidermidis* strains, isolated from dialysis biomaterial in the hospital, were in the range of 11-15 mm, five strains had zones >16 mm [Chaieb 2007]. Eugenol was active against the antibiotic-resistant bacteria, *Klebsiella pneumonia*, *Shigella ssp*. and *Proteus ssp*. [Nascimento 2000].

Synergistic and additive antibacterial effects
Synergistic interactions of eugenol with antibiotics against Gram-negative bacteria were reported in a model for antibiotic-resistant bacteria [Hemaiswarya 2009]. Combinations of clove oil and rosemary oil have produced mostly additive and rarely synergistic or even antagonistic effects [Fu 2007].

A selection of reported antibacterial activities of clove oil and eugenol against a series of oral, respiratory and antibiotic-resistant pathogens, Gram-positive as well as Gram-negative bacteria, is presented in Table 1. For the MIC values shown, those representing a strong antibacterial effect are in the range of 0.01% - 0.25% V/V.

Antifungal activities (Table 2)
Selected information about antifungal properties of clove oil is presented in Table 2. 48 h-Biofilms of *Candida albicans* were inhibited by eugenol treatment at 0.047 % V/V (IC_{50}) and at 0.188 % V/V (IC_{80}), using the tetrazolium salt reduction assay [He 2007].

Clove oil exhibited, in comparison, a slightly higher antifungal activity than rosemary oil. For example, *Candida albicans* was inhibited at 0.125 %V/V (MIC) by clove oil, as compared

TABLE 1 In vitro antibacterial activities of clove oil and eugenol

Author Year	Material Method	Bacteria	MIC* (%V/V)	MBC* (%V/V)
Saeki 1989	Oil and eugenol Oral bacteria	*Streptococcus species* *Actinomyces species* *Propionibacterium acnes* *Actinobacillus species* *Bacteroides species* *Capnocytophaga gingivalis* *Eikenella corrodens*	0.1 % 0.1 % 0.1 % 0.01 % 0.01 % 0.01 % 0.01 %	
Smith-Palmer 1998	Oil	*Escherichia coli* *Staphylococcus aureus* *Listeria monocytogenes* *Salmonella enteridis* *Campylobacter jejuni*	0.04 % 0.04 % 0.03 % 0.04 % 0.05 %	0.1 % 0.04 % 0.04 % 0.075 % 1 %
Nascimento 2000	Eugenol acts on antibiotic-resistant bacteria	*Klebsiella pneumoniae* *Shigella ssp.* *Proteus ssp.*	1.89 % 0.94 % 0.94 %	
Rhayour 2003	Oil and eugenol	*Escherichia coli* *Bacillus subtilis*	0.050 % 0.033 %	0.100 % 0.050 %
Prabuseenivasan 2006	Oil	*Staphylococcus aureus* *Bacillus subtilis* *Klebsiella pneumoniae* *Proteus vulgaris* *Pseudomonas aeruginosa* *Escherichia coli*	< 0.61 % < 0.30 % < 0.61 % < 0.30 % < 0.15 % < 0.15 %	
Fabio 2007	Oil Bacteria from respiratory infections	*Streptococcus pyogenes* *Streptococcus agalactiae* *Streptococcus pneumoniae* *Staphylococcus aureus* *Haemophilus influenzae* *Klebsiella pneumoniae* *Stenotrophomonas maltophilia*	1.25 % 1.25 % 1.25 % 1.25 % 1.25 % 5.00 % 1.25 %	
Fu 2007	Oil 3 Gram- positive and 3 Gram-negative bacteria	*Staphylococcus epidermidis* *Staphylococcus aureus* *Bacillus subtilis* *Escherichia coli* *Proteus vulgaris* *Pseudomonas aeruginosa*	0.250 % 0.125 % 0.125 % 0.125 % 0.125 % 0.500 %	0.250 % 0.250 % 0.250 % 0.125 % 0.250.% 0.500 %

* MIC = minimal inhibitory concentration; MBC = minimal bactericidal concentration;
For unit conversion to %V/V: density of clove oil =1.04 g/cm^3; of eugenol =1.06 g/cm^3.

to 0.250 %V/V (MIC) by rosemary oil [Fu 2007]. Clove oil was effective against isolates from hospitalised patients with antimycotic-resistant *Candida* species, *C. albicans* and *C. krusei* [Warnke 2009].

Mechanism of antifungal action
Clove oil and eugenol both exhibited antifungal activity towards various *Candida*, *Aspergillus* and dermatophyte species, and against fluconazole-resistant strains (MFC's ranged at 0.32-1.25 µl/mL and MIC's at 0.16-1.25 µl/mL). The fungicidal effect at concentrations just above the MIC values, measured by penetration of propidium iodide, resulted in extensive lesions of the fungal cell membrane and reduced ergosterol in the membrane [Pinto 2009].

The mode of action for the antifungal effects of clove oil and eugenol on the fungal virulence of *Aspergillus niger* and *Trichophyton rubrum* was investigated by measuring elastase and keratinase activitiy. Eugenol and clove oil inhibited fungal elastase by up to 78% and 68% respectively. Keratinase was slightly inhibited by both. Since proteinases contribute to fungal virulence by destroying host tissues and digesting immunologically important proteins, such as antibodies and complement factors, the anti-proteinase activity may reduce the pathogenesis of fungi [Khan 2011].

Antiviral activity
The lipid envelope of *Herpes simplex* virus type 1 and Newcastle disease virus has been destroyed by clove oil (1/500 dilution;0.2 %V/V) and replication inhibited. Non-enveloped viruses (*adenovirus*-3 and *poliovirus*-S) were not affected by clove oil [Siddiqui 1996]. Antiviral activity was exhibited by eugenol at 149.5 µg/ml (0.016 % V/V) for 2 isolated strains of *Herpes simplex* virus-1, however this concentration did not inactivate a standard HSV-1 strain. Eugenol reduced virus HSV-2 replication by 16.5 -100 % [Tragoolpua 2007].

Anti-inflammatory properties
A scintillation proximity-based assay demonstrated inhibition of COX-2 by eugenol (IC_{50} = 129 µM) [Huss, 2002]. COX-2 gene

TABLE 2 In vitro antifungal activities of clove oil and eugenol

Author Year	Material Method	Fungi	MIC* (% V/V)	MFC* (% V/V)
el-Naghy 1992	Oil 10 dermatophytes isolated from patients with dermatomycosis	*Trichophyton simii* *Trichophyton kyryngei* *Trichophyton rubrum* *Trichophyton equinum* *Chrysosporium keratinophilum* *Microsporum audouinii* *Microsporum canis* *Candida albicans* *Candida tropicalis* *Epidermophyton floccosum*	0.1 % 0.1 % 0.1 % 0.1 % 0.1 % 0.1 % 0.1 % 0.1 % 0.1 % 0.1 %	
Sinha 1993	Oil Aflatoxin formation	*Aspergillus flavus*	0.014 %	
Martini 1996	Eugenol	*Cladosporium herbarum* *Penicillium glabrum*	0.002 % 0.014 %	
Hussein 2000	Eugenol Aquatic molds	*Saprolegnia spp.myc* *Achlya klebsiana* *Aphanomyces piscicida*	0.047 % 0.024 % 0.012 %	0.094 % 0.047 % 0.024 %
Beg 2002	Oil	*Alternaria alternata*	0.05%	
Juglal 2002	Oil Mycotoxin, aflatoxin formation	*Aspergillus parasiticus* *Fusarium moniliforme*	0.01 % 0.05 %	
Bennis 2004	Eugenol	*Saccharomyces cerevisiae*	0.033 %	0.055 %
Ahmad 2005	Oil Isolate from vaginal candidiasis	*Candida albicans*	0.005 %	
Chami 2005a	Oil Surface alteration	*Saccharomyces cerevisiae*	0.033 %	0.05 %
Chami 2005b	Eugenol	*Candida albicans*	0.2 %	
Gayoso 2005	Oil and eugenol Fungi from onychomycosis	3 *Candida ssp.* *Trichophyton rubrum* *Trichophyton mentagrophytes* *Geotrichum candidum*	2% / 4% 2% / 4% 2% / 4% 2% / 4%	
He 2007	Eugenol	*Candida albicans*	IC50:0.04 7%	
Fu 2007	Oil	*Candida albicans* *Aspergillus niger*	0.125 % 0.062 %	0.250 % 0.125 %

* MIC = minimal inhibitory (fungostatic) concentration; MFC = minimal fungicidal concentration; For unit conversion to %V/V: density of clove oil =1.04 g/cm^3; of eugenol =1.06 g/cm^3; MW eugenol=197.

expression and PGE_2 production were inhibited by eugenol (IC_{50} = 0.37 µM) in mouse macrophages [Kim SS, 2003]. Eugenol inhibited 5-lipoxygenase activity (IC_{50} = 26 µM) and leukotriene C_4 formation (IC_{50} = 30 µM) by stimulated Human PMN (polymorphonuclear neutrophils) [Raghavenra 2006].

Induction of apoptosis
Reactive oxygen species-mediated apoptosis was induced by eugenol at 40 µM in HL-60 leukemia cells. Eugenol (at 700µM for 50% loss of viability) was also reported to induce apoptosis in mast cells, an indication of its anti-allergic potential. It is suggested that eugenol induces apoptosis *in vitro* by depleting intracellular thiols [Park 2005; Yoo 2005].

In vivo experiments

Clove oil, and its major constituents eugenol and β-caryophyllene, have been investigated for their local anaesthetic, antibacterial, antifungal, antioxidant, immunomodulatory, anti-allergic and insect repellent activities.

Antibacterial activity
Bacterial (*Klebsiella pneumonia*) colonization of the lungs was decreased ($p<0.01$) after 15 days of feeding with clove oil (0.5 mL of 1% w/v oil, once daily), when compared to control mice given saline 0.5 mL [Saini 2009].

Antifungal activity
To immunosuppressed rats with artificial vaginal candidiasis, 500 µL eugenol (equivalent to 2-fold MIC; approx. 20 mg/kg per day) was applied twice daily by topical intravaginal route (as prophylactic treatment, from 2 days before until 3 days after inoculation with *C. albicans*) and was compared to the effect of nystatin and further controls. Prophylactic eugenol reduced the colony count of *C. albicans* in vaginas of infected rats by 98.9% 10 days after inoculation, with negative culture results in 2/9 rats and significantly lower levels in 7/9 rats compared to control. In a second experiment, treatment with 500 µL eugenol

began 3 days after inoculation and was continued for 7 days. Two of 9 rats were cured completely and 7/9 showed an 84% reduction of the *C. albicans* colony count in the vagina. The positive control, nystatin, used at 10-fold MIC, confirmed the validity of the rat model [Chami 2004].

In the immunosuppressed (dexamethasone/tetracycline treated) rat model of experimental oral candidiasis, topical treatment in the oral cavity, twice daily with 0.5 mL of 0.4% eugenol dispersed in viscous 0.8% agar solution (20µg/kg/d), was started 3 days post-infection and continued for 8 days. Eugenol significantly ($p<0.05$) reduced the number of colonies sampled from the oral cavity, compared to the number of colonies in untreated rats. Nystatin was similarly effective and confirmed the fungicidal effect of eugenol [Chami, Bennis 2005].

Mice with an artificial urogenital infection were treated successfully with clove oil. Vaginal administration of a liposome preparation of clove oil, on days 2, 4, 6 and 8 after intra-vaginal inoculation with *Candida albicans*, was nearly as effective as a liposome preparation of nystatin in eliminating the fungal infection. Both the clove oil and nystatin formulations reduced the vaginal titre of *C. albicans* in vaginal lavage fluid significantly, when tested on day 10 against a negative control ($p<0.001$). At 14 days post-infection topical clove oil had completely eliminated the fungal burden in treated mice [Ahmad 2005].

Anti-anaphylactic, anti-allergic potential and immunomodulatory activity
Intraperitoneal treatment with eugenol at 50-100 mg/kg provided protection against PAF-induced shock from i.v. carrageenan-induced rat paw oedema in rabbits ($p\leq0.001$) [Saeed 1995].

In rats with chemically-induced anaphylactic shock, eugenol was anti-anaphylactic at an intraperitoneal dose of 10 mg/kg b.w. Accordingly, in rat peritoneal mast cells, histamine release and TNF-α production were reduced *in vitro* by eugenol at 10µg/mL [Kim 1997].

Immunomodulatory functions of clove oil were tested in mice (previously immunized with sheep red blood cells) after oral treatment once daily for 7 days with 100, 200 and 400 mg/kg clove oil. The oil increased the white blood cell count and also enhanced delayed-type hypersensitivity significantly ($p<0.001$ for all doses). It restored cellular and humoral immune reponses in cyclophosphamide-immunosuppressed mice in a dose-dependent manner ($p<0.001$ for 200 and 400 mg/kg) [Carrasco 2009].

Eugenol (at 700µM and at 10 µg/kg b.w.) was considered to exhibit an anti-allergic potential, resulting from induction of apoptosis in mast cells of rats (*in vitro* and *in vivo* respectively) [Park 2005].

Antioxidant activity in an aflatoxicosis rat model
Rats fed an aflatoxin-contaminated diet were either treated orally with clove oil (5 mg/kg b.w.) or untreated, for 30 days. Resulting haematological and biochemical changes were typical for aflatoxicosis. Clove oil treatment resulted in protection against aflatoxicosis, i.e. significantly restored ($p<0.05$) parameters that were altered by aflatoxin in rats [Abdel-Wahhab 2005].

Anaesthetic use
In the rabbit conjunctival reflex test, local β-caryophyllene treatment, by drops into the conjunctival sac, permitted a concentration-dependent increase of the number of stimuli necessary to provoke the reflex during 5-15 minutes after application, at 10-1000 µg/mL. In a comparative test, chemically related caryophyllene oxide, at 3-1000 µg/mL was ineffective. Thus β-caryophyllene acted as a specific local anaesthetic to the rabbit eye [Ghelardini 2001].

Eugenol produced reversible dose-dependent (i.v. administration of single doses at 5-60 mg/kg) anaesthesia. The anaesthetic level was determined using the withdrawal reflex test by pinching the rats until the return of a positive response. Eugenol-induced loss of consciousness, in a dose–dependent manner, showed a mean recovery time of 167 +/- 42 sec at the highest dose level [Guenette 2006]. Induced thermal hypersensitivity was attenuated in rats treated with 40 mg/kg eugenol for 5 days ($p<0.01$) [Guenette 2007].

Gastroprotective activity
Clove oil and eugenol both displayed anti-ulcer activity. Gastroprotective effects were observed after intraduodenal administration of clove oil and eugenol (100 and 250 mg/kg) in rat models of indomethacin- and ethanol-induced ulcers. At the same doses, eugenol and clove oil also stimulated the production of mucus ($p<0.01$), an important gastro-protective factor [Santin 2011].

Insect repellent activity
Tick nymphs (*Ixodes ricinus*) were exposed to clove oil on filter paper discs (0.5 mL, undiluted). Clove oil acted as a long lasting repellent (from 4h to 8h) for 68% to 78% of the nymphs, compared to 0% repellency observed for the controls [Thorsell 2006].

Pharmacological studies in humans

Studies in humans as insect repellent
Clove oil (in 25% - 100% preparations) administered to the skin of 2 human subjects was investigated as a repellent for the prevention of bites from 2 mosquito species (*Anopheles albimanus* and *Aedes aegypti*). These applications provided protection from 1.5h up to 3.5h, in a dose-dependent manner. From a group of 15 essential oils clove oil was the only one that could selectively prevent bites of *Anopheles albimanus*. Clove oil, in the dilution range of 25% - 100%, was 7 times more effective compared to a synthetic repellent (25% Deet, N,N-diethyl-3-methylbenzamide) [Barnard 1999].

A controlled study investigating clove oil application to the skin (4 groups: 10%, 50%, undiluted oil and solvent controls) was conducted in 3 human subjects (who agreed to be test objects) for prevention of mosquito bites. The undiluted oil fully prevented bites for 2 to 4 hours in 3 mosquito species (*Aedes aegypti*, *Anopheles dirus* and *Culex quinquefasciatus*). Dose dependency was shown, 50% oil prevented bites for 1.5h [Trongtokit 2005].

Clinical studies

A randomized, single-blind, controlled, comparative clinical trial was conducted for 6 weeks. Clove oil (as a 1% cream) was applied anorectally 3 times daily, and tested against a positive control (5% lignocaine cream), in 55 patients with chronic anal fissure (clove oil n=30; lignocaine n=25). After a 3-month follow-up, significantly more of the clove oil group (60%) than the lignocaine control group (12%) were symptom-free ($p<0.001$). Significant decreases in anal sphincter pressures were also reported for the clove oil patients. The majority of clove oil patients did not require additional systemic analgesics during the study, presumably because of the anaesthetic effect of 1% clove oil. This demonstrated a further advantage when compared to the control group who had received the traditional treatment of stool softeners and the topical anaesthetic 5% lignocaine cream [Elwakeel 2007].

Pharmacokinetic properties

Pharmacokinetics in animals

Absorption, metabolism, elimination
Pharmacokinetics of orally administered eugenol were assessed in rats following administration by gavage of 40 mg/kg for 5 successive days. Blood samples were collected over 24h. Eugenol in blood peaked rapidly, but mean $T_{1/2}$ values of eugenol in plasma and blood were quite long, at 14.0 h and 18.3 h respectively, suggesting a potential accumulation following repeated administration [Guenette 2007].

Eugenol is rapidly metabolized and excreted. Over 70% of an oral dose was excreted in the urine of rabbits. Eugenol had little or no effect on microsomal enzyme induction [Opdyke 1975b].

Blood and urinary samples from rats given a single intravenous dose of 20 mg/kg eugenol were collected over 1h for pharmacokinetic assessment. Mean systemic clearance from plasma and blood was 157 and 204 ml/min/kg respectively. Glucuronide and sulphate conjugate metabolites were identified in urine [Guenette 2006].

Pharmacokinetics in humans

Absorption, metabolism, elimination
Eugenol was rapidly absorbed and metabolized after oral administration to male and female healthy volunteers with 95% of the dose recovered in the urine within 24h. Most were phenolic conjugates (99%), plus nine metabolites of eugenol [Fischer 1990].

Dental healthcare/ anaesthetic
In vivo drug release studies were conducted with 6 healthy human volunteers using a eugenol-containing mucoadhesive layer (200 mg tablet contained 10 mg eugenol) stuck to the gums. The tablets remained on the gums up to 8h. Eugenol was completely released at 6h in 1 of 3 tested formulations [Jadhav 2004].

Preclinical safety data for clove oil and eugenol

Clove oil

Acute toxicity
In rats, the acute oral LD_{50} value of clove oil was 2.65-3.72 g/kg. In rabbits, the acute dermal LD_{50} was reported as 5g/kg [Opdyke 1975a].

There were no signs of toxicity, or alterations in food and water consumption, in rats during the 14 day period following a single oral dose of clove oil (2000 mg/kg b.w.).

Repeated dose and chronic toxicity studies
Rats tolerated daily oral doses of 35-70 mg clove oil well for 8 weeks. Higher doses led to inactivity and weight loss [Opdyke 1975a].

An estimated acceptable daily intake of eugenol up to 2.5 mg/kg b.w. for humans was established at the 26th and confirmed at the 65th Joint FAO/WHO Committee on Food Additives [Reynolds 1989].

Irritation of animal skin: Applied undiluted to backs of mice, clove oil was not irritating. Applied undiluted to abraded rabbit skin for 24h under occlusion it was mildly irritating [Opdyke 1975a].

Irritation of human skin: In 2 of 25 normal subjects, 20% clove oil in vaseline caused erythema in a closed-patch test on the skin. In 30 normal subjects tested at 2%, and in 35 subjects with dermatoses tested at 0.2%, no reaction was evoked. Tested at 5% in vaseline it produced no irritation after a 48h closed-patch test in humans [Opdyke 1975a].

Sensitization (human test): In a maximization test, clove oil at 5% in vaseline produced no sensitization reactions in 25 volunteers. In another study sensitization caused by clove oil was attributed to eugenol [Opdyke 1975a].

Human dental pulp: A zinc oxide-clove oil paste damaged the dental pulp in human dental cavities [Opdyke 1975a].

Mutagenicity and carcinogenicity
Clove oil was cytotoxic for HEP-2 cells (human carcinoma-derived) at ID_{50} = 7.31 µg/ml [Saenz 1996]. Clove oil and eugenol were both found to be cytotoxic to human fibroblasts and to a human transformed dermal endothelial cell line at concentrations as low as 0.03%V/V and 0.06%V/V, respectively. β-caryophyllene was not cytotoxic to these cells [Prashar 2006].

Reproductive toxicity
Motility and viability of human spermatozoa were investigated *in vitro*. Clove oil tested for immediate spermicidal activity was minimally effective at a concentration of 0.5 % V/V. Eugenol had a similar minimal effect at 0.33 %V/V [Buch 1988]. Female mating mice on a diet with additional 0.25% clove oil for 2 weeks, exhibited a slight but significant increase in embryo cell death at 0.8%, compared to control mice at 0.3% ($p<.0.01$) on day 4 of pregnancy [Domaracky, 2007].

Eugenol

Acute toxicity
Oral LD_{50} of eugenol in rats was 2.68 g/kg; in mice 3.00 g/kg; in guinea pigs 2.13 g/kg. Minor liver damage occurred in rats given oral doses of 900 mg/kg 4 times daily. The highest *no effect oral dose* in dogs was 200 mg/kg b.w. [Opdyke 1975b].

There were no signs of toxicity, or alterations in food and water consumption, in rats during the 14 day period following a single oral dose of eugenol (2000 mg/kg b.w.).

Repeated dose and chronic toxicity studies
After repeated application of a 5% eugenol emulsion to the mucosa of Heidenhain's pouch (mucous membranes) in dogs, the gastric mucous barrier showed degenerative and reparative changes [Opdyke 1975b].

Short term toxicity in rats: feeding male and female rats for 19 wk (0%, 0.1% and 1% eugenol in the diet, 3 groups of 20 rats) exerted no effect on growth, haematology or organ weights and histology [Opdyke 1975b].

The effect of one drop of topically applied, undiluted eugenol, when administered to a round 3mm diameter area of rat oral/labial mucosa for 1 minute, was observed by histological procedures after periods of 15 minutes up to 6 hours. This treatment resulted in denaturation of cytoplasmic proteins, loss of staining capacity of the epithelium, loss of cell boundaries, swelling and cell necrosis. In addition, vesicle formation, edema in the corium and striated muscle dissolution were observed. It was concluded that undiluted eugenol is injurious to labial mucous membranes [Kozam 1978].

Irritation of human skin: Mild irritation was observed in a study involving 25 human subjects topically treated with 8% eugenol

in vaseline for 48 h in a closed-patch test. A patch test using undiluted eugenol for 24h produced no reaction in 20 human subjects [Opdyke 1975b].

Sensitization (human test) by eugenol was tested in 25 human volunteers: a maximization test with 8% eugenol in vaseline, applied topically, produced no sensitization reactions [Opdyke 1975b].

Mutagenicity and carcinogenicity
Carcinogen (diethyl-nitrosamine)-induced microsomal degranulation of rat liver microsomes could be protected by 3mM eugenol [Selvi 1998]. Eugenol acted as an anti-mutagen in the *Salmonella typh*. mutagen-induced SOS response test (mutagen furylfuramide and others). Eugenol suppressed 29.9% of the SOS-inducing activity at 0.60 µM/ml [Miyazawa 2001]. Eugenol at 2500 µM induced chromosomal aberrations in V79 chinese hamster cells (3.5% aberrant cells). In the presence of biotransformation mix S9, aberrations increased dose-dependently to 15%. An increase of endoreduplicated cells was observed (up to 5% at 2000 µM eugenol) [Maralhas 2006].

Cell viability
Eugenol was metabolized by isolated myeloperoxidase and inhibited the oxidative burst in phorbol-stimulated human polymorphonuclear neutrophils. Intracellular glutathione levels decreased by 90% when the cells were exposed to 100 µM eugenol [Thompson 1989]. A review discussing the clinical use of eugenol, in dental materials, suggests that prolonged exposure to concentrations of 0.001 mol/L eugenol and higher may be toxic to mammalian cells, as well as to vital dental tissue, and should be avoided [Markowitz 1992]. Rat liver mitochondria membrane potential was decreased to 50% at 7.5 µmoles/mg protein for eugenol. Mitochondrial NADH oxidase was inhibited by eugenol in a dose dependent fashion [Usta 2002].

Clinical safety data
The total number of human patients, subjects and volunteers in studies, where either clove oil or eugenol were administered by cutaneous, oromucosal or anorectal route, is 276. In these studies no adverse reactions were reported, except for mild skin irritations by 8% eugenol and erythema caused by 20% clove oil [Opdyke 1975a; Opdyke 1975b; Fischer 1990; Jadhav 2004; Barnard 1999; Trongtokit 2005 and Elwakeel 2007].

A double-dummy, controlled, randomized clinical study involving 73 human subjects was conducted in 2 groups. The clove bud group consisted of 37 healthy volunteers; clove bud powder (containing 15-20% clove oil) was administered topically and investigated for its effect as an anaesthetic on the buccal mucosa. The positive control group (36 volunteers) received a benzocaine gel (20%). Treatments were on both sides of the mouth (dummy-placebo and active agent in each subject). Adverse effects were observed only in the clove bud group; 4 of 37 clove patients developed aphthous-like ulcers at the application site of the clove bud preparation. This adverse event could not be directly attributed to the clove oil content of the clove bud powder [Alqareer 2006].

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The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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The Scientific Foundation for Herbal Medicinal Products

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Centaury

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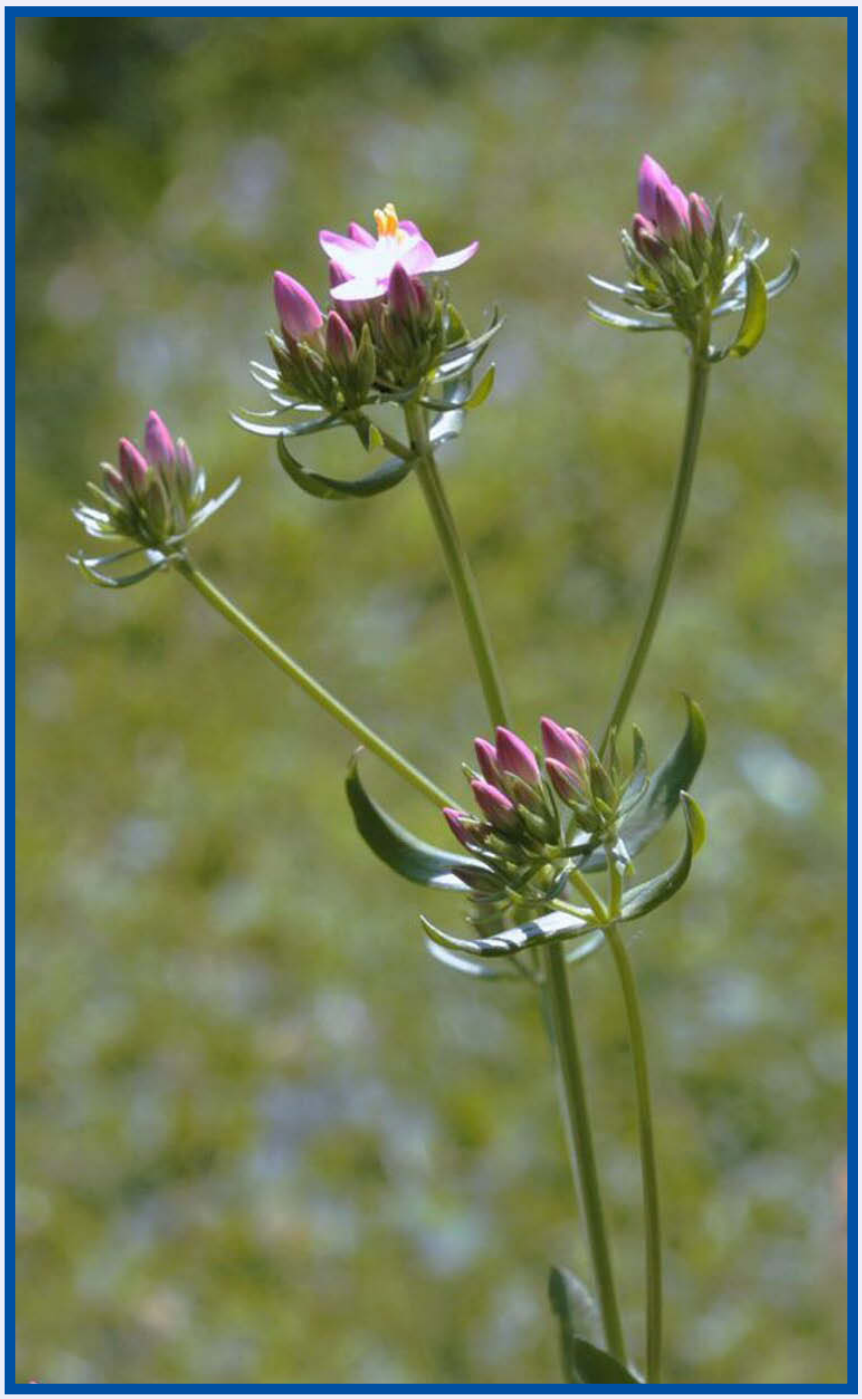

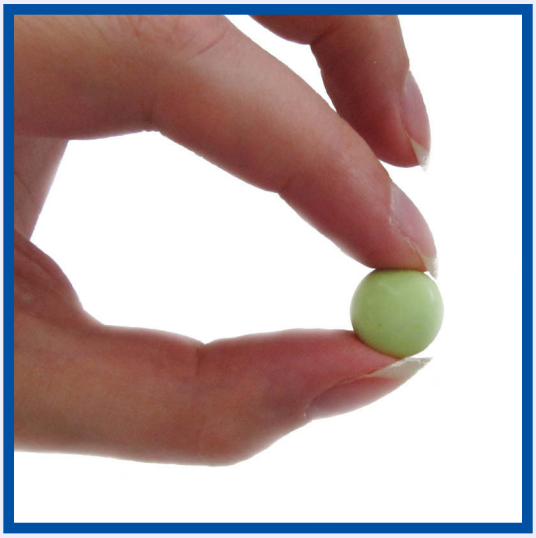

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ON PHYTOTHERAPY

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

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2015

Centaury

DEFINITION

Centaury consists of the whole or cut dried flowering aerial parts of *Centaurium erythraea* Rafn. s.l. including *Centaurium majus* Zeltner and *Centaurium suffruticosum* (Griseb.) Ronniger [syn. *Erythraea centaurium* Pers., *C. umbellatum* Gilib., *C. minus* Garsault].

The material complies with the European Pharmacopoeia [Centaury].

Fresh material may also be used, provided that when dried it complies with the European Pharmacopoeia.

CONSTITUENTS

The characteristic, bitter-tasting constituents are secoiridoid glucosides, principally swertiamarin (2-8%) and smaller amounts (0.1 to 0.5%) of gentiopicroside (gentiopicrin) and sweroside, with bitterness values of about 12,000 [van der Sluis 1985a, 1985b, Schimmer 1994a, Hoffmann-Bohm 2010, Aberham 2011]. Two intensely bitter *m*-hydroxybenzoyl esters of sweroside, centapicrin and deacetylcentapicrin, with bitterness values of about 4,000,000, are also present [Sakina 1976, van der Sluis 1985b, Schimmer 1994a]. Other iridoids include centauroside (a dimeric secoiridoid), secologanin, 6′-*m*-hydroxybenzoyl-loganin [Takagi 1982], dihydrocornin (a cyclopentane iridoid), gentioflavoside [Do 1987] and the secoiridoid alkaloid gentianine [Rulko 1972, Bishay 1978].

Various methoxylated xanthones [Nestha 1982, 1983, 1984, van der Sluis 1985a, 1985b, Schimmer 1994a, Valentao 2000, 2002, Hoffmann-Bohm 2010] including eustomin (1-hydroxy-3,5,6,7,8-pentamethoxyxanthone) and 8-demethyleustomin [Schimmer 1996].

Other constituents include phenolic acids, such as *p*-coumaric, *o*-hydroxyphenylacetic, ferulic, protocatechuic, sinapic, vanillic [Hatjimanoli 1977, Dombrowicz 1988, Sharaf 2003], hydroxyterephthalic and 2,5-dihydroxyterephthalic acids [Hatjimanoli 1988]; flavonoids [Sharaf 2003]; phytosterols (β-sitosterol, stigmasterol, campesterol and others) [Popov 1969, Aquino 1985, Loizzo 2008]; 5-formyl-2,3-dihydroisocoumarin [Valentao 2003a]; amino acids [Petlevski 2002]; small amounts of essential oil (up to 0.02%) [Jovanović 2009, Jerković 2012] and triterpenoids [Bellavita 1974, Loizzo 2008, Jäger 2009].

CLINICAL PARTICULARS

Therapeutic indications

Dyspeptic complaints; lack of appetite [Centaurium 1983, Bisset 1994, Leung 1996, Newall 1996, Hoffmann-Bohm 2010].

Posology and method of administration

Dosage

Adults: 1-4 g of the drug as a maceration, infusion or decoction in 150 mL of water, up to 3 times daily [Centaurium 1983, Bisset 1994, Leung 1996, Newall 1996, Hoffmann-Bohm 2010]; 2-4 mL of liquid extract (1:1, ethanol 25 % V/V), up to 3 times daily [Centaurium 1983, Newall 1996, Hoffmann-Bohm 2010]; tincture (1:5, ethanol 70 % V/V), 2-5 g daily [Hoffmann-Bohm 2010].

Children: proportion of adult dose according to age or body weight, in ethanol-free dosage forms.

The dosage may be adjusted according to the bitterness sensitivity of the individual.

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Method of administration
For oral administration in liquid preparations. For lack of appetite, administered half to one hour before meals [Hoffmann-Bohm 2010]; for dyspepsia, taken after meals.

Duration of administration
No restriction. If symptoms persist, medical advice should be sought.

Contra-indications
As with other drugs containing bitter substances, peptic ulcers are a contra-indication for centaury [Hoffmann-Bohm 2010].

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy or lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antioxidant and radical scavenging activities
A lyophilized hot water extract of centaury (7.5:1) exhibited antioxidant activity as shown by scavenging of superoxide radical in the NADH/PMS system (IC_{50} 120.2 µg/mL) as well as xanthine oxidase inhibitory activity (IC_{50} 73.2 µg/mL) [Valentao 2001].

In the hydroxyl radical assay a lyophilized infusion (1:100; yield 13.4%) showed an IC_{50} of 44.4 µg/mL [Valentao 2003b].

An infusion of powdered centaury (1:110; 100 µL) led to a 73.3% inhibition of desoxyribose decomposition [Gião 2008].

Methanolic extracts demonstrated moderate to low radical scavenging and antioxidant activities in various assays [Šiler 2014, Tusevski 2014].

Antimicrobial activity
In a panel of 17 bacteria gentiopicroside was most active against *Serratia marescens* with a MIC of 6.3 µg/mL [Kumarasamy 2003a]. In a similar study on 14 different bacteria swertiamarin showed the highest activity against *Citrobacter freundii* (MIC 5 µg/mL) [Kumarasamy 2003b].

Other effects
A chloroform extract containing mainly terpenes and fatty acids moderately inhibited α-amylase and α-glucosidase with IC_{50} values of 64.9 and 74.9 µg/mL respectively. The effect against angiotensin converting enzyme was weak [Loizzo 2008].

In vivo experiments

Anti-inflammatory activity
In the air pouch granuloma test in rats a dry aqueous extract of centaury (3.8:1), applied topically as 5% and 10% creams, exhibited significant transdermal anti-inflammatory activity compared to placebo ($p<0.01$). The anti-inflammatory activity of the extract was also demonstrated in Freund's adjuvant-induced polyarthritis, in rats treated orally with doses of 10-500 mg per day ($p<0.01$) [Berkan 1991].

A dry ethanolic extract, administered orally to rats at 100 mg/kg b.w., inhibited carrageenan-induced paw oedema by 40 % compared to control ($p<0.01$) [Capasso 1983].

Gastroprotective effects
Male Sprague-Dawley rats were treated intragastrically with a 50% ethanolic extract at 100 mg/kg b.w. for 7 days and additionally on day 7 with 200 mg/kg acetylsalicylic acid (ASA) 4 hours before sacrifice. Controls received saline during the same period and 200 mg/kg ASA on day 7. The extract led to a 77% inhibition of gastric lesions ($p<0.05$). The increase in catalase and malondialdehyde and the decrease in reduced glutathione caused by ASA were improved by the extract. The augmentation of myeloperoxidase activity, as an index of neutrophil infiltration into gastric mucosal tissue due to ASA, was significantly reduced ($p<0.05$) as compared to control [Tuluce 2011].

Effects on hyperglycaemia and lipid profiles
Male C57BL/6J mice received a high fat diet to induce type-2 diabetes. Simultaneous intragastric treatment with a dry extract (80% ethanol, yield 17.3 %) at a dose of 2 g/kg b.w. daily for 20 weeks resulted in a significant reduction of the mean fasting blood glucose level ($p<0.05$) from week 14 until week 20 as compared to untreated animals on the high fat diet. At the end of the experiment, plasma insulin concentrations, insulin resistance calculated by the homeostasis model assessment and plasma triglyceride levels were also significantly lower ($p<0.05$). No significant differences were observed between the treated group on high fat diet and controls on standard diet [Hamza 2010].

In a similar experiment type-2 diabetes was first established by 17 weeks of high fat diet. After this period treatment was started with the extract (80% ethanol, yield 17.3 %) at 2 g/kg b.w. daily for 18 weeks. At the end of the study body weight, mean fasting blood glucose level, plasma insulin concentration and insulin resistance were significantly better ($p<0.05$) as compared to untreated animals on the high fat diet. Administration of the extract also improved hypertriglyceridaemia and hypercholesterolaemia ($p<0.05$) without influencing HDL-cholesterol [Hamza 2011].

In streptozotocin (STZ)-induced diabetic male Wistar rats, treatment with 200 mg/kg b.w. of a lyophilized aqueous extract (1:10; yield 12%) for 30 days resulted in a significant reduction of serum glucose ($p<0.001$) and increase of serum insulin levels ($p<0.01$). Elevated triglycerides and total cholesterol in the diabetic rats were decreased significantly ($p<0.001$) by the extract. Markers of oxidative stress such as pancreatic malondialdehyde, protein carbonyl content and GSH as well as the decline in antioxidant enzymes in the pancreas (SOD, catalase, GSH peroxidase) due to the induced diabetes were improved significantly ($p<0.001$ to $p<0.01$) by the extract. Histological examination of the pancreas revealed a significant ($p<0.01$) increase in number and diameter of islets in the extract group as compared to the untreated diabetic group [Sefi 2011].

The effects of centaury on blood glucose levels, hepatic glycolytic

and gluconeogenic enzymes and their substrates, as well as on the lipid profile, were studied in male Wistar rats. Ten days after induction of diabetes with STZ the animals were treated intragastrically with a methanolic dry extract (yield 23.1%) for 12 days with 125, 250 or 500 mg/kg b.w. per day (short term treatment s.t.t.) or for 45 days with 250 mg/kg b.w. per day (long term treatment l.t.t.). Glibenclamide (2.5 mg/kg b.w. intragastrically) served as positive control. During s.t.t. blood glucose concentrations were significantly ($p<0.05$) reduced by all doses as compared to the start of the treatment. In l.t.t. the positive effect on blood glucose regulation was most pronounced up to day 30. The extract and glibenclamide normalised the diabetes-induced increases in liver glucose concentration, glucose-6-phosphatase and fructose-1,6-biphosphatase activity, as well as the decreases in liver glycogen content and glycogen phosphorylase activity. Increased triglycerides, LDL, HDL and cholesterol levels in diabetic rats were restored to the levels of untreated normal rats by all doses of the extract in s.t.t. [Stefkov 2014].

Hepatoprotective effects
A dried methanolic extract (6.2:1) was tested for its hepatoprotective effects in male Wistar rats receiving the extract intragastrically at a dose of 300 mg/kg b.w. for 6 days or at a single dose of 900 mg/kg before challenge with paracetamol. The strong increase in SGPT, SGOT and LDH levels induced by the acute administration of paracetamol was markedly attenuated by both pretreatments. The extract alone (300 mg/kg b.w. for 6 days; no paracetamol challenge) slightly reduced LDH but did not change SGPT and SGOT as compared to the untreated control. Histopathological examination of the livers confirmed the hepatoprotective effects of the extract [Mroueh 2004].

Isolated gentiopicroside significantly inhibited the production of TNF in carbon tetrachloride-induced and bacillus Calmette-Guérin/lipopolysaccharide-induced models of hepatic injury in mice after i.p. injection at 30 mg/kg ($p<0.05$) or 60 mg/kg b.w. ($p<0.01$) daily for 5 days [Kondo 1994].

Other effects
The antipyretic activity of a dry aqueous extract of centaury (3.8:1) was demonstrated in rats after intragastric administration of 50-100 mg in a yeast-induced fever test ($p<0.05$) [Berkan 1991].

A diuretic effect was shown in rats after oral administration of 8% or 16% aqueous extract of centaury at 10 mL/kg b.w. daily for one week. From the fifth day of treatment urine volume was increased significantly ($p<0.05$) with the lower dose, and both doses led to a significant increase of sodium, chloride and potassium excretion ($p<0.01$ – $p<0.001$). At the end of the treatment a diminution in creatinine clearance was observed ($p<0.05$ for the lower dose) [Haloui 2000].

Pretreatment of rats with a 50% ethanolic extract at an intragastric dose of 100 mg/kg b.w. for 7 days significantly ($p<0.05$) improved the increases in ALAT, ASAT, creatinine and potassium induced by administration of 200 mg/kg of ASA on day 7 [Ozkol 2011].

Larvae of red flour beetles (*Tribolium castaneum*) on a diet containing 10% of a methanolic extract gained significantly ($p<0.05$) less weight over 8 days than untreated control and starved larvae. Larval mortality was 62% after ten days and emergence of adult insects reduced to 33.7% as compared to 0% and 100%, respectively, in untreated control. Treated larvae had a significantly reduced α-amylase activity ($p<0.05$). Progeny production of F1 adults was completely suppressed by the extract [Jbilou 2008].

Isolated swertiamarin showed anticholinergic activity, significantly inhibiting carbachol-induced contractions of the proximal colon in rats in a dose-dependent manner ($p<0.05$) after oral administration at 150 mg/kg and 300 mg/kg b.w. [Yamahara 1991].

Clinical studies
No published clinical data currently available.

Pharmacokinetic properties
By anaerobic incubation with a mixture, and with individual strains, of human intestinal bacteria, swertiamarin was converted to three metabolites: erythrocentaurin, 5-hydroxymethylisochroman-1-one and gentianine. This demonstrated that orally administered swertiamarin can be transformed into a nitrogen-containing, biologically active substance by flora of the human gastrointestinal tract [El-Sedawy 1989].

Preclinical safety data

Acute toxicity
A dried aqueous decoction (1:10; yield not specified) administered orally at doses of 1 to 15 g/kg b.w. to male and female mice did not cause any signs of toxicity at any dose level. After i.p. administration at doses of 2 to 14 g/kg b.w. adverse effects such as anorexia, piloerection and hypoactivity were observed at higher doses from 8 g upwards; asthenia, diarrhoea and convulsions occurred in both sexes at doses above 12 g/kg b.w. The i.p. LD_{50} was calculated as 12.13 g/kg [Tahraoui 2010].

Isolated iridoids were shown to be toxic in the brine shrimp lethality assay (LC_{50} values: gentiopicroside 24 µg/mL, swertiamarin 8.0 µg/mL, sweroside 34 µg/mL, podophyllotoxin as control 2.7 µg/mL) [Kumarasamy 2003a,b].

Repeated dose and chronic toxicity studies
A dried aqueous decoction (1:10; yield not specified) was administered orally to male and female Wistar albino rats at 100, 600 and 1200 mg/kg b.w. daily for 90 days. No lethality or significant differences in body weight changes or in animal behaviour were noted in any of the groups. Blood cell counts were unaffected and haematological parameters remained within normal limits throughout treatment. The only change was a slight but significant decrease in mean red blood cell volume (600 mg/kg: $p<0.05$ after 30 days and $p<0.01$ after 60 and 90 days; 1200 mg/kg: $p<0.05$ after 90 days; compared to control). In the biochemical profiles plasma creatinine, urea, total proteins, total bilirubin, cholesterol, ALAT and ASAT remained unchanged. Blood glucose and triglycerides were significantly reduced ($p<0.05$) at doses of 600 and 1200 mg/kg from 60 days until the end of treatment [Tahraoui 2010].

Mutagenicity and carcinogenicity
Centaury extracts have been evaluated in the Ames mutagenicity test using *Salmonella typhimurium* strains TA98 and TA100 (with and without activation by S9 mix), with rather conflicting results. An inspissated extract showed weak mutagenicity in TA98, but negative results were obtained with a fluid extract and a tincture in strains TA98 and TA100 [Schimmer 1994b]. In another study, a fluid extract and a tincture showed weak mutagenicity in strain TA100, but not in strain TA98 [Göggelmann 1986].

An ethanolic extract of centaury at 200 µL/plate displayed markedly antimutagenic potency in *Salmonella typhimurium* strains TA98 and TA100, inhibiting mutagenicity induced by 2-nitrofluorene (2-NF) and 2-aminoanthracene (2-AA) by over 50 %. Furthermore, isolated eustomin at 50 µg/plate showed strong inhibition, 76 % against 2-NF and 64 % against 2-AA in strain TA100; 8-demethyleustomin was also active, with results of 43 % and 39 % respectively, but no inhibition was detected from secoiridoid or polar fractions of centaury [Schimmer 1996].

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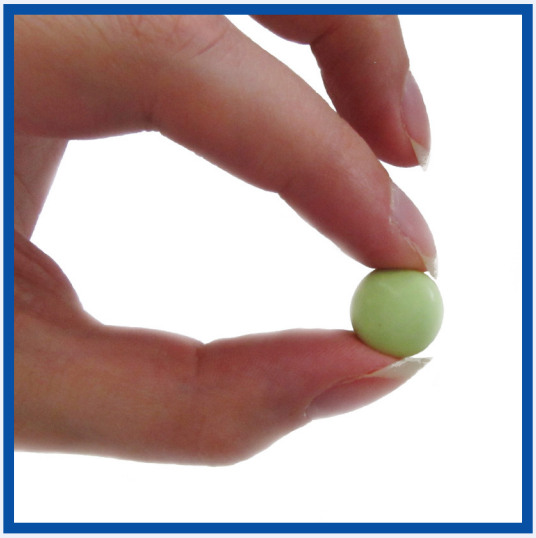

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FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

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We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

CHAMOMILLAE ROMANAE FLOS 2019

Roman Chamomile Flower

DEFINITION

Roman chamomile flower consists of the dried flower-heads of the cultivated double flowered variety of *Chamaemelum nobile* (L.) All. (syn. *Anthemis nobilis* L.). It contains not less than 7 mL/kg of essential oil.

The material complies with the monograph of the European Pharmacopoeia [Chamomile Flower, Roman].

CONSTITUENTS

The main characteristic constituents are the essential oil (0.6-2.4%) [Zwaving 1982], flavonoids [Herisset 1971, 1973, 1974] and derivatives such as apigeninglycosides [Chao-Mei 2007; Tschan 1996], acidic polysaccharides (3.9%) [Lukacs 1990], catechins [Herisset 1970], coumarins [Zwaving 1982], hydroperoxides such as 1-β-hydroperoxyisonobilin and allylhydroperoxides [Rücker 1989; Mayer 1987], octulosonic acid derivatives [Zhao 2014], β-amyrin and sterols such as taraxasterol, pseudotaraxasterol and β-sitosterol [Power 1914; Zyczyńska-Baloniak 1971; Fauconnier 1996].

The essential oil contains approximately 75%-85% aliphatic esters of angelic, tiglic, isovaleric and isobutyric acids, where angelates represent approximately 65% of the oil with isobutyl angelate (25%-32%) predominating [Omidbaigi 2003, 2004]. The main flavonoid glycoside (1.5-2.4%) is chamaemeloside (apigenin 7-glucoside-6''-(3'''-hydroxy-3'''-methyl-glutarate) [Chaumont 1969; Tschan 1996; Chao-Mei 2007] among other phenolic compounds [Avula 2013].

Other constituents include cerotic, stearic, palmitic, oleic and linoleic acids [Power 1914; Duarte 2005; Bail 2009; Guimarães 2013]. Monoterpenes (estragol, α-pinene) and sesquiterpenes of the germacranolide type including nobilin [Benešová 1964, 1970], 3-epinobilin, 1,10-epoxynobilin, 3-dehydronobilin and hydroxyisonobilin [Holub 1977; Tognolini 2006]. Spiroethers and the coumarin herniarin have also been identified [Wichtl 2009; Chao-Mei 2007].

CLINICAL PARTICULARS

Therapeutic indications

Internal use
Symptomatic treatment of mild dyspeptic complaints, such as bloating, nausea, flatulence and lack of appetite [Ellingwood 1919; Felter 1922; Rápóti 1974; Lukacs 1990; Bradley 1992; Rivera 1995; Duarte 2005; Wichtl 2009].

External use
For minor inflammations of the oral mucosa and skin in wounds and abrasions; as an itch-relieving agent [Bradley 1992; Bruneton 1999; Wichtl 2009]; for eye irritation or discomfort [Rivera 1995].

These indications are plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Internal use
Adult daily dose
Preparations equivalent to 1.5-12 g of the drug [Ellingwood 1919; Bradley 1992; Wichtl 2009].
Liquid extract (DER 1:1 in 70% alcohol): 1–4 mL three times daily [Barnes 2007].

Not recommended for children below 12 years.

External use
Topical use as a 3% infusion [Wichtl 2009] in poultices or mouthwashes, or as semi-solid preparations containing 5-15% of the drug or equivalent [Bradley 1992]. As a bath additive, 50 g are added to 10 litres of water; 'liquid rubs' are applied as poultices or washes 2 to 3 times daily [Gruenwald 2004].

Method of administration
Oral administration; oromucosal and topical applications.

Duration of use

Internal use
No restriction.

External use in poultices or mouthwashes
No restriction.

If symptoms persist or worsen, medical advice should be sought.

Contraindications
Persons with known sensitivity to plants of the Asteraceae family [Blumenthal 1998; Brinker 2001, 2008]. Nobilin and its derivatives are reported as the probable contact allergens of Roman chamomile [Benner 1973; McGeorge 1991; Bossuyt 1994; Hausen 1997; Pereira 1997; Giordano-Labadie 2000; Maddocks-Jennings 2004].

Special warnings and special precautions for use
None required.

Interaction with other medicinal products and other forms of interaction
As a precaution, Roman chamomile should not be used concomitantly with aspirin, warfarin or other substances possessing anticoagulant activitiy [Bratman 2003; Heck 2000].

Since apigenin is a ligand for the central benzodiazepine receptor, Roman chamomile should not be used concurrently with diazepam or other benzodiazepines, as this may potentiate their action [Paladini 1999; Viola 1995].

Some constituents may inhibit cytochrome P450 3A4 enzymes [Budzinski 2000]. Roman chamomile essential oil exhibited ≤40% inhibition of CYP3A4 activity [Cochrane 2015].

Pregnancy and lactation
In accordance with general medical practice it should not be taken orally during pregnancy and lactation without medical advice [Farnsworth 1975; Mills 2005].

Effects on ability to drive and use machines
None known.

Undesirable effects
None known.

Overdose
No case of overdose reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antimicrobial activities
The essential oil and various pure compounds were tested for antimicrobial activity at concentrations in the range of 1-1000 ppm. The oil and several of its constituents showed high antimicrobial activity (MIC=60 µg/mL) against *Salmonella sp.* and *E. coli*. The main constituent, isobutyl angelate, showed high activity (MIC=60 µg/mL) against *Staphylococcus aureus* and *Klebsiella pneumoniae*. The oil and most of the constituents tested also showed lower activity (MIC=600 µg/mL) against *S. aureus, Enterococcus faecali, Pseudomonas aeruginosa, Proteus vulgaris, K. pneumoniae* and the yeast *C. albicans* [Duarte 2005; Bail 2009].

The essential oil showed inhibitory activity on selected bacteria, fungi and viruses [Chao 2000], antibacterial activity against clinically isolated *Porphyromonas gingivalis* from periodontitis [Saderi 2005] and inhibition of methicillin-resistant *S. aureus* (MRSA) [Chao 2008].

A dry methanolic extract (100 mg), dissolved in 5 ml of 2% DMSO and then diluted to various concentrations in water, exhibited significant nematocidal activity when 5 mg/mL ($p<0.05$) and 10 mg/mL ($p<0.001$) were added to wells containing a 10 µl *Steinernema feltiae* nematode suspension (30-40 nematodes/well) [Al-Marby 2016].

A dichloromethane extract exhibited promising *in vitro* antiprotozoal activity against *Trypanosoma brucei rhodesiense* (IC_{50} 1.43 ± 0.50 µg/mL) and *Leishmania donovani* (IC_{50} 1.40 ± 0.07 µg/mL) [De Mieri 2017].

Antioxidant activities
Roman chamomile oil exhibited the highest antioxidant activity when compared with eleven aromatic plants typical of the Mediterranean area [Piccaglia 1993; Povilaityte 2000].

Antiplatelet activity
The essential oil exhibited a modest and dose-dependent inhibition of platelet aggregation in guinea pig platelet-rich plasma induced by three agents: ADP, arachidonic acid and a thromboxane A2 agonist. Fibrin clot retraction induced by thrombin was decreased by less than 50% by the oil at 300 µg/mL [Tognolini 2006].

Cytostatic activities
Nobilin, 1,10-epoxynobilin, 3-dehydronobilin and hydroxyisonobilin, as isolated constituents from Roman chamomile flower, showed *in vitro* cytostatic activity against human HeLa (cervix carcinoma cell line) and KB (nasopharyngeal carcinoma) cell lines. ED_{50} values for hydroxyisonobilin were 0.5 µg/mL and 1.23 µg/mL for HeLa and KB cell lines respectively [Holub 1977; Samek 1977; Grabarczyk 1977].

A study comparing the effects of a methanolic extract, a decoction and an infusion, on the growth of five human tumour cell lines (MCF-7, NCI-H460, HCT-15, HeLa and HepG2), demonstrated that the extract was more potent than the infusion in all the tested cell lines, presenting GI_{50} values (fifty percent of maximum inhibition of cell proliferation) that ranged from 82.52 to 168.40 µg/mL for the MCF-7 and HepG2 cells respectively. The decoction showed no antitumour activity at the maximum concentration (400 µg/mL) used [Guimarães 2013].

An ethyl acetate fraction obtained from Roman chamomile aerial parts demonstrated growth inhibitory activity on several cancer cell lines but had a minimal effect on normal cells. The strongest cytotoxic activity was obtained after 72 hours in MCF-7 breast cancer cells, with an IC_{50} of 0.002 mg/ml. The fraction also exhibited antiproliferative activity on breast cancer cells and induced apoptosis by activating the mitochondrial apoptotic pathway [Kandelous 2016].

Other activities
A hydroethanolic extract, various fractions, the essential oil and 4 component flavonoids were examined for their effect on rat, guinea pig and human gastrointestinal smooth muscle preparations. The extract demonstrated a transient stimulation of smooth muscle contraction as well as a more sustained relaxing effect on precontracted smooth muscle (histamine-stimulated contraction; and pre-treated with atropine and tetrodotoxin), while the oil exhibited only the relaxing effect [Sandor 2018].

Six caffeoyl derivatives possessing a rare octulosonic acid skeleton were identified and isolated from the ethyl acetate fraction of a methanolic extract. At a concentration of 30 μM, two compounds showed an increase in LXR (liver X receptor) activity (1.59- and 1.22-fold). Another three compounds increased PPARγ activity (1.68-, 1.59- and 2.09-fold), while PPARα was activated by two of the compounds only (1.43- and 1.35-fold at 30 μM). All 6 of the compounds increased NAG-1 (NSAIDs-activated gene-1) activity by 2-3 fold at 50 μM. No cytotoxicity was observed for any of these compounds against mouse macrophages (RAW 264.7) or human hepatoma cells (HepG2) up to a concentration of 50 μM. A methanolic extract demonstrated inhibition of intracellular oxidative stress (45% inhibition of ROS generation at 200 μg/mL) and inhibition of NF-κB activity (50% inhibition at 17 μg/mL). The extract was also found to activate NAG-1 (2.2-fold at 50 μg/mL), PPARα (1.8-fold at 25 μg/mL), and PPARγ (3.0-fold at 25 μg/ mL) [Zhao 2014].

In vivo experiments

Anti-inflammatory effects
Polysaccharides isolated from an aqueous extract (10 mg/kg i.p.) reduced rat paw oedema by 36-38% compared to untreated controls. Indomethacin was used as a positive control and showed 48.6% inhibition [Lukacs 1990].

In the same model, Roman chamomile oil (350 mg/kg b.w. i.p.) reduced oedema by 23-39% after two hours and 38-43% after 3 hours. The positive control indomethacin (5 mg/kg b.w. i.p.) demonstrated 74% and 67% inhibition respectively [Melegari 1988; Rossi 1988].

Antimicrobial and wound healing activity
An ointment prepared with 5% of a dried ethanolic extract in a eucerin base gel (0.5 g/day for 2 weeks) applied to incision wounds in rats experimentally infected with *Pseudomonas aeruginosa*, eradicated the infection significantly ($p<0.05$) faster and reduced healing time compared with a tetracycline ointment and with the base gel [Kazemian 2018].

Diuretic and hypotensive effect
In spontaneously hypertensive rats a single oral dose of a dry aqueous extract (140 mg/kg b.w.) produced a significant ($p<0.05$) reduction in systolic blood pressure (SBP) after 24 h. Daily oral administration (140 mg/kg b.w.) for 3 weeks produced a significant ($p<0.01$) reduction in SBP on day 8 of treatment. The hypotensive effect was associated with a significant ($p<0.01$) increase in urinary output and excretion of electrolytes, from day 8 to the end of treatment [Zeggwagh 2009].

Hypoglycaemic effect
A single oral dose of an aqueous extract (20 mg/kg b.w.) signifcantly reduced blood glucose levels from 6.0 ± 0.3 mmol/l to 4.9 ± 0.09 mmol/l ($p<0.05$) 6 h after administration in normal rats and from 21.1 ± 1.3 mmol/l to 14.5 ± 0.9 mmol/l ($p<0.001$) in streptozotocin-induced diabetic rats. Furthermore, after daily administration of the same dose for 15 days, blood glucose levels were decreased from 6.1 ± 0.06 mmol/l to 4.6 ± 0.17 mmol/l ($p < 0.01$) and from 21.1 ± 1.31 mmol/l to 13.7 ± 0.90 mmol/l ($p<0.01$) in normal and diabetic rats respectively. Basal plasma insulin concentrations remained unchanged after treatment in both normal and diabetic rats suggesting a mechanism independent of insulin secretion [Eddouks 2005].

Psychomotor activity
Roman chamomile essential oil (though not other extracts) significantly ($p<0.05$) increased the ambulatory activity of mice in the 60 mins following an i.p. injection of 400, 1600 and 3200 mg/kg b.w. when compared to an olive oil control. Three main constituents were isolated and identified as having a significant effect, isobutyl angelate, isoamyl angelate and 2-methylbutyl isobutyrate. However, a combination of these three constituents did not fully reproduce the activity of the essential oil suggesting other constituents also play a role in the ambulatory effect. Dopamine antagonists (chlorpromazine and haloperidol) attenuated the effects of the isolated constituents [Umezu 2017].

Clinical studies
In a randomised, double-blind, placebo-controlled study involving 55 patients with oral lichen planus, the effect of a gel containing 2% chamomile (chamomile preparation not futher defined; n=26) was compared to a placebo base gel (n=29), when applied topically to the affected areas of the oral cavity at 0.5 ml three times per day for 4 weeks. In the chamomile group, 92% of patients showed some improvement with 5 patients showing a complete resolution, while in the placebo group only 19.2% showed a response to treatment and none showed complete resolution. The chamomile group also exhibited significant improvements compared to baseline in pain ($p<0.001$), burning sensation ($p<0.001$), itching ($p=0.011$), Thongprasom Index ($p=0.001$) and Oral Health Impact Profile 14 ($p<0.001$); while there were no significant changes found in the placebo group [Lopez Jornet 2016].

Pharmacokinetic properties
No data available.

Preclinical safety data

Single dose toxicity
The acute oral LD_{50} of Roman chamomile flower oil was more than 5 g/kg b.w. in rats. Applied to the backs of hairless mice it produced no irritating effects. On intact or abraded rabbit skin for 24 hours under occlusion, the oil was only mildly irritating and lacked phototoxic effects [Opdyke 1974].

Genotoxicity and mutagenicity
Roman chamomile oil possessed *in vitro* genotoxic properties in the *Bacillus subtilis* rec-assay and Salmonella/microsome reversion assay [Zani 1991].

Clinical safety data

Reproductive and developmental toxicity
After case-control analysis of 424 women with low weight newborns (< 2500 g), no statistically significant associations were found between the use of Roman chamomile tea during the last 2 trimesters of pregnancy, either alone or in combination with other herbal products, and the risk of low birth weight [Moussally 2012].

Allergic reactions
Two cases of contact dermatitis of the nipple were reported in breast-feeding women after using a Roman chamomile containing ointment [McGeorge 1991].

Sensitization to Roman chamomile (dried flowers, oil) has been reported [Benner 1973; Hausen 1997], and has been discussed in relation to compresses containing Roman chamomile in patients with a known positive allergic reaction to a sesquiterpene lactone mix [Bossuyt 1994; Pereira 1997; Giordano-Labadie 2000].

An idiosyncratic allergic reaction (head rush, tachycardia and nausea) was reported after inhaling Roman chamomile oil that had been dropped on to a strip in an aromatherapy class exercise [Maddocks-Jennings 2004].

A study evaluating which constituents of two commercial Compositae mixes would be the most useful as screening agents involved 76 patients testing positive to Compositae mix 6% in petrolatum and 29 patients testing positive to Compositae mix in 5% petrolatum. All patients were tested with the constituents of the respective mixes, but none were found to be positive to Roman chamomile [Paulsen 2012].

A safety assessment review of various Roman chamomile preparations (flower extract, oil, powder and water) used in cosmetics at a concentration of up to 10% (flower water) concluded these ingredients are safe at these concentrations, when used in non-sensitizing formulations [Johnson 2017].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Online Series, 2019
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHAMOMILLAE ROMANAE FLOS	Roman Chamomile Flower	Online Series, 2019
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Online Series, 2018

JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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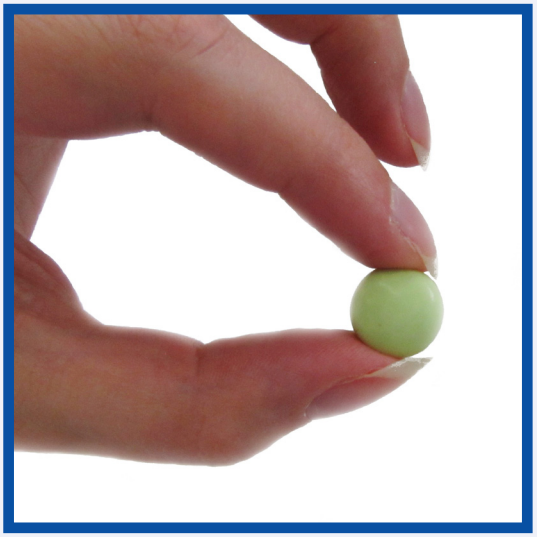

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

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Members of ESCOP
Board of Supervising Editors
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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin

i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection

CIMICIFUGAE RHIZOMA 2011

Black Cohosh

DEFINITION

Black cohosh consists of the dried rhizomes and roots of *Actaea racemosa* L. [*Cimicifuga racemosa* (L.) Nutt.] harvested in summer or autumn.

To comply with the monograph of the United States Pharmacopeia [Black Cohosh USP] the material should contain not less that 0.4 per cent of triterpene glycosides, expressed as 23-*epi*-26-deoxyactein ($C_{37}H_{56}O_{10}$; M_r 660.8) and calculated with reference to the dried drug.

A draft monograph intended for inclusion in the European Pharmacopoeia has been published [Black Cohosh]; it requires not less that 1.0 per cent of triterpene glycosides, expressed as monoammonium glycyrrhizate ($C_{42}H_{65}NO_{16}$; M_r 840) and calculated with reference to the dried drug.

CONSTITUENTS

Triterpene glycosides with a highly oxidized cycloartane-type skeleton, glycosylated at the C-3 position. Acetylacteol, cimigenol and shengmanol represent the major aglycones, and arabinose and xylose the dominant sugar moieties [Bedir 2000, Bedir 2001, Chen 2002a, Chen 2002b, Chen 2007, He 2000, He 2006, Li 2006, Mimaki 2006, Nuntanakorn 2006, Shao 2000, Watanabe 2002, Wende 2001].

The major glycosides have been identified as actein [Chen 2002b, Ganzera 2000, He 2000, He 2006, Li 2002], cimicifugoside[1] [He 2000, He 2006, Piancatelli 1971, Shao 2000], cimiracemoside A[2] [Bedir 2000, Ganzera 2000, He 2006] and 23-*epi*-26-deoxyactein [Chen 2002b, He 2006], formerly known as 27-deoxyactein [Berger 1988, He 2000, Shao 2000]. Cimigenol-3-*O*-α-L-arabinoside has been proposed as a species-specific marker glycoside for *C. racemosa* [He 2006]. A chlorine-containing triterpene glycoside might be an artefact [Chen 2007].

Phenylpropanoids (min. 0.5% expressed as caffeic acid), principally derivatives of hydroxycinnamic acids:

- Caffeic, ferulic and isoferulic acids [He 2006, Kruse 1999].
- Hydroxycinnamic acid esters of fukiic acid, e.g. fukinolic acid, cimicifugic acid A, cimicifugic acid B and cimicifugic acid G [He 2006, Kruse 1999, Nuntanakorn 2006].
- Hydroxycinnamic acid esters of piscidic acid, e.g. cimicifugic acid D, cimicifugic acid E and cimicifugic acid F [He 2006, Kruse 1999, Stromeier 2005].
- Other phenylpropanoid esters such as methyl caffeate [Chen 2002c], caffeoylglycolic acid, cimiciphenol, cimiciphenone [Stromeier 2005], petasiphenone [Jarry 2007, Stromeier 2005] and cimiracemates A-D (phenylpropanoid ester dimers) [Chen 2002c].

Other constituents include a cyclic guanidine alkaloid, cimipronidine (also proposed as a potential marker for *C. racemosa*) [Fabricant 2005], N_ω-methylserotonin [Powell 2008], starch, fatty acids, resin and tannins [Beuscher 1995].

[1] Cimicifugoside (cimigenol 3-O-β-D-xyloside) as isolated by He et al. [He 2000], Shao et al. [Shao 2000] and earlier by Piancatelli and coworkers [Piancatelli 1971] from *Cimicifuga racemosa*; in some literature it is called cimigoside or cimifugoside. A different 'cimicifugoside' has been isolated from the rhizoma of *Cimicifuga simplex* by Kusano et al. [Kusano 1998]; it is the 3-*O*-xyloside of an aglycone with a cyclolanost-7-ene structure.

[2] Cimiracemoside A (cyclolanost-7-ene 3-*O*-β-D-xyloside) as isolated by Bedir and Khan [Bedir 2000] and mentioned later by Ganzera et al. [Ganzera 2000] and He et al. [He 2006]. At almost the same time a different 'cimiracemoside A', identified as 21-hydroxycimigenol-3-*O*-α-arabinoside, was isolated from black cohosh by Shao et al. [Shao 2000].

CLINICAL PARTICULARS

Therapeutic indications
Climacteric symptoms such as hot flushes, profuse sweating, sleep disorders and nervous irritability [Osmers 2005, Bai 2007, Nappi 2005, Wuttke 2003b, Hernández Muñoz 2003, Kaiser 2008, Frei-Kleiner 2005, Oktem 2007].

Posology and method of administration

Dosage

Adult daily dose
Isopropanolic (40%V/V) [Osmers 2005, Bai 2007, Nappi 2005, Lehmann-Willenbrock 1988] or ethanolic (40-60%V/V) [Wuttke 2003b, Hernández Muñoz 2003, Kaiser 2008, Frei-Kleiner 2005] extracts corresponding to 40-140 mg of black cohosh; other corresponding preparations.

Method of administration
For oral administration.

Duration of administration
No restriction.

In efficacy studies patients have been treated with black cohosh extracts for periods of 3 months [Osmers 2005, Bai 2007, Nappi 2005, Wuttke 2003b, Kaiser 2008, Juliá-Mollá 2009], 6 months [Briese 2007, Fischer 2006, Liske 2002, Lehman-Willenbrock 1988, Pethö 1987] or 12 months [Geller 2009, Briese 2007, Raus 2006, Hernandez-Muñoz 2003].

In placebo-controlled studies with treatment periods of 3-12 months no significant differences in adverse effects were evident between black cohosh groups and placebo groups [Shams 2010, Geller 2009, Kaiser 2008, Reed 2008, Osmers 2005, Wuttke 2003b].

Contra-indications
Hypersensitivity to black cohosh.

Special warnings and special precautions for use
The use of black cohosh in patients with pre-existing breast cancer should be approached with caution. The evidence from pharmacological studies, *in vitro*[3] and *in vivo*[4], suggests that black cohosh extracts do not influence the latency or development of breast cancer and may have inhibitory effects[5]. However, contradictory results have been obtained in isolated *in vitro* experiments [Liu ZP 2001]. Clinical experience suggests a lack of risk [Wuttke 2003b, Low Dog 2003, Borrelli 2008b, Obi 2009].

A doctor should be consulted in the event of menstrual disorders or the reappearance of menstruation and if symptoms persist or new symptoms appear.

Black cohosh should only be used with caution in patients with pre-existing liver disease. Patients should stop taking black cohosh immediately if signs and symptoms occur suggesting a liver injury (icterus, dark urine, upper stomach pain, nausea, loss of appetite, tiredness) [EMEA 2007, Mahady 2008].

Interactions with other medicaments and other forms of interaction
None known.

Ethanolic (75% and 80%) extracts and a 40%-isopropanolic extract from black cohosh concentration-dependently inhibited the activity of four CYP450 enzymes (1A2, 2D6, 2C9 and 3A4) *in vitro* with IC_{50} values ranging from 21.9 to 65.0 µg/ml. Isolated triterpene glycosides were found to be weakly active (IC_{50}: 25-100 µM), while fukinolic acid and cimicifugic acids A and B were somewhat more active (IC_{50}: 1.8-12.6 µM) [Huang 2010].

Pregnancy and lactation
Black cohosh should not be used during pregnancy and lactation [Lepik 1997, Dugoua 2006, Barnes 2007].

Effects on ability to drive and use machines
None known.

Undesirable effects
In rare[6] cases gastrointestinal disorders (dyspepsia, diarrhoea), allergic skin reactions (urticaria, pruritus, skin rash) or facial and peripheral oedema. Rarely[6], an increase in liver enzymes (transaminases) may occur.

Liver injury associated with the use of black cohosh preparations has been reported with a very rare[6] frequency, but a causal relationship has not been proven to date [Lieberman 1998, Liske 1998, EMEA 2007, Hudson 2007, Borrelli 2008b, Firenzuoli 2008, Mahady 2008, Mazzanti 2008, Teschke 2009a, Teschke 2009b, Teschke 2010].

Overdose
Nothing of relevance reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Influence on breast cancer cells
The majority of studies evaluating the effects of black cohosh extracts on human oestrogen receptor-positive (ER^+) and -negative (ER^-) breast cancer cell lines have demonstrated inhibition of proliferation, or at least a lack of proliferative effects:

- An isopropanolic extract from black cohosh significantly inhibited proliferation of the oestrogen receptor-positive human breast cancer cell line 435 at concentrations of 2.5-25 µg/ml [Nesselhut 1993].

[3] [Nesselhut 1993, Zava 1998, Dixon-Shanies 1999, Liu J 2001, Amato 2002, Bodinet 2002, Zierau 2002, Lupu 2003, Morris 2003, Bodinet 2004, Einbond 2004, Hostanska 2004a, Hostanska 2004b, Pockaj 2004, Rockwell 2005, Stromeier 2005, Einbond 2006, Garita-Hernandez 2006, Al-Akoum 2007, Einbond 2007a, Einbond 2007b, Gaube 2007, Hostanska 2007, Rice 2007, Stute 2007, Einbond 2008, Omer-Adam 2008]

[4] [Freudenstein 2002, Davis 2008, Seidlová-Wuttke 2008]

[5] [Nesselhut 1993, Dixon-Shanies 1999, Bodinet 2002, Zierau 2002, Bodinet 2004, Einbond 2004, Hostanska 2004a, Hostanska 2004b, Einbond 2006, Garita-Hernandez 2006, Al-Akoum 2007, Einbond 2007a, Einbond 2007b, Gaube 2007, Hostanska 2007, Rice 2007, Stute 2007, Einbond 2008, Omer-Adam 2008]

[6] The stated frequency of the specified adverse reactions is the incidence rate in patients exposed to black cohosh preparations within appropriate limits of dosage and treatment duration, defined in accordance with a convention adopted by the European Commission: very common (≥1/10); common (≥1/100 to <1/10); uncommon (≥1/1,000 to <1/100); rare (≥1/10,000 to <1/1,000); very rare (<1/10,000) [European Commission].

- A 40%-isopropanolic extract from black cohosh at dilutions corresponding to native dry extract at 1-100 µg/ml significantly and dose-dependently inhibited proliferation of the oestrogen receptor-positive human breast adenocarcinoma cell line MCF-7 under oestrogen-deprived conditions ($p<0.05$) and also inhibited oestrogen-induced proliferation of the cells ($p<0.05$ vs. oestradiol 10^{-8} M control). Furthermore, the proliferation-inhibiting effect of tamoxifen at 10^{-6} M was enhanced by the extract. The results suggested a non-oestrogenic or oestrogen-antagonistic effect of the black cohosh extract [Bodinet 2002].

- Neither of two black cohosh extracts (one ethanolic, the other isopropanolic) exhibited oestrogenic activity in proliferation assays of oestrogen receptor-positive MCF-7 cells; the extracts antagonized oestradiol-induced stimulation of proliferation of the cells at concentrations > 1 µg/ml [Zierau 2002].

- A 50%-ethanolic extract of black cohosh (2 g/10 ml) at a concentration of 2 µl/ml did not stimulate proliferation of oestrogen receptor-positive T47D human breast cancer cells, assessed by measuring the increase/decrease in total protein in cell cultures for 9 days [Zava 1998].

- A similar 50%-ethanolic extract significantly inhibited the growth of oestrogen receptor-positive T47D human breast cancer cells at concentrations of 0.1 and 1% V/V [Dixon-Shanies 1999].

- In S30 breast cancer cells the oestrogen-inducible gene presenelin-2 was not up-regulated in the presence of a methanolic extract from black cohosh [Liu J 2001].

- The growth of oestrogen-dependent human breast cancer MCF-7 cells was not stimulated by 48-hour treatment with an alcoholic extract from black cohosh (extraction solvent not further defined) at dilutions from 1:500 to 1:5000. In contrast, in the same test, growth of MCF-7 cells was induced concentration-dependently by dong quai (*Angelica sinensis*; 27-fold at 1:500 dilution, $p<0.001$) and ginseng (16-fold at 1:500 dilution, $p<0.05$); however, since these herbal drugs did not show any oestrogenic activity as measured in a transient gene expression assay and an *in vivo* bioassay in mice, the authors considered the effect on MCF-7 cells to be independent of oestrogenic activity [Amato 2002].

- Black cohosh (extraction solvent not further defined) at concentrations from 0.1 to 100 times the maximum possible serum concentration of the herb did not stimulate the proliferation of oestrogen-sensitive (T-47D) or -insensitive (HCC-1937) breast cancer cells [Morris 2003].

- A 40%-isopropanolic extract not only failed to increase the proliferation of MCF-7 cells under oestrogen-deprived conditions in an MCF-7 test culture system but significantly inhibited proliferation ($p<0.001$) at dilutions corresponding to black cohosh root at 0.04-40 µg/ml. In the presence of oestradiol (10^{-7} M) the extract inhibited oestrogen-induced proliferation and showed oestrogen-antagonistic activity [Bodinet 2004].

- Three different black cohosh extracts (a 50%-ethanolic extract standardized to 3.0% of triterpene glycosides, a liquid extract containing 2.5% of triterpene glycosides and a further liquid extract) were tested for their effects on the growth of EMT6 mouse mammary tumour cells, which are neither oestrogen- nor progesterone-dependent. None of the black cohosh extracts altered the growth or viability of EMT6 cells [Rockwell 2005].

- A 60%-ethanolic dry extract (4.5-8.5:1) from black cohosh was evaluated for growth-inhibitory activity in MCF-7 and T47D human breast cancer cell cultures and, at the gene expression level, for oestrogen-antagonistic effects utilizing an ERE-luciferase reporter assay. At concentrations of 10-200 µg/ml the extract inhibited cell proliferation (measured by incorporation of ^{3}H-thymidine into DNA de novo synthesis), markedly inhibited luciferase activity driven by the cyclin D1 promoter and increased transcriptional activity of the p21 gene promoter [Garita-Hernandez 2006].

- The ethyl acetate (EtOAc) fraction of an 80%-methanolic extract from black cohosh and the triterpene glycoside actein were tested, alone and in combination with chemotherapy agents, for inhibition of the growth of the ER^{-} Her2 overexpressing breast cancer cell line MDA-MB 453. Actein and the EtOAc fraction inhibited growth of the cancer cells and caused synergistic inhibition of tumour cell proliferation even at low concentrations (0.1-10 µg/ml) when combined with 5-fluorouracil, paclitaxel or doxorubicin [Einbond 2006].

- Breast cancer cell cultures (MCF-7 and MDA-MB-231) were exposed to various concentrations of black cohosh (root powder containing actein 1.08 mg/g, 27-deoxyactein 0.819 mg/g and cimifugosides 1.59 mg/g), oestradiol (E2) and tamoxifen to examine effects on cell proliferation using the sulforhodamine B (SRB) dye solution test. E2 at 10^{-10} to 10^{-8} M markedly stimulated the proliferation of MCF-7 cells ($p<0.01$). Black cohosh alone had no stimulatory effect, but significantly inhibited proliferation ($p<0.001$ at 1 mg/ml). Adding black cohosh at 0.001-1 mg/ml to E2 at 10^{-9} M resulted in dose-dependent inhibition of the E2 proliferative effect. Combinations of black cohosh with increasing tamoxifen concentrations further inhibited MCF-7 cell growth. Neither E2 nor tamoxifen had any detectable effect on MDA-MB-231 cells. However, black cohosh at 1 mg/ml inhibited MDA-MB-231 cell proliferation ($p<0.05$) and this inhibitory effect was enhanced by combinations of black cohosh with increasing tamoxifen concentrations [Al-Akoum 2007].

- The anti-invasive effects of a 40%-isopropanolic extract and its two major fractions, triterpene glycosides (TTGs) and cinnamic acid esters (CAE), on the highly invasive potential of MDA-MB-231 cells were studied in a membrane invasion culture system. Suppression of invasion by the extract reached 51.8% at 77.4 µg/ml. TTG and CAE reduced cell invasion by 34% and 25.5% respectively at 5 µg/ml [Hostanska 2007].

- A 60%-ethanolic dry extract dose-dependently inhibited not only the growth of MCF-7 and MDA-MB-231 breast cancer cells at 50 µg/ml and 100 µg/ml ($p<0.05$) but also the conversion of oestrone sulphate to active oestradiol in both cell lines ($p<0.05$) [Rice 2007].

- The effects of a 40%-isopropanolic extract on steroid sulphatase (STS) activity were studied in normal breast tissue obtained from pre- and postmenopausal women undergoing reduction mammoplasty. STS protein expression was studied by immunohistochemistry and western blotting. Treatment with the black cohosh extract at 0.1 mg/ml significantly reduced local oestrogen formation ($p<0.05$) [Stute 2007].

- Various black cohosh extracts induced inhibition of the growth of two human breast cancer cell lines: MDA-MB-453 (ER^-, Her2 over-expressing) and MCF-7 (ER^+, Her2 low). Using a Coulter counter assay IC_{50} values for inhibition of cell growth at 96 hours were determined as 30 µg/ml for a 100%-methanolic extract, 120 µg/ml for an 80%-methanolic extract, 50 µg/ml for a 70%-ethanolic extract and 200 µg/ml for a 40%-isopropanolic extract [Einbond 2007a].

- Extracts, fractions and isolated compounds from black cohosh were tested for growth inhibitory activity on the human breast cancer cell line MDA-MB-453 using several test systems. The results suggested that the growth inhibitory activity of black cohosh extracts is related to their triterpene glycoside composition. The effects of actein on MDA-MB-453 (ER^-, Her2 over-expressing) and MCF-7 (ER^+, Her2 low) human breast cancer cells were examined by fluorescent microscopy. Treatment with actein (IC_{50}: 8.4 µM) altered the distribution of actin filaments and induced apoptosis [Einbond 2008].

- Six marketed black cohosh preparations were evaluated for their content of characteristic marker substances and their effects on the proliferation of MCF-7 human breast cancer cells. Wide variations in content of triterpene glycosides and polyphenolic compounds were found. None of the products stimulated the proliferation of MCF-7 cells, measured by incorporation of ^{3}H-thymidine [Omer-Adam 2008].

In contrast, proliferative effects of black cohosh have been demonstrated in other studies.

- Using the MTT assay, 50%-ethanolic and 96%-ethanolic extracts from black cohosh at concentrations of 0.1-10 µg/ml and 0.01-1 µg/ml respectively caused proliferation of oestrogen receptor-positive MCF-7 human breast cancer cells; this effect was antagonized by the specific anti-oestrogen ICI 182,780 [Winterhoff 2002].

  In another study using the MTT assay, after determination of the optimum concentration of black cohosh for the culture of human breast cancer MCF-7 cells, growth curves of MCF-7 cells in the presence of black cohosh at 4.75 µg/litre or 17β-oestradiol at 0.3 nM were observed for 5 days. Cell growth had doubled after 32.1 and 31.7 hours in the presence of black cohosh and 17β-oestradiol respectively, compared to 35.3 hours in the blank control. Furthermore, from indirect immunofluorescence assay by flow cytometry, black cohosh at 4.75 µg/litre significantly increased oestrogen receptor levels in the MCF-7 cells ($p<0.01$ compared to control) [Liu ZP 2001].

  However, the suitability of the MTT assay for testing the ability of herbal extracts to influence cell proliferation has been questioned on the basis that it may lead to false positive results [Brugisser 2002].

- Fukinolic acid isolated from black cohosh increased the proliferation of oestrogen-dependent MCF-7 cells by up to 120% at 5×10^{-7} M ($p<0.05$) and 126% at 5×10^{-8} ($p<0.001$). While these effects were equivalent to the activity of oestradiol at a lower concentration of 10^{-10} M, fukinolic acid showed no significant activity at 5×10^{-9} M [Kruse 1999]. The oestrogenic activity of fukinolic acid and the 50% ethanolic extract could not be confirmed in subsequent experiments [Stromeier 2005].

A number of physiological mechanisms have been proposed for the anti-proliferative effects of black cohosh, as described below.

Cell cycle arrest by an 80%-methanolic extract [Einbond 2004] and induction of apoptosis by a 40%-isopropanolic extract [Hostanska 2004a] have been proposed as mechanisms of action for the anti-proliferative effect of black cohosh on human breast cancer cells. After evaluation of black cohosh-induced inhibition of proliferation of MCF-7 breast cancer cells by enzymatic assay using a tetrazolium salt, screening in the relevant dose range revealed annexin V expression and caspase activation indicative of apoptosis. Metabolic activation of the cells (with rat liver S9 homogenate containing P450 enzymes) did not abrogate or weaken the capacity of black cohosh to induce apoptosis, the extent of which (3-4 fold greater than in untreated control cells) was comparable to that of staurosporin. Induction of apoptosis was shown to be attributable to both triterpene glycosides and cinnamic acid esters [Hostanska 2004b].

Induction of apoptosis by black cohosh was observed in oestrogen receptor-negative as well as oestrogen receptor-positive cell lines. The authors suggested that this effect is independent of oestrogen receptors but is, however, of considerably higher specificity than found in parallel investigations with non-malignant cells [Hostanska 2004a]. Black cohosh inhibited proliferation and induced apoptosis in human prostate carcinoma cell lines, the effect being independent of androgen receptor status [Hostanska 2005]. In parallel, significant binding of compounds in a 50%-ethanolic black cohosh extract to the aryl hydrocarbon receptor ($p<0.05$ versus control) has been demonstrated; activation of this receptor is known to inhibit the growth of prostate cancer cells [Jarry 2005].

Two tests were performed to confirm the absence of intrinsic oestrogenic activity of black cohosh using MCF-7, T47D and MDA-MB-231 cells: an RNAse protection assay, which tests for regulation of the expression of oestrogen-responsive genes, and an oestrogen-responsive-element (ERE)-luciferase assay, which determines modulation of oestrogen receptor function by transactivation of the ERE. The rate of colony formation of oestrogen receptor-expressing breast cancer cells (Ishikawa cells) was used as an *in vitro* tool to investigate the propensity of tumour cells to metastasise. These assay systems did not show malignancy-increasing potential of black cohosh extracts [Lupu 2003].

Using a proliferation assay and gene expression microarray analysis it was clearly shown that a dichloromethane dry extract from black cohosh and isolated constituents inhibited the proliferation of MCF-7 human breast cancer cells. Gene expression profiling with the extract allowed identification of 431 regulated genes. The extract-induced expression pattern differed from those of 17β-oestradiol or tamoxifen (an oestrogen receptor antagonist). Enrichment of gene expression in an anti-proliferative and apoptosis-sensitizing manner was observed, as well as an increase in mRNAs coding for gene products involved in several stress response pathways. Thus antiproliferative and proapoptotic (but not oestrogenic) gene expression was demonstrated at the transcriptional level. Triterpene glycosides and their aglycones were identified as active principles [Gaube 2007].

In another experiment changes were identified in gene expression of MDA-MB-453 and MCF-7 human breast cancer cells induced by treatment with a pure methanolic black cohosh extract at 40 µg/ml. Six hours after treatment there was an increase in expression of ER stress (GRP78), apoptotic (GDF15), lipid biosynthetic (INSIG1 and HSD17B7) and phase 1 (CYP1A1) genes and after 24 hours a decrease in expression of cell cycle (HELLS and PLK4) genes. The microarray results were confirmed with RT-PCR. Thus the extract activated genes that enhance apoptosis and repressed cell cycle genes [Einbond 2007a].

In a comparable experimental procedure MDA-MB-453 cells were treated with actein at two concentrations, 20 or 40 µg/ml, for 6 or 24 hours. Several genes that play a role in stress response pathways were induced, whereas the cell cycle genes cyclin E2 and cell division cycle 25A were repressed. The results suggested that actein induces two phases of the integrated stress response, the survival phase and the apoptotic phase, depending on the concentration and duration of treatment [Einbond 2007b].

Oestrogen receptor affinity/activity
It is now known that there are at least two oestrogen receptors, ERα and ERβ. Many plant compounds show a greater affinity for binding to ERβ than to ERα [Kuiper 1998].

In 1985 a chloroform fraction from a methanolic extract of black cohosh was reported to exhibit binding to oestrogen receptors of the rat uterus; formononetin, reported as isolated from this fraction, exhibited weak binding to the receptors (1.15% of that of 17β-estradiol) [Jarry 1985b]. However, no formononetin has been detected in black cohosh in more recent investigations [Jiang 2006a, Jiang 2006b, Avula 2009, Kennelly 2002, Struck 1997]. A trace of formononetin (3.3 ppm) was reported in one other study [Panossian 2004], but none was detected in a subsequent study with a limit of detection as low as 0.08 ppm [Jiang 2006a].

A commercial black cohosh extract (not further defined) at 1:20 dilution to full strength did not inhibit [^{3}H]-oestradiol binding to cytosolic oestrogen receptor from livers of adult ovariectomized rats [Eagon 1996].

In a radioimmunoassay for determination of oestrogenic compounds, increasing sample volumes (5-100 µl) of two different 60%-ethanolic extracts from black cohosh dose-dependently displaced radioactively-labelled oestradiol bound to a specific oestradiol antiserum, paralleling the curve obtained with non-labelled oestradiol. This indicated competition with oestradiol for binding sites on the antibody molecules [Jarry 1995].

A methanolic extract from black cohosh gave negative results in assays for oestrogenic activity: no competitive binding affinity for recombinant human oestrogen receptors (ERα and ERβ) and no induction of alkaline phosphatase activity or up-regulation of progesterone receptor mRNA in cultured Ishikawa cells (an ER-positive endometrial adenocarcinoma cell line) [Liu J 2001].

In other investigations using oestrogen-dependent reporter gene assays or transcriptional activation assays black cohosh again showed no oestrogenic activity [Lupu 2003, Garita-Hernandez 2006].

The binding properties of three triterpene glycosides (cimicifugoside, cimiracemoside F and 27 deoxyactein) to the ligand binding domain of the oestrogen receptor ERβ were investigated by affinity ultrafiltration and LC/MS detection. Oestradiol and the phyto-oestrogens daidzein and genistein were used as positive controls. Neither the triterpene glycosides nor their enzymatically prepared aglycones bound to the ERβ, except for the aglycone of 27 deoxyactein, which showed weak binding affinity (4%). Metabolites of the aglycones, prepared by incubation with female human liver microsomes, were also found not to bind significantly to the ERβ [Onorato 2001].

An alcoholic extract (not further defined) from black cohosh at 1:500 dilution did not significantly transactivate human oestrogen receptors ERα or ERβ in a transient gene expression assay using HeLa cells co-transfected with an oestrogen-dependent reporter plasmid, whereas 17β-oestradiol at 10^{-9} M increased transcriptional activity by human ERα and ERβ by 20-fold and 10-fold respectively [Amato 2002].

To determine whether active substances present in a dry 58%-ethanolic extract of black cohosh bind to either of the two known subtypes of the oestrogen receptor (ERα and ERβ), subtype-specific oestrogen receptor ligand-binding assays were conducted using recombinant human ERα or ERβ. Although the extract displaced radiolabelled oestradiol from binding sites of cytosol preparations from porcine or human endometrium (containing both ERα and ERβ), no such displacement of oestradiol by the extract was achieved when either recombinant human ERα or ERβ was used. Dopaminergic activity of the black cohosh extract was demonstrated in an assay using recombinant human dopamine D_2 receptor protein and application of countercurrent chromatography to the extract enabled separation of oestrogenic and dopaminergic activities in two distinct fractions [Jarry 2003].

Expression patterns of two oestrogen-regulated gene products, truncated oestrogen receptor product 1 (TERP-1) and prolactin (PRL), were investigated in a rat pituitary gland-derived somatolactotrophic cell line. Treatment for 21 days with an isopropanolic extract from black cohosh or oestradiol induced significant upregulation of the two marker genes ($p<0.05$). This effect could be inhibited by simultaneous treatment with the antagonist faslodex. However, in contrast to the *in vitro* results, the extract did not mimic these gene regulatory properties in *in vivo* experiments using ovariectomized rats [Wober 2005].

Osteoprotective effects
Increased bone resorption mediated by osteoclasts is central to the pathogenesis of osteoporosis and under pathological conditions cytokines, particularly RANKL and TNFα, are often increased, leading to enhanced osteoclastogenesis. It has been demonstrated *in vitro* that 25-acetylcimigenol xyloside isolated from black cohosh potently blocks osteoclastogenesis induced by either RANKL or TNFα. This suppression of osteoclastogenesis results from abrogation of the NF-κB and ERK pathways induced by either RANKL or TNFα. Furthermore, 25-acetylcimigenol xyloside has been shown to attenuate TNFα-induced bone loss *in vivo* (see In vivo experiments) [Qiu 2007].

RANKL effects are physiologically counterbalanced by the glycoprotein osteoprotegerin, a potent inhibitor of bone resorption. In primary human osteoblasts a 40%-isopropanolic extract from black cohosh at concentrations between 0.75 and 750 µg/litre significantly upregulated the antiresorptive cytokine osteoprotegerin ($p<0.001$) in a similar manner to oestradiol or raloxifene, increased osteoprotegerin protein secretion ($p<0.001$) and enhanced two osteoblastic differentiation markers ($p<0.001$), bone-specific alkaline phosphatase activity and osteocalcin expression. These effects were completely abolished by co-incubation with faslodex, a selective oestrogen receptor down-regulator [Viereck 2005].

Neurovegetative pathways
It has been demonstrated that black cohosh may contain (as yet unidentified) compounds with dopaminergic effects [Winterhoff 2002; Jarry 2003]. Further studies have shown that extracts from black cohosh bind to GABA, serotonin, dopamine and acetylcholine receptors. Follow-up investigations into the cellular functions which might be induced by receptor binding, revealed, for example, partial agonistic effects of extracts at the serotonin receptor and inhibition of catecholamine secretion [Winterhoff 2003, Burdette 2003, Jarry 2003, Woo 2004, Nisslein 2006].

Black cohosh has been shown to act as a mixed competitive ligand and partial agonist of the serotonin receptor. Receptor subtype-specific IC_{50} values were 2.4 µg/ml for the human 5-HT_7 receptor and 13.9 µg/ml for the rat 5-HT_{1A} receptor. Via the 5-HT_7 receptor black cohosh also elevated intracellular cAMP levels; this effect was reversed by the specific antagonist methiothepin, suggesting receptor-mediated agonistic properties for black cohosh [Burdette 2003].

The binding of a 40%-isopropanolic extract from black cohosh to 21 sub-types of three classes of CNS receptors (dopamine, 5-HT and GABA) was evaluated by displacement of radio-labelled receptor ligands. The extract showed the highest affinities (IC_{50} values less than 15 µg/ml) towards 5-HT_{1A}, 5-HT_{1D}, 5-HT_7 and $GABA_A$ receptors; it did not influence serotonin transport nor interfere with serotonin secretion or release [Nisslein 2006].

It has also been demonstrated that black cohosh behaves as a mixed competitive ligand and partial agonist at the human μ opiate receptor expressed in Chinese hamster ovary cells. At 0.3 mg/ml three different extracts from black cohosh, prepared with 100% methanol, 75% ethanol or 40% isopropanol, effectively displaced the binding of a μ opiate receptor-specific ligand (a synthetic peptide) to the receptor. The 75%-ethanolic extract displaced this ligand with an IC_{50} of 165.9 µg/ml and, in a different assay, was shown to act as an agonist at the human μ opiate receptor. Because the opiate system in the brain is intimately associated with mood, temperature and sex hormone levels, the authors considered these results to be consistent with the effects of black cohosh in alleviating some menopausal symptoms [Rhyu 2006].

Black cohosh extracts (100%-methanolic and 75%-ethanolic) and their constituents were evaluated for serotonergic activity using 5-HT_7 receptor binding, cAMP induction and serotonin selective re-uptake inhibitor (SSRI) assays. Crude extracts displayed 5-HT_7 receptor binding activity and induced cAMP production. Triterpenoids and phenolic acids bound weakly to the 5-HT_7 receptor but exhibited no cAMP or SSRI activity. In contrast, N_ω-methylserotonin (identified as a minor constituent of black cohosh) showed 5-HT_7 receptor binding (IC_{50}: 23 pM), induced cAMP (EC_{50}: 22 nM) and blocked serotonin re-uptake (IC_{50}: 490 nM) [Powell 2008].

Cimicifugoside inhibited catecholamine secretion, as well as increases in calcium (IC_{50}: 18 µM) and sodium (IC_{50}: 2 µM), induced in bovine adrenal chromaffin cells by the nicotinic acetylcholine receptor agonist DMPP (1,1-dimethyl-4-phenylpiperazinium iodide). The results suggested that cimicifugoside binds to the nicotinic acetylcholine receptor in a non-competitive way [Woo 2004].

Vasoactive effects
Isolated fukinolic acid and cimicifugic acid D at a concentration of 3×10^{-4} M caused sustained relaxation of rat aortic strips precontracted with norepinephrine; cimicifugic acids A, B and E and fukiic acid showed no vasoactivity at this concentration [Noguchi 1998].

Antioxidant activity
A methanolic extract from black cohosh scavenged 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals with an IC_{50} of 99 µg/ml, and at concentrations up to 200 µg/ml dose-dependently inhibited DNA single-strand breaks and oxidation of DNA bases induced by menadione. Of nine hydroxycinnamic acid derivatives identified as antioxidants in the extract, six reduced menadione-induced DNA damage in cultured S30 breast cancer cells, the most potent being methyl caffeate, caffeic acid and ferulic acid [Burdette 2002].

Hepatic effects
A 60%-ethanolic dry extract (4.5-8.5:1) from black cohosh was tested for cytotoxicity, mitochondrial toxicity and apoptosis/necrosis using the human liver cancer cell line HepG2. Cytotoxicity was apparent at concentrations ≥ 75 µg/ml and mitochondrial β-oxidation was impaired at concentrations ≥ 10 µg/ml. The mitochondrial membrane potential was decreased at concentrations ≥ 100 µg/ml and oxidative phosphorylation was impaired at concentrations ≥ 300 µg/ml. The mechanism of cell death was predominantly apoptosis [Lüde 2007].

The growth of human HepG2 liver cancer cells was not inhibited by 75%- or 80%-ethanolic extracts or a 40%-isopropanolic extract from black cohosh at concentrations up to 50 µg/ml [Huang 2010]. In another study actein was found to inhibit the growth of HepG2 cells with an IC_{50} of 27 µg/ml (40 µM) [Einbond 2009].

In vivo experiments

Influence on breast cancer
As an oestrogen receptor-positive breast cancer model, mammary tumours were induced in female rats by intragastric administration of 7,12-dimethylbenz[*a*]anthracene (DMBA), then the animals were ovariectomized, allowed to recover, randomized into groups and treated orally for 6 weeks with an isopropanolic extract from black cohosh at daily dose levels of 0.714, 7.14 or 71.4 mg/kg (comparable to 1, 10 or 100 times the human therapeutic dose respectively) or with mestranol (an oestrogenic steroid) at 450 µg/kg/day as a positive control, or vehicle only as a negative control. Six weeks later, whereas tumour growth had been stimulated in the mestranol-treated group ($p<0.05$), no significant differences in the number or size of tumours could be detected between the black cohosh groups and the vehicle-only control group. A trend towards tumour reduction was observed in the black cohosh groups compared to the control. Serum levels of prolactin, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), organ weights and endometrial proliferation were unaffected by the extract in comparison with the vehicle-only group [Freudenstein 2002].

Sexually mature female MMTV-*neu* transgenic mice were used in another study; these mice develop primary and metastatic mammary tumours by spontaneous activation of the proto-oncogene *neu* (c-erbB2, the rodent homologue of HER2), the most common oncogene of breast cancer. An isopropanolic extract from black cohosh, administered orally to the mice in the diet at an amount corresponding to 40 mg of rhizome and root per 1800 calories of diet (correlating to 40 mg/day in women) from 2 months of age until the maximum age of 16 months, did not alter the incidence of mammary tumours compared to controls: 118 out of 127 mice (92.9%) in extract-treated animals; 123 out of 131 mice (93.9%) in control animals. Tumour latency was also comparable in the two groups. However, mice treated with black cohosh had a significantly higher incidence of lung metastatic lesions that were grossly visible at necroscopy ($p<0.002$) and detected by histopathology ($p<0.001$). It has not yet been determined whether black cohosh accelerated the development of metastatic disease or increased its incidence, nor whether the observed metastatic effects in this transgenic model could predispose to the development of tumours [Davis 2008]. These effects require further investigation.

After treatment of ovariectomized rats with a 60%-ethanolic extract from black cohosh at 30 mg/day for 3 months no proliferative or oestrogenic effects of the extract on the mammary gland were evident from morphometric analysis [Seidlova-Wuttke 2008].

Influence on tumours
As an animal model of endometrial cancer, highly metastasizing, hormone-responsive RUCA-I rat endometrial adenocarcinoma cells were subcutaneously implanted into ovariectomized rats of the syngeneic DA/Han strain. The rats (5-6 per group) were then given one of four treatments for 5 weeks: an isopropanolic liquid extract from black cohosh (an amount corresponding to herbal drug at 60 mg/kg), tamoxifen (subcutaneously at 200 µg/kg), a combination of the extract and tamoxifen, or isopropanol only as a control. Tumour growth was comparable in rats treated with the extract and those in the control group whereas tamoxifen, in its role as an endometrium-specific oestrogen-agonist, accelerated tumour growth and metastasis formation. The mean tumour mass was 550 mg in animals treated with the black cohosh extract, 660 mg after tamoxifen, 590 mg after the tamoxifen-extract combination and 570 mg in the control group. Pulmonary metastases were found in all groups. In the control and tamoxifen groups respectively, 3 and 4 of the animals showed pulmonary metastases; additionally 2 abdominal metastases were found in tamoxifen-treated animals. In the extract group 2 animals and in the tamoxifen-extract combination group 1 animal produced metastases. The extract did not influence the growth of primary tumours and the tendency to metastasize was reduced, irrespective of whether black cohosh was administered alone or in combination with tamoxifen [Nisslein 2004].

Endocrine activities
A 60%-ethanolic black cohosh dry extract administered intraperitoneally to ovariectomized rats at 2 × 12 mg per animal (approx. 2 × 48 mg/kg body weight) daily caused a significant decrease in serum concentration of luteinizing hormone (LH) after 3 days; the mean LH level was 332 ng/ml in extract-treated animals compared to 781 ng/ml in control animals ($p<0.01$). However, no significant differences in LH from control levels were evident after 1 or 14 days, and no significant changes were observed in serum levels of follicle stimulating hormone (FSH) or prolactin [Jarry 1985a, Jarry 1995].

The chloroform-soluble dry fraction from a methanolic extract of black cohosh (crude drug to chloroform fraction ratio approx. 35:1), administered intraperitoneally to ovariectomized rats in 9 divided doses over 4 days (a total dose of 108 mg per animal corresponding to approx. 568 mg/kg body weight), reduced serum levels of LH from 657 ng/ml (injection vehicle as control) to 339 ng/ml [Jarry 1985b].

Oral administration of a 50%-ethanolic black cohosh extract at 6, 60 or 600 mg/kg body weight/day to immature mice for 3 days followed by uterus weight tests, and subcutaneously to mature ovariectomized rats followed by vaginal smear tests, did not reveal any oestrogenic effects [Einer-Jensen 1996]. In another study, oral administration of a 50%-ethanolic extract from black cohosh to ovariectomized female mice at 50-600 mg daily for 3 days did not produce any oestrogenic effects (cornification) in vaginal smears [Knüvener 2000].

Treatment of ovariectomized mice with 500 µl of an alcoholic black cohosh extract (not further defined) by oral gavage daily for 4 days did not increase uterine weight compared to untreated controls, whereas 17β-oestradiol administered subcutaneously at 100 µg/kg/day caused a 1.7-fold increase [Amato 2002].

Ovariectomized rats were treated for 17 days with a 40%-isopropanolic extract, alone or in combination with the anti-oestrogen fulvestrant, and the effects were investigated by vena cava and uterine gene expression analysis using real-time PCR. Treatment with the extract or fulvestrant, or a combination of both, did not lead to significant changes in uterine weight. Black cohosh showed no or only slight anti-oestrogenic effects on gene expression in the uterus [Kretzschmar 2005].

In contrast, when black cohosh was administered to immature female mice by gavage at 75, 150 or 300 mg/kg body weight daily for 14 days, uterine weights (measured when oestrus was observed) increased with the increasing dosage of black cohosh and the duration of oestrus was significantly extended in the 300 mg/kg group ($p<0.05$) [Liu ZP 2001].

The partly conflicting results of experiments described above may be explained by the concept of the Selective Estrogen Receptor Modulator (SERM) [Kuiper 1998, Winterhoff 2002, Wuttke 2003a, Wuttke 2003b, Seidlová-Wuttke 2003b]. Since oestrogen receptors are expressed differently in various organs, oestrogenic effects of a substance need to be assessed for each individual tissue; the same substance can exert oestrogenic and non- or anti-oestrogenic effects depending on the specific tissue. This may explain observations of both oestrogenic and anti-oestrogenic effects of black cohosh [Boblitz 2000].

Effects on the nervous system
In a systematic review of 15 *in vitro* and 15 *in vivo* studies it was concluded that black cohosh exerts a central activity rather than a hormonal effect [Borrelli 2003].

Hot flush equivalents in ovariectomized rats (rapid and short-term increases of more than 1°C in subcutaneous body temperature, measured by an implanted temperature sensor and transmitter) were reduced in frequency by oestradiol valerate (2 mg/kg/day; $p<0.05$) or the antidopaminergic drug veralipride (100 mg/kg/day), indicating a response and sensitivity comparable to hot flushes in women. Oral administration of a 58%-ethanolic dry extract of black cohosh to the rats at 100 mg/kg body weight daily for 5 days also significantly reduced the number of hot flush equivalents per day ($p<0.05$ on days 3-5); the frequency returned to pre-treatment levels after cessation of treatment [Winterhoff 2003].

In a subsequent study using the same extract and a similarly implanted sensor and transmitter, subcutaneous temperatures measured at 5-minute intervals in individual *intact* rats were found to be stable for periods of 3 hours. Following ovariectomy, temperature pulses appeared with peaks occurring every 20-40 minutes, but these peaks were not seen in rats treated with the extract at 25 mg/animal/day in food. Over a study period of 3 weeks ovx rats had a significantly higher mean temperature than intact ($p<0.05$) or ovx + extract-treated rats ($p<0.001$), but there was no significant difference between the body temperatures of intact and ovx + extract-treated rats [Kapur 2010].

An extract of black cohosh orally administered to mice at 25-100 mg/kg body weight caused a distinct and dose-dependent fall in body temperature one hour after gavage, 100 mg/kg having an effect comparable to that of bromocriptine (a dopamine D_2 agonist) given intraperitoneally at 5 mg/kg. The fall in body temperature was inhibited by pretreatment with the dopamine D_2/D_3 receptor antagonist sulpiride at 100 mg/kg, but not by the selective D_1 receptor antagonist SCH 23390.
Furthermore, the extract prolonged ketamine-induced sleeping time in mice, an effect which also occurred after bromocriptine injection but could be blocked by sulpiride and was unchanged by SCH 23390. These results indicated central activity of the extract mediated by central D_2 receptors [Löhning 1999].

Osteoprotective effects
Ovariectomized female rats fed on a soya-free diet were given one of three treatments daily for 12 weeks: a 40%-isopropanolic extract from black cohosh corresponding to 4.5 mg/kg of

triterpene glycosides, raloxifene (a known SERM; 3 mg/kg intragastrically) or soya-free diet only. Post-mortem femoral bone density and bone mineral density at the lumbar vertebral site were determined by computed tomography. Mechanical resistance to fracture of the femoral head was assessed in a material testing device. Urinary pyridinoline (PYR) and deoxypyridinoline (DPY), breakdown products of collagen during bone resorption serving as specific markers of bone loss, were determined by HPLC at baseline and at weekly intervals. The black cohosh extract induced a significant improvement in overall bone quality score ($p<0.05$) and significantly reduced the urinary content of PYR and DPY ($p<0.05$). Comparable results were obtained in the raloxifene group [Nisslein 2003].

Treatment of ovariectomized rats with a black cohosh extract, added to food at 33 mg/day per animal for 3 months, had osteoprotective effects; compared to ovariectomized controls, significant reductions in loss of bone mineral density in tibia ($p<0.05$) and reduced serum levels of osteocalcin (a marker of bone turnover produced by osteoblasts; $p<0.05$) were observed. The extract also significantly reduced paratibial (foreleg) and abdominal fat (both $p<0.05$) and hence body weight ($p<0.05$), and also serum concentrations of the hormone leptin (an indicator of lipocyte activity; $p<0.05$). Oral 17β-oestradiol at 0.325-0.35 mg/day per animal had similar and somewhat stronger effects but, in contrast to the black cohosh extract, also reduced serum crosslaps (a marker of bone turnover produced by osteoclasts; $p<0.05$) and significantly increased uterine weight ($p<0.05$). Furthermore, oral 17β-oestradiol influenced the expression of oestrogen-regulated uterine genes, thereby inhibiting ERβ ($p<0.05$) and stimulating IGF-1 (insulin-like growth factor, which stimulates proliferation of the endometrium; $p<0.05$). Since the black cohosh extract exerted significant oestrogenic effects in the bone (particularly in osteoblasts, cells which rebuild absorbed bone) and fat tissue of ovariectomized rats, but not in the uterus, it appeared to contain organ-specific SERMs with effects in some, but not all, oestrogen-sensitive organs [Seidlova-Wuttke 2003a, Seidlova-Wuttke 2003b].

Female 12-week-old rats were ovariectomized (n = 36) or sham-operated (n = 12) and, under the same anaesthesia, all 48 animals underwent a standardized metaphyseal tibial osteotomy (a cut through the bone) and osteosynthesis by bridging with a five-hole, T-shaped titanium plate. The healing process was then investigated over a period of 35 days during which the sham-operated rats were fed a phytoestrogen-free diet, while groups of 12 ovariectomized rats received the same phytoestrogen-free diet, alone or supplemented with 17β-oestradiol 3-benzoate (approx. 0.03 mg/kg body weight per day) or a 50% V/V-ethanolic black cohosh dry extract (approx. 24.9 mg/animal/day). Black cohosh induced a high rate of metaphyseal callus formation. The biomechanical properties and amount of new callus indicated that fracture healing was still in progress, therefore gene expression of osteoblasts was comparatively high. However, osteoporotic metaphyseal fracture healing was improved more by oestrogen than by black cohosh and the authors considered the 5-week duration of treatment to be too short for black cohosh to show its full potential [Kolios 2010a].

In a related study by the same authors, another 36 female 12-week-old rats were ovariectomized and over a period of 10 weeks on a standard diet of phytoestrogen-free food they developed severe osteopenia with about 40% loss of bone mineral density in the proximal tibia. All animals subsequently underwent the metaphyseal tibial osteotomy and T-plate osteosynthesis, and in groups of 12 were treated for 35 days with the standard diet, alone or supplemented with 17β-oestradiol or the black cohosh extract. Black cohosh did not exhibit positive effects in severe (senile) osteopenic fracture healing, as it had done in early (postmenopausal) osteoporotic fracture in rats [Kolios 2010b].

From a review in 2003 it was concluded that in ovariectomized rats a black cohosh extract showed many of the beneficial effects of 17β-oestradiol, including effects in the brain/thalamus to reduce serum LH levels, effects in the bone to prevent osteoporosis and oestrogenic effects in the urinary bladder, but had no uterotrophic effect [Wuttke 2003a].

25-Acetylcimigenol xyloside dissolved in dimethyl sulfoxide (DMSO) was administered intraperitoneally to mice at 12 mg/kg body weight for 5 days, with or without supracalvarial injection of TNFα, while other groups received TNFα + DMSO, DMSO only, or no treatment. Histological assessment of the percentage of bone surface covered by osteoclasts in the calvaria of each group revealed that the extent of osteoclastogenesis induced by TNFα + DMSO was significantly reduced in mice treated with 25-acetylcimigenol xyloside + TNFα + DMSO ($p<0.04$), while DMSO alone had no impact [Qiu 2007].

Hepatic effects

After oral administration of a 60%-ethanolic black cohosh dry extract to rats at daily dose levels of 1, 10, 100, 300 and 1000 mg/kg body weight for 21 days, electron microscopy of liver sections revealed microvesicular steatosis of hepatocytes only in animals treated with the extremely high daily dose of 1000 mg/kg.

It is important to note that the abstract in the original paper incorrectly cited the daily dose causing microvesicular steatosis as "> 500 µg/kg body weight"; a correction was published three years later [Lüde 2007].

No effects on liver morphology or hepatic function indices were evident in rats after administration by gavage of a 50%-ethanolic dry extract of black cohosh at 300 mg/kg/day for 30 days [Mazzanti 2008].

Actein was administered orally to rats at 35.7 mg/kg body weight, while control groups received only water, and separate groups of animals were tested after 6 hours and 24 hours. Using labelled complementary DNA (cDNA) generated from the liver tissue of each study animal, chemogenomic analyses indicated that actein elicited stress- and statin-associated responses in the liver, altering the expression of cholesterol and fatty acid biosynthesis genes and various other genes. Consistent with these findings, a 0.6-fold decrease in levels of cholesterol ($p<0.018$) and free fatty acids ($p<0.012$) in the liver was found after 24 hours, with no change in triglyceride content [Einbond 2009].

Other effects

In the tail suspension test in mice (a behavioural test for antidepressant activity) a 58%-ethanolic dry extract from black cohosh, administered by gavage at 50 or 100 mg/kg, reduced the period of immobility ($p<0.05$), as did imipramine at 30 mg/kg ($p<0.05$); this effect was observed 60 minutes after a single dose and also after 8 days of treatment [Winterhoff 2003].

In studies of gene expression in the *vena cava* of ovariectomized rats, treatment of the animals for 17 days with a 40%-isopropanolic extract (a daily amount corresponding to black cohosh at 60 mg/kg body weight) did not cause any changes in mRNA expression of ERα, clusterin, NOS3 (endothelial NO synthase), KI67, PCNA and VEGF. In contrast, a slight downregulation of COX2 and ACE (angiotensin converting enzyme) mRNA expression was found. As these effects were not abrogated by co-treatment with the antiestrogen fulvestrant, they cannot be attributed to oestrogen receptor interactions alone. Furthermore, unlike oestradiol, black cohosh treatment slightly upregulated C3 mRNA expression suggesting that this effect is also not mediated by oestrogen receptor interaction. Therefore, long-term administration of black cohosh did not change mRNA

expression of specific oestrogen-sensitive genes in the vascular system [Kretzschmar 2005].

Another study investigated whether extracts from black cohosh altered the response of EMT6 mouse mammary tumour cells to radiation or to four drugs commonly used in cancer therapy. The extracts increased the cytotoxicity of doxorubicin and docetaxel and decreased the cytotoxicity of cisplatin, but did not alter the effects of radiation or 4-hydroperoxycyclophosphamide. The interaction between black cohosh and cisplatin was seen only at a high concentration (100 times the therapeutic level), which can be regarded as not clinically relevant [Rockwell 2005].

Pharmacological studies in humans

Serum levels of pituitary gonadotropins (FSH and LH), prolactin, oestradiol, cortisol, lipids and liver transaminases (glutamate-oxalacetate transaminase and glutamate-pyruvate transaminase) were determined, and endometrial thickness measured by ultrasonography, at baseline and at the end of a 3-month randomized study in which postmenopausal women with climacteric complaints received daily either a 40%-isopropanolic black cohosh extract (corresponding to 40 mg of dried rhizome, n = 32) or an oestradiol treatment (n = 32). In the black cohosh group after 3 months total cholesterol was unchanged, while a slight but significant increase in HDL-cholesterol ($p<0.04$) and a decrease in LDL-cholesterol ($p<0.003$) were observed. No significant changes were evident in triglycerides, FSH, LH, prolactin, oestradiol or cortisol, and liver function and endometrial thickness were not affected. Efficacy data from this study are summarized under Clinical studies [Nappi 2005].

In conjunction with a placebo-controlled clinical study [Newton 2006, summarized under Clinical studies], in which a total of 235 peri- and postmenopausal women were assigned to daily treatment for 12 months with black cohosh extracts corresponding to 160 mg (alone) or 200 mg (in combination with other herbal ingredients) of herbal drug, the opportunity was taken to examine the effect of black cohosh on various serum parameters between baseline and the 3-month stage. No significant differences were found between the herbal and placebo groups with respect to serum total cholesterol, LDL, HDL, triglycerides, glucose, insulin or fibrinogen [Spangler 2007].

In a 12-week randomized, double-blind study in postmenopausal women neither a 58%-ethanolic dry extract from black cohosh (daily dose equivalent to 2 × 20 mg of crude drug, n = 20) nor placebo (n = 20) influenced endometrial thickness, measured by transvaginal ultrasound, whereas conjugated oestrogens (CE, 2 × 0.3 mg daily, n = 22) increased endometrial thickness by more than 1 mm. The black cohosh extract slightly increased the amount of superficial cells in vaginal smears (10% increase from baseline; $p = 0.0542$) compared to a significant increase of 38% after CE ($p<0.01$) and a decrease of 10% after placebo.

Analysis of markers of bone metabolism in serum indicated beneficial effects in both the black cohosh and CE groups. Serum levels of "CrossLaps" (metabolic products of bone-specific collagen-1α1, generally accepted as markers of bone degradation) showed little change after black cohosh, whereas treatment with CE produced a significant decrease in CrossLaps after 4 and 12 weeks ($p<0.05$) compared to an increase in the placebo group. On the other hand serum levels of bone-specific alkaline phosphatase, a metabolic marker for bone formation, increased significantly in the black cohosh group over the 12 weeks ($p<0.05$ vs. placebo) while increasing only slightly in the CE group and remaining almost unchanged in the placebo group. Combined as a Bone Turnover Index, i.e. log (bone-specific alkaline phosphatase/CrossLaps), the beneficial effects on bone metabolism after black cohosh or CE were comparable and significant ($p = 0.0138$ vs. placebo in both cases). No significant effects were observed on coagulation markers or liver enzymes in the blood. Efficacy data from this study are summarized under Clinical studies [Wuttke 2003b, Wuttke 2006b].

Treatment of 45 healthy postmenopausal women with a 40%-isopropanolic extract of black cohosh (corresponding to 40 mg of the herbal drug) daily for 3 months also had a favourable effect on bone markers, producing a significant decrease in urinary concentration of N-telopeptides ($p<0.05$), a marker of bone resorption, and an increase in serum alkaline phosphatase ($p<0.05$), a marker of bone formation, in comparison with 37 women participating as untreated controls. These biochemical changes would be consistent with a small protective effect against the increased bone loss that occurs after menopause.

However, addition of serum from treated women to a murine osteoblastic cell line did not confirm any effect on osteoblasts; no differences between groups were observed with respect to effects on the activity of alkaline phosphatase or the expression of three genes related to osteoblastic differentiation and function.

Compared to baseline, no significant differences were found after 3 months in routine serum biochemical parameters, lipid profiles or hormone levels (oestradiol, testosterone, FSH and parathyroid hormone) within or between the two groups of women [García-Pérez 2009].

The effects of black cohosh on circulating and breast-specific oestrogenic markers were investigated during the course of an open clinical study in which 61 women (aged 36-62 years) with daily menopausal symptoms, natural or following surgery, took a black cohosh extract (2 × 40 mg) daily for 12 weeks followed by a 12-week wash-out phase (a total of 24 weeks of assessment). Specimens of blood and nipple aspirate fluid were collected at baseline and at 4,12 and 24 weeks. Serum oestradiol, pS2 protein (a marker of oestrogenic activity), FSH and LH showed no significant changes during black cohosh treatment. Nipple aspirate fluid was assayed for changes in oestrogenic markers and the epithelial cells in it were studied for changes in proliferation; no significant effects on pS2 or cellular morphology were detected. Efficacy data from this study are summarized under Clinical studies [Ruhlen 2007].

Physiological parameters were evaluated over the course of a clinical study involving peri- and post-menopausal women with climacteric symptoms, who were randomized to daily treatment for 24 weeks with a 40%-isopropanolic black cohosh extract at dose levels corresponding to either 39 mg (n = 76) or 127.3 mg (n = 76) of herbal drug. No significant changes in serum levels of oestradiol, FSH, LH, prolactin or sexual hormone binding globulin were detected in either group compared to pre-treatment levels. Vaginal cytological parameters also remained unchanged [Liske 2002].

In contrast, in an earlier placebo-controlled study in menopausal women (mean age 52 years), a significant decrease was reported in serum LH levels ($p<0.05$) in the verum group (n = 55) compared to the placebo group (n = 55) after daily oral administration of 8 mg of a hydroethanolic extract of black cohosh for 2 months. FSH levels remained similar in the two groups [Düker 1991].

A pilot study using positron emission tomography neuroimaging to examine central opioid function in 5 postmenopausal women demonstrated that a black cohosh 40%-isopropanolic extract (2 × 20 mg taken daily for 30 days) had a direct neuropharmacological action on the brain and that the effects are mediated via alterations in μ-opioid receptor binding potential. The brain regions affected comprise areas which show similar responses to oestrogen, areas involved in emotional and cognitive function

and areas involved in the placebo response. Black cohosh did not exhibit direct central oestrogen-like effects [Reame 2008].

Clinical Studies

According to the EMEA's *Guideline on Clinical Investigation of Medicinal Products for Hormone Replacement Therapy of Oestrogen Deficiency Symptoms in Postmenopausal Women* enrolled subjects should have a defined minimum number of hot flushes per day at baseline (at least 5 moderate to severe hot flushes) to justify a need for treatment. The proposed primary endpoint for efficacy is the frequency of moderate to severe hot flushes. As a secondary endpoint the Kupperman Menopausal Index or other fully validated scales can be used. A duration of treatment of 3 months is generally recommended for symptom efficacy evaluation [European Medicines Evaluation Agency 2005].

Note: Throughout this section the abbreviation "KM Index" refers to the Kupperman Menopausal Index

Clinical studies performed with a 40%-isopropanolic dry extract of black cohosh (daily dose corresponding to 40 mg of herbal drug)

In a double-blind, placebo-controlled study 304 patients with climacteric complaints (including a median of 28 hot flushes per week) were randomized to treatment with the black cohosh extract (n = 153) or placebo (n = 151) daily for 3 months. Assessment of efficacy from changes in the Menopause Rating Scale (MRS) from baseline to end of treatment showed a significant difference in favour of the active treatment after 3 months ($p = 0.027$). A relevant increase in the treatment difference with declining baseline FSH level was also observed. Analysis of MRS subscores revealed that "hot flushes" ($p = 0.007$), "atrophy" ($p = 0.012$) and "psyche" ($p = 0.019$), but not "soma", decreased significantly in the black cohosh group compared to the placebo group [Osmers 2005].

A randomized, double-blind study conducted in 5 centres in China involved 244 patients aged 40-60 years with menopausal complaints. At baseline the interval since last regular menstruation was more than 5 months. The patients were treated with either the black cohosh extract (n = 122) or tibolone (2.5 mg/day, n = 122) for 12 weeks. The efficacy of tibolone (a synthetic steroid used as HRT) in climacteric complaints has been extensively confirmed by placebo-controlled studies [Borrelli 2008a]. A benefit-risk balance was the primary endpoint, calculated as a composite from the KM Index total score and the frequency of adverse events. KM Index total scores decreased substantially in both groups with no significant differences between groups at the 4-week and 12-week time points; from 24.7 at baseline the scores after 12 weeks were 7.7 in the black cohosh group and 7.5 in the tibolone group. Since the incidence of adverse events was significantly lower in the black cohosh group ($p<0.001$) the benefit-risk balance for black cohosh was non-inferior and even superior to tibolone ($p_{non\text{-}inferiority}<0.0001$ and $p_{superiority} = 0.0088$ in per protocol patients). KM Index responder rates were similar in the two groups (84% and 85%) [Bai 2007].

In another randomized study 64 postmenopausal women with climacteric complaints received daily for 3 months either the black cohosh extract (n = 32) or low-dose transdermal oestradiol (25 µg, n = 32) every 7 days plus dihydrogesterone at 10 mg/day for the last 12 days of the 3-month oestradiol treatment. The patients recorded the number of hot flushes per day in a diary. Other climacteric symptoms (vasomotor and urogenital symptoms) using the Greene Scale, and anxiety and depression using the Symptom Rating Test, were evaluated at baseline and after 3 months. Both treatments significantly reduced the number of hot flushes per day ($p<0.001$) and vasomotor symptoms ($p<0.001$) by the end of the first month, and this positive effect was maintained throughout the 3 months of observation without any significant difference between treatments. Identical effects were evident in the black cohosh and oestradiol groups for anxiety ($p<0.001$) and depression ($p<0.001$) [Nappi 2005].

In an open observational study 6,141 women with climacteric complaints were treated (in 1,287 outpatient gynaecologists' practices, with the choice of treatment at the discretion of participating physicians) over a period of approx. 6 months with either a mono-preparation containing the black cohosh extract (recommended daily dose: 2 tablets, corresponding to 2 × 20 mg of dried rhizome; n = 3,027) or a combination preparation, tablets each containing 3.75 mg of the black cohosh extract and 70 mg of an ethanolic St. John's wort extract 3.5-5:1 (recommended daily dose: 2 × 1-2 tablets; n = 3,114). The patients (average age 52 years) had been suffering from climacteric complaints for 2.5 years on average; 27% had previously been treated with HRT and 8% with herbal remedies; 8% reported breast cancer during the anamnesis. Changes of dose or study therapy were permitted if considered necessary by the physician. Concomitant use of HRT therapy was not allowed during the study period. Drop-out rates were 15% with monotherapy and 11% with combination treatment, thus 5,373 patients were analyzed after 6 months.

Efficacy was evaluated using the Menopause Rating Scale (MRS) with assessments at 0, 3 and 6 months; a subset of patients (n = 736) treated for a further 6 months was also analysed after 12 months of therapy. The primary efficacy variable was the MRS "psyche" subscore, which covers symptoms of depressive mood, nervousness and nervous irritability, and generally impaired performance and memory. At baseline the MRS total score was assessed as less "severe" in the mono-product group (0.30 ± 0.17) than in the herbal combination group (0.36 ± 0.17). The "psyche" subscore was also differently distributed: symptoms were "mild" (0.31 ± 0.22) in the mono-group, compared to "moderate" (0.42 ± 0.23) in the group receiving the combination with St. John's wort (the latter treatment being mainly used for patients with more pronounced mood complaints).

Symptom scores improved substantially from baseline with both treatments. The MRS "psyche" subscore decreased under monotherapy to 0.21 ± 0.18 after 3 months and to 0.16 ± 0.14 after 6 months, and under combination therapy to 0.26 ± 0.18 and 0.19 ± 0.15 respectively. After adjustment for differences in baseline scores, the reductions from baseline were still significantly greater in the combination group ($p<0.001$ after 3 months). In the subset of patients followed up after 12 months the improvement from both therapies was maintained. In this study, therefore, both treatments were effective but the combination of black cohosh and St. John's wort was superior to black cohosh alone in alleviating climacteric mood symptoms [Briese 2007].

A total of 2,016 Hungarian women aged 40-65 years with moderate menopausal complaints were enrolled in a post-marketing surveillance study of the effects of the black cohosh extract. The inclusion criteria were a KM Index score ≥ 20 and refusal (74.5%) or contraindication (25.5%) of oestrogen therapy. The daily dose of extract corresponded to 40 mg of black cohosh and the severity of complaints was evaluated on commencement and at the end of weeks 4, 8 and 12. Data from 1,876 patients were completely evaluated. From the mean KM Index score of 28.00 points at week 0, the average decrease in the Index was 17.64 points after 12 weeks ($p<0.001$): 8.12 points after 4 weeks, a further 5.56 points after 8 weeks and a further 3.96 points after 12 weeks. The most favourable changes in weighted symptom scores were decreases of 6.32 in hot flushes,

2.86 in night sweating, 2.27 in insomnia and 2.0 in anxiety ($p<0.001$ in each case). Improvements were also experienced in palpitations (0.84), depression (0.72) and headache (0.69). The KM Index scores of almost half of the women included in the study decreased by more than 18; no improvement was evident in 11 patients (0.55%) and symptoms became more severe in 6 (0.30%) [Vermes 2005].

Women with menopausal complaints (n = 502; mean age 56 years; 79% no longer menstruating) enrolled in an open 3-month observational study were treated with the black cohosh extract, and the severity of climacteric symptoms was assessed on a visual analogue scale (VAS) and by a modified KM Index. VAS scores for hot flushes, profuse sweating, insomnia, nervousness/irritability and mood swings were included in the assessment. The percentage of hot flush symptoms classified as "very severe" decreased from a baseline of 51.9% to 16.3% after therapy. Conversely, the percentage of mild hot flushes increased from 16.2% initially to 59.9% under therapy. Mean values of the modified KM Index were 5.1 before and 2.9 after therapy. A beneficial effect was experienced by 73.8% of patients and 69.8% continued therapy after completing the study [Schmidt 2005].

A 3-month observational study carried out in Spain evaluated the effects of the black cohosh extract on the quality of life of 122 post-menopausal women who were experiencing climacteric symptoms; three sub-groups were assessed according to age range: 45-49 years (n = 35), 50-54 years (n = 54) and 55-59 years (n = 33). Before and after treatment each patient completed the Cervantes Scale, a 31-item self-reporting questionnaire designed to create a patient profile of health-related quality of life in four domains or categories: menopause and health (15 items, including vasomotor symptoms), psyche/mental (9 items), sexuality (4 items) and couple relationship (3 items). Compared to baseline the global quality of life score, and scores in the "menopause and health" and "psyche/mental" domains, had improved substantially across the entire population and in women of all three age groups at the end of 3 months of treatment. Improvement in the "sexuality" domain score was evident across the entire population but not in individual age ranges, while no changes were found in the "couple relationship" domain [Juliá Mollá 2009].

In an open observational study breast cancer patients undergoing tamoxifen therapy were also treated for climacteric complaints with the 40%-isopropanolic extract for 6 months, usually at the recommended dosage. Efficacy was assessed after 1, 3 and 6 months by means of the Menopause Rating Scale (MRS II; self-assessment scale) and tolerability was evaluated by each patient using a 5-point scale. Data from 47 patients (mean age 56 years; three-quarters postmenopausal) were available for intention-to-treat analysis. The initial tumour diagnosis dated back 8.6 months on average. All the women had previously undergone surgery, mainly breast-preserving; most had previously been treated with radiation therapy and hormones, and about half had undergone chemotherapy. On inclusion in the study metastases had already been diagnosed in 4 patients. Concomitant illnesses were documented in 29 women. Adjuvant treatment with tamoxifen had started on average 4 months after the first tumour diagnosis and 4 months prior to inclusion in the study. On commencement of therapy the dominant symptoms, of moderately severe intensity, were hot flushes and profuse sweating, followed by sleep disorders and physical and mental exhaustion. Mental symptoms (depressive moods, irritability and anxiety) were of mild intensity. MRS total scores of approximately two-thirds of the patients indicated "severe symptoms."

Most patients rated the efficacy of black cohosh as "good" to "satisfactory". The responder rate (very good, good or satisfactory efficacy) was 68.1% after 1 month and 70.2 % after 6 months. At the end of the black cohosh treatment the MRS total score had decreased substantially (from 17.6 to 13.6), as had subscores for "vegetative somatic complaints" and "mental complaints". The tolerability of the extract was rated as "very good" or "good" by 90% of the patients [Fischer 2006].

Women who reported at least 14 hot flushes per week were enrolled in an open, 4-week pilot study with the black cohosh extract; 21 patients (aged 38-80 years, mean 56 years) completed the study, of whom 13 had a history of breast cancer and 6 were also taking tamoxifen or raloxifene. At baseline (after keeping a diary in the previous week) the patients reported an average of 8.3 hot flushes per day. After 4 weeks the mean hot flush frequency had decreased by 50% to 4.2 per day, while the weekly hot flush score had decreased by 56%. The patients reported less trouble with sleeping, less fatigue and less abnormal sweating [Pockaj 2004].

Clinical studies performed with a 40%-isopropanolic dry extract of black cohosh (comparison between 39 and 127 mg of herbal drug/day)

In a randomized, double-blind study, 152 peri- and postmenopausal women outpatients (aged 42-60 years) with a KM Index score ≥ 20 (i.e. climacteric symptoms of at least moderate severity) were assigned to daily treatment for 24 weeks with the black cohosh extract at dose levels corresponding to either 39 mg (n = 76) or 127.3 mg (n = 76) of unprocessed drug; the study was not placebo-controlled. From the intention-to-treat group (n = 152) 123 patients completed the first 12 weeks and 116 completed 24 weeks.

Similar therapeutic effects were evident after 2 weeks in the two treatment groups; after 12 weeks the initial median KM Index scores of 30.5 (39 mg group) and 31.0 (127.3 mg group) had decreased to 8 and 7 respectively, and this was maintained to the end of the 6-month study period. The number of responders (defined by a KM Index score < 15) after 3 months was 70% in the 39 mg group and 72% in the 127.3 mg group. No significant difference in results was evident between the two groups from intention-to-treat or per-protocol analysis, indicating that a daily dose corresponding to 39 mg of unprocessed drug was sufficient. Self-Rating Depression Scale (SDS) scores supported the KM Index scores, reflecting an improvement in the state of menopausal depression during therapy in both groups without significant group differences [Liske 2002].

Clinical studies performed with a 58%-ethanolic dry extract of black cohosh (daily dose corresponding to 40 mg of herbal drug)

In a randomized, double-blind, placebo-controlled, three-armed multicentre study, post-menopausal women aged 40-60 with well-defined postmenopausal hormone levels and climacteric complaints were treated daily for 12 weeks with the black cohosh extract (n = 20) or 2 × 0.3 mg of conjugated oestrogens (CE, n = 22) or placebo (n = 20). Out of 97 patients initially randomized as intention-to-treat, almost one-third were subsequently found to have hormone levels not in accordance with the protocol and data on only 62 women were considered evaluable.

With regard to the primary efficacy criterion, the Menopause Rating Scale (MRS; 10 criteria, rated by the patients), superiority to placebo was evident after both the black cohosh extract ($p = 0.0506$) and CE ($p = 0.0513$). Although reductions in total scores did not quite reach significance due to a high placebo effect, evaluation of various combinations of MRS subscores revealed significant improvements (all $p<0.05$) in "major climacteric complaints" (4 criteria), "mental score" (4 criteria) and "atrophy"

(4 criteria) in the black cohosh group, in "hot flushes" (3 criteria) in the CE group, and in "somatic complaints" (5 criteria) in both the black cohosh and CE groups. Patients' diaries showed a decline in mean daily number of sweating episodes, reaching significance after 12 weeks in patients treated with black cohosh ($p<0.05$ vs. placebo) but not those treated with CE. Significant improvements in sleeping behaviour were also evident, the average number of nightly wake-up periods decreasing over 12 weeks in black cohosh- and CE-treated patients (both $p<0.05$ vs. placebo) [Wuttke 2003b, Wuttke 2006a].

Results from this study relating to effects on the endometrium and bone metabolism are summarized under Pharmacological studies in humans.

The effects of exercise training with and without black cohosh on bone mineral density (BMD) and 10-year coronary heart disease (CHD) risk were evaluated in 128 early postmenopausal women who were randomly assigned to one of three groups for a period of 12 months:

- Exercise group (n = 43). An initial 6-week conditioning period, then blocks of 6 weeks of high-intensity/high-impact exercise focussed primarily on bone strength, interspersed by blocks of 10 weeks of moderate exercise focussed on CHD parameters. Both blocks consisted of three weekly supervised group classes of 60 minutes.
- Exercise + black cohosh group (n = 43). The same exercise programme and the black cohosh extract - daily for 3 months, then discontinued for 3 months (as recommended by the manufacturer), with a further 3-month course thereafter and then abstinence.
- Control group (n = 42). A low-frequency and low-intensity exercise protocol focussed on well-being without causing physical adaptations; just 60 minutes of exercise weekly for 10 weeks, followed by 10 weeks of rest and so on.

Exercise group and control group participants received daily placebo tablets and all participants were given calcium and vitamin D supplements.

BMD at the lumbar spine decreased in the control group ($p<0.001$ compared to baseline) but was maintained in both exercise groups, which differed significantly from the control group (exercise group, $p = 0.001$; exercise + black cohosh group, $p = 0.005$) but not from each other. No significant differences in BMD between groups were evident at the femoral neck. The Framingham Risk Calculator (with scores based on age, total and HDL cholesterol, blood pressure, diabetes and smoking status) was used to calculate 10-year CHD risk, which increased significantly compared to baseline in the control group ($p = 0.007$) and in the exercise + black cohosh group ($p = 0.018$) but not in the exercise group ($p = 0.60$); however, no significant differences between groups were observed. As a secondary endpoint, menopausal complaints, assessed using the Menopause Rating Scale, significantly decreased in both exercise groups (exercise group, $p<0.001$; exercise + black cohosh group, $p = 0.003$) and increased marginally in the control group. The authors concluded that 12 months of fairly strenuous weekly exercise had favourably affected BMD and menopausal symptoms, and to a lesser extent 10-year CHD risk, and that under these conditions black cohosh did not enhance the positive effects [Bebenek 2010].

A randomized open study assessed the effects of black cohosh on hot flushes caused by tamoxifen (20 mg daily) in young premenopausal breast cancer survivors (aged 35-52 years) who had undergone surgery (lumpectomy or mastectomy), irradiation therapy and/or chemotherapy. Patients randomized to the "usual-care" group (n = 46) took tamoxifen only (for a period of 5 years), while those randomized to the intervention group (n = 90) commenced 12 months of treatment with the black cohosh extract 15 days before starting with tamoxifen. The number and severity of hot flushes was significantly reduced in the black cohosh group ($p<0.01$), with almost half of the patients (46.7%) free from hot flushes at the end of the study compared to none in the tamoxifen-only group. Severe hot flushes were reported by 24.4% of patients in the black cohosh group compared to 73.9% in the tamoxifen-only group [Hernández-Muñoz 2003].

In an open study primarily designed to investigate the safety of black cohosh, 400 postmenopausal women (aged 50-75 years) with symptoms including moderate to severe hot flushes were treated with the extract daily for 12 months. From self-assessment of their climacteric complaints using the Menopause Rating Scale II, baseline total scores decreased by about 50% over the course of treatment. The 4-week weighted score of hot flushes (number of hot flushes per day multiplied by a factor according to severity) decreased by 80.7% from baseline [Raus 2006].

In another open study, designed primarily to evaluate the effects of black cohosh on the liver, 100 healthy postmenopausal women (mean age 50 years), of whom 87 completed the study, were assigned to daily treatment with the extract for 12 months. Based on the participants' self-assessment, the prevalence, daily frequency and severity of hot flushes significantly decreased over the 12-month period of treatment ($p<0.001$ for each) [Nasr 2009].

Clinical studies performed with a 60%-ethanolic dry extract of black cohosh (DER 4.5-8.5:1)

In a randomized, double-blind, placebo-controlled study 180 women with menopausal complaints and a baseline KM Index score ≥ 20 were assigned to one of three daily treatments for 12 weeks: 13 mg of the black cohosh extract (as two tablets, each containing 6.5 mg; n = 60); one tablet containing 6.5 mg of the extract + one placebo tablet (n = 60); or two placebo tablets (n = 60). The most common complaints were hot flushes (89%), irritability and sleep disturbances (both 83%). KM Index scores, the primary efficacy variable, decreased significantly in both verum groups compared to placebo ($p<0.0001$ for 13 mg of extract, $p<0.001$ for 6.5 mg) and improvement in the 13 mg group was significantly superior to that in the 6.5 mg group ($p<0.01$). Responder rates (i.e. ≥ 50% decrease in the KM Index score) were found to be 63% in the 13 mg group, 38% in the 6.5 mg group and 7% in the placebo group. Quality of life scores, assessed on a visual analogue scale, improved in a comparable manner [Kaiser 2008].

A similar (or the same) extract was used in an earlier randomized, double-blind, placebo-controlled study, but only at the lower daily dose; 122 menopausal women (mean age 52 years) experiencing at least 3 hot flushes per day were treated with 6.5 mg of the extract (n = 81) or placebo (n = 41) daily for 12 weeks. There were two primary efficacy measures, the KM Index and a weekly weighted score of hot flushes, and secondary efficacy variables such as the Menopause Rating Scale. Primary efficacy analysis in the intention-to-treat population (median initial KM Index score < 20) did not demonstrate superiority of the black cohosh extract compared to placebo. However, in the subgroup of patients (n = 53) with a KM Index score ≥ 20 (i.e. with climacteric complaints of at least moderate intensity) improvements in this Index demonstrated significant superiority of black cohosh ($p<0.018$); reductions in scores of 47% and 21% were observed in the black cohosh (n = 35) and placebo (n = 18) groups respectively. The results for weekly weighted scores of hot flushes ($p<0.052$) and the Menopause Rating Scale ($p<0.009$) were in the same direction [Frei-Kleiner 2005].

Clinical study performed with a 70%-ethanolic dry extract of black cohosh (daily dose corresponding to 128 mg of herbal drug)

Women experiencing at least 35 menopausal vasomotor symptoms per week were assigned to one of four daily treatments for 12 months in a randomized, double-blind study: 128 mg of the 70%-ethanolic black cohosh dry extract, standardized to 7.27 mg of triterpene glycosides (n = 22), or 398 mg of a 70%-ethanolic dry extract of red clover (n = 22), or 0.625 mg of conjugated equine oestrogens + 2.5 mg of medroxyprogesterone acetate (CEE/MPA) as a positive control (n = 23), or placebo (n = 22). The women maintained diaries throughout the study, each day recording the number of vasomotor symptoms (hot flushes and night sweats) as well as the intensity (mild, moderate or severe) of hot flushes. Over the 12-month intervention period the average number of vasomotor symptoms decreased across all groups: black cohosh group, 34% reduction; red clover, 57%; CEE/MPA, 94%; placebo, 63%. There were no statistically significant differences in the number of vasomotor symptoms per week or hot flushes alone between placebo and black cohosh (or red clover) groups after 3, 6, 9 or 12 months. Vasomotor intensity was significantly higher in the black cohosh group than in the placebo group at the 6-month ($p = 0.008$) and 9-month time points ($p = 0.02$). The black cohosh extract was therefore ineffective in this study. In the CEE/MPA control group, relative to placebo, significant reductions in the number of vasomotor symptoms per week ($p<0.0001$ to $p = 0.0004$), vasomotor intensity ($p<0.001$ to $p<0.05$) and hot flushes per week ($p<0.0001$ to $p = 0.0001$) were recorded at all time points [Geller 2009].

Clinical study performed with a 70%-ethanolic dry extract of black cohosh (daily dose corresponding to 160 mg of herbal drug)

In a 12-month double-blind study 351 peri- and postmenopausal women aged 45-55 years were randomly assigned to one of five daily treatments: placebo (n = 84); a 70%-ethanolic extract of black cohosh (corresponding to 160 mg of crude drug; n = 80); a different black cohosh extract (corresponding to 200 mg of crude drug) in combination with eight other herbal ingredients (n = 76); the same herbal combination plus counselling to ensure increased consumption of dietary soya (n = 79); or conjugated equine oestrogens (0.625 mg daily, n = 32) with additional medroxyprogesterone acetate 2.5 mg for those women with an intact uterus (n = 29) [Note: Following premature ending of the Women's Health Initiative study in 2002 (due to concerns about the safety of HRT) participants in this arm were given the option of finding out if they had been assigned to hormone therapy; 16 were unblinded and 1 discontinued use of the study drug, but all remained enrolled]. Women with and without hysterectomy (without bilateral ovariectomy) and at least two vasomotor symptoms (hot flushes and/or night sweats) per day over 2 weeks were included.

The frequency and intensity of vasomotor symptoms were self-assessed and symptoms were recorded at baseline and after 3, 6 and 12 months. Additionally, the Wiklund Menopause Symptom Scale (rating the severity of 13 menopausal symptoms) was used, particularly the mean Wiklund Vasomotor Symptom subscale. At baseline the average number of vasomotor symptoms per day was 6.5 and the average intensity (mild, moderate or severe on a scale of 1 to 3) was 1.8. Of 183 women in menopausal transition at baseline 79 experienced no menstruation during the study. The primary outcome parameters, frequency and intensity of vasomotor symptoms and the Wiklund Vasomotor Symptom subscale score, decreased between baseline and 3 months in all groups. However, differences between any of the herbal groups and placebo were not significant at 3, 6 or 12 months. In contrast, the number of vasomotor symptoms per day and Wiklund Vasomotor Symptom subscale scores decreased significantly (both $p<0.001$ compared to placebo) in HRT-treated patients over the study period [Newton 2006].

Clinical studies performed with extracts not fully defined in the respective papers

In a randomized, double-blind, placebo-controlled cross-over study 132 patients aged 32-76 (64% with a history of breast cancer) who had experienced more than 14 hot flushes per week for at least one month were randomly assigned to daily treatment for 4 weeks with either a black cohosh extract (corresponding to 2 × 20 mg of crude drug; extraction solvent not stated) or placebo, followed by a further 4-week period on the alternative treatment. As co-medication 44% of the patients continued taking tamoxifen, 1% raloxifene and 11% an aromatase inhibitor; the co-medication of 8% was unknown and 36% took no other medication. Data from 116 patients were analysed. Each patient kept a daily hot flush diary (frequency and severity) and a weekly symptom experience diary (rating various symptoms and impressions from 0 to 10), and the Greene Climacteric Scale was completed at baseline and after 4 weeks of both treatments. The primary endpoint was the difference in average hot flush scores (a combination of average daily hot flush frequency and severity) between the baseline week and the last study week of the first treatment period.

Patients on black cohosh reported a mean decrease of 15% in hot flush scores during the fourth treatment week, compared to a 31% decrease for patients on placebo. Thus no significant difference in efficacy between black cohosh and placebo treatment was evident from primary endpoint results after 4 weeks ($p = 0.10$), and cross-over analysis did not suggest any benefit for black cohosh ($p = 0.98$). Furthermore, there were no differences in any of the symptoms evaluated in symptom experience diaries or in overall quality of life, and no significant decrease in either arm for any parameter of the Greene Climacteric Scale [Pockaj 2006].

Patients who had previously received chemotherapy and radiation therapy for breast cancer and were experiencing daily hot flushes participated in a randomized, double-blind, placebo-controlled study to assess the effect of black cohosh (2 tablets of a commercial product daily for 2 months; no further details given) on the frequency and intensity of hot flushes on a 1 to 3 scale. Some patients also used tamoxifen concomitantly for the management of breast cancer. Of the 85 study participants, 42 (of whom 29 used tamoxifen) were assigned to treatment with black cohosh and 43 (of whom 30 used tamoxifen) were assigned to placebo; 69 patients completed the study. Both black cohosh and placebo groups reported a decline in the frequency (about 27% overall) and intensity of hot flushes during the study, but differences between groups were not significant. Both groups reported improvements in seven menopausal symptoms (heart palpitations, excessive sweating, headaches, poor sleep, depression and irritability or nervousness) during the study, but only sweating was significantly reduced in the black cohosh group compared to the placebo group ($p = 0.04$). Small changes in serum levels of follicle-stimulating hormone and luteinizing hormone also did not differ between groups [Jacobson 2001].

In a randomized study 120 women (average age 53 years) with post-menopausal symptoms were assigned to daily treatment for 6 months with either a black cohosh extract (40 mg, extraction solvent not stated, n = 60) or fluoxetine (20 mg, n = 60), with interim assessments after 1, 2 and 3 months. The daily number and severity (graded from 0 to 3) of hot flushes and night sweats were recorded in diaries to calculate monthly scores (number × severity to obtain a daily score, all daily scores added together to

yield the score for the month). A modified KM Index (including vaginal dryness), Beck's Depression Scale and a quality of life questionnaire (RAND-36) were also used in assessments at baseline and after 3 months of treatment.

KM Index and Beck's Depression Scale scores decreased significantly within 3 months in both groups ($p<0.001$ compared to baseline) with significantly greater reduction in KM Index score in the black cohosh group ($p = 0.02$) and in Beck's Depression Scale score in the fluoxetine group ($p = 0.01$). Compared to month 1 scores, month 6 hot flush scores and night sweat scores had decreased significantly in both groups ($p<0.001$ for both scores in both groups), but to a significantly greater extent in the black cohosh group ($p<0.001$ for both scores); by the end of 6 months of therapy the hot flush score had decreased by 85% in the black cohosh group compared to 62% in the fluoxetine group. Quality of life improved in all parameters except pain in both treatment groups [Oktem 2007].

In an open study, 61 post-menopausal women (aged 36-62 years) with daily symptoms, natural or following surgery, took a black cohosh extract (2 × 40 mg capsules daily, each standardized to 1 mg of 23-*epi*-26-deoxyactein; extraction solvent not stated) for 12 weeks followed by a 12-week wash-out phase (a total of 24 weeks of assessment); 48 subjects were available for assessment after 4 weeks, 46 after 12 weeks and 45 after 24 weeks. Menopausal symptoms, assessed using the KM Index, improved in 83% of the women by week 4 (at least 1 point reduction in KM Index score) and at 12 weeks at least 21% of patients had a 50% reduction in their KM Index score. During the wash-out phase KM Index scores increased by at least 1 point in 73% of the patients [Ruhlen 2007].

In another open study, 50 postmenopausal women were treated daily for 6 months with a black cohosh extract (type and amount not stated). An excellent response was reported in subjective complaints assessed by the KM Index and Hamilton Anxiety Scale; only 10% of patients had no relevant improvement after 3 months. Endometrial thickness (measured by endovaginal ultrasound) remained unchanged after 6 months [Georgiev 1997].

Patients with a DSM-IV diagnosis of anxiety disorder due to menopause were randomized to treatment with 2-4 × 32 mg of a black cohosh extract (5.6% triterpene glycosides, extraction solvent not stated; n = 15) or placebo (n = 13) daily for up to 12 weeks in a double-blind, placebo-controlled study. No significant anxiolytic effect of black cohosh was evident from the primary outcome measure, Hamilton Anxiety Rating Scale (HAM-A) scores ($p = 0.29$), or the proportion of subjects with a reduction at least 50% in baseline HAM-A scores by the end of the study period ($p = 0.79$). The authors noted that the extremely small sample size of this preliminary study limited their ability to identify clinically meaningful differences between groups [Amsterdam 2009].

Clinical studies performed with a 40%-isopropanolic dry extract of black cohosh (8 mg/day)

The corresponding amounts of herbal drug were not stated in the respective papers, but were within the range of 40-140 mg [Boblitz 2000]

In a randomized, double-blind, three-armed study, women aged 46-58 years received one of the following treatments daily for 12 weeks: 8 mg of the black cohosh extract (n = 30) or 0.625 mg of conjugated oestrogens (n = 30) or placebo (n = 20). The patients suffered from menopausal complaints such as hot flushes, sweating, palpitations, depressed mood and sleep disorders; they also had vaginal complaints due to oestrogen deficiency. Effects on climacteric symptoms were assessed by the KM Index and Hamilton Anxiety Scale (HAMA). Compared to placebo the extract produced a significant decrease in KM Index scores (mean value from 34 to 14; $p<0.001$) and the HAMA ($p<0.001$). Vaginal cytology parameters also improved significantly ($p<0.001$). The extract significantly increased the degree of proliferation of the vaginal epithelium from 2-3 to 3-4 on the Schmitt scale for vaginal cytology ($p<0.01$ compared to placebo) [Stoll 1987]. This study has been described as questionable because of low methodological quality and, in finding no difference between placebo and 0.625 mg of oestrogen, a result that contradicts a large body of data [Borelli 2008a].

In an open, randomized, comparative study 60 women under 40 years of age who had undergone hysterectomy but retained at least one intact ovary and complained of climacteric symptoms were treated daily for 24 weeks with 1 mg of oestriol (n = 15) or 1.25 mg of conjugated oestrogens (n = 15) or an oestrogen-progesterone sequence preparation (n = 15) or 8 mg of the black cohosh extract (n = 15). Using a modified KM Index for assessment at 4, 8, 12 and 24 weeks, statistically significant decreases in climacteric symptoms were observed in all treatment groups ($p<0.01$). Conjugated oestrogens and the oestrogen-progesterone combination appeared to be slightly superior but differences between the groups were not statistically significant [Lehmann-Willenbrock 1988].

In another open study, 50 women with climacteric symptoms, who had previously been treated every 4-6 weeks with intramuscular injections of a combination of oestradiol valerate 4 mg and prasterone nantate 200 mg, were treated with the black cohosh extract for 6 months. During this time 28 patients (56%) required no further injections, 21 patients (44%) required one injection and 1 patient required two injections. The KM Index decreased on average from 17.6 to 9.2 ($p<0.001$) [Pethö 1987].

Clinical studies performed with a 60%-ethanolic dry extract of black cohosh (80 drops/day)

The corresponding amounts of herbal drug were not stated in the respective papers, but were within the range of 40-140 mg [Boblitz 2000]

In an open, randomized, three-armed study 60 women (aged 45-60 years) with climacteric symptoms received the black cohosh extract (n = 20) or 0.6 mg of conjugated oestrogens (n = 20) or 2 mg of diazepam (n = 20) daily for 12 weeks. All three forms of treatment led to reductions in a modified KM Index (which included hot flushes, nocturnal sweating, nervousness, headache and palpitations), the Hamilton Anxiety Scale and a Self-assessment Depression Scale. Conjugated oestrogens and (in contrast to other studies) the black cohosh extract showed a trend towards oestrogenic stimulation, with increased proliferation of the vaginal epithelium as shown by increases in karyopyknosis and eosinophil indices [Warnecke 1985].

In an open study in 50 women aged 45-60 years with climacteric complaints (mainly neurovegetative), the black cohosh extract reduced symptoms assessed as moderate by the KM Index to "requiring no therapy" after 12 weeks ($p<0.001$) [Vorberg 1984].

In another open study, in 36 women aged 45-62 years with climacteric symptoms, treatment with the black cohosh extract for 12 weeks led to a significant average decrease in KM Index scores compared to initial values ($p<0.001$) [Daiber 1983].

In an open, multicentre, drug-monitoring study of 629 patients, menopausal symptoms such as hot flushes, profuse sweating, headache, vertigo, nervousness and depression improved in over 80% of cases after 6-8 weeks of treatment with the black cohosh extract [Stolze 1982].

Pre-1970 clinical studies
From 1957 to 1964 a number of case reports and uncontrolled studies were published describing the successful treatment of numerous women with climacteric symptoms or menstrual disorders with black cohosh extracts [Kesselkaul 1957, Schotten 1958, Földes 1959, Stefan 1959, Stiehler 1959, Brücker 1960, Heizer 1960, Starfinger 1960, Görlich 1962, Langfritz 1962, Schildge 1964].

Clinical reviews
In a systematic review of the clinical efficacy of black cohosh in the treatment of menopausal symptoms, published in 2002, only randomized, blind and controlled studies were considered. Four studies involving a total of 226 women met the inclusion criteria [Warnecke 1985, Stoll 1987, Lehmann-Willenbrock 1988, Jacobson 2001] and two were placebo-controlled [Stoll 1987, Jacobson 2001]. The authors concluded that, in spite of plausible mechanisms of action, the clinical efficacy of black cohosh for the treatment of menopausal symptoms had not been convincingly demonstrated [Borrelli 2002].

An update by the same authors in 2008 evaluated more recent evidence from randomized, double-blind studies; only mono-preparations of black cohosh in comparison with placebo or a standard drug were considered and studies involving medically (drug)-induced menopause or women with bilateral hysterectomy were excluded. Six studies involving a total of 1112 peri- and post-menopausal women met the inclusion criteria [Osmers 2005, Bai 2007, Wuttke 2003b + Wuttke 2006a, Frei-Kleiner 2005, Newton 2006, Stoll 1987]. The authors considered that the efficacy of black cohosh in reducing menopausal symptoms was still not supported by fully conclusive evidence and further investigations are desirable [Borrelli 2008a].

Another systematic review of black cohosh in the treatment of menopausal symptoms, based on searches up to November 2007, considered 12 randomized studies using mono-preparations:

- Placebo-controlled studies [Osmers 2005, Wuttke 2003b, Frei-Kleiner 2005, Newton 2006, Pockaj 2006, Jacobson 2001, Stoll 1987].
- Studies comparing black cohosh with reference medications of known therapeutic efficacy: tibolone [Bai 2007], fluoxetine [Oktem 2007] or oestrogen therapy [Nappi 2005, Lehmann-Willenbrock 1988, Warnecke 1985].

Two out of seven placebo-controlled studies [Osmers 2005, Stoll 1987] and all five comparisons with reference medications were found to suggest significant therapeutic benefit from black cohosh. The authors concluded that black cohosh alone may not have a meaningful effect on menopausal symptoms in most cases [Palacio 2009].

Clinical review with meta-analysis
Four randomized placebo-controlled studies [Osmers 2005, Wuttke 2003b, Frei-Kleiner 2005, Newton 2006] were included in a systematic review of the efficacy of black cohosh mono-preparations for the treatment of menopausal symptoms in peri- and/or postmenopausal women with no history of breast cancer. In three of the studies [Osmers 2005, Wuttke 2003, Frei-Kleiner 2005] black cohosh was found to be efficacious in reducing the frequency of vasomotor symptoms, but no significant improvement compared to placebo was evident in the fourth study [Newton 2006].

Only two of the studies [Frei-Kleiner 2005, Newton 2006] afforded data suitable for meta-analysis. From the pooled data, the combined estimate of rate difference for improvement of vasomotor symptoms (defined as the difference between black cohosh and placebo groups with respect to the change in proportion of subjects with vasomotor symptoms between baseline and 12 weeks) was calculated as 11%. Although this was only moderately positive, one of the two studies on which the calculation was based had not shown superiority over placebo [Newton 2006], and a further relevant and highly positive study [Kaiser 2008] was not taken into account [Shams 2010].

Pharmacokinetic properties
Single doses of 32, 64 or 128 mg of a 75%-ethanolic black cohosh extract, containing 1.4, 2.8 or 5.6 mg respectively of 23-*epi*-26-deoxyactein, were administered orally to 15 healthy menopausal women, assigned to one of three dosage groups with 5 women per group. Blood samples and urine were collected over a period of 24 hours from administration. In all three groups the serum concentration of 23-*epi*-26-deoxyactein increased rapidly during the first hour, reaching a maximum after 2.0-2.9 hours; the serum half-life was 2.1-3.0 hours and the area under the curve increased linearly with dosage; less than 0.01% was recovered in urine. Since only small amounts of the ingested 23-*epi*-26-deoxyactein were detected intact in blood or urine and no metabolites were detected, it appeared likely that this glycoside undergoes degradation in the stomach; *in vitro* tests indicated a half-life of 80 minutes in simulated gastric fluid [van Breemen 2010].

In rats treated orally with actein at 35.7 mg/kg body weight the serum level of actein reached a peak of 2395 ng/litre at 6 hours then declined to 102 ng/litre within 24 hours; in urine collected for the first 24 hours the concentration of actein was 777 ng/litre [Einbond 2009].

Preclinical safety data

Chronic toxicity
A 40%-isopropanolic dry extract of black cohosh, administered to rats for 6 months as a daily dose equivalent to the unprocessed drug at 535.5 mg/kg body weight (approximately 700 times the human therapeutic dose), produced no outward signs of toxicity or histopathological changes [Boblitz 2000].

Mutagenicity
In the Ames test a 40% V/V-isopropanolic dry extract of black cohosh revealed no mutagenic potential at up to the equivalent of 30.3 mg of unprocessed drug per plate [Boblitz 2000]. A 60% V/V-ethanolic dry extract of black cohosh also gave a negative result in the Ames test [UKPAR].

Acute toxicity of actein
Minimum lethal single doses of actein were determined [Genazzani 1962] as:
Mouse, intraperitoneal: > 500 mg/kg
Rat, oral: > 1000 mg/kg
Rabbit, intravenous: > 70 mg/kg

Subchronic toxicity of actein
Minimal lethal doses of actein administered daily for 30 days were determined [Genazzani 1962] as:
Mouse, intraperitoneal: > 10 mg/kg
Rabbit, oral: > 6 mg/kg

Clinical safety data
Doses of up to 200 mg of black cohosh or corresponding amounts of extracts have been used without apparent adverse effects [Liske 2002, Cimicifuga-UK GSL 1990].

Adverse events reported in clinical studies
In two 12-week randomized studies using a 60%-ethanolic dry extract (4.5-8.5:1) of black cohosh no significant differences were reported between verum and placebo groups in the prevalence or intensity of adverse events. In the first study the frequencies were 17/83 patients (20%) in the black cohosh group (6.5 mg of extract per day) and 10/44 (23%) in the placebo group [Frei-

Kleiner 2005]. The frequencies in the second study, 4/60 (7%) in both 6.5 mg/day and 13 mg/day groups, and 6/60 (10%) in the placebo group, bore no relationship to dosage [Kaiser 2008]. In another study the proportion of patients with adverse effects judged to have at least a "possible" causal relationship with the study medication was assessed as 6/153 (3.9%) in the black cohosh group and 7/151 (4.6%) in the placebo group [Osmers 2005].

Adverse events were reported in every arm of a study in which patients were treated for 12 months with HRT, black cohosh or placebo; none were severe in the black cohosh arm. Menstrual disorders were reported more frequently in the HRT group than in the placebo group ($p<0.001$; HRT, 59% of patients; placebo, 20%; black cohosh, 13%), as was breast discomfort ($p = 0.04$; HRT, 16% of patients; placebo, 4%; black cohosh, 0%) [Newton 2006]. In the same study black cohosh was found to have no effects on vaginal epithelium, the endometrium or reproductive hormones such as oestradiol, FSH, LH and SHBG [Reed 2008].

In another 12-month study, no significant differences were evident between menopausal women who took 128 mg of a 70%-ethanolic black cohosh dry extract daily and those who took placebo with respect to adverse events (none serious) or any of a wide range of safety parameters tested, including breast and endometrial screening, liver function, blood count and lipid profile [Geller 2009].

No significant differences in adverse effects between black cohosh groups and placebo groups were found in a systematic review of nine randomized, placebo-controlled studies, in four of which mono-preparations of black cohosh were used [Osmers 2005, Wuttke 2003b, Frei-Kleiner 2005, Newton 2006]; the other five involved black cohosh combined with other active ingredients [Shams 2010].

During 6 months of treatment with a black cohosh extract (40 mg/day) or fluoxetine (20 mg/day) all reported adverse events were mild, but the frequency was significantly lower in the black cohosh group (7/40 patients) than in the fluoxetine group (13/40) ($p<0.001$) [Oktem 2007].

In an open clinical study 19/61 (31%) of the patients, who took 2 × 40 mg of a black cohosh extract daily for 6 months, reported adverse events: 12 gastrointestinal, 5 reproductive (vaginal dryness, nipple tenderness), 4 headaches, 1 of dysphagia and 1 of fatigue. None were reported to be serious but they led to 5 dropouts [Ruhlen 2007].

A retrospective case-control study carried out in a USA-based population of 949 breast cancer cases and 1,524 controls evaluated whether the use of herbal preparations to manage menopausal symptoms was associated with breast cancer risk. The authors concluded that the use of black cohosh had a significant breast cancer protective effect (adjusted odds ratio 0.39, 95% CI: 0.22-0.70). This association was similar among women who reported use of either black cohosh or specifically a 40%-isopropanolic dry extract [Rebbeck 2007].

In a German case-control study, associations between patterns of use of herbal preparations and the incidence of breast cancer were investigated in 10,121 postmenopausal women (3,464 cases and 6,657 controls). Information was collected in face-to-face interviews supported by a list of brand names and the preparations evaluated contained black cohosh, St. John's wort, "phytoestrogens" (red clover or soy isoflavones), agnus castus or certain other herbal active ingredients. In the 9.9% of these women who had ever used a herbal preparation the risk of invasive breast cancer was found to decrease by approximately 26% overall, and by 4% per year of use ($p = 0.03$), irrespective of a healthy lifestyle (physical activity and diet) or the histological type and receptor status of the tumour. Risk estimates did not differ significantly between different types of herbal active ingredients. For a 40%-isopropanolic dry extract of black cohosh (in a mono-preparation or a combination with St. John's wort) the inverse association with invasive breast cancer was found to have an odds ratio of 0.80 (95% CI: 0.63-1.00); for other black cohosh preparations the odds ratio was 0.96 (95% CI: 0.64-1.45). These findings supported the hypothesis of a protective effect of black cohosh against breast cancer in postmenopausal women [Obi 2009].

The clinical safety of black cohosh was investigated in an open study by determining its effects on mammographic breast tissue density and, from collection of breast cells by fine needle aspiration biopsy and assessment using the Ki-67 marker, on breast cell proliferation. The baseline status of 74 postmenopausal women with menopausal symptoms was compared by blinded observers with their status after 6 months of treatment with a 40%-isopropanolic extract (corresponding to 40 mg/day of black cohosh). None of the mammography results indicated a change in breast tissue density over the period of treatment and there was no increase in breast cell proliferation. No change was observed in mean endometrial thickness, and laboratory parameters and vital functions remained normal. Adverse events, possibly related to the treatment, were reported by 12 women (16%), but none were severe [Linden Hirschberg 2007].

Anonymous data relating to breast cancer patients (including those with oestrogen receptor-positive tumours) who had been treated between 1992 and 2003 in 1,511 medical practices in Germany were evaluated in a retrospective cohort study; the mean age at diagnosis was 61.4 years. The main endpoint was disease-free survival following a diagnosis of breast cancer in relation to the use of an isopropanolic black cohosh extract, alone or in combination with a St. John's Wort extract. A total of 18,861 women were included in the analysis, of whom 1,102 were categorized as black cohosh users and 17,759 served as a control group. There was a significant association between black cohosh and tamoxifen use with tamoxifen patients 67% more likely to have been placed on black cohosh than their non-tamoxifen counterparts ($p<0.0001$).

The mean observation period between diagnosis and last database entry was 3.6 years in the control group but longer, 4.6 years, for black cohosh users. The rate of recurrence in the control group (3,148 out of 17,759, i.e. 17.7%) was higher than in black cohosh patients (113 out of 1,102, i.e. 10.3%). In the control group 14% of women developed a recurrence within 2 years of first diagnosis while this proportion was reached in the black cohosh group only after 6.5 years. The data showed that black cohosh was not associated with an increase in risk of recurrence but associated with prolongation of disease-free survival [Henneicke-von Zepelin 2007].

The endometrial safety of a 58%-ethanolic black cohosh extract, taken for 12 months at a daily dose corresponding to 2 × 20 mg of herbal drug, was assessed from biopsy samples of endometrial tissue in an open prospective study involving 400 postmenopausal women (aged 50-75 years) with symptoms including moderate to severe hot flushes. No cases of endometrial hyperplasia or more serious adverse endometrial outcomes occurred and endometrial thickness, measured by endovaginal ultrasonography, did not increase compared to baseline. Mammograms from 138 patients were available for evaluation and showed no increase in breast density, except in the case of one woman found to have breast cancer unrelated to the medication. No clinically relevant changes in hormone levels

were observed during the study. From a total of 752 recorded adverse events 318 (42%) were assessed as possibly and 8 (1%) as probably related to the black cohosh medication, the majority (295) of these being due to increased serum lipids. The intensity of adverse events was mild in about 88% of cases, moderate in 10.5% and severe in 1.9%. None of eight adverse events assessed as serious were considered to have a causal relationship with the medication; four resulted from an accident and the others included a planned cholecystectomy, acute bronchitis and surgery [Raus 2006].

In a 3-month surveillance study involving 502 women with menopausal complaints the tolerability of black cohosh was generally assessed as very good [Schmidt 2005] and in a subsequent 6-month observational study of over 6000 women more than 90% of physicians and patients rated the tolerability as excellent or good [Briese 2007].

Effects on the liver

The European Medicines Agency (EMA, formerly EMEA) assessed all available case reports (up to 2007) associating hepatotoxicity and black cohosh with regarding to causality. In 26 of the 43 cases there was insufficient documentation to support a causal relationship between black cohosh use and hepatotoxicity. The association was deemed unrelated in 5 cases and unlikely in 7 cases, leaving only 5 cases classified as having "possible" or "probable" causality. Based on these findings, the HMPC recommended the following advice: "Patients should stop taking Cimicifugae racemosae rhizoma (black cohosh, root) and consult their doctor immediately if they develop signs and symptoms suggestive of liver injury (tiredness, loss of appetite, yellowing of the skin and eyes or severe upper stomach pain with nausea and vomiting or dark urine)" [EMEA 2007].

Four cases of hepatotoxicity classified in the EMEA's assessment of June 2006 as having "possible" or "probable" causality with the use of black cohosh were re-evaluated using the qualitative and quantitative causality assessment of the updated system of the Council for International Organizations of Medical Sciences (CIOMS); the fifth case was reported at a later date and appeared in the EMEA's updated assessment (Rev 1) of May 2007. Due to incomplete data, one of the four cases was not assessable. Of the remaining three, one patient had a favourable course under continued steroid therapy and the final diagnosis was autoimmune hepatitis; the other two patients required liver transplants following final diagnoses of herpetic hepatitis. Using a causality assessment in the form of a diagnostic algorithm it was concluded that there was no evidence for a causal relationship between treatment with black cohosh and observed liver disease in the four patients.

Even in the five possible or probable cases it was found that the products in question, the co-medications and the adverse reactions were not precisely defined in the original reports. Duplicated cases could not easily be identified because information such as duration of intake, dosage, co-medication or medical history was often lacking or contradictory [Teschke 2009a, Teschke 2009b, Hudson 2007].

The Expert Committee of the US Pharmacopeia reviewed and analysed information relating to black cohosh from human clinical case reports, adverse event reports, animal pharmacological and toxicological data, historical use, regulatory status and contemporaneous extent of use. Reports were obtained from various sources including the EMEA, Health Canada, the Australian Therapeutic Goods Administration and the US FDA. Case reports associating liver damage with the use of black cohosh were evaluated using the Naranjo causality algorithm scale; 30 non-duplicate cases were assigned "possible" causality, but none were assigned probable or certain causality [Mahady 2008].

The position regarding black cohosh and suspected hepatoxicity has been summarized in a 2010 review [Teschke 2010]. While the original case reports of hepatotoxicity were generally of poor quality, with major inconsistencies and confounding variables, and no definite causal relationship was established [Teschke 2009a, Teschke 2009b, Teschke 2010], further case reports have appeared [Mahady 2009, Mahady 2010, Guzman 2009, Vannacci 2009, Pierard 2009]. From a consumer protection standpoint some regulatory authorities (in the European Union, the USA and Australia, for example) have taken the view that a safety signal is discernible and hence black cohosh products should be labelled to include a cautionary statement [EMEA 2007, Mahady 2008, Mahady 2009, Mahady 2010].

To evaluate the effects of black cohosh on liver function 100 healthy postmenopausal women (mean age 50 years), of whom 87 completed the study, were treated daily for 12 months with a 58%-ethanolic extract (corresponding to 40 mg of dried rhizome). Women with abnormal liver function were excluded and menopausal state was confirmed from follicle-stimulating hormone (FSH) levels. From assessments at baseline and after 12 months no significant changes were observed over the treatment period in hepatic blood flow or liver function tests (prothrombin time and concentration, serum albumin, bilirubin, γ-glutamyltransferase, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase), nor in body weight or blood pressure [Nasr 2009].

Over a 10-year period a black cohosh extract (2.5% actein, extraction solvent not stated) at high daily doses of 500 mg or 1000 mg, alone or in combination with other herbal ingredients, was prescribed to 798 women with menopause-related complaints by an Italian clinic of natural medicine without any reported adverse effects. In response to a public statement (2006) by the EMEA concerning a possible link between black cohosh and cases of hepatotoxicity, 158 patients who had taken the extract continuously for at least 12 months were identified and contacted, and 107 menopausal women were subsequently followed up by telephone (33/107) or by physical examination including evaluation of blood samples and parameters of liver function (74/107). No sign of hepatic disease, or worsening of already altered but stable liver function parameters, was detected in any of the 107 patients [Firenzuoli 2008].

Following the administration of single doses of 32, 64 or 128 mg of a 75%-ethanolic black cohosh extract to groups of 5 healthy menopausal women in a pharmacokinetic study no serum markers of liver function were found to have changed significantly from baseline within 12 hours [van Breemen 2010].

Interactions

In an open-label study randomized with respect to both medication and sequence 16 healthy young volunteers received, on separate occasions followed by 30-day wash-out periods: a black cohosh extract (2 × 20 mg/day; 14 days), a milk thistle extract (3 × 300 mg/day; 14 days) and, as positive controls for transport protein P-gp induction and inhibition respectively, rifampin (2 × 300 mg/day; 7 days) and clarithromycin (2 × 500 mg/day; 7 days). Digoxin (0.4 mg) was administered orally 24 hours before the start and on the last day of each treatment period, and serum digoxin concentrations were monitored over the next 24 hours. The black cohosh and milk thistle preparations had no significant effects on digoxin pharmacokinetics and hence appeared to pose no clinically significant risk for P-gp-mediated interactions [Gurley 2006a].

In a further study of similar design, the effects of black cohosh and milk thistle extracts on cytochrome P450 CYP3A enzyme activity were investigated in 19 healthy young volunteers. They

were randomly assigned to receive, on separate occasions followed by wash-out periods, a black cohosh extract (2 × 40 mg/day; 14 days), a milk thistle extract (3 × 300 mg/day; 14 days) and, as positive controls for CYP3A induction and inhibition respectively, rifampin (2 × 300 mg/day; 7 days) and clarithromycin (2 × 500 mg/day; 7 days). Midazolam (8 mg) was administered orally 24 hours before the start and on the last day of each treatment period, and serum midazolam and 1-hydroxymidazolam concentrations were monitored for 6 hours thereafter. In contrast to the marked effects caused by rifampin and clarithromycin, midazolam pharmacokinetics were unaffected by black cohosh and milk thistle, which appeared to have no clinically relevant effect on CYP3A activity [Gurley 2006b].

Other studies, of similar design and carried out by the same research group, have shown that black cohosh has no clinically relevant effects on human CYP1A2, CYP2E1, CYP3A4/5 [Gurley 2005] and CYP2D6 [Gurley 2008] enzymes.

Other human safety data
The authors of a review suggested that black cohosh should be used with caution during pregnancy, particularly during the first trimester where its purported labour-inducing effects could be of concern, and during lactation [Dugoua 2006].

Patients with unexplained infertility (n =119) were randomly divided into two groups. Both groups received clomiphene citrate (an ovulation-inducer) at 150 mg/day on days 3-7 and one group also received a black cohosh extract (corresponding to 120 mg/day of herbal drug) on days 1-12. When a leading follicle attained a diameter > 17 mm and serum oestradiol exceeded 200 pg/ml human chorionic gonadotrophin (HCG) was injected and scheduled intercourse recommended. The pregnancy rate was significantly higher in the black cohosh group ($p<0.01$). No safety issues were reported [Shahin 2008].

Safety reviews
Data from 13 clinical studies, 3 postmarketing surveillance studies, 4 series of cases and 8 single case reports, evaluated to assess the evidence for or against the safety of black cohosh, suggested that black cohosh is safe to use at the recommended dose. Attribution of causality of severe adverse events in case reports proved problematic and required further investigation [Borrelli 2008b].

A critical evaluation of the safety of black cohosh concluded that human clinical studies, postmarketing surveillance studies and uncontrolled reports involving over 2,800 patients demonstrated a favourable safety profile with a low incidence of adverse events (5.4%). Of the reported adverse events, 97% were minor and did not result in discontinuation of therapy. No severe adverse events were attributed to black cohosh [Low Dog 2003].

A systematic review of data from clinical studies and also from spontaneous reporting programmes of the World Health Organization and national regulatory bodies suggested that adverse events arising from the use of black cohosh are rare, mild and reversible, the most common being gastrointestinal upsets and rashes. It was concluded that, although definitive evidence is not available, black cohosh seems to be a safe herbal medicine [Huntley 2003].

In another review the author concluded that clinical studies and case reports have demonstrated good tolerance of, and low risk of side effects from, ethanolic and isopropanolic extracts of black cohosh [Foster 1999].

From a systematic review of 5 clinical studies in which breast cancer patients took black cohosh extracts for the treatment of hot flushes and other menopausal symptoms, as well as evidence from 21 preclinical studies relating to black cohosh and cancer, it was concluded that the use of black cohosh appears to be safe in breast cancer patients [Walji 2007].

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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The Scientific Foundation for Herbal Medicinal Products

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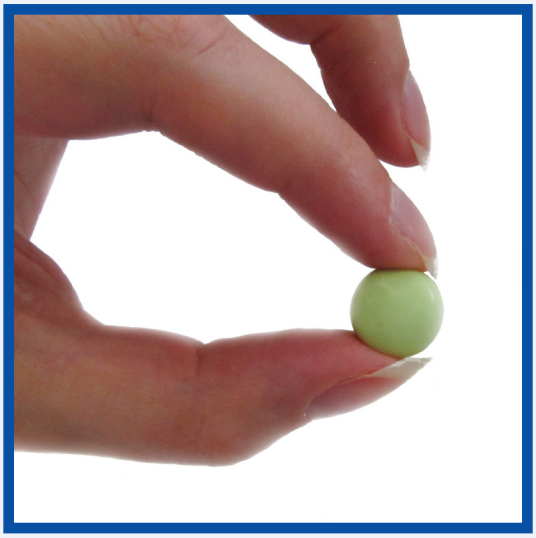

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
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Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

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Cola

DEFINITION

Cola consists of the whole or fragmented dried seeds, freed from the testa, of *Cola nitida* (Vent.) Schott et Endl. (*C. vera* K. Schum.) and its varieties, as well as of *Cola acuminata* (P. Beauv.) Schott et Endl., (*Sterculia acuminata* P. Beauv.). It contains not less than 1.5 per cent of caffeine (M_r 194.2) (dried drug).

The material complies with the monograph of the European Pharmacopoeia [Cola].

CONSTITUENTS

The main constituents are purine alkaloids such as caffeine (approximately 2%) and small amounts of theobromine (0.02 to 0.08%) and theopylline. Other constituents are catechins, proanthocyanidins, tannins (5 to 10%), up to 45% starch, sugars, proteins and minerals [Weinges 1964; Weinges 1967; Balansard 1987; Morton 1992; Seitz 1992; Adeyeye 1994: Boudjeko 2009; Bruneton 1998; Wichtl 2002; Atawodi 2007; Niemenak 2008; Burdock 2009].

Fresh material contains caffeine-catechin-complexes (approximately 90% of the caffeine present) [Maillard 1988; Seitz 1992; Wichtl 2002].

Primary amines such as methylamine and secondary amines such as dimethylamine have also been determined in quantities of up to 100 ppm [Atawodi 1995]. Thiamine and other B-vitamins are also present [Heinrich 2004].

CLINICAL PARTICULARS

Therapeutic indications
For the treatment of short-term physical and/or mental exhaustion. In these indications, the efficacy is plausible on the basis of human experience and long-standing use [Kerharo 1969; Etkin 1981; Bradley 1992].

Posology and method of administration

Dosage

Adults and children above 12 years:
Dried drug: 2-6 g per day, divided into up to 3 doses [Bradley 1992; Seitz 1992].
Liquid extract (1:1 in 60% ethanol), 0.6-1.2 mL three times daily [Bradley 1992].

Method of administration
For oral administration.

Duration of administration
If symptoms persist or worsen, medical advice should be sought.

Contra-indications
Gastric or duodenal ulcers [Ibu 1986].

Special warnings and special precautions for use
The intake of cola should be restricted in individuals with hypertension and cardiac disorders, because of the caffeine content [Bradley 1992; Barnes 2007].

Interaction with other medicaments and other forms of interaction
Due to the caffeine content, concomitant intake of caffeine-containing drinks or psycho-analeptic medicines may cause a stronger caffeine effect.

Pregnancy and lactation
There is evidence of effects of cola consumption by women on the head and chest measurements of their new born infants [Abidoye 1990]. The product should not be used during pregnancy or lactation without medical advice.

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Effects on ability to drive and use machines
None known.

Undesirable effects
Difficulties in falling asleep, excitation, nervousness. Gastrointestinal disturbances are possible [Atawodi 1995; Barnes 2007].

Overdose
Overdosage may result in enhanced effects as described under "Undesirable effects".

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments
An aqueous decaffeinated cola extract, containing approximately 40% oligomeric and polymeric procyanidins, showed oxygen radical scavenging activity as well as reduction of elastase liberation from polymorphonuclear neutrophils [Daels-Rakotoarison 2003].

In vivo experiments

Effects on locomotor activity
An experiment involving i.p. administration of an aqueous extract to mice resulted in no effect at the lower dose of 2.5 mg/kg b.w., yet significantly ($p<0.01$) increased locomotor activity at the medium dose of 5.0 mg/kg b.w. and slightly depressed locomotor activity at the highest dose of 10.0 mg/kg b.w. [Ajarem 1990].

Oral administration of a dried aqueous extract (1:2) at doses of 400 mg and 800 mg/kg b.w. resulted in significantly enhanced exploratory activity of rats during the first day only ($p<0.05$). The effect was similar but weaker than that following administration of caffeine at 15 mg/kg b.w., for which the effect was still noticeable the following day [Ettarh 2000].

For two weeks, three groups of 10 rats each received either 1 mL of distilled water (control group), 1 mL of a solution of caffeine corresponding to 20 mg/kg b.w. or 1 mL of a solution of cola extract at 320 mg/kg b.w.(extract not further specified; corresponding to 20 mg/kg b.w. of caffeine). Locomotor activity was significantly increased in both the caffeine and cola extract groups as compared to control ($p<0.05$). Onset of the effect in the cola extract group was slower and more gradual but more sustained than in the caffeine group [Scotto 1987].

Neurological effects
In a further study, three groups of 10 rats each received p.o. either 1 mL of distilled water, 20 mg/kg b.w. of caffeine in solution or cola extract containing an equivalent level of caffeine (320 mg/kg b.w.; extract not further specified) for 15 days. Cola treatment induced an increase in the cortical activity with a widening of the dominant frequency spectrum 7 to 10Hz band of EEG whereas caffeine alone induced a shift of the dominant frequency band towards higher frequencies [Vaille 1993].

Cardiovascular effects
Effects of cola on blood pressure were assessed in a 7 day experiment involving 4 groups of 10 rats each. Group 1 served as the control and consumed only normal rat feed (10.0 ± 0.5 g/day). Group 2 consumed normal feed (9.6 ± 1.0 g/day) mixed with ground cola (2:1). Group 3 consumed normal feed (10.5 ± 0.8 g/day) mixed with caffeine (1.0 g/120 g) at a level equivalent to the caffeine content of the cola in the feed of group 2. While group 4 consumed ground cola as the only feed (9.2 ± 1.2 g). Mean arterial pressure (MAP measured as mm Hg; control: 88.0 ± 1.2) was increased significantly in groups 2-4 (group 2: 110.8 ± 1.7, $p<0.001$; group 3: 108.4 ± 1.9, $p<0.001$; group 4: 108.6 ± 2.0, $p<0.001$) compared to the control; whereas there was no significant difference between groups 2-4. Furthermore, there were no significant differences in body weight change between all 4 groups [Osim 1993].

Gastrointestinal effects
Rats were treated with aqueous cola extracts (10 mg/100 g b.w.) either by gastric perfusion or by intravenous injection. Both administrations resulted in increased (60-70%) gastric acid secretion (measured by titration), the effect after gastric perfusion being slightly stronger [Ibu 1986].

Pharmacological studies in humans
Changes in parameters of optical performance were assessed in a study with 10 healthy volunteers who received a single oral dose of 30 g whole raw cola seed to be chewed. The parameters were pupil size, near point convergences, visual acuity, amplitude of accommodation and intraocular pressure. No changes were observed immediately after cola ingestion except for near point convergence and amplitude of accommodation which were improved. Exophoria was also changed to orthophoria in some subjects [Igwe 2007].

Pharmacokinetic properties
Differences in the pharmacokinetics of comparable doses of pure caffeine (20 mg/kg) with cola of comparable caffeine content (320 mg/kg extract) were observed in studies in matched samples of rats for single doses and exposure over 30 days (n=110 and 88 respectively). After single oral administration of caffeine solution and cola extract, analysis showed two peaks of caffeine concentration in both plasma and erythrocytes. However, in caffeine-treated animals these peaks occurred much later. The mean time to reach the first maximum concentration of caffeine after a single dose was 1 hour for cola and 1.5 hours for caffeine alone in both plasma and erythrocytes, to reach the second peak 3 and 8 hours (7 for erythrocytes) respectively. Caffeine when given alone was also eliminated in most measures at more than twice the rate as when given in cola. On chronic oral administration of caffeine and cola extract, the variations in plasma and erythrocyte caffeine concentrations show a peak of maximal concentration that also appears much earlier in animals treated by cola extract [Vaille 1993].

On EEG readings cola extracts demonstrated a more complex spectrum of effects consistent with the simultaneous presence of catechin, that partically antagonises the action of caffeine [Vaille 1993].

Preclinical safety data

Repeated dose and chronic toxicity studies
Male albino rats received p.o. either 1 mL of distilled water (control group) or 0.5 mL of an aqueous cola extract (100 g/200 mL) corresponding to 57 mg/kg b.w. every second day over 18 weeks (n = 24). After approximately 9 weeks of treatment, irritability, restlessness, local alopecia, diuresis and loss of appetite but no signs of weakness were observed. At the end of the study a decrease in body weight was found in the experimental rats. Increases in the weight of organs and of the levels of serum alkaline phosphatase, serum acid phosphatase and serum total cholesterol ($p<0.05$) were observed [Ikegwuonu 1981].

Reproductive toxicity
Three groups of 6 male albino rats received p.o. either 25 mg/kg b.w., 50 mg/kg b.w. or 100 mg/kg b.w. of an aqueous extract

once a day for 30 days. A fourth group received 1.0 mL of distilled water (control). On day 31 after the last dosing, blood samples were collected and the animals were then euthenised. Histological preparation of the testes was also carried out. There was a significant decrease in testosterone levels ($p<0.05$), sperm motility ($p<0.05$), sperm viability ($p<0.05$) and sperm count ($p<0.05$) in all groups relative to the control, but there was no significant change in sperm morphology. There was moderate to severe necrosis with the dissolution of numerous seminiferous tubules in testis [Oyedeji 2013].

Clinical safety data

The effects of cola ingestion during pregnancy were investigated in 100 women. The study showed that there was a correlation between cola consumption and head and chest circumference. Women who ingested cola gave birth to babies with smaller head and chest circumference than women who did not ingest cola. There was no correlation between cola consumption and birth weight [Abidoye 1990].

The concomitant consumption of cola with halofantrine hydrochloride (an antimalarial drug) was investigated in 15 healthy males in a randomised crossover design study. A single dose of 500 mg halofantrine was orally administered alone or with 12.5 g powdered cola nut. C_{max} for halofantrine decreased from 179 ± 119 to 98 ± 32 ng/mL ($p = 0.021$), and the AUC was reduced from 17,450 ± 4,611 to 11,821 ± 4,069 ng•h/mL ($p <0.001$) when co-administered with cola. C_{max} of the active metabolite, desbutylhalofantrine, decreased from 124 ± 41 to 62 ± 23 ng/mL ($p <0.001$) and the AUC from 13,341 ± 4,749 to 7,359 ± 3,018 ng•h/mL ($p <0.001$) when co-administered with cola.There was no significant difference for t_{max} and $t_{1/2}$ [Kolade 2008].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Second Edition, 2003
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
BALLOTAE NIGRAE HERBA	Black Horehound	Supplement 2009
BETULAE FOLIUM	Birch Leaf	Second Edition, 2003
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Second Edition, 2003
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Supplement 2009
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Second Edition, 2003
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Second Edition, 2003
FUMARIAE HERBA	Fumitory	Supplement 2009
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Supplement 2009
GRINDELIAE HERBA	Grindelia	Supplement 2009
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Second Edition, 2003
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Second Edition, 2003
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003

LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Supplement 2009
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Second Edition, 2003
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Second Edition, 2003
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Second Edition, 2003
RATANHIAE RADIX	Rhatany Root	Supplement 2009
RHAMNI PURSHIANI CORTEX	Cascara	Second Edition, 2003
RHEI RADIX	Rhubarb	Second Edition, 2003
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Second Edition, 2003
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Second Edition, 2003
SALICIS CORTEX	Willow Bark	Second Edition, 2003
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Second Edition, 2003
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VIOLAE HERBA CUM FLORE	Wild Pansy	Supplement 2009
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

E/S/C/O/P Monographs

Online Series

The Scientific Foundation for Herbal Medicinal Products

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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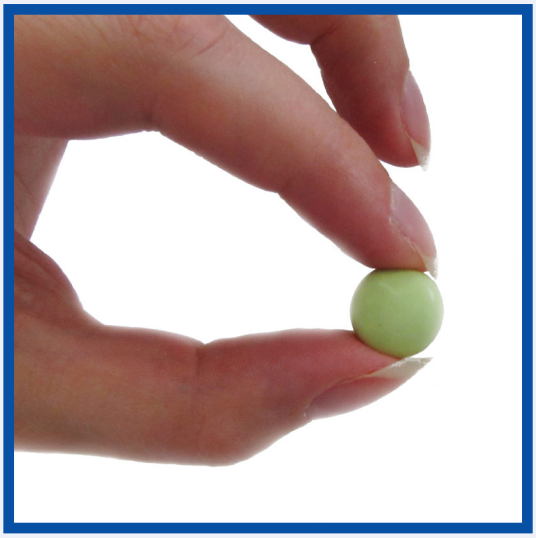
The Scientific Foundation for Herbal Medicinal Products

Echinaceae angustifoliae radix

Narrow-leaved Coneflower Root

2019



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MONOGRAPHS

***The* Scientific Foundation for**
Herbal Medicinal Products

ECHINACEAE ANGUSTIFOLIAE RADIX
Narrow-leaved Coneflower Root

2019

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Second Edition, Supplement 2009

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

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Plant illustrated on the cover: *Echinacea angustifolia*

FOREWORD

It is a great pleasure for me, on behalf of my colleagues in ESCOP, to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in the production of ESCOP monographs and other activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

ECHINACEAE ANGUSTIFOLIAE RADIX 2019

Narrow-leaved Coneflower Root

DEFINITION

Narrow-leaved coneflower root consists of the dried, whole or cut, underground parts of *Echinacea angustifolia* DC. It contains not less than 0.5 per cent of echinacoside ($C_{35}H_{46}O_{20}$; M_r 786.5), calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Narrow-leaved Coneflower Root].

CONSTITUENTS

The characteristic constituents are:

Caffeic acid derivatives (1.0-1.4%), principally echinacoside (0.3-1.3%), cynarin (1,3-dicaffeoylquinic acid; 0.1%) and chlorogenic acid [Bauer 2007; Perry 2001; Pellati 2005; Bradley 2006].

Alkamides (0.01-0.5%), principally isomeric dodeca-2*E*,4*E*,8*Z*,10*E/Z*-tetraenoic isobutylamides together with undeca-2*E/Z*-ene-8,10-diynoic acid isobutylamides, dodeca-2*E*-ene-8,10-diynoic acid isobutylamide and others [Bauer 1988, 1989a; Bauer 2007; Laasonen 2002; Bradley 2006].

Polysaccharides and glycoproteins [Wagner 1985; Beuscher 1995; Bradley 2006].

A small amount of essential oil (up to 0.1% V/m) is also present [Voaden 1972; Heinzer 1988; Mazza 1999].

CLINICAL PARTICULARS

Therapeutic indications

Internal use
Adjuvant therapy and prophylaxis of recurrent infections of the upper respiratory tract (common cold) [Melchart 1998; Bauer 2007; Bradley 2006].

Posology and method of administration

Dosage
Adult daily dose: 3 x 1 mL of extract (1:11, ethanol 30% V/V), 3 × 60 drops of tincture (1:5, ethanol 55% V/V) [Bauer 2007; Melchart 1998]; other equivalent preparations.

Method of administration
For oral administration.

Duration of administration
The duration of treatment should not exceed 8 weeks.

Contra-indications
Known hypersensitivity to plants of the Asteraceae (Compositae).

As with all immunostimulants, not recommended in cases of progressive systemic disorders such as tuberculosis, diseases of the white blood cell system, collagenoses, multiple sclerosis and other autoimmune diseases, AIDS or HIV infections [Bauer 2007, Ardjomand-Woelkart 2016].

Special warnings and special precautions for use
If the symptoms worsen or high fever occurs during the use of the medicinal product, medical advice should be sought.

There is a possible risk of allergic reactions in atopic patients. These patients should consult their doctor before using Echinacea.

Interaction with other medicaments and other forms of interaction
None reported in clinical studies.

E/S/C/O/P MONOGRAPHS

Only weak effects on the cytochrome P450 enzymes CYP1A2 and CYP3A4 were observed in *in vitro* assays [Modarai 2007; Woelkart 2007].

Pregnancy and lactation
Although there is no evidence of harmful effects during pregnancy [Gallo 2000; Perri 2006], in accordance with general medical practice the product should not be used during pregnancy or lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
Echinacea can trigger allergic reactions in atopic patients.

Allergic reactions due to Echinacea e.g. hypersensitive skin reactions are rather rare and mostly not serious [Ardjomand-Woelkart 2016].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Influence on immune functions
A 90% ethanolic extract (1:10) at a concentration of 1 µg/mL enhanced the phagocytosis index of human granulocytes by 17%; no effect was observed at concentrations of 1 ng/mL or lower. The chloroform-soluble fraction from the ethanolic extract increased phagocytosis by 34% at 0.1 µg/mL, while the water-soluble fraction stimulated phagocytosis by a maximum of only 17% at 1 µg/mL [Bauer 1988, 1989b].

Three fresh root extracts, a 50% ethanolic tincture, a cold water infusion and a hot water infusion, were tested using human blood in several assays of immune function: monocyte secretion of TNF-α, IL-10 and IL-12, and proliferation of peripheral blood mononuclear cells. All the extracts increased TNF-α production. The tincture caused a significant ($p=0.005$) increase and the cold water extract a non-significant increase in IL-12 production compared to controls on day 1 post-extraction. However, effects on peripheral blood mononuclear cell proliferation were minimal [Senchina 2005]. Other experiments with an extract (50% ethanol) showed an increase in peripheral blood mononuclear cells and tended to increase IL-10 production [McCann 2007].

A 55% ethanolic and an aqueous extract (80°C) inhibited IL-8 activity in both uninfected human epithelial cells and cells infected with rhinovirus (RV 14). A markedly enhanced IL-8 production was found for an aqueous extract (40 °C) in virus-stimulated cells [Vimalanathan 2009].

A root extract (standardized to contain > 4% of echinacoside, > 5% of high molecular weight polysaccharides of MW ca. 200 kDa and < 0.1% of isobutylamides), from which lipo-polysaccharides (LPS) had been removed to avoid non-specific responses of immunocompetent cells, produced significant and dose-dependent increases ($p<0.01$ at 1-10 µg/mL) in IFN-γ production in a murine T-lymphocyte cell culture stimulated by anti-CD3. A high molecular weight polysaccharide fraction from the extract produced a greater increase. Neither the extract nor the polysaccharide fraction had any effect on immune-stimulating activity in a murine macrophage cell line, indicated by a lack of effect on nitric oxide (NO) production. In contrast, the original LPS-containing extract was effective in activating the macrophages [Morazzoni 2005].

Significant ($p<0.05$) inhibition of prostaglandin E_2 production in LPS-stimulated RAW 264.7 mouse macrophages was shown for an ethanolic extract at a concentration of 15 µg/mL [LaLone 2007].

A dose-dependent inhibition of NO production and TNF-α release in RAW 264.7 macrophages was demonstrated (10-200 µg/mL) for a combination of extracts from narrow-leaved coneflower root obtained with various solvents. In macrophages incubated overnight with the extract at 100-200 µg/mL, and subsequently stimulated with *Salmonella enterica* subspecies *enterica* serovar Typhimurium, phagocytosis was significantly ($p<0.04$) enhanced and bacterial killing over 24 hours significantly ($p<0.026$) inhibited [Zhai 2007a].

An alkamide fraction and individual alkamides inhibited LPS-mediated activation of murine macrophages with IC_{50} values of 6 µg/mL for the fraction and 3-25 µg/mL for the individual compounds. Individual alkamides alone did not activate the macrophages. However, when the macrophages were pre-incubated with the total alkamide fraction and then stimulated with LPS, NO production was reduced when compared to cells treated only with LPS [Chen 2005].

Isolated alkamides (isomeric dodeca-2*E*,4*E*,8*Z*,10*E/Z*-tetraenoic acid isobutylamide, dodeca-2*E*,4*E*,8*Z*-trienoic acid isobutylamide and dodeca-2*E*,4*E*-dienoic acid isobutylamide) produced a dose-dependent induction of TNF-α mRNA in monocytes/macrophages. This effect was mediated by the cannabinoid receptor CB2 and also by multiple signal transduction pathways [Gertsch 2004]. The binding affinity of alkamides isolated from a carbon dioxide extract for rodent cannabinoid receptors CB1 and CB2 was evaluated in a standard receptor binding assay. Most of the alkamides showed affinities for CB2 receptors with Ki values lower than 20 µM [Woelkart 2005].

Further experiments showed that dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide and dodeca-2*E*,4*E*-dienoic acid isobutylamide bind to the CB2 receptor more strongly than endogenous cannabinoids. Both alkamides (50 nM) up-regulated constitutive IL-6 protein expression to 130-160% of the level seen in controls, while 500 nM of dodeca-2*E*,4*E*-dienoic acid isobutylamide inhibited expression. LPS-induced TNF-α, IL-1β and IL-12p70 were inhibited by 50 nM of the two alkamides in a CB2-independent manner [Raduner 2006].

Antioxidant activity
Methanolic and ethanolic (1:10, 85%) extracts exhibited antioxidant activity, including free radical scavenging and metal chelating [Sloley 2001; Hu 2000].

In the DPPH assay, an 80%-methanolic extract, cynarin and echinacoside, gave EC_{50} values of 231 µg/mL, 11.0 and 6.6 µM respectively [Pellati 2004].

Caffeic acid derivatives such as echinacoside inhibited free radical-induced degradation of collagen in a dose-dependent manner [Maffei Facino 1995].

Antimicrobial and antiviral activity
A high molecular weight fraction (M_r > 10,000 D) containing polysaccharides and glycoproteins exhibited antiviral activity against *Herpes simplex* virus type 1 in a plaque reduction assay [Beuscher 1995]. Antiviral activity was also shown for

echinacoside against vesicular stomatitis virus in L-929 mouse cells in a plaque reduction assay [Cheminat 1988].

An ethanolic (70%) extract, and an ethyl acetate fraction thereof, showed activity against rhinovirus, *Herpes simplex* virus and influenza virus [Hudson 2005].

The n-hexane fraction from a 95%-ethanolic extract of fresh root exhibited marginal near-UV light (300-400 nm)-activated phototoxicity and hence antifungal activity against the fungal pathogens *Candida shehata*, *Cryptococcus neoformans* and *Fusarium oxysporum*, but not against *Candida albicans* [Merali 2003].

Other effects
Alkamides have been shown to exert anti-inflammatory activity by inhibiting the enzymes 5-lipoxygenase (5-LOX) and cyclo-oxygenase; their IC_{50} values depended on the particular structure and ranged from 8 to 83% and 23 to 75% respectively [Wagner 1989; Müller-Jakic 1994]. A 70%-ethanolic extract inhib-ited 5-LOX activity with an IC_{50} of 0.44 µg root/mL [Merali 2003].

Treatment of H4 human neuroglioma cells with a carbon dioxide extract for 48 hours led to significant suppression of PGE_2 formation ($p<0.001$). Experiments with eight different alkamides isolated from the extract demonstrated a direct inhibitory effect by three alkamides: undeca-2*Z*-ene-8,10-diynoic acid isobutylamide, dodeca-2*E*-ene-8,10-diynoic acid isobutylamide and dodeca-2*E*,4*Z*-diene-8,10-diynoic acid 2-methylbutylamide. All three alkamides were shown to suppress COX-2 activity but none inhibited COX-2 mRNA and protein expression. The results suggested that certain alkamides may inhibit COX-2-dependent PGE_2 formation at sites of inflammation [Hinz 2007].

Ethanolic extracts from narrow-leaved coneflower root and a 1:1 blend of purple coneflower root and narrow-leaved coneflower root inhibited human cytochrome P450 3A4 (CYP 3A4) activity with relative IC_{50} values of 1.05% and 6.73% respectively (expressed as percentage of full strength of stock solutions after serial dilutions), compared to 0.04% for St. John's wort [Budzinski 2000]. Other experiments demonstrated CYP enzyme-mediated degradation of alkamides in human liver microsomes. Metabolism was dependent on both the structure of individual compounds and the combination present in the assay [Matthias 2005].

A hexane extract (3 to 30 µg/mL) was reported as inhibiting Pgp activity in the human proximal tubular cell line HK-2 [Romiti 2008].

A hexane extract exhibited concentration-dependent (3-300 µg/mL) cytotoxic activity in human pancreatic (MIA PaCa-2) and colon cancer (COLO320) cells with IC_{50} values of 82.86 and 31.78 µg/mL respectively [Chicca 2007].

In vivo experiments

Phagocytosis-stimulating effects
In the carbon clearance test in mice, oral administration of a 90% ethanolic extract (1:10) daily for 2 days at 0.5 mL/kg b.w. caused a 1.7-fold increase in phagocytosis [Bauer 1988, 1989b].

Immunomodulating effects
An extract (standardized to contain > 4% of echinacoside, > 5% of high molecular weight polysaccharides of MW ca. 200 kDa and < 0.1% of isobutylamides), administered orally to mice at 1 g/kg/day one day before and for six days after infection with Candida albicans, reduced mortality by 30% ($p<0.01$). In *C. albicans*-infected mice treated orally with cyclosporin A (10 mg/kg/day for 5 days) to cause immunosuppression, as well as with the extract orally at 1 g/kg/day for 7 days, mortality was reduced by 40% ($p<0.01$) [Morazzoni 2005].

To demonstrate that Echinacea treatment restored mild stress-induced immune changes to normal levels, BALB/c mice were given an extract obtained with various solvents (130 mg/kg b.w. p.o.) once a day for 7 consecutive days. On the 4th day of treatment they were immunized with sheep red blood cells to induce an immune response; and immune cells were collected 12–15 hours after the last dose of extract. Compared to the vehicle only (5% ethanol) and no treatment control groups, treatment with the extract demonstrated a significant increase in the percentage of peripheral blood lymphocytes ($p=0.001$) and the splenic lymphocyte subpopulation percentage ($p=0.004$); and more specifically in the CD49 cell subset ($p<0.05$). *Ex vivo* experiments with stimulated splenocytes showed a significant increase in the production of IFN-γ ($p<0.05$), IL-4 ($p=0.046$), IL-2 ($p=0.037$) and IL-10 ($p=0.057$). There was significantly reduced production of TNF-α ($p=0.004$) and IL-1β ($p=0.007$) [Zhai 2007b].

Rats which were injected with the antigen keyhole limpet haemocyanin and re-exposed to the same antigen on days 14 and 35, were then treated orally with an extract (3.3 g/L, not further specified) in their drinking water over a period of 6 weeks. The animals showed a significant augmentation of their primary and secondary IgG responses to the antigen compared to control on days 7 ($p=0.04$), 21 ($p=0.005$) and 27 ($p=0.002$) of treatment. After 31 days, there was no longer a significant difference between the Echinacea-treated and control groups [Rehman 1999].

Pharmacological studies in humans
In a randomized, double-blind, placebo-controlled study, the effects of several extracts on experimentally-induced rhinovirus type 39 infections (a well-established model for study of the pathogenesis and treatment of common cold) were evaluated in 399 healthy young adult volunteers found by screening to be susceptible to this virus. Three different extracts, prepared from the same batch of *E. angustifolia* root but having distinct phytochemical profiles, were used: a supercritical carbon dioxide extract (73.8% alkamides, no polysaccharides), a 60%-ethanolic extract (2.3% alkamides, 48.9% polysaccharides) and a 20%-ethanolic extract (0.1% alkamides, 42.1% polysaccharides). The volunteers received 3 × 1.5 mL of the extract preparations (equivalent to 3 × 300 mg of root) or placebo daily, either as prophylactic pre-treatment (for 7 days before virus challenge) or treatment (from virus challenge to day 5), or in both phases. No significant effects of the extracts were observed on rates of infection or severity of symptoms [Turner 2005].

Combination preparation
The effects of a combination of Echinacea root dry ethanolic extracts on expression of leucocyte heat shock protein 70 (hsp70), erythrocyte haemolysis, plasma antioxidant status, standard serum chemistry, haematological values and plasma alkamide concentrations were studied in 11 healthy individuals aged 26-61 years. The volunteers were evaluated at baseline (day 1) and on day 15 after taking 2 tablets (each containing extracts equivalent to 675 mg of purple coneflower root + 600 mg of narrow-leaved coneflower root) daily for 14 days. Significant increases were found in leucocyte hsp70 expression after mild heat shock ($p = 0.029$) and in white cell counts ($p = 0.043$). A preventative effect against free radical-induced erythrocyte haemolysis ($p = 0.006$), indicative of an antioxidant effect, was also observed [Agnew 2005].

Clinical studies
A double-blind, placebo-controlled study evaluated the efficacy of root extracts from two *Echinacea* species in the prevention of upper respiratory tract infections in 289 volunteers. In randomized groups the volunteers were instructed to take 2 × 50 drops (about 2 × 1 mL) of an ethanolic extract (1:11 in 30% ethanol) of narrow-leaved coneflower root (group A, n = 100) or purple coneflower root (group B, n = 99), or placebo (group C, n = 90), daily on 5 days per week (Monday to Friday) for 12 weeks. Of the 244 participants fully complying with the protocol, 84, 85 and 75 were in groups A, B and C respectively. The average time to the occurrence of first upper respiratory tract infections was 66, 69 and 65 days in groups A, B and C respectively, and 32%, 29% and 37% of participants had at least one upper respiratory tract infection. Although suggesting a relative reduction of 20% in risk of infection in the purple coneflower root group and 13% in the narrow-leaved coneflower root group, the results were not significant; a prophylactic effect of the extracts was not evident [Melchart 1998].

Pharmacokinetic properties
In a randomized crossover study 11 healthy subjects received a single oral dose of 2.5 mL of a 60%-ethanolic extract in the morning following an overnight fast. C_{max} for dodeca-2*E*,4*E*,8*Z*,10*E/Z*-tetraenoic acid isobutylamides was approximately 8 ng/mL (slow absorber) or 11 ng/mL (fast absorber) [Woelkart 2005].

Combination preparation
Alkamides were rapidly absorbed and measurable in plasma from 9 healthy volunteers 20 minutes after ingestion of 4 tablets, each containing dry ethanolic extracts corresponding to 675 mg of purple coneflower root + 600 mg of narrow-leaved coneflower root, and remained detectable for up to 12 hours. The maximum plasma concentration of total alkamides (average 336 ± 131 ng eq/mL) was reached within 2.3 hours. Caffeic acid conjugates could not be identified in any plasma sample [Matthias 2005].

Preclinical safety data
In the Ames mutagenicity test conflicting results were obtained with three fluid extracts of narrow-leaved coneflower root. One fluid extract showed mutagenic potential in *Salmonella typhimurium* strains TA98 and TA100 (with or without S9 activation), while a second fluid extract gave positive results only in TA98 and a third one gave negative results in both strains [Schimmer 1994].

Clinical safety data
Several meta-analyses on clinical use and safety generally concluded that the use of Echinacea is safe, notwithstanding that several adverse reactions have been reported. However, accurate information to evaluate these reports, e.g. data on the Echinacea species used, plant parts, type of extract, dosage and co-administration of other medicines, is missing [Soon 2001; Mullins 2002; Huntley 2005; Gryzlak 2007; Freeman 2008; Kocaman 2008; Karsch-Völk 2014; Ardjomand-Woelkart 2016].

Mild adverse effects were reported by 18 out of 100 subjects who took 2 × 1 mL of a hydroethanolic extract of narrow-leaved coneflower root daily for 12 weeks in a randomized study, compared to 11 out of 90 subjects in the placebo group [Melchart 1998].

In a randomized study of experimental rhinovirus infections, 19 out of 437 volunteers (4%) reported adverse events during a prophylaxis phase; in 4 volunteers out of 164 (2%) in the verum group and 5 out of 274 (2%) in the placebo group the adverse effects were judged to be possibly related to the medication. During the treatment phase, 15 out of 315 (5%) in the verum group reported an adverse event possibly related to the treatment compared to 4 out of 104 (4%) in the placebo group. Gastrointestinal side effects were the most frequent [Turner 2005].

In a prospective controlled study involving 412 women, the pregnancy outcome was investigated following gestational exposure to (undefined) solid or liquid preparations of *Echinacea* (*angustifolia*, *purpurea* and in one case *pallida*). In the group of 206 women who used Echinacea products during pregnancy (112 in the first trimester), 13 spontaneous abortions and 6 major malformations (4 occurring after exposure to Echinacea in the first trimester) were reported, compared to 7 spontaneous abortions and 7 major malformations in the matching control group of 206 women. There was no significant difference found between the Echinacea and control groups in terms of pregnancy outcome, delivery method, maternal weight gain, gestational age, birth weight or fetal distress [Gallo 2000].

A systematic review of evidence from the use of Echinacea during pregnancy and lactation led to the conclusion that Echinacea (including *Echinacea angustifolia*) is not teratogenic [Perri 2006].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2019
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2018
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003

LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2019
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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The Scientific Foundation for Herbal Medicinal Products

The second edition of ESCOP Monographs, published as a hardback book in 2003 with a Supplement in 2009, has been widely acclaimed for its authoritative information on the therapeutic uses of herbal medicines. Monographs covering a total of 107 herbal substances include extensive summaries of pharmacological, clinical and toxicological data, and copious references to scientific literature form an important part of each text.

Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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European Scientific Cooperative On Phytotherapy

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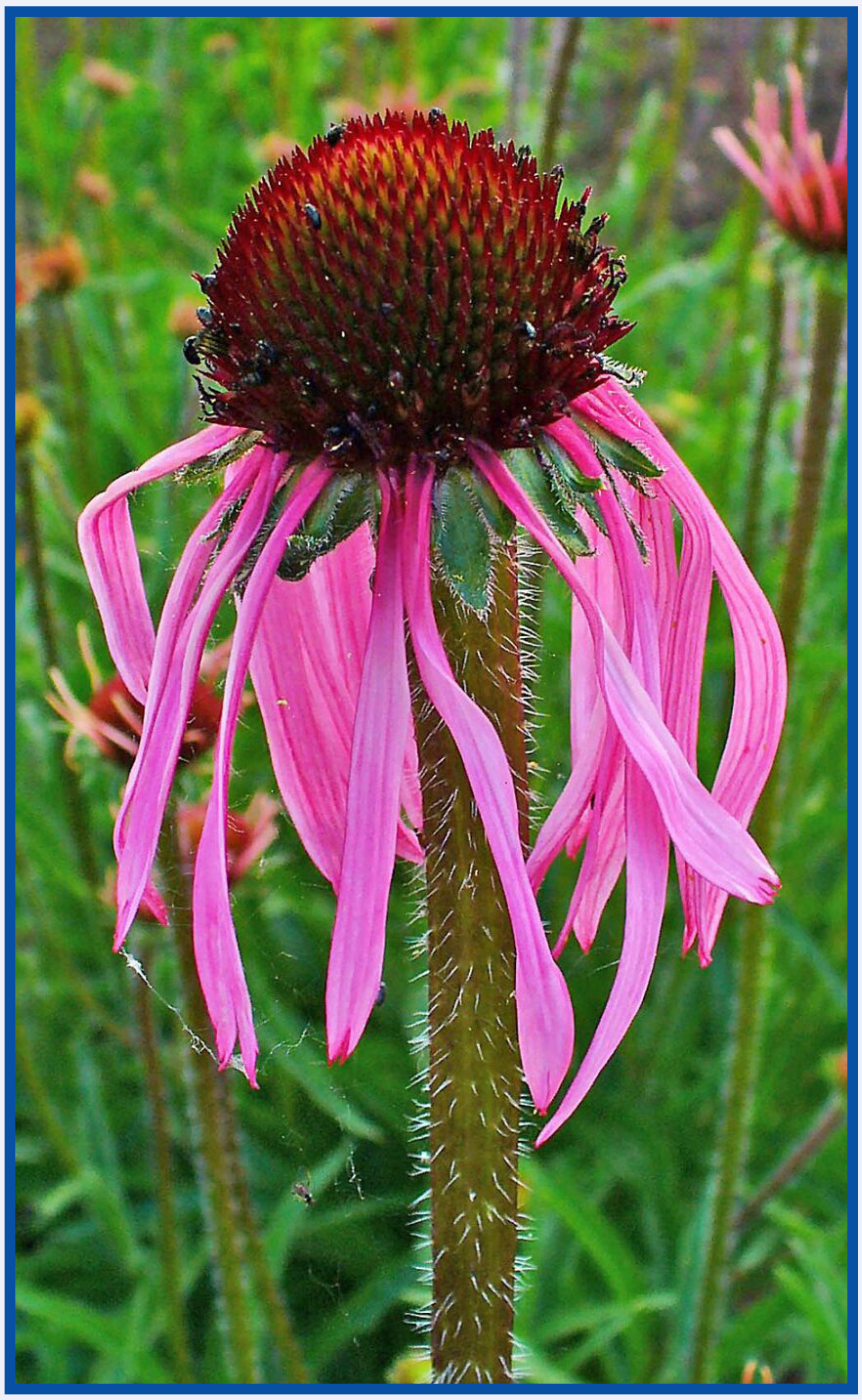
Online Series

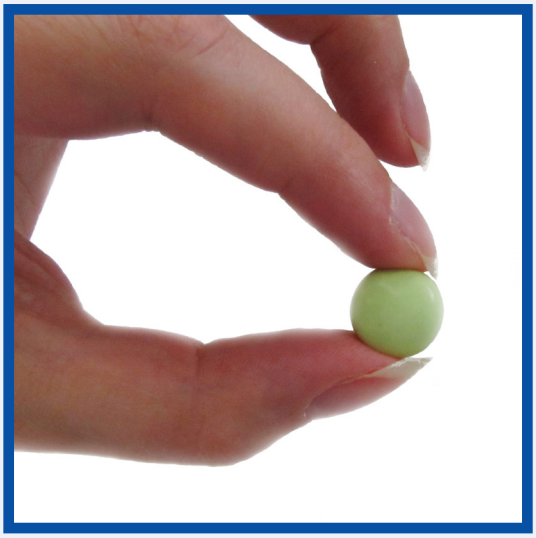
The Scientific Foundation for Herbal Medicinal Products

Echinaceae pallidae radix

Pale Coneflower Root

2018





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Herbal Medicinal Products

ECHINACEAE PALLIDAE RADIX
Pale Coneflower Root

2018

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
CCl_4	carbon tetrachloride
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase

INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

ECHINACEAE PALLIDAE RADIX 2018

Pale Coneflower Root

DEFINITION

Pale coneflower root consists of the dried, whole or cut, underground parts of *Echinacea pallida* Nutt. It contains not less than 0.2 per cent of echinacoside ($C_{35}H_{46}O_{20}$; M_r 786.5), calculated with reference to the dried drug.

The material complies with the European Pharmacopoeia [Pale Coneflower Root].

CONSTITUENTS

The characteristic constituents are:

- Caffeic acid derivatives, especially echinacoside (0.5-1.6%) [Bauer 1987a, 1987b, 1988a, 1989, 1990, 1994 2007; Brown 2010; Cheminat 1988; Pellati 2004, 2005; Thomson 2012].
- Essential oil (0.2-2.0%) [Bauer 1988a, 1990, 1994, 2007; Heinzer 1988] containing polyacetylenes and polyenes including alkenes such as pentadeca-1,8*Z*-diene [Bauer 2007; Heinzer 1988], and a range of unsaturated alkyl ketones (ketoalkenes, ketoalkenynes and ketoalkenonen) principally pentadeca-8*Z*-ene-2-one together with pentadeca-8*Z*,11*Z*-diene-2-one, pentadeca-8*Z*,13*Z*-diene-11-yne-2-one (Bauer keton 24), tetradeca-8*Z*-ene-11,13-diyne-2-one and others [Bauer 1987a, 1988a, 1989, 1990, 1994, 2007; Heinzer 1988; Pellati 2006; Morandi 2008; Thomsen 2012].
- 8-Hydroxyketoalkenynes, produced by oxidation of unsaturated alkyl ketones on storage of the dried drug. They include 8-hydroxytetradec-9*E*-ene-11,13-diyn-2-one, 8-hydroxypentadec-9*E*-ene-11,13-diyn-2-one and 8-hydroxypentadec-9*E*,13*Z*-dien-11-yn-2-one [Bauer 1987a, 1988a, 1989, 1990, 1994, 2007; Bradley 2006].
- A small amount of alkamides (0.001%): isomeric dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraneoic acid isobutylamides (Bauer alkamides 8) and various others [Bradley 2006; Chen 2005; LaLone 1009].
- Arabinans, arabinogalactan proteins and other polysaccharides [Beuscher 1995; Classen 2005; Thude 2005].

CLINICAL PARTICULARS

Therapeutic indications
Adjuvant therapy and prophylaxis of recurrent infections of the upper respiratory tract (common cold) [Bräunig 1993; Bradley 2006; Bauer 2009].

Posology and method of administration

Dosage

Adult daily dose: Hydroethanolic extracts corresponding to 900 mg of crude drug [Bradley 2006; Bräunig 1993; Bauer 2009]; other equivalent preparations.

Method of administration
For oral administration.

Duration of administration
The duration of treatment should not exceed 8 weeks.

Contra-indications
Known hypersensitivity to plants of the Asteraceae (Compositae).

As with all immunostimulants, not recommended in cases of progressive systemic disorders such as tuberculosis, diseases of the white blood cell system, collagenoses, multiple sclerosis and other autoimmune diseases, AIDS or HIV infections [Bauer 2007; Ardjomand-Woelkart 2016].

Special warnings and special precautions for use
If the symptoms worsen or high fever occurs during the use of the medicinal product, medical advice should be sought.

There is a possible risk of allergic reactions in atopic patients. These patients should consult their doctor before using Echinacea.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
Although there is no evidence of harmful effects during pregnancy [Gallo 2000; Perri 2006], in accordance with general medical practice the product should not be used during pregnancy or lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
Echinacea can trigger allergic reactions in atopic patients.

Allergic reactions due to Echinacea e.g. hypersensitive skin reactions are rather rare and mostly not serious [Ardjomand-Woelkart 2016].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Influence on immune function
A 90% ethanolic extract (1:10) at a concentration of 10 µg/mL enhanced the phagocytosis index of human granulocytes by 23%; no effect was observed at concentrations of 1 ng/mL or lower. The chloroform-soluble fraction from the ethanolic extract increased phagocytosis by 39% at 0.1 µg/mL, while the water-soluble fraction stimulated phagocytosis by a maximum of only 14% at 1 µg/mL [Bauer 1988b].

A 55% ethanolic and an aqueous extract weakly inhibited IL-8 activity in both uninfected and Rhinovirus (RV 14) infected human epithelial cells. By contrast a markedly enhanced IL-8 production was found for the hexane fractions of the hydro-ethanolic extract [Vimalanathan 2009].

A high molecular weight fraction (M_r > 10,000 Da) containing polysaccharides and glycoproteins enhanced the proliferation of mouse spleen cells and stimulated the production of interferon (IFNα/β) and immunoglobulin M as well as the number of antibody-producing cells in spleen cell cultures. It also increased the production of cytokines (such as IL-1, IL-6 and TNF-α) and nitric oxide in mouse macrophage cultures [Beuscher 1995; Bodinet 1999]. Incubation of this fraction with human monocytes enhanced the production of IL-1, IL-6 and TNF-α [Bodinet 1999].

An arabinogalactan-protein fraction from an aqueous extract showed a stimulated proliferation of C3H/HeJ mouse spleen cells at concentrations up to 100 µg/mL, a dose-dependent increase in IgM titres of NMRI mouse spleen cell cultures, a dose-dependent increase in IL-6 production and a dose-dependent increase in nitrite production in alveolar mouse macrophages [Classen 2006].

Three extracts of fresh pale coneflower root (a 50%-ethanolic tincture, a cold water infusion and a hot water infusion) were tested in several assays of immune functions in human blood: monocyte secretion of TNF-α, IL-10 and IL-12 and proliferation of peripheral blood mononuclear cells. The cold water extract enhanced TNF-α production on day 1, but a reduction was seen at day 4 for all the extracts. The cold water extract caused a significantly greater production of IL-12 than the control on day 1 ($p<0.05$). However, effects on T-cell proliferation were minimal [Senchina 2005].

A dose-dependent inhibition of NO production and TNF-α release in RAW 264.7 macrophages was demonstrated for a combination of extracts obtained with various solvents (10-200 µg/ml). Stimulation of the macrophages with *Salmonella* after an overnight incubation with the extract had no effect on phagocytosis but showed increased bactericidal activity ($p=0.002$ at 200 µg/ml) [Zhai 2007a].

Additional experiments with the same combination of extracts showed no direct inhibition of NO release. An immune blotting assay demonstrated that the extract (100 µg/mL) inhibited iNOS protein expression in LPS-treated macrophages. A dose-dependent enhancing effect on arginase activity by the extract (10-200 µg/mL) was seen in RAW 264.7 cells incubated with 8-bromo-cAMP. None of the doses (19-200 µg/mL) had an effect on cell viability [Zhai 2009a].

Significant inhibition ($p<0.05$) of prostaglandin E_2 production in LPS-stimulated RAW 264.7 mouse macrophages was shown for an ethanolic extract at a concentration of 15 µg/mL [LaLone 2007]. This inhibition was also reported for a 96% ethanolic extract (25 µg/mL); fractionation of this extract revealed Bauer ketones which inhibited PGE_2 production at a concentration of 1 µg/mL [LaLone 2009].

An alkamide fraction and individual alkamides inhibited LPS-mediated activation of murine macrophages with IC_{50} values of 6 µg/ml for the fraction and 3-25 µg/ml for individual compounds. Alkamides alone failed to activate the macrophages, while pre-incubation of macrophages with the alkamide fraction before LPS stimulation reduced NO production [Chen 2005].

Antioxidant activity
Methanolic and ethanolic (85%, 1:10) extracts exhibited anti-oxidant activity, including free radical scavenging and transition metal chelating [Hu 2000; Sloley 2001].

Caffeic acid derivatives such as echinacoside inhibited free radical-induced degradation of collagen in a dose-dependent manner [Maffei Facino 1995].

In the DPPH assay, an 80% methanolic extract and isolated echinacoside gave EC_{50} values of 167 µg/mL and 6.6 µM respectively [Pellati 2004].

Antimicrobial and antiviral activity
Extracts of pale coneflower root exhibited near UV-mediated phototoxic and antifungal activity, measured by inhibition of the growth of *Candida shehata, Cryptococcus neoformans, Candida albicans* (B-resistant strains) and *Trichophyton mentagrophytes*. The activity was attributed primarily to ketoalkenes and keto-alkynes [Binns 2000; Merali 2003].

An aqueous and two ethanolic (55% and 70%) extracts showed no activity against rhinovirus, *Herpes simplex virus* and influenza virus [Hudson 2005].

A high molecular weight fraction (M_r > 10,000 Da) containing polysaccharides and glycoproteins exhibited antiviral activity against *Herpes simplex* virus type 1 in a plaque-reduction assay [Beuscher 1995].

Antiviral activity of echinacoside against vesicular stomatitis virus in L-929 mouse cells was demonstrated in a plaque reduction assay [Cheminat 1988].

Other effects
A 95% ethanolic extract (1:10) inhibited 5-LOX activity with an IC_{50} of 1.08 µg/mL [Merali 2003].

A hexane extract (0.3 to 300 µg/mL) inhibited P-glycoprotein (Pgp) activity in the human proximal tubular cell line HK-2. The maximal inhibition was found with 3 µg/mL. At higher concentrations a reduction of cell viability was seen. Bioassay-guided fractionation revealed several polyacetylenes and polyenes with Pgp inhibiting activity [Romiti 2008]. An ethanolic extract (5 mg/mL, 96%) showed a significant ($p<0.01$) inhibition of Pgp activity [Qiang 2013].

A concentration-dependent (3-300 µg/mL) cytotoxic activity of a hexane extract in human pancreatic (MIA PaCa-2) and colon cancer (COLO320) cells was reported (IC_{50} 46.4 and 10.6 µg/mL respectively, $p<0.05$) [Chicca 2007]. Bioassay-guided fractionation led to the isolation of two polyacetylenes and three polyenes with IC_{50} values ranging from 9.3 to 35.6 µg/mL [Pelatti 2006].

The cytotoxic activity of two polyacetylenes and three polyenes was evaluated in MIA PaCa-2 and COLO320 cell lines. The five compounds caused a significant ($p<0.05$) dose-dependent decrease in MIA PaCa-2 and COLO320 cell viability after 72 h exposure (IC_{50} for the most active one, 32.17 and 2.34 µM in MIA PaCa-2 and COLO320 respectively). Pentadeca-(8*Z*,13*Z*)-dien-11-yn-2-one showed a significant ($p<0.05$) increase of caspase 3/7 activity (1.51 and 1.47 fold higher than the vehicle in MIA PaCa-2 and COLO320 cells respectively) as well as significantly enhanced intranucleosomal DNA fragments (2.97 and 2.32-fold higher than the vehicle in MIA PaCa-2 ($p<0.01$) and COLO320 cells ($p<0.05$), respectively) [Chicca 2008]. The compound also showed a concentration-dependent cytotoxicity on human leukaemia (Jurkat and HL-60), breast carcinoma (MCF-7) and melanoma (MeWo) cells. The ability to arrest the cell cycle in the G1 phase was demonstrated in HL-60 cells [Chicca 2010].

A concentration-dependent (5-75 µg/mL) inhibition of cell proliferation was found for a methanolic extract (not further specified) and isolated pentadeca-(8*E*,13*Z*)-dien-11-yn-2-one in Hela and C6 cell lines [Yaghoglu 2013].

A 55% ethanolic extract (not further specified) inhibited CYP3A4 activity with an IC_{50} of 66.08 µg/ml. An aqueous fraction thereof showed a lower activity (IC_{50} 169.6 µg/mL), whereas an ethanolic fraction alone had no activity [Modarai 2010].

In vivo experiments

Phagocytosis-stimulating effects
In the carbon clearance test in mice, oral administration of a 90% ethanolic extract (1:10) daily for 2 days at 0.5 ml/kg b.w. increased phagocytosis 2.2 fold. When chloroform and water fractions of this extract were administered separately at concentrations corresponding to their content in the original extract, the lipophilic fraction (2.6-fold increase) proved more active than the hydrophilic (1.3-fold increase) [Bauer 1988b].

Intravenous injection of 50, 100 or 500 µl of a high molecular weight fraction (M_r > 10,000 Da) containing polysaccharides and glycoproteins significantly increased the concentration of the cytokine IL-1 in the serum of mice ($p<0.05$) [Beuscher 1995]. A single oral administration of this fraction to mice at 3.7 mg per animal significantly enhanced antibody production in Peyer's patch cells [Bodinet 1999].

Immunomodulating effects
Male BALB/c mice received a preparation combining extracts using various solvents (130 mg/kg b.w. p.o., once a day for 7 consecutive days) and were immunized with sheep red blood cells. Four days later immune cells were collected. Compared to the vehicle only (5% ethanol) control and a no-gavage control, the preparation significantly ($p<0.001$) increased the percentage of lymphocytes in peripheral blood and significantly ($p=0.004$) increased splenic lymphocytes, but did not demonstrate a significant effect on splenic NK cell and B-cell subpopulations. Compared to the vehicle control, the preparation demonstrated a significant ($p<0.035$) increase in NK cell cytotoxicity and interferon-γ production, significantly ($p=0.05$) increased production of IL-4 and IL-10, and significantly reduced the production of TNF-α ($p=0.004$) and IL-1β ($p=0.007$) [Zhai 2007b].

In a further experiment, mice received the same extract preparation and treatment protocol, and 3 days before harvesting peritoneal exudate cells at the end of treatment, an inflammatory cell response was induced with 3% protease peptone i.p. There were no significant differences in IL-1β, IL-10 and NO production compared to the vehicle control group, in cells incubated with or without LPS [Zhai 2007a].

Wound healing effects
Topical application of an extract (100 mg dry ethanol extract (1:10 w/v)/mL gel) or echinacoside (0.4 mg/mL gel) to an abraded wound on rats showed a reduction of inflammation and erythema after 24 and 48 hours. After 72 hours no further signs of inflammation were observed in the two treated groups while the controls (vehicle treatment only) still showed clear signs. Compared to control a markedly enhanced wound-healing effect was seen after 48 and 72 hours in the extract and echinacoside treated groups. Histological examination showed that the injured area was covered by a crusty exudate and a generalised acute serous inflammation in the dermis of animals treated with the extract. After 48 hours new tracts of epidermis were detectable while the scab was also present. After 72 hours fully recovered epidermis was seen in the treated groups [Speroni 2002].

A preparation consisting of a combination of extracts obtained using various solvents (130 mg/kg b.w.) was administrated orally to hairless mice for 3 days prior to and 4 days after wounding with a dermal biopsy on the back. Mice were either exposed to 3 cycles of 12 h restraint stress prior to and 4 cycles after wounding (just before each dose of the preparation) or kept food and water deprived for the same 12 h but not restrained, or given food *ad lib* and kept unrestrained. Wound healing was accelerated by the preparation in the stressed mice but no effect was seen in the non-stressed mice compared to their controls [Zhai 2009b].

Clinical studies
In a randomized, double-blind study, 160 patients (83 males and 77 females) with influenza-like infections of the upper respiratory tract were treated for 8-10 days with either a hydroalcoholic liquid extract (not further specified) at a daily dose corresponding to 900 mg of dried root (n = 80) or placebo (n = 80). Significant improvements in four major symptoms, common cold, weakness, pain in arms and legs, and headache ($p<0.0001$), and in the overall symptom score ($p<0.0004$),

were observed in the verum group compared to the placebo group. Also, the duration of illness was significantly shorter in verum patients ($p<0.0001$): in those with putative bacterial infections, 9.8 days compared to 13.0 with placebo; in those with putative viral infections, 9.1 days compared to 12.9 with placebo [Bräunig 1993].

Pharmacokinetic properties
A single oral administration of dodeca-2*E*,4*E*,8*E*,10*E*/*Z*-tetraenoic acid isobutylamides (tetraenes) to male Sprague-Dawley rats (2.5 mg/kg) resulted in rapid absorption (T_{max} 15 minutes) and distribution in the liver and brain tissues within 8 minutes. The maximum amount of the tetraenes, calculated as $AUC_{0-\infty}$, in different brain parts, plasma and liver were 1764 to 6192, 794 and 1254 min x ng/mL respectively. $T_{0.5}$ was 1 and 4 hours in plasma and brain regions respectively [Woelkart 2009].

Preclinical safety data
No data available.

Clinical safety data
Several meta-analyses on clinical use and safety generally concluded that the use of Echinacea is safe. There are occasional reports of adverse reactions: however, accurate information to evaluate these reports e.g. data on the Echinacea species used, plant parts, type of extract, dosage and co-administration of other medicines, are missing [Soon 2001; Mullins 2002; Huntley 2005; Gryzlak 2007; Freeman 2008; Kocaman 2008; Karsch-Volk 2014; Ardjomand-Woelkart 2016].

In a prospective controlled study involving 412 women, the pregnancy outcome was investigated following gestational exposure to (undefined) solid or liquid preparations of *Echinacea* (*angustifolia*, *purpurea* and in one case *pallida*). Of 206 women who used Echinacea products during pregnancy, 112 used them in the first trimester. In the Echinacea group, 13 spontaneous abortions as well as 6 major malformations (4 of these occurring after exposure to Echinacea in the first trimester) were reported, compared to 7 spontaneous abortions and 7 major malformations in the control group. No statistically significant difference was seen between the groups in terms of pregnancy outcome, delivery method, maternal weight gain, gestational age, birth weight or fetal distress [Gallo 2000].

A systematic review of the evidence from the use of Echinacea during pregnancy and lactation concluded that Echinacea (including *Echinacea pallida*) is not teratogenic when used during pregnancy [Perri 2006].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Supplement 2009
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003

LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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Monographs

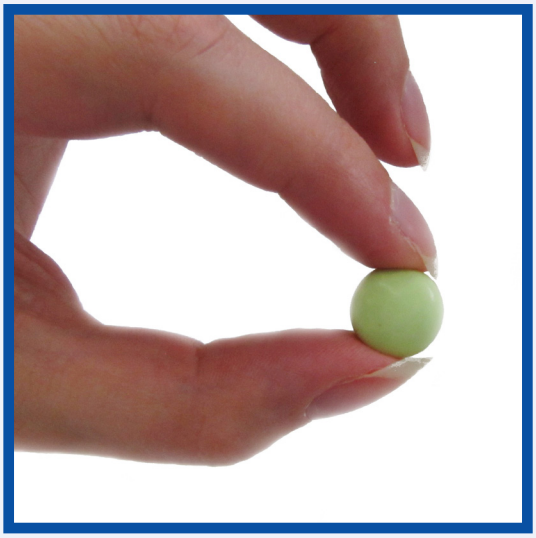
Online Series

The Scientific Foundation for Herbal Medicinal Products

Echinaceae purpureae herba

Purple Coneflower Herb

2021



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MONOGRAPHS

***The* Scientific Foundation for**
Herbal Medicinal Products

ECHINACEAE PURPUREAE HERBA
Purple Coneflower Herb

2021

E/S/C/O/P
European Scientific Cooperative
On Phytotherapy

ESCOP Monographs were first published in loose-leaf form progressively
from 1996 to 1999 as Fascicules 1-6, each of 10 monographs

Second Edition, completely revised and expanded

Second Edition, Supplement 2009

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

Edited by Roberta Hutchins and Simon Mills
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Plant illustrated on the cover: *Echinacea purpurea*

FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

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Abbreviations
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Information on the member organizations and people involved in the production of ESCOP monographs and other activities can be found on the website (www.escop.com):

Members of ESCOP
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ESCOP Scientific Committee
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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

ECHINACEAE PURPUREAE HERBA 2021

Purple Coneflower Herb

DEFINITION

Purple coneflower herb consists of the dried, whole or cut flowering aerial parts of *Echinacea purpurea* (L.) Moench. It contains not less than 0.1 per cent for the sum of caftaric acid ($C_{13}H_{12}O_9$; Mr 312.2) and cichoric acid ($C_{22}H_{18}O_{12}$; Mr 474.3) (dried drug).

The material complies with the monograph of the European Pharmacopoeia [Purple Coneflower Herb].

CONSTITUENTS

The characteristic constituents are:

Caffeic acid derivatives, predominantly cichoric acid (2,3-*O*-dicaffeoyl-tartaric acid) (0.5-5.7%) [Bauer 1988, 1998; Brown 2010; Pellati 2005; Iranshahi 2008; Upton 2007; Thomson 2012], 2-*O*-feruloyl-tartaric acid and 2-*O*-caffeoyl-3-*O*-coumaroyl-tartaric acid [Soicke 1988].

A series of alkamides (alkylamides) with isomeric dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides (0.02-0.53%) as the main compounds [Bauer 1988; Bohlmann 1983; Upton 2007; Thomson 2012].

Polysaccharides (PS) such as PS I (a 4-*O*-methyl-glucuronoarabinoxylan with an average MW of 35,000 D), PS II (an acidic arabinorhamnogalactan of MW 450,000 D) and a xyloglucan (MW 79,500 D) have been isolated from the herb. A highly-branched acidic arabinogalactan-protein (average MW of 1.2 × 106 D, with a protein content of less than 10%), inulin-type fructans (average MW 6,000 D) and a pectin-like polysaccharide have been isolated from the pressed juice from fresh herb [Heise 2015; Classen 2000, 2009; Bauer 1998; Proksch 1987; Stuppner 1985; Wagner 1981,1985].

A small amount of essential oil (up to 0.1% V/m) is also present [Mazza 1999; Pellati 2005].

Other constituents include flavonoids (0.48%) such as quercetin, kaempferol and isorhamnetin and their glycosides, found in the leaves [Malonga-Makosi 1983]; essential oil (0.08-0.32%) [Bomme 1992; Kuhn 1939; Neugebauer 1949] containing borneol, bornyl acetate, pentadeca-8-ene-2-one, germacrene D, caryophyllene, caryophyllene epoxide [Bos 1988] and a germacrene alcohol, germacra-4(15),5*E*,10(14)-triene-1β-ol [Bauer 1988] and as minor constituents, 13-hydroxy-octadeca-9*Z*,11*E*,15*Z*-trienoic acid, methyl-*p*-hydroxycinnamate, a labdane derivative and polyacetylenes [Becker 1985; Schulte 1967].

CLINICAL PARTICULARS

Therapeutic indications

Internal use
Adjuvant therapy and prophylaxis of recurrent infections of the upper respiratory tract (common colds) and also of the urogenital tract [Bauer 2007; Brinkeborn 1996; Coeugniet 1986; Goel 2004, 2005; Hoheisel 1997; Schulten 2001; Schöneberger 1992].

External use
As an adjuvant for the treatment of superficial wounds [Bauer 2007; Viehmann 1978].

Posology and method of administration

Dosage

Internal use
Adult daily dose: 6-9 mL of pressed juice; other equivalent preparations at

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comparable dosage [Bauer 2007; Brinkeborn 1996; Coeugniet 1986; Hoheisel 1997; Schulten 2001; Schöneberger 1992].

Children: Proportion of adult dose according to age or body weight.

External use
Semi-solid preparations with a minimum of 15% of pressed juice [Bauer 2007; Viehmann 1978].

Method of administration
For oral administration and topical application.

Duration of administration
The duration of continuous treatment should not exceed 8 weeks [Bauer 2007].

Contra-indications
Known hypersensitivity to plants of the Asteraceae (Compositae).

As with all immunostimulants, not recommended in cases of progressive systemic disorders such as tuberculosis, diseases of the white blood cell system, collagenoses, multiple sclerosis and other autoimmune diseases, AIDS or HIV infections [Bauer 2007; Ardjomand-Woelkart 2016].

External use
Hypersensitivity to plants of the Compositae.

Special warnings and special precautions for use
If the symptoms worsen or high fever occurs during the use of the medicinal product, medical advice should be sought.

There is a possible risk of allergic reactions in atopic patients. These patients should consult their doctor before using Echinacea.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
Although there is no evidence of harmful effects during pregnancy [Gallo 2000; Perri 2006], in accordance with general medical practice the product should not be used during pregnancy or lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
Echinacea can trigger allergic reactions in atopic patients.

Allergic reactions due to Echinacea e.g. hypersensitive skin reactions are rather rare and mostly not serious [Ardjomand-Woelkart 2016].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Influence on immune functions
A lyophilisate of the pressed juice at concentrations from 1.0 to 5.0 mg/mL significantly and dose-dependently increased the ratio of phagocytosing human granulocytes from 79% to 95% ($p<0.001$), and at 5.0 mg/mL increased the phagocytosis index of starch grains (mean number of phagocytosed grains per granulocyte) by more than 50% ($p<0.01$). At the highest tested dose, 12.5 mg/mL, the number of phagocytosing granulocytes and the phagocytosis index decreased, probably due to cytotoxic effects [Stotzem 1992].

Stimulation of phagocytosis has been verified in further assays [Bittner 1969; Fanselow 1984; Tympner 1981]. Similar results were reported with the alkamide fraction, polysaccharides and cichoric acid [Bauer 1989; Soicke 1988; Stimpel 1984].

Cultures of cytologically unchanged human bone marrow cells had a significantly enhanced mitotic index of granulo- and monopoesis after incubation for up to 72 hours with 0.2 or 2.0 mg/mL lyophilized pressed juice ($p=0.99$). In blood cultures of patients suffering from chronic myeloic leukaemia or osteomyelosclerosis, the number of mature granulocytes was mostly unchanged. In blood cultures of patients suffering from acute non-lymphatic leukaemia the total count of functionally mature granulocytes increased. More importantly, there was a highly significant reduction of granuloblasts due to a changed pattern of differentiation [Krause 1984].

Stimulation of T-lymphocyte populations has been observed in the T-lymphocyte transformation test. Lyophilized pressed juice stimulated incorporation of ^{3}H-thymidine at medium concentrations (50-500 µg/mL), while high concentrations (≥ 2500 µg/mL) showed a suppressive or cytotoxic effect [Hoh 1990].

In macrophage cultures from mice, lyophilized pressed juice immediately induced a sharp increase in chemiluminescence [Hoh 1990]. Similar findings were seen in granulocyte cultures from healthy human donors; a 24% increase in the formation of oxygen radicals has been demonstrated after 60 minutes of pre-incubation with lyophilized pressed juice at 50 µg/mL and suboptimal stimulation with zymosan, suggesting a significant increase in previously depressed granulocyte activity in accordance with the results of the chemiluminescence method [Krause 1986].

After elicitation of submaximal IL-2 production in human Jurkat T cells, treatment with a 95%-ethanolic extract resulted in dose-dependent suppression of IL-2 production, significant at 50 and 100 µg/mL ($p<0.0001$). The effect was due to alkamides, whereas the caffeic acid derivatives were not active [Sasagawa 2006].

Incubation of mice bone marrow-derived dendritic cells with a 70% ethanolic extract (50 and 150 µg/mL) for 48 h inhibited the expression of MHC class II, CD86, and CD54 receptors, and the activity of COX-2 [Benson 2010].

Pressed juice inhibited the release of rhinovirus-induced pro-inflammatory cytokines (such as IL-1 and IL-6) and cytokines in human bronchial epithelial cells. Treatment of uninfected cells stimulated the release of various pro-inflammatory cytokines [Sharma 2006]. Additional experiments showed that the pressed juice stimulated the expression of genes involved in immune response including several chemokines and cytokines [Altamirano-Dimas 2007].

A butanol subfraction of a 70% ethanolic extract (not further specified) showed a dose-dependent (10-100 µg/mL) down-regulation of mRNA expression of chemokines (e.g. CCL3 and CCL8) and their receptors (e.g. CCR1 and CCR9) in human immature dendritic cells. Additional experiments showed an

up-regulation of genes involved in the control of cytokines (IL-8, IL-1β and IL-18) and of chemokines (CXCL 2, CCL 5 and CCL 2) within 4 h after treatment [Wang 2006, 2008].

An arabinogalactan-protein isolated from pressed juice was identified as a stimulator of both the classical and alternative pathways of complement activation. Removal of the arabinose side chains resulted in a reduction of this effect [Alban 2002].

Cytotoxicity
High concentrations (≥ 2500 µg/mL) of lyophilized pressed juice were cytotoxic in the T-lymphocyte transformation test [Hoh 1990]. Furthermore, 100 µg of a polysaccharide fraction stimulated peritoneal macrophages and bone marrow macrophages to cytotoxicity against P-815 cells in the same manner as 10 units of macrophage activating factor (MAF). It also stimulated phagocytosis and interleukin formation [Stimpel 1984; Wagner 1985].

Antiviral and viral resistant activity
Incubation of mouse L-929 cells or HeLa cells with 20 µg/mL of a pressed juice preparation for 4-6 hours before viral challenge increased their resistance to influenza, herpes and vesicular stomatitis viruses by 50-80% for at least 24 hours. The presence of hyaluronidase eliminated this effect [Wacker 1978].

In the presence of DEAE dextran a lyophilisate of pressed juice at concentrations of 25-200 µg/mL exhibited antiviral activity against encephalomyocarditis and vesicular stomatitis viruses. No antiviral activity was observed with DEAE dextran or the lyophilisate alone [Orinda 1973].

A decoction and a 30%-ethanolic extract inhibited the intracellular propagation of $ECHO_9$ Hill virus in a monkey kidney cell culture [Skwarek 1996].

An aqueous extract (100 mg dried herb powder/mL) showed antiviral activity against 40 strains of herpes simplex virus (HSV-1 and HSV-2) which were either resistant or susceptible to acyclovir. The median ED50 was a 1:100 dilution (with a range of 1:50–1:400) for acyclovir-resistant strains of HSV-1 and a 1:200 dilution (with a range of 1:50–1:3200) for strains of HSV-2. For the acyclovir-suspectible strains of HSV-1 and HSV-2, the median ED_{50} of the extract was 1:100 and 1:200 respectively [Thompson 1998].

Wound healing activity
Fibroblast populated collagen lattice was used to study the influence of extracts on the collagen-contracting ability of mouse fibroblasts. An ethanolic extract (final ethanol concentration 65%) significantly ($p<0.05$) inhibited collagen contraction when added, at 10 µl or 30 µl per 2 mL of collagen gel, during preparation of the lattice. A corresponding amount of ethanol had no inhibitory effect. With increasing time between preparation of the gel and addition of the extract there was less inhibition of elongation of fibroblasts and of the process leading to collagen linking. No effect was observed when the extract was added one hour after gel formation [Zoutewelle 1990].

Hyaluronidase-inhibiting activity
Hyaluronidase-inhibiting activity has been demonstrated in a *Streptococcus mucosus* culture. Hyaluronidase-dependent decapsulation of the bacteria (through depolymerization of hyaluronic acid) was diminished by the addition of 0.1 mL pressed juice to 0.3 mL of the test solution [Büsing 1955].

Other effects
In high-throughput screening of CYP inhibition, an extract (50:1) mildly inhibited CYP3A4 activity with 7-benzyloxy-4-trifluoromethylcoumarin as the model substrate, but had mild inducing effects in the presence of resorufin benzyl ether. It had little effect on CYP2D6 but moderately inhibited CYP2C9 [Yale 2005].

Dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamide inhibited the CYP enzymes 2C19, 2D9 and 3A4 (IC_{50} values: 18.9, 6.8 and 1.9 µg/mL respectively) as did dodeca-2*E*,4*E*-dienoic acid isobutylamide (IC_{50} values: 23.4, 10.1 and 5.2 µg/mL respectively); these alkylamides did not induce CYP1A2 inhibition [Modarai 2007].

The same compounds were shown to bind to the CB2 receptor more strongly than endogenous cannabinoids. Both alkamides (50 nM) up-regulated constitutive IL-6 protein expression to 130-160% of controls, while 500 nM of dodeca-2*E*,4*E*-dienoic acid isobutylamide inhibited expression. LPS-induced TNF-α, IL-1β and IL-12p70 were inhibited by 50 nM of the two alkamides in a CB2-independent manner [Raduner 2006].

Caffeic acid derivatives characteristic of *Echinacea* species protected collagen from free radical-induced degradation in a dose-dependent manner; the IC_{50} for cichoric acid was 16.5 µM [Maffei 1995].

In vivo experiments

Immunomodulating effects
Intravenous injection of rabbits with 0.5 mL of pressed juice, followed by a second injection 24 hours later, initiated leucocytosis after transient leucopenia [Heuschneider 1970]. As well as blood leucocytosis, a migration of bone marrow granulocytes into peripheral blood was demonstrated in rabbits following a single injection of 1.5 mL of pressed juice. Within 6 hours the number of ^{3}H-labelled lymphocytes had increased from 7% to 40% and granulocytes from 34% to 89% [Choné 1965].

In the carbon clearance test in mice, an ethanolic extract was used as a solution of 5 mg extract per 30 mL of normal saline. After oral administration of this solution at 10 mL/kg b.w., three times daily for 2 days, carbon clearance was increased by a factor of 1.5 compared to the control. A chloroform-soluble fraction was even more active (factor 1.6), while the water-soluble fraction was less active (factor 1.3) [Bauer 1989].

Similarly, an ethanolic tincture administered orally to mice at 0.17 mL/kg three times daily for 2 days, stimulated carbon clearance by a factor of 2.1 [Bauer 1989].

In a study in female mice, an extract (250 mg per mL of glycerine-water 1:1) was administered by gavage at dosages of 0.4 and 0.8 mL/kg/day. Following immunization with sheep red blood cells (SRBC), 4 days of treatment with the extract produced a significant ($p<0.05$) enhancement of humoral immune responses compared to controls. In further experiments, 8 days of treatment with the same extract (0.6 mL/kg/day), and SRBC immunisation after day 4, did not enhance the immune response, whereas 4 days of treatment (starting 1 hour after SRBC immunization) did result in enhancement of immune response [Freier 2003].

Daily oral administration of the herb (50 mg, providing 0.5 mg of cichoric acid/kg b.w.) to 12-month-old male rats for 8 weeks resulted in a significant ($p<0.05$) transient increase in total white blood cell counts during the first 2 weeks. IL-2 levels increased significantly ($p<0.05$) in weeks 3 to 8 with the maximal effect from week 4 to week 6. Differential white blood cell counts showed an increase in monocytes and lymphocytes, while the numbers of neutrophils and eosinophils decreased ($p<0.05$) [Cundell 2003].

The protective effects of dried pressed juice (not defined further) against radiation-induced leukopenia in mice were investigated by evaluation of changes in peripheral blood cell count and peripheral blood antioxidant activity. Intra-abdominal administration (360 mg/kg every other day for at least 3 weeks) followed by whole-body radiation (two Gy) led to reduced leukopenia ($p=0.06$), faster recovery of blood cell counts ($p<0.01$) and an increase in peripheral blood antioxidant activity ($p<0.05$) compared to untreated animals.

The treatment also activated macrophages to stimulate IFN-γ production in association with secondary activation of T lymphocytes, which resulted in a decrease in IgG and IgM production. Immunostimulation was more pronounced in CD4 and CD8 subsets when compared to helper T cells and suppressor T cells. The pressed juice eliminated free radicals produced by irradiation, reduced cytotoxicity and prevented radiation-induced impairment of immunity [Mishima 2004].

In piglets receiving either 1.8% dried flowers or 20 mg/kg/day of flavomycin in their diet for 6 weeks no significant differences from control animals were observed in feed intake, daily weight gain, blood cell count, enzymes or proliferation of lymphocytes. Treatment of adult pigs in two phases, with 1.5% of the flowers or 4 mL/day of pressed juice (from herb) in weeks 1-3, and 1.5% of flowers or 6 mL/day of pressed juice in weeks 7-9, resulted in significantly ($p<0.03$) better feed conversion in echinacea-treated animals, but no significant changes were observed in the other parameters determined for the piglets. Antibody production in response to Swine erysipelas vaccination was enhanced significantly ($p<0.05$) after the flowers or pressed juice supplementation compared to controls [Maass 2005].

In mice, a polysaccharide-enriched extract showed a significant ($p<0.05$) increase of Delayed-Type Hypersensitivity response after 24 and 48 h treatment compared to control. At 72 h the differences were not significant [Ghaemi 2009].

After oral administration of pressed juice (10, 30 or 100 mg/kg b.w. daily for two weeks) to BALB/c mice, a significant reduction of splenocyte proliferation and splenic natural killer cell activity ($p<0.05$) as well as a significant increase of CD^{4+} and CD^{8+} T lymphocytes in the blood ($p<0.05$) were found. Serum cytokine levels, including IL-6, IL-10, and IL-17, as well as the mRNA expressions of these cytokines in spleen ($p<0.05$) were restored [Park 2018].

Cichoric acid, polysaccharides and alkamides from the herb and root were administered orally to rats twice daily for 4 days. Phagocytic activity and the phagocytic index, as well as TNF-α and NO release after stimulation with LPS, dose-dependently increased in alveolar macrophages. Enhanced release of TNF-α and IFN-γ was observed in spleen macrophages stimulated with phorbol myristate acetate and ionomycin [Goel 2002b].

In a similar experiment, three dose levels of cichoric acid, polysaccharides and alkamides were administered orally to rats twice daily for 4 days. Alkamides at the highest dose level (12 µg/kg b.w. per day) significantly ($p<0.05$) increased the phagocytic activity of alveolar macrophages by 60% and enhanced the phagocytic index ($p<0.05$). Dose-dependent increases in NO release and TNF-α production by LPS-stimulated alveolar macrophages were also observed with the alkamides. No significant changes in these parameters were observed with the other compounds. None of the components had any effect on the release of TNF-α, IFN-γ or IL-2 by splenocytes [Goel 2002a].

Other effects

Double-circle incisions in the left flank of anaesthetized guinea pigs were treated daily with 0.15 mL of an ointment containing pressed juice and covered with surgical gauze. By the 6th and 9th days following surgery the wound areas were significantly smaller ($p<0.05$) in comparison with untreated controls [Kinkel 1984].

Three groups of 10 mice were administered i.p. injections on days 0 and 21. One group received injections of 100 µL of an inoculum containing 10^5 pfu of live UV-inactivated HSV-1 strain (positive control), a second received PBS (negative control) and a third 100 µg of a polysaccharide-enriched extract. In an ocular lethal challenge performed three weeks after the last injection, all mice received an application of $5 \times 10^{5.5}$ pfu of wild type HSV-1 strain to each eye. The median survival time was significantly ($p<0.05$) greater in the extract and positive control groups compared to negative control, with 90% of mice from the extract group surviving longer than 7 days [Ghaemi 2009].

Male wistar rats were randomly divided into four groups of 10 rats each. One group received an ethanolic extract (60%; standardised to 3.7% polyphenolic compounds) at 50 mg/kg p.o., once daily for 3 days, another group received the same extract at the same dose for 10 days, while two control groups received a standard diet. Sixteen hours after the last administration, rats were decapitated and the mRNA expression level of major CYP450 enzymes was analysed. After 10 days of treatment with the extract, a significant ($p<0.05$) inhibition of the expression of CYP3A1/2, a significant ($p<0.05$) induction of CYP1A1 and CYP2D1 and no effect on CYP2E1 was found as compared to control [Mrozikiewicz 2010].

Pharmacological studies in humans

In a randomized, double-blind, prospective study, 42 healthy male athletes were treated orally with 8 mL of a preparation containing 80 g of pressed juice per 100 g or a magnesium hydrogen phosphate + magnesium hydrogen citrate preparation (providing 43 mg of Mg^{2+}) or placebo daily for 28 days preceding a triathlon competition to investigate the effects on exercise-induced whole body reactions. Compared to placebo, the pressed juice markedly reduced sIL-2R (soluble interleukin 2 receptor) in urine before the competition and enhanced the exercise-induced decrease in serum sIL-2R; it also enhanced exercise-induced increases in urinary IL-6 and serum cortisol. There was no incidence of respiratory infection in the pressed juice group compared to 3 out of 13 in the magnesium group and 4 out of 13 in the placebo group [Berg 1998].

A randomized, double-blind, placebo-controlled study involving 48 healthy volunteers evaluated the ability of pressed juice to prevent experimentally-induced infections with rhinovirus type 39. The volunteers received 3 × 2.5 mL of either the pressed juice or placebo daily for 7 days before and 7 days after intranasal inoculation with the virus. Clinical symptoms of rhinorrhea, congestion, sneezing, coughing, sore throat, headache, malaise and chills were evaluated. Viral culture and serological tests were also carried out to check for the presence of rhinovirus infection. From laboratory evidence there was little difference between groups in rates of infection: 92% in the verum group and 95% in the placebo group. Colds developed in 58% of echinacea recipients compared to 82% of placebo recipients ($p=0.114$) [Sperber 2004].

Clinical studies

Several reviews and meta-analyses of clinical studies using echinacea preparations have been published. A general conclusion has been that the preparations tested in clinical studies vary greatly. Some have shown efficacy in the early treatment of colds, while prophylactic use has also been suggested [David 2019; Blumenthal 2007; Linde 2006; Schoop 2006; Caruso 2005].

Meta-analyses
A meta-analysis [Schoop 2006] was carried out using the data from three experimental placebo-controlled studies in which echinacea treatment was evaluated following intranasal inoculation of a rhinovirus to experimentally induce a type of common cold. In two of these investigations [Sperber 2004; Turner 2000] the verum medication was pressed juice from the aerial parts of purple coneflower, while in the third study [Turner 2005] an extract from narrow-leaved coneflower (Echinacea angustifolia) was used. Although the individual studies had been unable to demonstrate significant superiority of the verum treatments, the larger sample size arising from pooled data revealed that the likelihood of experiencing a clinical cold was 55% higher under placebo than under echinacea treatment ($p<0.043$). The difference in total symptom scores between groups did not reach significance. This meta-analysis suggested that, in comparison with placebo, standardized extracts of coneflower were effective in the prevention of symptoms of the common cold after clinical inoculation of rhinoviruses.

A larger meta-analysis [Shah 2007] evaluated the data from 14 randomized, placebo-controlled studies in which a variety of echinacea-containing products had been used: 7 with *E. purpurea*, 1 with *E. angustifolia*, 1 with *E. pallida*, 4 with combinations of *Echinacea* species and one in which the type of echinacea was not specified. The data encompassed 1356 participants for the incidence and 1630 participants for the duration of common colds. Echinacea was found to significantly decrease the odds of developing a common cold by 58% ($p<0.001$) and the duration of colds by 1.4 days ($p=0.01$). A subgroup analysis on the data from 5 studies in which pressed juice from fresh purple coneflower herb was used indicated similar reductions in patients' odds to those in the overall analysis ($p=0.0009$) [Sperber 2004; Schulten 2001; Berg 1998; Hoheisel 1997; Schöneberger 1992].

Systematic review
A systematic review assessed the data from clinical studies in which preparations of purple coneflower had been used: 7 with pressed juice from purple coneflower aerial parts, 2 with an extract from various plant parts and 1 with a tincture made from 80% aerial parts and 20% root. The authors found some evidence that preparations based on the aerial parts of purple coneflower are effective for the early treatment of colds in adults, but the results were not fully consistent [Linde 2006].

Controlled studies
In a randomized, double-blind prospective study, 108 patients with a history of more than 3 colds or respiratory infections in the preceding year were treated daily for 8 weeks with 2 × 4 mL of either a preparation containing pressed juice (n=54) or placebo (n=54). During the treatment period 65% of patients in the verum group and 74% in the placebo group had at least one cold or respiratory infection. The average number of colds or respiratory infections per patient and the median duration of these ailments were 0.78 and 4.5 days respectively in the verum group compared to 0.93 and 6.5 days in the placebo group. Within a subgroup of 66 patients (29 verum, 37 placebo) who were especially susceptible to infections (T4/T8 cell ratio < 1.5), verum treatment reduced the average duration of infections by 29% to 5.34 days, compared to 7.54 days in patients given placebo. Although the incidence, duration and severity of colds and respiratory infections tended to be lower in the verum group, none of the results reached statistical significance [Schöneberger 1992].

In a randomized, double-blind, placebo-controlled study, 120 patients with initial symptoms of acute, uncomplicated upper airways infections were treated orally for up to 10 days with either a preparation containing 80 g of pressed juice per 100 g, at a dosage of 20 drops every 2 hours for the first day and thereafter three times daily (n = 60), or placebo (n = 60). The time taken to improvement was significantly shorter in the verum group ($p<0.0001$). In the subgroup of patients in whom common colds developed, the average time to improvement was 4 days with the expressed juice preparation (n=24) compared to 8 days with placebo (n=36) [Hoheisel 1997].

In a similar randomized, double-blind, placebo-controlled study, 80 patients with common cold were treated orally with 2 × 5 mL of pressed juice or placebo daily over a period of 10 days. The patients documented their symptoms and the intensity of symptoms in a daily questionnaire and the effect of treatment was assessed by the modified Jackson score. From intention-to-treat analysis the pressed juice reduced the median number of days of illness to 6, compared to 9 in the placebo group. Per-protocol analysis confirmed this result. The results were clinically relevant and statistically significant ($p=0.0112$ and 0.0180 for intention-to-treat and per-protocol analyses respectively) [Schulten 2001].

In a double-blind, placebo-controlled study, 282 healthy volunteers (aged 18-65 years) with a history of two or more colds in the previous year were randomized to receive either placebo or a 40%-ethanolic extract prepared from various parts of fresh purple coneflower plant and standardized to provide 1.0, 10.0 and 100 mg of alkamides, cichoric acid and polysaccharides respectively in a 4 mL dose. From the onset of symptoms of a cold, the volunteers were instructed to take 10 × 4 mL doses on the first day and 4 × 4 mL doses on each of the next six days. A total of 128 subjects contracted a common cold during the study, 59 in the verum group and 69 in the placebo group. In those who completed the study (n=111) the total daily symptom scores were 15.9 in the verum group and 20.7 in the placebo group, a significant ($p<0.01$) 23% lower in the verum group. The response rate to therapy was better in the verum group throughout the entire treatment period [Goel 2004].

The effect of the same preparation on immune response during common cold was evaluated in a subsequent randomized, double-blind, placebo-controlled study. Each dose of 5 mL of the 40%-ethanolic extract contained 1.25 mg of alkamides, 12.5 mg of cichoric acid and 127.5 mg of polysaccharides. Volunteers (n=150) aged 18 to 65 years, with a history of two or more common colds within the previous year, were recruited and instructed to start treatment from the onset of two symptoms of common cold, one being rhinitis. Treatment commenced with 8 × 5 mL doses on the first day, spaced equally throughout the day, followed by 3 × 5 mL doses on each of the next six days. At the time of recruitment and on days 3 and 8 of the cold fasting blood samples were collected in the morning. During the treatment period the volunteers were asked to complete a daily log, assessing the severity of their symptoms on a 4-point scale. Sixty-two volunteers contracted a common cold and completed the trial (26 verum and 36 placebo). Compared to the placebo group, a significant ($p<0.05$) decline in daily symptom score was evident in the verum group from day 3 onward, for the rest of the observation period. Significant increases were observed in the numbers of circulating total white blood cells, monocytes, neutrophils (all $p<0.01$ on day 3 compared to baseline) and NK cells. Furthermore, suppression of the cold-related increase in superoxide production by neutrophils, leading to a reduction in free radical generation, was observed in the later part of the common cold [Goel 2005].

Another double-blind, placebo-controlled study evaluated the efficacy of purple coneflower herb in upper respiratory tract infections (URTIs) in healthy children aged 2-11 years. The

participants were randomized to receive either dried pressed juice (31.5-53.6:1, in an alcohol-free syrup) or placebo during up to 3 URTIs over a 4-month period, with treatment to be started at the onset of symptoms and continued throughout the infection, for a maximum of 10 days. During URTIs children aged 2-5 years received 2 × 3.75 mL of the syrup per day (equivalent to 175 mg of dried pressed juice), while those aged 6-11 years received 2 × 5 mL per day. The primary outcome variables were duration and severity of symptoms, and adverse events recorded by parents; secondary variables included peak severity of symptoms, number of days of peak severity, number of days of fever, and overall assessment of severity of symptoms by the parents. Overall compliance was reported to be 80%. Data were analysed on 707 URTIs, 337 treated with echinacea and 370 with placebo, occurring in 407 children. The median duration of a URTI was 9 days.

No statistically significant differences were found in the duration of URTIs. However, an unexpected finding of the study was confirmed by a secondary analysis. Among 401 children experiencing at least one URTI, 55.8% of the children receiving purple coneflower herb developed a second URTI compared to 69.2% in the placebo group. This 28% reduction in risk of a subsequent URTI was significant (p = 0.01) [Taylor 2003; Weber 2005].

In a further randomized, double-blind, placebo-controlled study, patients received either 3 × 100 mg of a freeze-dried pressed juice in capsules (n=63) or placebo (n=65) daily, from the self-reported onset of common cold until the symptoms were relieved, or for up to 14 days. Symptoms such as sneezing, nasal congestion and discharge, headache, sore throat, cough or muscle aches were rated daily by the patients in a 4-point severity score system. No significant differences between verum and placebo groups were observed in total or individual symptom scores [Yale 2004].

Open studies
In a retrospective open study of 170 patients, intramuscular injection of 1-2 mL of diluted pressed juice (equivalent to 100-200 mg of pressed juice) on three successive days for the treatment of pertussis was comparable to antibiotics alone or in combination therapy. In a group of 63 patients treated only with the pressed juice 35% improved within 5 days of treatment and 81% within 10 days, whereas only 10% in a group of 30 patients treated with antibiotics alone improved within 5 days and 46% within 10 days. Out of a group of 77 patients treated with antibiotics plus intramuscular pressed juice, 9% improved within 5 days of treatment and 53% within 10 days [Baetgen 1984].

Similar results were obtained in a comparative retrospective open study of 1280 patients suffering from bronchitis. Results in a group treated only with pressed juice (intramuscularly, same dosage as in the previous study [Baetgen 1984]) were better than those in groups treated with antibiotics alone or with antibiotics plus intramuscular pressed juice [Baetgen 1988].

In an open comparative study involving 203 patients suffering from recurrent vaginal candidiasis, all patients were treated for 6 days with locally applied antifungal cream containing econazole nitrate. In addition, groups of patients received pressed juice for 10 weeks: 2 mL subcutaneously twice weekly (n=20); 2 mL intramuscularly twice weekly (n=60); 0.5-2 mL intravenously twice weekly (n=20); or 30 drops orally three times daily (n=60). Cell-mediated immunity, measured by an intracutaneous test with recall antigens before and 10 weeks after the start of treatment, showed significant improvement over this period in patients receiving the pressed juice. In the control group, patients (n=43) treated only with econazole nitrate had a very high relapse rate of 60.5% within 6 months, while adjuvant immunostimulation with the pressed juice significantly reduced relapse rates to 5-17% depending on the mode of administration [Coeugniet 1986].

Positive results were reported from clinical evaluation of 4598 patients with skin disorders, such as wounds, eczema, herpes simplex and burns, after topical application several times daily of an ointment containing pressed juice. Healing was achieved in 85% of cases [Bauer 2007].

Pharmacokinetic properties
A single oral administration of dodeca-2*E*,4*E*,8E,10*E*/*Z*-tetraenoic acid isobutylamides (tetraenes) to male Sprague-Dawley rats (2.5 mg/kg) resulted in rapid absorption (T_{max} 15 minutes) and distribution in the liver and brain tissues within 8 minutes. The maximum amounts of the tetraenes, calculated as $AUC_{0-\infty}$, in different brain parts, plasma and liver were 1764 to 6192, 794 and 1254 min x ng/mL respectively. $T_{1/2}$ was 1 and 4 hours in plasma and brain respectively [Woelkart 2007].

Preclinical safety data

Single dose toxicity
Single oral or intravenous doses of pressed juice caused no toxicity in rats or mice at the maximum administrable dose. The LD_{50} in rats is therefore over 15,000 mg/kg orally and over 5,000 mg/kg intravenously; and in mice, over 30,000 mg/kg orally and over 10,000 mg/kg intravenously [Mengs 1991].

Repeated dose toxicity
After oral administration of 0, 800, 2400 or 8000 mg/kg of pressed juice daily for 4 weeks to male and female rats, no relevant differences between the groups were evident from laboratory or necropsy results [Mengs 1991].

Effects on offspring
Female pigs (sows) received 0%, 1.2% or 3.6% of purple coneflower dried flowers in their diet from day 85 to day 110 of gestation and 0%, 0.5% or 1.5% up to day 28 of lactation, while a control group received alfalfa meal supplementation. No significant differences were found between groups with regard to feed intake, body weight, body temperature, plasma enzymes, blood cell count or proliferation of lymphocytes in sows and piglets. Colostrum composition was unaffected, as was daily weight gain and performance of the suckling piglets during lactation and a further 4-week observation period [Maass 2005].

Mutagenic potential

In vitro experiments
No significant increase in revertant numbers was observed at concentrations of up to 5000 µg per plate when preparations containing pressed juice were tested in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538, with or without S-9 metabolic activation [Mengs 1991].

The same preparations produced no statistically significant increase in the frequency of mutations in the mouse lymphoma assay at concentrations up to 5000 µg/mL, with or without S-9 metabolic activation [Mengs 1991].

There was no evidence of a clastogenic effect in human lymphocyte cultures with the lyophilized product at concentrations of up to 5000 µg/mL, with or without S-9 metabolic activation [Mengs 1991].

No significant differences were observed in the frequency of morphologically transformed colonies between controls

and groups of Syrian hamster embryo (SHE) cells treated with a preparation of lyophilized pressed juice at 5-55 µg/mL, equivalent to 80-880 µg of pressed juice per mL. Benzo[*a*]pyrene positive controls showed significant increases in malignant transformed cells [Mengs 1991].

In vivo experiments
A single oral dose of 25 g/kg of a preparation containing 80 g of pressed juice per 100 g and ethanol (22%) administered to mice did not increase the number of micronucleated polychromatic erythrocytes (PCE) compared to negative controls. Positive controls treated with cyclophosphamide did show a significant increase in micronucleated PCEs [Mengs 1991].

Clinical safety data

Several meta-analyses on clinical use and safety generally concluded that the use of echinacea is safe notwithstanding that several adverse reactions are reported. However, accurate information to evaluate these reports e.g. data on the echinacea species used, plant parts, type of extract, dosage and co-administration of other medicines are missing [Soon 2001; Mullins 2002; Huntley 2005; Gryzlak 2007; Freeman 2008; Kocaman 2008; Karsch-Völk 2014; Ardjomand-Woelkart 2016].

In controlled clinical studies involving oral treatment with purple coneflower herb preparations adverse event rates were comparable to placebo (<15%) and the preparations were very well tolerated [Brinkeborn 1999; Schulten 2001; Goel 2004]. In two studies no adverse events were observed in the purple coneflower herb group [Hoheisel 1997; Berg 1998]. Only one study reported a higher rate, with adverse events occurring in 20.4% of patients [Schöneberger 1992].

In children treated for URTIs with purple coneflower herb or placebo there was no overall difference between groups in the rate of adverse events except for the occurrence of rash: 7.1% in the verum group, 2.7% in the placebo group (p=0.008) [Taylor 2003].

Out of 63 patients with common cold receiving 3 × 100 mg daily of a freeze-dried pressed juice only a few reported adverse events, the most prevalent being dry mouth, headache and nausea [Yale 2004]

After topical application of an ointment containing pressed juice several times daily, adverse events were observed in only 2.3% of 4598 patients in an uncontrolled study. None were of a serious nature, being mainly pains, burning or itching [Viehmann 1978].

In a prospective controlled study involving 412 women, the pregnancy outcome was investigated following gestational exposure to (undefined) solid or liquid preparations of *Echinacea* (*angustifolia*, *purpurea* and in one case *pallida*). Of 206 women who used Echinacea products during pregnancy, 112 used them in the first trimester. In the Echinacea group 13 spontaneous abortions as well as 6 major malformations (4 of these occurring after exposure to Echinacea in the first trimester) were reported compared to 7 spontaneous abortions and 7 major malformations in the control group. No statistical difference was seen in terms of pregnancy outcome, delivery method, maternal weight gain, gestational age, birth weight or fetal distress [Gallo 2000].

A systematic review of the evidence from echinacea use during pregnancy and lactation concluded that echinacea (including *Echinacea purpurea*) is not teratogenic when used during pregnancy [Perri 2006].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Online Series, 2019
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Online Series, 2021
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Online Series, 2019
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Online Series, 2019
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHAMOMILLAE ROMANAE FLOS	Roman Chamomile Flower	Online Series, 2019
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Online Series, 2021
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Online Series, 2021
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HELICHRYSI FLOS	Sandy Everlastin Flower	Online Series, 2019
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013

HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Online Series, 2020
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Online Series, 2020
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Online Series, 2020
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Online Series, 2021
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Online Series, 2020
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Online Series, 2020
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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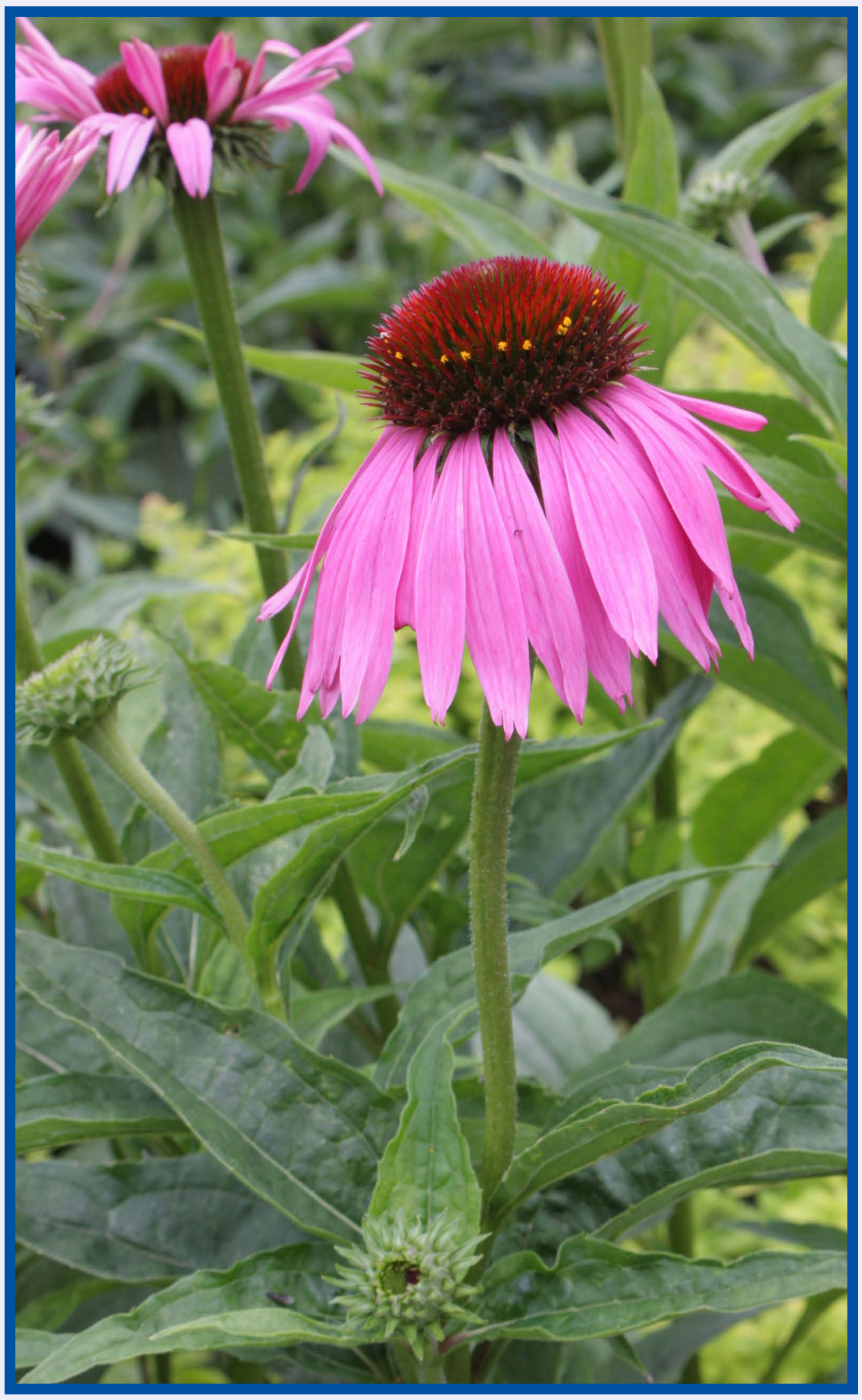
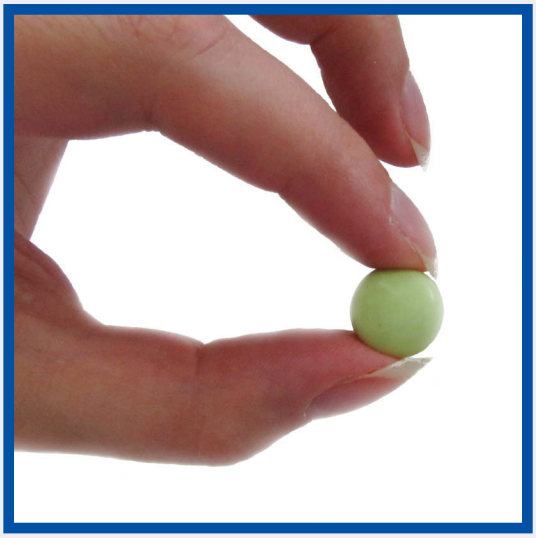
The Scientific Foundation for Herbal Medicinal Products

Echinaceae purpureae radix

Purple Coneflower Root

2021





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***The* Scientific Foundation for**
Herbal Medicinal Products

ECHINACEAE PURPUREAE RADIX
Purple Coneflower Root

2021

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ON PHYTOTHERAPY

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Second Edition, Supplement 2009

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

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Plant illustrated on the cover: *Echinacea purpurea*

FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
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Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
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Information on the member organizations and people involved in the production of ESCOP monographs and other activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

ECHINACEAE PURPUREAE RADIX 2021

Purple Coneflower Root

DEFINITION

Purple coneflower root consists of the dried, whole or cut underground parts of *Echinacea purpurea* (L.) Moench. It contains not less than 0.5 per cent for the sum of caftaric acid ($C_{13}H_{12}O_9$; *M*r 312.2) and cichoric acid ($C_{22}H_{18}O_{12}$; *M*r 474.3) (dried drug).

The material complies with the monograph of the European Pharmacopoeia [Purple Coneflower Root].

CONSTITUENTS

The main characteristic constituents are:

Caffeic acid derivatives (0.6-2.8%), principally cichoric acid (2,3-*O*-dicaffeoyltartaric acid, 0.5-2.4%) and caftaric acid (2-*O*-caffeoyltartaric acid, 0.2-0.8%). Alkamides (0.5-0.7%), principally isomeric dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides [Bauer 1988a, 1989a, 2007; Perry 2000, 2001; Stuart 2003; Laasonen 2002; Pellati 2004; Bradley 2006; Cech 2006; LaLone 2009].

Polysaccharides such as fructans and glycoproteins [Beuscher 1990, 1995; Dalby-Brown 2005; Wack 2006].

Polyacetylenic compounds [Schulte 1967] and a small amount of essential oil (up to 0.1% V/m) [Heinzer 1988; Mazza 1999].

CLINICAL PARTICULARS

Therapeutic indications

Internal use
Adjuvant therapy and prophylaxis of recurrent infections of the upper respiratory tract (common cold) [Bauer 2007; Bräunig 1992; van Hellemont 1994].

Posology and method of administration

Dosage

Adult daily dose: 3 × 60 drops of a tincture (1:5, ethanol 55% V/V), 3 × 300 mg of dried root [Bauer 2007; Bräunig1992]; other equivalent preparations at comparable dosage.

Children: Proportion of adult daily dose according to age or body weight.

Method of administration
For oral administration.

Duration of administration
The duration of continuous treatment should not exceed 8 weeks.

Contra-indications
Known hypersensitivity to plants of the Asteraceae (*Compositae*).

As with all immunostimulants, not recommended in cases of progressive systemic disorders such as tuberculosis, diseases of the white blood cell system, collagenoses, multiple sclerosis and other autoimmune diseases, AIDS or HIV infections [Bauer 2007, Ardjomand-Woelkart 2016].

Special warnings and special precautions for use
If the symptoms worsen or high fever occurs during the use of the medicinal product, medical advice should be sought.

There is a possible risk of allergic reactions in atopic patients. These patients should consult their doctor before using echinacea.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
Although there is no evidence of harmful effects during pregnancy [Gallo 2000; Perri 2006], in accordance with general medical practice the product should not be used during pregnancy or lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
Echinacea can trigger allergic reactions in atopic patients.

Allergic reactions due to echinacea e.g. hypersensitive skin reactions are generally rare and mostly not serious [Ardjomand-Woelkart 2016].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Influence on immune functions
An ethanolic extract enhanced phagocytosis by 33% in the granulocyte smear test at a concentration of 10 µg/ml. Aqueous and lipophilic fractions from the extract showed immunostimulatory activity [Bauer 1989b].

In a simulated digestion protocol, a root powder increased cytokine secretion by murine macrophages. In contrast, extracts standardized to 4% of phenolic acids (such as chlorogenic and cichoric acids) were inactive with respect to macrophage activation [Rininger 2000].

Peritoneal macrophages were isolated from mice treated orally with a root powder at 100 mg/kg b.w. twice daily for 3 days. Western blot analysis of these macrophages after stimulation with LPS and interferon-γ showed a significant decrease of 57% in COX-2 expression ($p<0.05$) while iNOS expression was unaffected [Mattace Raso 2002].

A 50% ethanolic tincture partially or completely inhibited the release of pro-inflammatory cytokines such as IL-1 and IL-6 in a human bronchial epithelial cell line stimulated by rhinovirus type 14. Treatment of uninfected cells produced a different response and stimulated the release of pro-inflammatory cytokines [Sharma 2006].

Three extracts of fresh purple coneflower root (a 50% ethanolic tincture, a cold water infusion and a hot water infusion) were tested in four immune assays using human blood: secretion of TNF-α, IL-10 and IL-12 by monocytes, and proliferation of peripheral blood mononuclear cells. All three extracts significantly ($p<0.05$) stimulated TNF-α production, while effects on T-cell proliferation were minimal [Senchina 2005].
Other experiments with a 50% ethanolic extract demonstrated an increase in peripheral blood mononuclear cells and significantly ($p\leq 0.05$) increased IL-10 production [McCann 2007].

Significant ($p<0.05$) inhibition of prostaglandin E_2 production in LPS-stimulated RAW 264.7 mouse macrophages was shown for an ethanolic extract at a concentration of 15 µg/mL [LaLone 2007].

An alcoholic extract (not further defined; 10 to 200 µg/mL) concentration-dependently inhibited NO production and TNF-α release in LPS-stimulated RAW 264.7 macrophages. In RAW 264.7 macrophages incubated overnight in the extract and subsequently stimulated with salmonella, there was a significant ($p<0.037$ at 100-200 µg/mL) decrease in bactericidal activity as measured by an increase in internalized salmonella bacteria survival, but no significant effect on phagocytosis [Zhai 2007a].
Additional experiments with the same extract showed no direct inhibition of NO release. An immune blotting assay demonstrated that the extract (100 µg/mL) inhibited iNOS protein expression in LPS-treated macrophages. The extract (10-200 µg/mL) exhibited a concentration-dependent enhancing effect on arginase activity in RAW 264.7 cells incubated with 8-bromo-cAMP. None of the concentrations (19-200 µg/mL) had an effect on cell viability [Zhai 2009].

Various extracts (ethanolic 55% and 70%, water and ethyl acetate) inhibited IL-6 and IL-8 activity in both uninfected human epithelial cells and cells infected with rhinovirus (RV 14). The secreted IL-8 level was usually higher than IL-6 [Vimalanathan 2009].

Incubation of bone marrow-derived dendritic cells from mice with an aqueous extract (1:5 (w/v), 150 and 450 µg/mL) for 48 h resulted in an increase of the expression of MHC class II, CD86, and CD54 cell surface receptors and of the production of IL-6 and TNF-α [Benson 2010].

Seventeen ethanolic extracts (75%, 1:5 (m/V), root material of various ages and locations), as well as 4 alkylamides, were tested for their ability to inhibit the production of cytokines, chemokines, and PGE_2 from RAW 264.7 cells infected with the H1N1 influenza A strain PR/8/34. A dose-dependent suppression of the production of TNF-α and PGE_2 from infected cells was found for the alkylamides. In contrast, the extracts showed a range of effects from suppression to stimulation of inflammatory mediator production. Precipitation of the extracts with ethanol removed the stimulatory activity [Cech 2010].

A 75% ethanolic extract and its fractions inhibited the production of TNF-α, CCL1 and CCL5 by LPS-stimulated RAW 264.7 macrophages. The inhibition correlated with the content of alkylamides and other not specified constituents [Todd 2015].
The same extract (51±8.2 µg alkylamides/mg) and a fraction with a high alkylamide content (310±42 µg/mg) demonstrated significant inhibition ($p<0.05$, $p<0.001$ respectively) of de-granulation and calcium influx in A2318 stimulated RBL-2H3 cells [Gulledge 2018].

In concanavalin A-stimulated murine splenocytes, a glycerine extract (not further specified) increased TNF-production dose-dependently, while a modest increase of IFN-γ was found. No effect on production of IL-2 or cell survival was seen [Cadiz 2019].

A high molecular weight fraction (MW > 10,000 D) containing polysaccharides and glycoproteins enhanced the proliferation of mouse spleen cells; stimulated the production of cytokines such as IFN α/β in spleen cell cultures and IL-1, IL-6 and TNF-α in mouse macrophage cultures; increased immunoglobulin M production and the number of antibody-producing cells as well as NO production of macrophages. Incubation of this fraction with human monocytes also enhanced the production of IL-1, IL-6 and TNF-α [Beuscher 1990, 1995; Bodinet 1999].

Several alkamides isolated from dried roots inhibited COX-I and COX-II enzymes in the range of 36–60% and 15–46% respectively at 100 µg/mL. The inhibitory activity was compared to that of the positive controls acetylsalicylic acid, ibuprofen, naproxen, celecoxib and rofecoxib [Clifford 2002].

An alkamide fraction and individual alkamides inhibited LPS-mediated activation of murine macrophages with IC_{50} values of 6 µg/ml for the fraction and 3-25 µg/ml for individual compounds. Alkamides alone failed to activate the macrophages, while pre-incubation of the macrophages with the alkamide fraction before LPS stimulation reduced NO production [Chen 2005].

Further experiments showed that dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide and dodeca-2*E*,4*E*-dienoic acid isobutylamide bound to the cannabinoid CB2 receptor more strongly than endogenous cannabinoids. Both alkamides (50 nM) up-regulated constitutive IL-6 protein expression to 130-160% of the level seen in controls, while 500 nM of dodeca-2E,4*E*-dienoic acid isobutylamide inhibited expression. LPS-induced TNF-α, IL-1β and IL-12p70 were inhibited by 50 nM of the two alkamides in a CB2-independent manner [Raduner 2006].

Alkamides from a chloroform extract showed both partial and inverse agonist activity for CB1 receptors in [35S] GTPc S-binding experiments on rat brain membrane preparations. Compared with arachidonyl-20-chloroethylamide (ACEA) there was a very weak G-protein modulating activity. Upon co-administration with ACEA, several of the compounds inhibited the agonist [Hohmann 2011].

Antioxidant activity
Methanolic and ethanolic (1:10, 85%) extracts exhibited free radical scavenging and metal chelating activity [Sloley 2001; Hu 2000].

A sub-fraction, as well as isolated mixtures of alkylamides and caffeic acid derivatives from an 80% ethanol extract, showed a prolongation of the log phase of the Cu (II)-catalysed oxidation of human LDL. The maximum amount of oxidised LDL was the same in all cases [Dalby-Brown 2005].

In the DPPH assay, an 80%-methanolic extract, cichoric acid and caftaric acid showed EC_{50} values of 134 µg/ml, 6.6 µM and 20.5 µM respectively [Pellati 2004].

Caffeic acid derivatives such as cichoric acid protected collagen from free radical-induced degradation in a dose-dependent manner [Maffei Facino 1995].

Antiviral activity
A decoction and a 30% ethanolic extract inhibited the propagation of $ECHO_9$ Hill virus in monkey kidney cell cultures [Skwarek 1996].

An aqueous and two ethanolic (55% and 70%) extracts showed activity against rhinovirus, *Herpes simplex* virus and influenza virus [Hudson 2005].

A high molecular weight fraction (MW > 10,000 D) containing polysaccharides and glycoproteins exhibited antiviral activity against *Herpes simplex* virus and influenza virus [Beuscher 1995].

Wound healing activity
Fibroblast-populated collagen lattice was used to study the influence of an extract on the collagen contracting ability of C3H10T1/2 mouse fibroblasts. An ethanolic extract (65% V/V) dose-dependently inhibited collagen gel contraction when added at the time of preparation of the gel. With increase of elapsed time between gel preparation and addition of extract, there was less inhibition of elongation of fibroblasts and of the processes leading to collagen linking. No effect was observed when the extract was added one hour after gel preparation [Zoutewelle 1990].

Other effects
A hexane fraction of an ethanolic extract exhibited UV-mediated antifungal activity against *Candida* spp. and *Saccharomyces cerevisiae* [Binns 2000].

An ethanolic tincture (prepared from 95% herb and 5% root) inhibited the activity of the cytochrome P450 (CYP) enzymes 1A2, 2C19, 2D9 and 3A4 (IC_{50} values: 30.21, 60.87, 69.40 and 22.18 µg/ml respectively) [Modarai 2007].

A concentration-dependent (3-300 µg/mL) cytotoxic activity of a hexane extract in human pancreatic (MIA PaCa-2) and colon cancer (COLO320) cells was reported (IC_{50} 62.9 and 25.4 µg/mL respectively, $p<0.05$) [Chicca 2007].

An ethanolic extract (not further specified) and dodeca-2(*E*),4(*E*)-dienoic acid isobutylamide showed a significant accumulation of lipid droplets ($p<0.01$) and cellular triglycerides ($p<0.05$) in insulin-induced adipocyte differentiation of mouse 3T3-L1 pre-adipocytes as compared to control. A dose-dependent increase in expressions of peroxisome proliferator-activated receptor γ and C/EBPα in adipocytes was found as compared with control cells [Shin 2014].

Alkamides exerted anti-inflammatory activity in the 5-lipoxygenase assay [Müller-Jakic 1994; Wagner 1989]. A fraction consisting of ten polyunsaturated alkamides inhibited the effect of 5-lipoxygenase by 92.5% at 50 µM [Wagner 1989].

CYP enzyme-mediated degradation of alkamides was observed in human liver microsomes. Metabolism was dependent on both the structure of individual compounds and the combination in which they are present [Matthias 2005a].

In vivo experiments

Phagocytosis-stimulating effects
A purified glycoprotein fraction and polysaccharide fractions stimulated the activity of mouse macrophages in the carbon clearance test [Wagner 1985].

A 3-fold increase in phagocytosis in the carbon clearance test in mice was demonstrated after oral administration, at 10 ml/kg three times daily for 2 days, of a solution containing approx. 5 mg of an ethanolic extract in 30 ml of physiological saline. When chloroform and aqueous fractions of this extract were administered separately, the lipophilic fraction proved to be more active than the hydrophilic [Bauer 1989b, Bauer 1988b].

Cichoric acid as well as alkamide and polysaccharide fractions from ethanolic extracts were orally administered to rats twice daily for 4 days. Dose-dependent increases in phagocytic activity and the release of TNF-α and NO by the alveolar macrophages were found. At higher concentrations, enhanced release of cytokines (such as TNF-α and IFN-γ) was observed in rat spleen macrophages [Goel 2002].

Immunomodulating effects
Oral administration of an extract (0.45 mg/day, not further specified) to 7-week-old mice for 2 weeks resulted in a doubling of the number of natural killer (NK) cells and monocytes in the bone marrow and in the spleen [Sun 1999]. Oral administration

of the same amount of extract to ageing mice (15-16 months old) led to a 30% increase in the absolute number of NK cells and a 20% increase in the total functional activity of NK cells in the spleen [Currier 2000]. Oral administration of the extract to mice injected with leukaemia cells increased their survival time compared to controls [Currier 2001]. In leukaemic mice, the extract (0.45 mg/kg b.w.) in the diet increased the life span and the number of NK cells compared to controls [Currier 2002].

Mice were fed a normal diet or a diet supplemented with an extract (not further specified, 2 mg/mouse/day) from the age of 7 weeks until 13 months. At 10 months the verum group had a 100% survival rate compared to 79% in the control group, and at approximately 13 months of age the respective figures were 74% and 46%. Furthermore, the number of NK cells was significantly elevated in the verum group both in bone marrow ($p<0.01$) and in the spleen ($p<0.004$) [Brousseau 2005].

Oral administration of root powder to mice at 100 mg/kg b.w. twice daily for 3 days significantly ($p<0.01$) inhibited carrageenan-induced paw oedema. No significant changes were observed in haematological parameters, except for a significant ($p<0.01$) increase in granulocyte count [Mattace Raso 2002].

Oral administration of root powder (1.5% total polyphenols) to mice at 30 and 100 mg/kg/day for 14 days increased resistance of spleen lymphocytes to apoptosis. Both dose levels produced a significant decrease in Fas-Ag expression ($p<0.01$ at 30 mg/kg; $p<0.001$ at 100 mg/kg) and a significant ($p<0.001$) increase in Bcl-2 expression [Di Carlo 2003].

Male BALB/c mice received an alcoholic extract (130 mg/kg b.w. p.o. once a day for 7 consecutive days) and were immunized with sheep red blood cells 4 days prior to collection of immune cells. *Ex vivo* experiments with splenocytes showed significantly increased IL-2 and IFN–*g* production (both $p<0.035$) and with Con A stimulated splenocytes a significantly ($p=0.05$) increased IFN-γ production. The production of IL-1β was also significantly ($p<0.05$) increased [Zhai 2007b].

Mice received the same extract and treatment regimen in a further experiment, 3 days before collecting peritoneal exudate cells an inflammatory cell response was induced (3% protease peptone i.p.). Incubation of these cells with and without LPS showed no significant differences in IL-1β and IL-10 production compared to control groups (no gavage or vehicle). A significant ($p<0.05$) decrease in NO production was seen at 4 hours, but not at 24 hours after infection [Zhai 2007a].

Stimulation of the cell defence mechanism
In mice, production of IL-1 and IL-6 was enhanced by intravenous doses (50, 100 and 500 µg/animal) of a purified high molecular weight fraction containing glycoproteins and polysaccharides [Beuscher 1995]. Oral administration of this fraction to mice significantly enhanced antibody production in Peyer's plaque cells [Bodinet 1999].

Other effects
A significant ($p=0.02$) reduction in prostate weight was observed when rats were given a 50% ethanolic extract at 50 mg/kg b.w. daily for 8 weeks. Blood analysis showed an increase in lymphocytes after 8 weeks, but no differences in neutrophils, eosinophils or monocytes [Skaudickas 2003].

Pharmacological studies in humans
In a double-blind study, 24 healthy male volunteers took 3 × 30 drops of an ethanolic extract or placebo daily for 5 days. By day 5 a significant increase of 120% in phagocytosis was observed in the verum group compared to 20% in the placebo group. The effect was transient and phagocytic activity returned to normal within 6 days [Jurcic 1989].

Clinical studies
Several reviews and meta-analyses of clinical studies using echinacea preparations have been published. A general conclusion has been that the preparations tested in clinical studies vary greatly. Some have shown efficacy in the early treatment of colds, while prophylactic use has also been suggested [Caruso 2005; Linde 2006; Schoop 2006; Blumenthal 2007; David 2019].

In a double-blind, placebo-controlled study 180 patients aged 18-60 years with influenza, randomized into three groups of 60, were given a tincture (1:5, ethanol 55%) at daily dosages corresponding to 450 mg or 900 mg of dried root, or placebo. After 3-4 days and 8-10 days there was no statistical difference in symptoms between the group taking the 450 mg dose and the placebo group. In contrast, the group taking the 900 mg dose showed a highly significant ($p<0.0001$) reduction in symptom score at both time points [Bräunig1992].

In a randomized, double-blind, placebo-controlled study, volunteers took 1 mL of an ethanolic extract (1:11 in 30% ethanol, n = 99) or placebo (n = 90), once daily on 5 days per week (Monday to Friday) for 12 weeks. The average time to occurrence of first upper respiratory tract infection was 69 days in the treatment group and 65 days in the placebo group. In the treatment group, 29% had at least one upper respiratory tract infection, with 37% in the placebo group [Melchart 1998].

Pharmacokinetic properties

Root extracts from two Echinacea species
Alkamides were rapidly absorbed and measurable in plasma from 9 healthy volunteers 20 minutes after ingestion of 4 tablets, each containing dry ethanolic extracts corresponding to 675 mg of purple coneflower root + 600 mg of narrow-leaved coneflower root and remained detectable for up to 12 hours. The maximum plasma concentration of total alkamides (average 336±131 ng eq/ml) was reached within 2.3 hours. Caffeic acid conjugates could not be identified in any plasma sample [Matthias 2005b].

Preclinical safety data
No data available on purple coneflower root.

Clinical safety data
Several meta-analyses on clinical use and safety have generally concluded that the use of echinacea is safe, notwithstanding that several adverse reactions have been reported. However, accurate information to evaluate these reports e.g. data on the echinacea species used, plant parts, type of extract, dosage and co-administration of other medicines are missing [Soon 2001; Mullins 2002; Huntley 2005; Gryzlak 2007; Freeman 2008; Kocaman 2008; Karsch-Völk 2014; Ardjomand-Woelkart 2016].

No adverse events were reported in a study in which groups of 60 patients with influenza received oral treatment daily for 10 days with a tincture at dosages corresponding to 450 mg or 900 mg of dried root [Bräunig1992].

In another clinical study, 10 out of 99 subjects who took 2 × 1 ml of a hydroethanolic extract daily for 12 weeks reported adverse effects compared to 11 out of 90 in the placebo group. None of the adverse effects were serious or required therapeutic action [Melchart 1998].

In a prospective controlled study, the pregnancy outcome was investigated following gestational exposure to (undefined) solid

or liquid preparations of *Echinacea (angustifolia*, *purpurea* and in one case *pallida*). In the group of 206 women who used echinacea preparations during pregnancy (112 in the first trimester), 13 spontaneous abortions and 6 major malformations (4 occurring after exposure to echinacea in the first trimester) were reported, compared to 7 spontaneous abortions and 7 major malformations in the matching control group of 206 women. There were no statistical differences between the study and control groups for any of the end points analysed: pregnancy outcome, delivery method, maternal weight gain, gestational age, birth weight or fetal distress [Gallo 2000].

A study involving 12 healthy non-smoking volunteers assessed the effects of a root extract (not further specified) on the activity of CYP1A2 (substrate: caffeine), CYP2C9 (substrate: tolbutamide), CYP2D6 (substrate: dextromethorphan) and CYP3A (substrate: midazolam). After a control phase during which the volunteers received a single dose of each substrate orally (and midazolam also intravenously on a different day), 4 × 400 mg of the extract was taken daily for 8 days. After six days the substrates were administered and blood and urine samples were collected as during the control phase. The extract reduced the oral clearance of substrates of CYP1A2, but not of substrates of CYP2C9 or CYP2D6, and it selectively modulated the catalytic activity of CYP3A at hepatic and intestinal sites [Gorski 2004].

A systematic review of the evidence from use of echinacea during pregnancy and lactation concluded that echinacea (including *Echinacea purpurea*) is not teratogenic [Perri 2006].

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E/S/C/O/P
MONOGRAPHS

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ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Online Series, 2021
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Online Series, 2019
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Online Series, 2019
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHAMOMILLAE ROMANAE FLOS	Roman Chamomile Flower	Online Series, 2019
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Online Series, 2021
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Online Series, 2021
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HELICHRYSI FLOS	Sandy Everlastin Flower	Online Series, 2019
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013

HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Online Series, 2020
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Online Series, 2020
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Online Series, 2020
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Online Series, 2021
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Online Series, 2020
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Online Series, 2020
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

E/S/C/O/P Monographs

Online Series

The Scientific Foundation for Herbal Medicinal Products

The second edition of ESCOP Monographs, published as a hardback book in 2003 with a Supplement in 2009, has been widely acclaimed for its authoritative information on the therapeutic uses of herbal medicines. Monographs covering a total of 107 herbal substances include extensive summaries of pharmacological, clinical and toxicological data, and copious references to scientific literature form an important part of each text.

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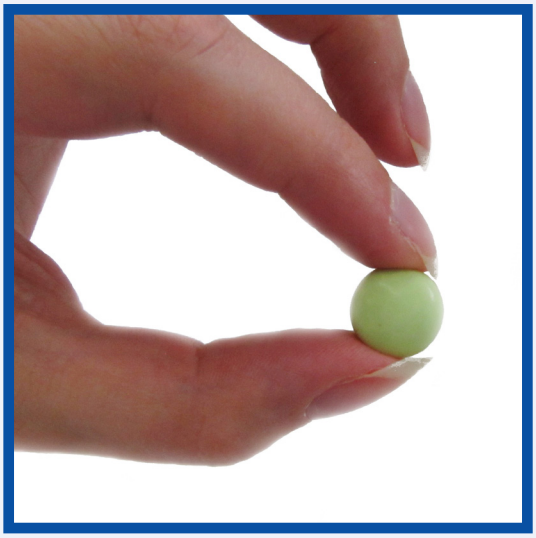
The Scientific Foundation for Herbal Medicinal Products

Epilobii herba
Willow herb

2024



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MONOGRAPHS

The Scientific Foundation for Herbal Medicinal Products

EPILOBII HERBA
Willow herb

2024

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Second Edition, completely revised and expanded

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Plant illustrated on the cover: *Epilobium angustifolium*

FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books*, ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009,* but are not available online for copyright reasons.

After purchase of a single monograph*,* the specific items to be downloaded are:

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Notes for the Reader
Abbreviations
The monograph text
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Information on the member organizations and people involved in the production of ESCOP monographs and other activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-kB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-b	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

EPILOBII HERBA

2024

Willow herb

DEFINITION

Willow herb consists of the whole or cut dried aerial parts of *Epilobium parviflorum* Schreb (syn. *Chamaenerion parviflorum*) and *E. angustifolium* L (syn. *Chamaenerion angustifolium*) (Onagraceae) collected before or during flowering time.

The materials comply with the monograph of the Czech Pharmaceutical Codex [Epilobii herba 1993; Kartnig 2014].

CONSTITUENTS

For *E. angustifolium* and *E. parviflorum* the main characteristic constituents are:

- Tannins and related compounds (4% to more than 10%), mainly oenothein B, oenothein A (dimeric and trimeric macrocyclic ellagitannins), tri-, tetra-, and penta-*O*-galloylglucose [Wichtl 2016; Granica 2014; Kartnig 2014].
- Flavonoids (1-2%): kaempferol, quercetin, myricetin, and their glycosides (e.g. kaempferol-3-*O*-rhamnoside, quercitrin, isoquercitrin, quercetin-3-*O*-glucuronide, myricitrin) [Slacanin 1991; Wichtl 2016; Granica 2014; Kartnig 2014; Monschein 2015].
- Phenolic acids and their derivatives: e.g. chlorogenic acid, neochlorogenic acid, coumaroylquinic acids, cinnamic acid, caffeic acid, ferulic acid, gallic acid and ellagic acid [Hevesi Toth 2009a; Ruszová 2013].
- Steroids (ca. 0.4%) and triterpenes (ca. 1.5%): e.g. cholesterol, campesterol, β-sitosterol, ursolic and oleanolic acid, pomolic acid [Wichtl 2016; Frolova 2014; Granica 2014; Kartnig 2014; Kukina 2014].
- Other constituents: e.g. linoleic acid, palmitic acid, stearic acid, eicosenoic acid, behenic acid, arachidic acid [Wichtl 2016; Granica 2014; Kartnig 2014].

CLINICAL PARTICULARS

Therapeutic indications

For symptomatic treatment of mild micturition disorders related to benign prostatic hyperplasia, irritable bladder [Epilobii herba 1993; Wichtl 2016; Kartnig 2014].

In these indications, the efficacy is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Adult dose
3-4 g daily as an aqueous herbal infusion in two divided doses [Epilobii herba 1993].

Method of administration
For oral administration.

Duration of use
No restriction.
If symptoms persist or worsen, medical advice should be sought.

Contra-indications
None known.

Special warnings and special precautions for use
All cases of difficulty in micturition require clarification by a physician and regular medical checks in order to rule out the need for other treatment, e.g. surgical intervention. Consultation with a physician is particularly necessary in cases of blood in the urine or acute urine retention.

E/S/C/O/P MONOGRAPHS

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
Not applicable.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No case of overdose reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Hormonal activity
In a screening study, an undefined aqueous extract of *E. parviflorum* inhibited 5α-reductase enzyme activity by 90% at 10 µL/mL [Lesuisse 1996].

Aromatase inhibitory activity was found to be 70% for oenothein A and 33% for oenothein B at 50 µM; aminoglutethimide was used as a positive control (37% inhibition at 50 µM). Against 5α-reductase, oenothein A had an IC_{50} of 1.24 µM and oenothein B an IC_{50} of 0.44 µM; finasteride was used as a positive control (IC_{50} = 5 nM) [Ducrey 1997].

Antiproliferative activity, enzyme and PSA secretion inhibitory activities
An undefined ethanolic extract of *E. angustifolium* was investigated using human prostatic epithelial cells (PZ-HPV-7) by MTT-assay. Cells were exposed to 19, 190, and 1900 µg of dry extract per mL medium. A significant ($p<0.05$) antiproliferative effect was observed at the highest concentration after 24 h (68% growth inhibition (microscopic cells count) and 43.6% (MTT viability assay)) and 48 h (67% inhibition (microscopic cell count) and 76.6% (MTT viability assay)) compared with untreated controls [Vitalone 2001].

Undefined extracts prepared from *E. angustifolium* (using water and organic solvents) showed inhibitory activity against ACE, neutral endopeptidase (NEP) and aminopeptidase N (APN) enzymes. The strongest inhibition in case of NEP activity was demonstrated by the 70% methanolic and ethyl acetate fractions with IC_{50} values reaching 10 µg/mL. Inhibition of APN and ACE was much weaker, with IC_{50} values of 80 µg/mL (APN-butanolic extract) and 150 µg/mL (ACE-ethyl acetate extract). Among the isolated compounds, oenothein B showed the highest inhibitory potency against NEP with an IC_{50} of 20 µM. Phosphoramidon was used as a positive control (IC_{50} 9 nM) [Kiss 2004].

The effects on cell proliferation and the induction of NEP of an aqueous extract (DER 1:10, lyophilized) of *E. angustifolium* herb were investigated in PC-3 prostate cells (expressing low levels of NEP) and SK-N-SH human neuroblastoma cells (expressing high levels of NEP). NEP activity was dose-dependently increased in PC-3 cells by 138, 150 and 166% when compared to control (100%) at concentrations of 25, 50, and 100 µg/mL respectively. Cell proliferation was inhibited in the PC-3 cells by 93, 83 and 69% at the same concentrations. The increase in NEP activity was greater in the naturally higher NEP expressing SK-N-SH cells, increasing by 139, 309 and 2679% respectively, with reductions in cell proliferation of 84, 80 and 74% respectively. These improvements are stated as being significant when compared to control values by the authors but without p values being specified [Kiss 2006a, Kiss 2006b].

Hormone dependent prostate cancer cells (LNCaP) were exposed to 20, 50 and 70 µg/mL of lyophilized aqueous extracts (DER 4-5:1, extraction at 40°C) prepared from *E. angustifolium* and *E. parviflorum*. The extract from *E. parviflorum* most potently reduced cell proliferation (IC_{50} 32.2 µg/mL). PSA secretion (expressed in ng of PSA per mL/mg protein) was reduced from 325.6 ng/mL (control) to 84.6 ng/mL by the *E. parviflorum* extract. The extract of *E. parviflorum* also reduced arginase activity to ~40 mUnits of urea/mg protein compared to control (~65 mUnits of urea/mg protein). In all three test systems the inhibition was significant ($p<0.05$) and concentration-dependent. Incubation of the cells with the same extracts resulted in concentration-dependent significant ($p<0.01$) increases in the proportion of late apoptotic cells compared to control; results indicated activation of the mitochondrial pathway [Stolarczyk 2013a; Stolarczyk 2013b].

LNCaP cells were exposed to oenothein B. Maximum inhibition of proliferation with an IC_{50} value of 7.8 µM, reduction of PSA secretion (IC_{50} 21.9 µM) and inhibition of arginase activity (IC_{50} 19.2 µM) were observed [Stolarczyk 2013b].

The main constituents (oenothein B, myricetin-3-*O*-rhamnoside and quercetin-3-*O*-glucuronide) of *Epilobium* species, and ellagitannins' metabolites (urolithins) were investigated for their effects on proliferation of LNCaP cells, and against PSA secretion and arginase activity. Flutamide was used as a positive control for LNCaP cell proliferation (IC_{50} 52.9 µM) and PSA secretion (IC_{50} 206.2 ng/mL). L-NAME was used as a positive control for arginase activity (IC_{50} 53.2 ng/mL) as flutamide was inactive. Among the tested compounds, the strongest antiproliferative activity was shown by oenothein B (IC_{50} 7.8 µM), while among urolithins, urolithin C proved to be the most active (IC_{50} 35.2 µM). Oenothein B reduced PSA secretion to 59.0 ng/mL (IC_{50} 21.9 µM) and urolithin C to 100.7 ng/mL. Oenothein B reduced arginase activity to 1.1 mUnits of urea/mg protein (IC_{50} 19.2 µM) [Stolarczyk 2013b].

A lyophilized *E. angustifolium* aqueous extract containing 15% oenothein B was tested on selected prostate cancer cell lines (PZ-HPV-7, LNCaP), hormone-independent prostate cancer cells (DU 145), and normal human skin fibroblast cells (NHDFs). Incubation with the extract (20, 50, 70 µg/mL) resulted in a concentration-dependent and significant ($p<0.05$ and $p<0.001$) reduction of proliferation of PZ-HPV-7 (~34% at 10 µM) and LNCaP (~12% at 10 µM) cells without affecting the viability of NHDFs. The proliferation of DU 145 cells was slightly inhibited (69% of control proliferation at a concentration of 50 µg/mL) [Piwowarski 2017].

Oenothein B at concentrations of 2 µM, 5 µM and 10 µM concentration-dependently and significantly reduced the proliferation of LNCaP cells (~19% at 10 µM; $p<0.05$ at 5 µM, and $p<0.001$ at 10 µM) and of PZ-HPV-7 cells (~33% at 10 µM; $p<0.05$ at 5 µM, and $p<0.001$ at 10 µM) without affecting the viability of NHDFs cells. The proliferation of DU 145 cells was not affected [Piwowarski 2017].

Anti-inflammatory activity
Anti-inflammatory activity of undefined aqueous (A) and ethanolic (E) extracts of *E. parviflorum* were tested in the COX-1 and COX-2 assay at a concentration of 250 µg/mL. Indomethacin was used as a positive control [20 µM (COX-1) and 200 µM (COX-2)]. Inhibition of COX-1- [58% (A) and 96% (E)] and COX-2- [<10% (A)

and 59% (E)] catalysed prostaglandin biosynthesis was observed [Steenkamp 2006].

An undefined aqueous-acetone (80% V/V) extract of *E. parviflorum* was investigated in a COX-inhibition assay. Reduction of LPS-stimulated PGE2-release of macrophages (IC_{50} 1.4 µg/mL) was comparable to that of the positive control indomethacin (IC_{50} 21 µM). The anti-inflammatory effect of the extract was also investigated in the TBARS-assay. The extract inhibited lipid peroxidation at concentrations over 0.20 mg/mL (IC_{50} 2.37 mg/mL). However, it seemed to be pro-oxidant at lower concentrations [Hevesi Tóth 2009b].

The effects of lyophilized aqueous extracts (DER 1:10, extraction at 40 °C) of *E. angustifolium* and *E. parviflorum* on inhibition of hyaluronidase and lipoxygenase activity and the release of elastase and myeloperoxidase, were investigated. Hyaluronidase and lipoxygenase were inhibited by both extracts with IC_{50} values of 3.3 and 24 µg/mL (*E. angustifolium*), and 4.0 and 28 µg/mL (*E. parviflorum*), respectively. Elastase release was inhibited by both extracts with an IC_{50} > 50 µg/mL, and myeloperoxidase release was inhibited by the extracts of *E. parviflorum* (IC_{50} > 50 µg/mL) and *E. angustifolium* (34 µg/mL). For oenothein B, the IC_{50} values were 1.1 µM for hyaluronidase, 15 µM for lipoxygenase and 7.7 µM for myeloperoxidase. Heparin (hyaluronidase inhibition: IC_{50} 62 µg/mL), fisetin (lipoxygenase inhibition: IC_{50} 180 µM), quercetin (elastase inhibition: IC_{50} 20 µM) and indomethacin (myeloperoxidase inhibition: IC_{50} 15.4 µM) were used as positive controls. All extracts significantly ($p<0.01$) attenuated LTB4 release. None of the tested extracts showed cytotoxicity at concentrations of 3.125–50 µg/mL on human skin fibroblast cells [Kiss 2011].

The elastase, tyrosinase and lipoxygenase inhibitory activities of an aqueous dry extract (DER 1:25) of *E. angustifolium* leaves were evaluated. EC_{50} values were found to be 42.7 µg/mL (elastase); 33.0 µg/mL (tyrosinase) and 0.6 µg/mL (lipoxygenase) [Onar 2012].

Immunomodulatory activity

Immunomodulatory activity of oenothein B was investigated using an interleukin-18 (IL-18) activation assay and the K562 chronic myeloid leukemia assay. In the IL-18 activation assay, human and bovine lymphocytes were exposed to 0, 10, 20 and 40 µg/mL of oenothein B, while in the K562 assay 20 µg/mL was used. Stimulation of innate lymphocytes (NK and various T cells) and enhancement of the production of interferon γ(IFNγ) by bovine and human NK cells alone and in combination with IL-18 were detected. Furthermore, oenothein B enhanced the production of IFNγ by human T cells.

In another study, the effect of age on the immunostimulation of T cells by oenothein B was tested in human and bovine T cells. Cells yielded from neonate, young and adult donors, could be stimulated by oenothein B. However, the production of certain cytokines (e.g. IL-2Rα, IFNγ) by T cells, in response to oenothein B treatment, was enhanced only in adults. In addition, oenothein B induced granulocyte macrophage colony-stimulating factor production in human adult T cells, but not cord blood cells [Ramstead 2012; Ramstead 2015].

Antioxidant activity

Analysis of the radical scavenging capacity of a hot water extract (not further specified) of *E. parviflorum* evaluated by the $ABTS^{\bullet+}$ assay resulted in an IC_{50} of 1.1 µg/mL [Arredondo 2004].

Five different water-soluble extracts, prepared from the aerial parts of *E. angustifolium* (not further specified), were screened in 6 assays [iron(III) to iron (II) reducing activity, iron(II) chelation activity, DPPH scavenging, ascorbate–iron(III)-catalyzed phospholipid peroxidation, non-site-specific as well as site specific hydroxyl radical mediated 2-deoxy-D-ribose degradation] for antioxidant and radical scavenging activity. Ascorbic acid and Trolox were used as positive controls. In all test-systems, such properties were detected. The potency of the two most active extracts may be explained by their relatively high content of phenolic substances, as determined by the Folin-Ciocalteu reagent method [Shikov 2006].

The antioxidant and radical scavenging capacities of extracts prepared from various parts (root, leaf and stalk) of *E. angustifolium* with 1 mol/L or 0.1 mol/L K_2HPO_4 (pH=7.0) were evaluated. SOD enzyme activity was detected only in the leaves (17.21 U/mg protein). The leaf extract also exhibited the highest GPX (11.07 U/mg protein) and CAT (11.20 U/mg protein) activities. Substantial differences in the levels of MDA, $O2^{\bullet-}$, $OH^{\bullet}$, GSH and total flavonoid content were observed between the plant parts. The highest quantities of GSH (0.291 µmol/mg protein) and flavonoids (62.45 mg/mg protein) were recorded in the leaf extract. The results obtained in the DPPH assay suggested that the radical scavenging capacity (95.57%) and the total reducing power obtained by FRAP assay (46.51 FRAP units) were the highest in the leaves relative to the other plant parts [Štajner 2007].

E. parviflorum and *E. angustifolium* aqueous acetone extracts (80% v/v; not further specified) have been evaluated with respect to their radical scavenging effect using the ABTS assay. Trolox (EC_{50} 7.96 µM) and ascorbic acid (EC_{50} 14.29 µM) were used as positive controls. The extracts demonstrated high radical-scavenging activity (*E. parviflorum*: EC_{50} 1.71 µg/mL; *E. angustifolium*: EC_{50} 2.17 µg/mL) [Hevesi Tóth 2009a].

The radical scavenging effect of an undefined aqueous acetone (80% v/v) extract of *E. parviflorum* was investigated. In the DPPH assay, the extract showed a stronger antioxidant activity (2 µM, calculated by the average molecular weight of the extract components (MW 331.07)) than the reference substances Trolox (4 µM) and ascorbic acid (5 µM) [Hevesi Tóth 2009b].

Lyophilized aqueous extracts (not further specified) of *E. angustifolium* (EA) and *E. parviflorum* (EP) were investigated for antioxidant activity by evaluation of ROS production in formyl-met-leu-phenylalanine (fMLP)- and 4β-phorbol-12β-myristate-13α-acetate (PMA)-induced human neutrophils, and for radical scavenging activity in the xanthine-xanthine oxidase assay. The extracts significantly reduced the production of ROS in the activated neutrophils with IC_{50} values of 5 and 30 µg/mL (EA), and 5 and 24 µg/mL (EP), showing no significant differences between the two species. For the radical scavenging activity, IC_{50} values of 5 µg/mL ($O_2^{\bullet-}$), 3.6 µg/mL (H_2O_2) and 25 µg/mL (HClO) for EA, and 4 µg/mL ($O_2^{\bullet-}$), 2.2 µg/mL (H_2O_2) and 33 µg/mL (HClO) for EP were determined. Oenothein B showed a radical scavenging activity with IC_{50} values 0.9 µg/mL ($O_2^{\bullet-}$), 0.7 µg/mL (H_2O_2) and 10 µg/mL (HClO) [Kiss 2011].

The antioxidant activity of a dried aqueous extract of *E. angustifolium* (not further specified) was measured using various methods. Superoxide radical scavenging activity of the extract was determined as EC_{50} 127.15 mg/mL (7.41 mg/mL for Trolox); hydroxyl radical scavenging activity was shown to be 2.64 mg/mL (2.34 mg/mL for ascorbic acid); DPPH activity was measured as 2.70 mg/mL (2.59 mg/mL for ascorbic acid); and ABTS activity was determined as 0.052 mg/mL (0.051 mg/mL for Trolox) [Onar 2012].

The total phenolic content (TPC) and the radical scavenging activities (RSA) (expressed as rutin equivalents) of *E. angustifolium* collected from various locations were investigated. Extracts were prepared from fresh and dried plant samples with 75% ethanol. It was observed that the TPC content and the RSA of the extracts prepared from the fresh plant were higher (TPC: 180.2-324.3

mg/g rutin equivalents; RSA: 238.6-557.1 mg/g rutin equivalents) than those yielded from the dried ones (TPC: 70.6-126.8 mg/g; RSA: 119.9-124.8 mg/g) [Kaškonienė 2015].

Antimicrobial activity
A dried ethanolic extract of *E. angustifolium* (not further specified) showed antimicrobial activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* with MIC values of 81 µg/mL and 162 µg/mL respectively. Moreover, the alcoholic extract inhibited the growth of the fungi, *Microsporum canis*, at 10 µg/mL. The action was cytocidal [Battinelli 2001].

E. coli growth was inhibited totally by an ethanol extract (125 µg/mL; not further specified) of *E. parviflorum*. However, the extract was less potent than the control compound, ciprofloxacin (0.1 µg/mL) [Steenkamp 2006].

A hot water extract of *E. angustifolium* root (not further specified) was tested against several fungal strains. Activity was observed against *Candida glabrata* and *C. lusitaniae* at concentrations of 25 and 50 mg/L respectively [Webster 2008].

Undefined extracts of *E. angustifolium* showed bacterial growth inhibitory activity in all tested strains of *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The tested concentrations were not shown, but the results were comparable to or more effective than 250 µg/mL vancomycin or tetracycline [Bartfay 2012].

Anti-aging effect
A 75% isopropanol extract of *E. angustifolium* (DER 10:1) was investigated in a specific anti-aging (photoprotective) model by measuring luminol-enhanced chemiluminescence. The antioxidant capacity of the extract in this model was ~2000 µmol/L, compared with Trolox (~8000 µmol/L). Furthermore, the extract at 10 µg/mL revealed a protective effect ($p<0.05$) on the viability of serum deprivation-induced senescent normal human dermal fibroblasts (NHDF). The treatment also led to a reduction ($p<0.05$) in connective tissue growth factor gene expression levels. The extract (10 µg/mL) inhibited TIMP-1 and -2 (tissue inhibitor of matrix metalloprotease) production and completely inhibited Hyal-2 gene expression in UV-irradiated NHDF [Ruszová 2013].

In vivo experiments

Hormonal activity
The anti-androgen effect of a dried aqueous extract of *E. angustifolium* (DER 20:6.3) was investigated at 40 mg/kg/day p.o. for 20 days in intact and testosterone-stimulated, castrated male Wistar rats. An anti-androgenic effect was observed in the intact rats as demonstrated by a significant ($p<0.01$) inhibitory effect on the weight of seminal vesicles. A pro-androgenic effect was observed in castrated rats, with the extract inducing a significant ($p<0.05$) increase in the weight of prostate, seminal vesicles and levator ani muscle compared to castrated rats not given the extract [Hiermann 1997].

Antiproliferative activity
The antiproliferative effect of a lyophilized aqueous extract of *E. angustifolium*, containing 15% of oenothein B, was investigated in rats. The extract was administered orally to rats at doses of 50, 100 or 200 mg/kg b.w. per day for 80 days. On day 20 of treatment, rats were i.p. implanted with LNCaP prostate cancer cells.

In rats fed with a standard diet only, implantation with the LNCaP cells resulted in the occurrence of prostatic adenoma in 71% (5 out of 7) of the animals. A considerably lower incidence of prostatic adenoma was observed in rats administered the extract, with 13% (1 out of 8) of the animals developing a tumour at 50 mg/kg b.w., and 25% (2/8) in both the 100 mg/kg and 200 mg/kg groups [Piwowarski 2017].

Anti-inflammatory activity
The anti-inflammatory activity of aqueous extracts of *E. angustifolium* and *E. parviflorum* was investigated in two animal models of inflammation. The ratios of herbal substance to extract solvent were 1:50 and 1:30 respectively for a prostaglandin release in perfused rabbit ear model and the carrageenan-induced rat paw oedema model in female Sprague-Dawley rats. Oral administration of 1 mL of the *E. angustifolium* extract (corresponding to 33 mg/mL dry herb/100 g b.w.) significantly ($p<0.001$) attenuated rat paw oedema compared to control, and was comparable to indomethacin (2 mg/kg p.o.); while the *E. parviflorum* extract was inactive. Inhibition of release of pro-inflammatory prostaglandins was much greater in the *E. angustifolium* extract group (1.25, 2.5 and 5.0 mg/mL; calculated as equivalents to the herbal substance) than the *E. parviflorum* group (5.0 mg/mL) in the perfused rabbit ear model [Hiermann 1986].

The flavonoid myricetin-3-*O*-glucuronide, from a hot water extract of the leaves of *E. angustifolium*, exhibited a strong, concentration-dependent anti-inflammatory effect in the carrageenan-induced rat paw oedema model (ED_{50} 15 µg/kg for the flavonoid and 10 mg/kg for indomethacin) as well as in adjuvant arthritis as a chronic model of inflammation (18.1 and 20.6% inhibition on the left and right paw respectively at 150 µg flavonoid/kg; 18.0 and 19.4% inhibition for 3 mg/kg indomethacin) [Hiermann 1991; Hiermann 1998].

Analgesic activity
An ethanolic extract of *E. angustifolium* (not further specified) was tested for analgesic activity in the hot plate and writhing tests. Concentrations of 0, 47.5, 95, 190 and 380 mg/kg b.w. were applied s.c. to Swiss ICR (CD1) male mice. An analgesic effect was observed in the writhing test at 95 mg/kg ($p<0.05$), while at doses ≥190 mg/kg the activity was similar to that of lysine acetylsalicylate (300 mg/kg s.c.) [Tita 2001].

Effect on CYP isoenzymes
A standardized dried aqueous extract of *E. angustifolium* (0.91% flavonoids, 24.36% phenolic compounds, 0.09% sterols and 0.01% tannins), administered to rats at 40 mg/kg b.w./day p.o., significantly ($p<0.05$) inhibited the expression of CYP3A1 mRNA, whereas it had no significant effect on the genes encoding CYP7A1, after 21 days of treatment [Kujawski 2009]. In a similar study, concurrent administration of the same extract (100 mg/kg b.w./day) with testosterone to castrated male Wistar rats significantly ($p<0.01$) ameliorated the testosterone-induced increase in CYP3A1 mRNA [Kujawski 2010]. In a further similar study, administration of the same extract (100 mg/kg/day) significantly increased the RafA mRNA ($p=0.076$), Mapk1 and Mapk3 mRNA levels ($p<0.05$ for both) and augmented the mRNA level-increasing effect of testosterone [Kujawski 2014].

Pharmacological studies in humans

Photoprotective effect
The photoprotective effect of a carbomer (0.5%) gel containing 3% of an *E. angustifolium* extract [prepared with 75% V/V of isopropanol (ratio of dried herbal substance:extraction solvent 1:5), and dissolved in 80% of 1.3-butanediol (200 mg/mL)] was tested in eight healthy volunteers. The preparation or a control gel without the extract were applied 3 times/day for 14 days to the forearm. The erythema indices were determined 24 and 48 hours after a single UV exposure of 1.25-times of the minimal erythema dose. Erythema indices decreased significantly ($p<0.05$) in the verum group [Ruszová 2013].

Pharmacokinetic properties
No data available.

Preclinical safety data

Acute toxicity
In an acute toxicity experiment, the LD_{50} value of *E. angustifolium* extract for mice was established as 1.4 mg/kg b.w. s.c. after 24 h [Tita 2001].

Repeated dose toxicity
Hydroalcoholic extracts prepared from *E. angustifolium* and *E. parviflorum* were tested for cytotoxic activity in Wistar rats orally administered 1.5 mL of extract for 10 days. Neither extract exhibited cytotoxic effects in the brain, hypothalamic-hypophyseal-adrenal axis, liver, kidney, spleen and thymus of the rats. *E. angustifolium* extract had only a moderate influence on the increase of oxidoreductase levels in liver and kidneys [Roman 2010].

Mutagenicity and carcinogenicity
The potential genotoxic and mutagenic properties of pomolic acid, isolated from *E. angustifolium*, were investigated using the SOS chromotest and the Ames test. Neither pomolic acid nor its metabolites induced DNA modifications in *Salmonella typhimurium* (TA98 and TA102) and *Escherichia coli* (PQ37) cells [Frolova 2014].

Clinical safety data
In a phase II randomized, double-blind, placebo controlled clinical trial, 32 men with benign prostatic hyperplasia were treated with a combination preparation including *E. parviflorum* extract for 3 months. The preparation was well tolerated and there were no adverse reactions reported [Coulson 2013].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Online Series, 2019
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Online Series, 2021
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Online Series, 2019
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Online Series, 2019
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHAMOMILLAE ROMANAE FLOS	Roman Chamomile Flower	Online Series, 2019
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Online Series, 2021
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Online Series, 2021
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EPILOBII HERBA	Willow Herb	Online Series, 2024
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Online Series, 2023
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil´s Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HELICHRYSI FLOS	Sandy Everlastin Flower	Online Series, 2019
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John´s Wort	Online Series, 2018

JUGLANDIS FOLIUM	Walnut Leaf	Online Series, 2024
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Online Series, 2020
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Online Series, 2021
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passionflower Herb	Online Series, 2023
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Online Series, 2020
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Online Series, 2021
PRUNI AFRICANAE CORTEX	Pygeum Bark	Online Series, 2020
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher´s Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Online Series, 2021
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TILIAE FLOS	Lime Flower	Online Series, 2022
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Online Series, 2020
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Online Series, 2020
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (well-established use) or on experience and historical use of that product (traditional use) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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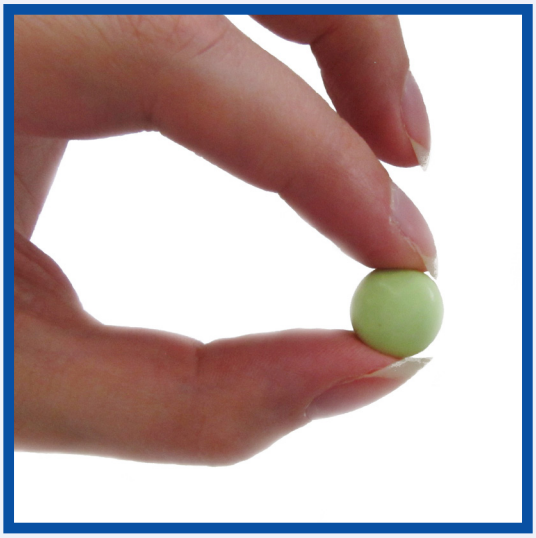
Online Series

The Scientific Foundation for Herbal Medicinal Products

Equiseti herba
Equisetum stem

2018



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MONOGRAPHS

***The* Scientific Foundation for**
Herbal Medicinal Products

EQUISETI HERBA
Equisetum stem

2018

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Second Edition, completely revised and expanded

Second Edition, Supplement 2009

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Equiseti herba - Equisetum stem

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

Edited by Simon Mills and Roberta Hutchins
Cover photographs by Simon Mills (*Equisetum arvense*) and Martin Willoughby
Cover and text design by Martin Willoughby
Typeset in Optima by Roberta Hutchins

Plant illustrated on the cover: *Equisetum arvense*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
CCl_4	carbon tetrachloride
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase

INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

EQUISETI HERBA

2018

Equisetum stem

DEFINITION

Equisetum stem consists of the whole or cut, dried sterile aerial parts of *Equisetum arvense* L.. It contains not less than 0.3 per cent of total flavonoids, expressed as isoquercitroside ($C_{21}H_{20}O_{12}$; M_r 464.4), calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Equisetum stem].

CONSTITUENTS

The characteristic constituents are flavonoids (0.2-0.9%) [Veit 1989; Veit 1991], silica (1.3 – 10%) [Piekos 1975, Bruneton 1999, Bisset 2001, Bye 2010], caffeic acid esters [Veit 1991a, Weidner 1991], styrylpyrone glucosides [Veit 1993, Veit 1995], sterols [D'Agostino 1984], essential oil [Radulovič 2006, Fons 2013] and thiaminase [Fabre 1993].

CLINICAL PARTICULARS

Therapeutic indications
Irrigation of the urinary tract, especially in cases of inflammation and renal gravel, and as an adjuvant in the treatment of bacterial infections of the urinary tract. Topically as a haemostatic [Ożarowski 1987, Classen 2015], for wound healing [Asgharikhatooni 2015]. In these indications, the efficacy is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Adult daily dose for internal use: Decoction of 5-6 g in 600 mL water, divided into 2-4 portions per day with an additional amount of liquid [Ożarowski 1987]; 370 mg dry extract (4-7:1; water) 3 times daily [Classen 2015].
Topical use: 3% ointment of hydroalcoholic extract [Asgharikhatooni 2015].

Method of administration
For oral and topical administration.

Duration of use
If symptoms persist or worsen, medical advice should be sought.

Contra-indications
Known hypersensitivity to equisetum stem.

Special warnings and precautions for use
Equisetum stem should be used with caution in patients with oedema due to impaired heart function [Hiller 2009].

Interaction with other medicinal products and other forms of interaction
None reported.

Pregnancy and lactation
In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
Not known.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

E/S/C/O/P MONOGRAPHS

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antibacterial activity
The antibacterial activity of an aqueous dry extract (not further specified) against *Escherichia coli* and biofilm formation was studied at concentrations of 0.125, 0.25, 1.0, 5.0, 10.0 and 20.0 mg/mL. Compared to 100% cell survival in the control group, the percentage of surviving *E. coli* cells incubated with the extract was reduced to 82% at 0.125 mg/mL and 50% at 20 mg/mL. *E.coli* biofilm formation was significantly ($p<0.05$) reduced by the extract at a concentration of 0.125 mg/mL [Wojnicz 2012].

The antimicrobial activity of the essential oil, prepared by hydrodistillation of dried equisetum stem, against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus enteritidis*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans* was studied using ampicillin and doxycyclin as positive controls. The results showed a broad spectrum and strong antimicrobial activity against all tested strains, similar to the positive controls [Radulović 2006].

The antimicrobial activity of a hydroalcoholic dry extract against *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, *S. enteritidis*, *A. niger* and *C. albicans* was studied using ampicillin and nystatin as positive controls. The effect of 5 µg of the extract per disc was comparable to the activity of 30 µg of ampicillin or nystatin [Milovanović 2007].

Antioxidant activity
Examination of ethyl acetate, n-butanol and aqueous extracts (not further specified) showed that the highest total radical scavenging capacity towards DPPH radicals and NO was exhibited by the ethyl acetate extract (EC_{50}=2.4 µg/mL and 90 µg/mL respectively), and the lowest by the aqueous extract (EC_{50}=37 µg/mL and EC_{50}=333 µg/mL respectively). The *n*-butanol extract showed the highest total reducing power expressed by Ascorbate Equivalent Antioxidant Capacity (13.4 µg/mL) compared to the ethyl acetate and aqueous extracts (both 1.5 µg/mL) [Mimica-Dukic 2008].

The radical scavenging capacity of an aqueous extract (1 g of fresh plant material in 5 mL 0.1 mol/L K_2HPO_4) towards DPPH and NO radicals was 51% and 9% respectively, compared to control [Štajner 2006].

Anti-inflammatory effects
An extract (1 part of dry equisetum stem with 9 parts of 36% ethanol V/V; 0.05 to 0.8 µg/mL) demonstrated dose-dependent significant reductions in IL-2 cytokine production and proliferation of immunocompetent cells ($p<0.05$ to 0.001) [Gründemann 2014].

Activity on bone tissue
In a study to observe the effects of equisetum on bone marrow cells and on bacterial infection to which they are prone, various hydromethanolic (1:1) extracts (differentiated by length of extraction time - not further specified) were tested over 21 days on human bone marrow osteoblastic cells (hBMC) and hBMC cultured over hydroxyapatite disc. At day 21, there were a range of effects with a significant dose-dependent increase in the cell population and activity against *S. aureus* at 50 and 100 µg/ml for the shorter-term extract. Longer extraction times and higher doses did not convey such benefits: different levels of silica in the extracts were postulated as part of the explanation [Bessa Pereira 2012].

A hydromethanolic (1:1) dry extract (not further specified) at concentrations of 0.016 to 500 µg/mL in DMSO was tested on both non-stimulated and stimulated human peripheral blood mononuclear cells (PBMC), used as osteoclast precursor cells, or a co-culture of PBMC with human bone marrow osteoblastic cells (hBMC). The extract dose-dependently reduced osteoclast development and function, both in stimulated osteoclast precursor cell cultures and in the co-culture of osteoclastic and osteoblastic cells [Costa-Rodrigues 2012].

Antiproliferative activity
n-Butanolic, methanolic, ethyl acetate and aqueous extracts were investigated for their antiproliferative activity against the human cancer cell lines HeLa, HT-29 and MCF7. The activity was dependent on cell lines, type of extract and concentration. The ethyl acetate extract exhibited the greatest antiproliferative effect [Cetojević-Simin 2010].

In vivo experiments

Anti-inflammatory effects
A 50% hydroethanolic extract (not further specified) at 50 mg/kg b.w. significantly ($p<0.05$) reduced carrageenan-induced paw oedema in mice by 25% and 29% at 2 h and 4 h respectively after carrageenan administration. A dose of 100 mg/kg b.w. significantly ($p<0.05$) reduced paw oedema by 30% at only 2 h after carrageenan administration [Do Monte 2004].

Effects on central nervous system
Aged rats demonstrate significant impairments in short- and long-term retention compared to young animals. A study aimed to show whether equisetum could reverse this impairment. Wistar rats aged 80 weeks were treated with a 50% hydroethanolic dry extract (not further specified) at a dose of 50 mg/kg b.w. i.p. or saline for 8 weeks. A group of untreated 8 week old rats was used as a control. Long-term retention was significantly ($p<0.05$) improved and short-term retention somewhat improved after administration of the extract, while cognitive performance in a modified version of the Morris Water Maze was ameliorated. No differences were found between the three groups (young controls, aged controls, and treated animals) with regard to the open field and elevated plus maze tests [Dos Santos Jr. 2005a].

The same extract was studied in male Wistar rats at doses of 50, 100, 200 and 400 mg/kg i.p. in various test systems including the open field test, rota-rod, elevated plus maze, barbiturate-induced sleeping time and pentylenetetrazole seizures compared to control. The extract showed dose-dependent significant effects ($p<0.05$ to 0.01) in the open-field test, and at doses of 200 and 400 mg/kg b.w significantly ($p<0.01$) increased the number of falls in the rota-rod reducing the time of permanence in the bar, and also at these doses increased barbiturate-induced sleeping time by 46% and 74% respectively. In the pentylenetetrazole-seizure test, all doses significantly ($p<0.05$ to 0.01) increased the latency until the first convulsion, diminished the severity of convulsion, and protected animals from death. The 200 and 400 mg/kg doses significantly reduced the percentage of animals developing convulsion by 50% ($p<0.05$) and 25% ($p<0.01$) respectively. On the contrary, no effect was seen in the elevated plus maze test [Dos Santos Jr. 2005b].

The anxiolytic effects of petroleum ether, chloroform, ethanolic and aqueous extracts were studied in mice using the elevated plus-maze model. The ethanolic extract (50 and 100 mg/kg b.w. i.p.) had a comparable effect to diazepam, significantly ($p<0.05$) increasing the time-spent and the percentage of open arm entries. At 100 mg/kg it also prolonged the ketamine-induced total sleeping time and decreased the locomotor activity [Singh 2011].

Antidiabetic effect
A methanolic extract (not further specified) administered to diabetic rats at doses of 50, 100, 250 and 500 mg/kg b.w. p.o. for 5 weeks, significantly ($p<0.0001$) reduced plasma glucose levels compared to untreated controls. In comparison to 5 mg/kg b.w. of glibenclamide, no significant difference was observed. The urinary creatinine level in rats treated with the extract and with glibenclamide was significantly ($p<0.001$) higher compared to control, whereas the microalbumin level was lower [Safiyeh 2007a].

Diabetic rats were treated with *n*-hexane, dichloromethane or methanolic extracts (not further specified) for 5 weeks. The methanolic extract at doses of 50, 250 and 500 mg/kg b.w. showed a significant reduction ($p<0.0001$) of plasma glucose levels compared with untreated controls. The extract showed no significant difference compared to glibenclamide [Safiyeh 2007b].

The treatment of streptozotocin-induced diabetic rats with a methanolic extract (not further specified) at doses of 50 and 250 mg/kg b.w. p.o. or 5 mg/kg b.w. of glibenclamide for 5 weeks showed a significant ($p<0.0001$) hypoglycaemic effect. The histological examination showed a regeneration of the streptozotocin-induced necrosis of the pancreas by the extract comparable to glibenclamide [Safiyeh 2007c].

Wound healing effect
The effect of 5% and 10% equisetum ointments (not further specified) on dermal wounds in rats was studied. The groups treated with equisetum showed a significantly higher wound closure ratio, higher dermal and epidermal regeneration, angiogenesis and granulation tissue thickness after 14 days as compared to the untreated control groups ($p<0.05$) [Ozay 2010]. The same preparations were tested in the treatment of dermal wounds in streptozotocin-induced diabetic rats. The same parameters as in the previous study showed significant improvement in 14-days old wounds as compared to control ($p<0.05$) [Ozay 2013].

Pharmacological studies in humans

Diuretic effect
In a double-blind, randomized trial, the diuretic effect of a dry extract containing 0.026% of total flavonoids (not further defined) was investigated using a 3 phase cross over protocol. Six groups (n=6 each) of healthy male volunteers were alternately administered for 4 consecutive days with the extract at 300 mg 3 times daily, hydrochlorothiazide 25 mg/day and placebo (cornstarch 900 mg/day) with a 10 day washout period between treatments. Each group was given the treatments in a different order. The diuretic effect was assessed by monitoring the water balance (difference between fluid intake and excreted urine) over a 24 h-period. Diuretic activity was significantly ($p<0.001$) stronger than in the placebo group and equivalent to hydrochlorothiazide ($p=0.566$), without causing significant changes in the elimination of electrolytes [Carneiro 2014].

Wound healing effect
In a randomized, placebo-controlled, double-blind study, the effects of a 3% hydroalcoholic extract (not further specified) in an ointment on wound healing, reduction of inflammation and pain relief were examined in 108 nulliparous mothers after episiotomy. The effects of the treatment compared with the placebo ointment were assessed at 5±1 and 10±1 days post-episiotomy. Significant differences were observed between the treated and control groups for erythema at baseline ($p<0.04$) and 10 days after intervention ($p<0.001$), and for oedema at baseline ($p<0.002$) and 5 and 10 days after intervention ($p<0.001$ for both). Pain intensity had decreased on day 5 by 14% in the treated group and increased in the control group by 34%. On day 10, pain intensity was reduced by 86% and 13% in the treated and control groups respectively. The number of acetaminophen pills (0.5 g) used during the 10 day study period by treated and control groups were 6.8 ± 4.4 and 11.6 ± 7.1 respectively [Asgharikhatooni 2015].

Pharmacokinetic properties
No data available.

Preclinical safety data

Toxicity
The subchronic toxicity of powdered equisetum stem administered with the diet to female and male F344 rats was evaluated for 13 weeks. Significant alterations ($p<0.05$) were observed in haematological parameters including increased mean corpuscular haemoglobin (MCH) in males at a dose of 3.8 g as well as increased platelet count (PLT) and decreased MCH in the females at a dose of 7.9 g. Significant decreases ($p<0.01$) in serum calcium concentrations were observed at doses of 7.9 and 24.2 g in males. The intake did not significantly influence macroscopic and histopathological parameters or weight of organs [Tago 2010].

Oral administration of dried equisetum stem at doses of 30, 50 or 100 mg/kg b.w. for 14 days did not cause important changes in the morphology and hepatic function in rats [Baracho 2009].

Wistar rats at the age of 8 weeks (10) and 80 weeks (22) were treated with a hydroalcoholic dry extract (not further specified) at a dose of 50 mg/kg b.w. i.p. for 8 weeks. There was no toxicity observed during treatment [Dos Santos Jr. 2005a].

Clinical safety data
No data available.

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E/S/C/O/P
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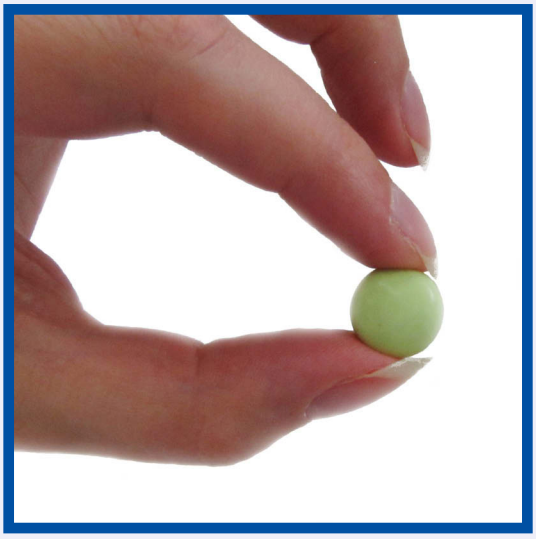

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***The* Scientific Foundation for**
Herbal Medicinal Products

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Black Cohosh

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Filipendulae ulmariae herba - Meadowsweet

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

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Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

FILIPENDULAE ULMARIAE HERBA 2015

Meadowsweet

DEFINITION

Meadowsweet consists of the whole or cut, dried flowering tops of *Filipendula ulmaria* (L.) Maxim. (= *Spiraea ulmaria* L.). It contains not less than 1 mL/kg of essential oil, calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Meadowsweet].

CONSTITUENTS

Flavonoids, up to 6% in the flowers, particularly spiraeoside (quercetin 4′-glucoside), and approx. 3-4% in the flowering herb, including hyperoside, other quercetin derivatives and kaempferol 4′-glucoside [Hörhammer 1955, 1956; Shelyuto 1977; Scheer 1987; Lamaison 1991, 1992; Poukens-Renwart1992; Fecka 2009].

Glycosides of salicylaldehyde (monotropitin = gaultherin), of methyl salicylate (spiraein) and of salicyl alcohol (isosalicin) (up to 0.5% in total) [Thieme 1965, 1966; Meier 1987a, 1987b, 1993].

Steam distillation of the dried flowers yields a small amount (0.2%) of volatile oil (arising from the phenolic glycosides during drying and storage), of which about 75% is salicylaldehyde [Kozhin 1971; Lindeman 1982; Meier1987b]. Previous findings, however, stated that such compounds are only present in fresh flowers [Piette 1981]; after steam distillation of fresh flowering tops, the proportion of salicylaldehyde in the oil was 36% [Valle 1988].

Ellagitannins (10-15%) derived from galloyl-4,6-hexahydroxydiphenoyl-β-D-glucose units, the major one being the dimeric rugosin D [Gupta 1982; Haslam 1982,1996; Meier 1993; Okuda 1993; Wichtl 1997, 2009; Fecka 2009].

The flowers also contain a heparin-like substance, which is bound to plant proteins in the form of a complex [Kudrjashov 1990, 1992].

CLINICAL PARTICULARS

Therapeutic indications

Filipendula is used as supportive therapy for the common cold [Schilcher 1992; Meier 1993; Wichtl 1997; Zeylstra 1998; Schulz 2001; Wichtl 2009]. Meadowsweet is also used to enhance the renal elimination of water [Decaux 1941; Leclerc 1976; Valnet 1976; Meier 1993; Wichtl 1997, 2009; Zeylstra 1998], although published scientific evidence does not adequately support this indication.

Posology and method of administration

Dosage
Unless otherwise prescribed, the daily dose as a tea infusion is:

Adults: 2-6 g of the drug daily [Mills 2000; Wichtl 2009].
Children 1-4 years of age: 1-2 g daily [Dorsch 1998].
Children 4-10 years of age: 2-3 g daily [Dorsch 1998].
Children 10-16 years of age: adult dose [Dorsch 1998].

Liquid extract (1:2), 3-6 mL daily; tincture (1:5), 7.5-15 mL daily [Mills 2000].

Method of administration
For oral administration.

Duration of administration
No restriction.

Contra-indications
Due to the presence of salicylates, the drug should not be used in cases of hypersensitivity to salicylates [Meier 1993; Wichtl 2009].

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported. The level of salicylate derivatives makes interaction with anticoagulant agents unlikely.

Pregnancy and lactation
No data available. In accordance with general medical practice, the products should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antimicrobial activity
Combined 70%-ethanolic and aqueous extracts (1 mL corresponding to 1 g of herb), tested at 5% in the culture medium, inhibited the growth of *Staphylococcus aureus haemolyticus*, *Streptococcus pyogenes haemolyticus*, *Escherichia coli*, *Shigella flexneri*, *Klebsiella pneumoniae* and *Bacillus subtilis* [Csedö 1993].

A tincture from the flowers (70% ethanol, diluted 1:10 and 1:25) inhibited the growth of *Staphylococcus aureus* and *S. epidermis* at both concentrations, and of *Proteus vulgaris* and *Pseudomonas aeruginosa* at the higher concentration only. No effect was seen with *E. coli* or *Klebsiella* [Hintz 1983].

Dried ethanolic extracts (96% and 40% V/V) showed antimicrobial activity at concentrations from 0.1 – 0.8 mg/mL in Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*). Higher concentrations were needed to inhibit growth of Gram-negative bacteria (0.6 – 6mg/mL; *Salmonella enteritidis* and *Escherichia coli*) and fungi (6 – 48mg/mL; *Penicillium expansum* and *Rhizopus arrhizus*). The 40% ethanolic extract contained higher amounts of phenolic compounds and showed better results in the inhibition of tested bacteria and fungi [Gniewosz 2013].

A methanolic extract showed a MIC_{50} of 0.08% on the Gram negative pathogens *Proteus vulgaris* and *Klebsiella pneumoniae* [Denev 2014]

Anti-inflammatory activity
Extracts (50% ethanolic) of meadowsweet flowers and leaves inhibited the activity of the proteolytic enzyme elastase by 100% and 92% respectively, measured by spectrophotometry using an amino acid-nitroanilide substrate and attributed to the tannin content of the materials [Lamaison 1990].

An aqueous, lyophilized extract of meadowsweet leaves inhibited prostaglandin biosynthesis from ^{14}C-arachidonic acid (i.e. inhibited cyclooxygenase) in bovine seminal vesicle microsomes by 36% at 0.2 mg/mL, a relatively low level of activity compared to 88% inhibition by indometacin at 2.8 µM. The same extract strongly inhibited PAF-induced exocytosis (and hence release of the enzyme elastase) in neutrophils from human peripheral blood by 93% at 0.25 mg/mL [Tunòn 1995].

An aqueous extract was incubated with human faeces to convert ellagitannins into urolithins by the gut microflora. Results of inhibition studies in monocyte-derived macrophages showed a significant decrease of TNF-α production. The most potent derivative urolithin A reached an inhibition of 29.2% ±6.2 ($p<0.05$) at 0.625 µM. Urolithin C significantly decreased Interleukin-6 production by 25.5% ±4 as well at 10 µM ($p<0.001$) compared to stimulated but untreated cells [Piwowarski 2014].

An aqueous extract as well as isolated constituents were investigated for their ability to inhibit production of inflammatory biomarkers in monocyte derived macrophages. Apigenin and quercetin significantly reduced the production of TNF-α in the stimulated cells at the highest concentration (50 µM) by 89% ±4 and 84% ±2 and of Interleukin-6 by 97% ±3 and 96% ±1, respectively ($p<0.05$). The extract, containing 3 µM quercetin, reduced production of TNF-α by 34% ±11, IL-1β by 45% and IL-6 by 16% ±11. [Drummond 2013].

Immunomodulatory activity
Dry extracts from meadowsweet flowers and herb exhibited strong inhibitory activity towards the classical pathway of the complement system, ethyl acetate (IC_{50}: 5.4 µg/mL) and methanolic (IC_{50}: 14.6 µg/mL) extracts of the flowers, and the methanolic extract (IC_{50}: 14.5 µg/mL) of the herb, being the most effective. As the ethyl acetate extract of the flowers retained its activity after treatment with skin powder, it was concluded that complement inhibitory activity of this fraction is not attributable to tannins [Halkes 1997a].

In the same series of experiments, meadowsweet dry extracts from flowers and herb prepared with ether, ethyl acetate, methanol or water inhibited luminol-dependent chemiluminescence (an indicator of the production of reactive oxygen species) generated by zymosan-stimulated human polymorphonuclear leukocytes (PMNs) with IC_{50} values of 66.3 µg/mL, 42.3 µg/mL, 39.8 µg/mL and 37.4 µg/mL respectively for the flower extracts and 81.9 µg/mL, 44.5 µg/mL, 59.5 µg/mL and 60.8 µg/mL respectively for the herb extracts. The extracts also inhibited T-cell proliferation, with IC_{50} values of 100.4 µg/mL, 173.9 µg/mL and 210.5 µg/mL respectively for ethyl acetate, methanol and aqueous extracts of the flowers, and 195.1 µg/mL, 150.0 µg/mL and 195.2 µg/mL respectively for ether, ethyl acetate and methanolic extracts of the herb [Halkes 1997a].

Various extracts of meadowsweet flowers were investigated for *in vitro* modulatory activity towards the classical pathway of complement activation. The highest inhibitory activity was found in the ethyl acetate extract (IC^{50}: 2.9 µg/mL), followed by the ether (IC^{50}: 9.8 µg/mL), light petroleum (IC^{50}: 12.0 µg/mL), methanol (IC^{50}: 12.8 µg/mL) and aqueous (IC^{50}: 53.5 µg/mL) extracts. A purified fraction from the ethyl acetate extract (constituents not identified) also showed strong inhibitory activity (IC^{50}: 0.46 µg/mL), even stronger than that of isolated flavonoids (of which quercetin was the most potent with an IC^{50} of 16.7 µg/mL) [Halkes 1997b].

A decoction of the flowers enhanced the growth-stimulating activity of mice peritoneal macrophages *in vitro* and *in vivo* [Bespalow 1992].

A decoction (1g/200 mL) was investigated for growth inhibition of non-small cell lung cancer (NCI-H460) cells. The anti-proliferative potential was measured by reduction of the percentage of bromodeoxyuridine positive cells. The treatment showed an effect on the cell cycle profile by increase of cells in G1 proliferation phase. The extract had no effect on programmed cell death but a significant increase of the protein p21 was found ($p<0.05$) [Lima 2014].

Antihistaminic effect
Four isolated ellagitannins (rugosin A, rugosin D, rugosin A methyl ester and tellimagradinin II) were tested for inhibition of human histidine decarboxylase which catalyses the formation of histamine from histidine. The inhibition constant values (Ki) of the non-competitive reaction ranged from 0.35-1µM, comparable with the potency of the substrate analogue inhibitor histidine methyl ester (Ki 0.46 µM) [Nitta 2013].

Affinity for proteins
Isolated rugosin D showed a high capacity for binding to bovine serum albumin (BSA), even higher than penta-O-galloyl-β-D-glucose, the tannin having the highest protein binding capacity in the simple galloyl-D-glucose series [46], whereas 1,2,3-tri-O-galloyl-4,6-hexahydroxydiphenoyl-β-D-glucose had a weaker effect and 2,3-di-O-galloyl-4,6-hexahydroxydiphenoyl-β-D-glucose a low effect [Beart 1985].

Antioxidant effect
A methanolic extract inhibited xanthine oxidase (XO) with an IC_{50} value of 6.2 µg/mL ± 0.6, compared to allopurinol and its main metabolite oxypurinol of 2.6µg/mL ±0.9 and 1.0 µg/mL ±0.2 respectively [Kazazi 2009].

In vivo experiments

Anticoagulant and fibrinolytic effects
A heparin-like complex found in meadowsweet flowers showed some anticoagulant and fibrinolytic properties after intramuscular and intravenous administration to animals, the effect being neutralized by protamine sulphate [Kudrjashov 1990, 1991].

Intestinal effects/effects on gastric ulcers
Orally administered decoctions of meadowsweet flowers (1:10 and 1:20) at doses of 0.5 or 2.5 mL/100 g respectively reduced the formation of stomach lesions induced by fixation, immobilisation or subcutaneous injection of reserpine to rats and mice. The decoctions also prevented acetylsalicylic acid-induced lesions of the stomach and promoted healing of ethanol-induced stomach lesions in rats [Barnaulov 1980].

An ethanolic spissum extract from meadowsweet, administered to mice (50-1000 mg i.p.), rats (2500 mg/kg orally) and rabbits (15-30 mg/kg i.v.) as a 5% aqueous solution and a decoction (1:20), showed a positive effect on the permeability of vessels provoked by histamine in the trypan blue test system [Barnaulov 1977].

Quercetin-3'-glucoside from meadowsweet, orally administered as a dose of 0.5 mL of a 5% solution per 10 g b.w., reduced by 50% the occurrence of serious lesions of the rat stomach provoked by immobilisation and intraperitoneal injection of reserpine (2.5 mg/kg) [Barnaulov 1984].

CNS effects
In various animals, a 5% aqueous solution of an ethanolic spissum extract and a decoction (1:20) from meadowsweet showed suppressive effects on the central nervous system, such as reduction of motor activity and rectal temperature, myorelaxation and potentiation of the activity of narcotic agents, and prolongation of the life time of mice in closed cages [Barnaulov 1977].

Anticarcinogenic effects
Long-term oral administration of a decoction of meadowsweet flowers inhibited the growth of brain and spinal cord tumours induced by transplacental administration of N-ethyl-N-nitroso-urea in rats. It did not affect the development of cervical and vaginal tumours induced by intravaginal application of 7,12-dimethylbenz[*a*]anthracene in mice but, when applied intravaginally, the decoction inhibited cervical and vaginal carcinogenesis induced by this compound. Tubal oral administration of the decoction suppressed the growth of transplanted sarcoma-180 as well as the growth and metastasis of transplanted Lewis' carcinoma in mice [Bespalov 1992].

Local administration of a decoction of meadowsweet flowers resulted in a 39% decrease in the frequency of squamous-cell carcinoma of the cervix and vagina induced in mice by 7,12-dimethylbenz[*a*]anthracene treatment [Peresun`ko 1993].

Isolated rugosin D was administered as a single i.p. dose of 10 mg/kg b.w. to 6 female mice; 4 days later and at weekly intervals thereafter for 60 days the mice were injected intraperitoneally with 10^5 sarcoma-180 tumour cells. Antitumour activity was calculated as the percentage increase in life-span, %ILS = 100 × (mean survival days of the treated group - mean survival days of the vehicle control group/mean survival days of the vehicle control group). The vehicle control group had a mean survival period of 12.9 days. After 10 mg of rugosin D, the %ILS value was 171.5 and one animal showed no tumours on day 60. The authors suggested that the antitumour activity was likely to be through potentiation of the immunity of host animals rather than direct activity on the tumour cells [Miyamoto 1987].

Clinical studies
After local application of an ointment containing a decoction of meadowsweet flowers to 48 patients with cervical dysplasia, a positive response was recorded in 32 patients (67%), including 25 cases (52%) of complete regression of dysplasia. No recurrence was observed within 12 months in 10 completely cured patients [Peresun`ko 1993].

Pharmacokinetic properties
Ellagitannins are converted into urolithins by decarboxylation of the lactone rings by the gut microflora [Piwowarski 2014].

Preclinical safety data
For an ethanolic spissum extract of meadowsweet as a 5% aqueous solution, the i.p. LD_{50} in mice and i.v. LD_{50} in rabbits were determined as 1770 mg/kg and 75.7 mg/kg respectively. For a decoction (1:20), the i.p. LD_{50} in male and female mice, and the i.v. LD_{50} in rabbits, were found to be 535 mg/kg, 1050 mg/kg and 141.5 mg/kg respectively [Barnaulov 1977].

Pharmacological studies of meadowsweet flowers and their extracts in rats and rabbits did not show any influence on liver function [Barnaulov 1984].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
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GINSENG RADIX	Ginseng	Second Edition, 2003
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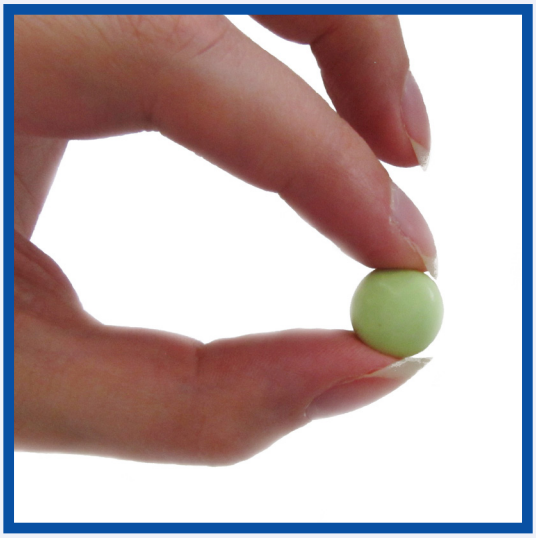

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FOREWORD

It is a great pleasure for me, on behalf of my colleagues in ESCOP, to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

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Board of Supervising Editors
ESCOP Scientific Committee
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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

FOENICULI AETHEROLEUM 2019

Bitter-Fennel Fruit Oil

DEFINITION

Bitter-fennel fruit oil is obtained by steam distillation from the ripe fruits of *Foeniculum vulgare* Miller subsp. *vulgare* var. *vulgare* [Bitter-fennel fruit oil].

The material complies with the monograph of the European Pharmacopoeia [Bitter-fennel fruit oil].

CONSTITUENTS

Bitter-fennel fruit oil contains predominantly anethole (55.0-75.0%) and fenchone (12.0-25.0%) with not more than 6% of estragole. It also contains α-pinene, limonene, camphene, *p*-cymene, β-pinene, β-myrcene, α-phellandrene, sabinene, γ-terpinene and terpinolene [Brand 2007, Tóth 1967; Trenkle 1972; Tschiggerl 2010].

Chemotypes with essential oil containing a high amount of estragole (79-94%) and very little *trans*-anethole (<1%) have been identified [Miralda 1999; Miguel 2010]. These chemotypes do not comply with the monograph of the European Pharmacopoeia.

Sweet fennel oil from the fruit or herb of *Foeniculum vulgare* Miller subsp. *vulgare* var. *dulce* (Miller) Thellung also exists. It contains more than 75% anethole with not more than 6% of estragole and not more than 8% of fenchone [Tóth 1967;Trenkle 1972]. Sweet fennel oil does not comply with the monograph of the European Pharmacopoeia.

CLINICAL PARTICULARS

Therapeutic indications

Dyspeptic complaints such as mild, spasmodic gastro-intestinal ailments, bloating and flatulence [Brand 2007].

Catarrh of the upper respiratory tract [Brand 2007; Brayfield 2014].

In these indications, efficacy is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Adult daily dosage: 0.1-0.6 ml, or equivalent preparations [Brand 2007]. Fennel honey with 0.5 g fennel oil/kg:10-20 g, or equivalent preparations [Brand 2007].

Children: Fennel syrup or fennel honey: *1-4years of age*, 3-6g; *4-10years of age,* 6-10g; *10-16 years of age*, adult dose [Dorsch 2002].

See note on safety concerns regarding estragole under preclinical safety data.

Method of administration
For oral administration.

Duration of administration
No restriction. If symptoms persist, consult your doctor.

Contra-indications
Persons with known sensitivity to anethole should avoid the use of fennel oil [Andersen 1978, Franks 1998].

Special warnings and special precautions for use
Persons with known sensitivity to anethole should avoid the use of fennel oil [Andersen 1978, Franks 1998].

E/S/C/O/P MONOGRAPHS

Interaction with other medicaments and other forms of interaction
None reported for humans.

Pregnancy and lactation
Preparations containing the essential oil or alcoholic extracts should not be used during pregnancy and lactation. Mild oestrogenic activity and antifertility effects of anethole (the major constituent of the essential oil) have been demonstrated in rats [Tisserand 1995, Dhar 1995].

Effects on ability to drive and use machines
None known.

Undesirable effects
Rare cases of contact dermatitis caused by anethole-containing preparations [Andersen 1978, Franks 1998].

Overdose
No toxic effects reported [Keller 1992].

PHARMACOLOGICAL PROPERTIES

As the type of fennel oil is rarely specified, it is referred to here as fennel oil.

Pharmacodynamic properties

In vitro experiments

Antimicrobial effects
Fennel oil inhibited the growth of *Escherichia coli* (MIC: 0.5% V/V), *Staphylococcus aureus* (MIC: 0.25%), *Salmonella typhimurium* (MIC: 1.0%) and *Candida albicans* (MIC: 0.5%) using the agar dilution method [Hammer 1999]. Significant antibacterial activity of the oil (10 µl of undiluted oil added to wells in agar plates) has been demonstrated against *Brevibacterium linens*, *Clostridium perfringens*, *Leuconostoc cremoris* and *Staphylococcus aureus* [Ruberto 2000]. Earlier studies also demonstrated antibacterial activity of the oil [Ramadan 1972, Afzal 1981].

Essential oil of bitter fennel fruit inhibited the food-born micro-organisms *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella enteridis* using the disc diffusion method with tetracycline and rifampicin as positive controls [Soylu 2009].

Fennel oil inhibited *Escherichia coli* (MIC:259.3µg/mL) and *Bacillus subtilis* (MIC:62.6 µg/mL). The MIC values for the positive control amoxicillin were 80.3µg/mL (*E. coli*) and 20.0 µg/mL (B. subtilis). The oil also showed antifungal activity against *Aspergillus niger* (MIC: 80.6 µg/mL), *Fusarium solani* (MIC: 91.1 µg/mL) and *Rhizopus solani* (MIC: 110.3µg/mL). For the positive control flumequine MICs were 30.3, 40.4 and 43.9 µg/mL respectively [Anwar 2009].

Using the disc diffusion method, fennel oil at 100 µg/disc showed antimicrobial activity against Gram-positive and Gram-negative bacteria and against *Candida albicans*, comparable to the activity of ofloxacin 6 µg/disc and miconazole nitrate 50 µg/disc. The MIC values tested in the agar dilution assay were 0.4-2.5% [Gulfraz 2008].

Antioxidant activity
Antioxidant activity of fennel oil against lipid peroxidation was demonstrated in the thiobarbituric acid reactive species assay and in a micellar model system [Ruberto 2000].

Free radical scavenging activities of fennel oil were demonstrated in the DPPH assay with an IC_{50} value of 32.32 µg/mL. The value for the positive control butylated hydroxytoluene (BHT) was 19.00 µg/mL.The oil also inhibited linoleic acid oxidation by 45.05%, while the value for BHT was 92.07% [Anwar 2009].

Anticoagulant activity
The anticoagulant properties of various compounds isolated from fennel were evaluated using a platelet aggregometer with acetylsalicylic acid as a positive control. Aggregation of rabbit platelets was induced by collagen. IC_{50} values for fenchone, estragole and acetylsalicylic acid were determined to be 3.9, 4.7 and 34.9 µM respectively [Lee 2006].

Fennel oil and anethole inhibited platelet aggregation in guinea pig plasma induced by arachidonic acid, collagen, adenosine diphosphate and U46619 (IC_{50} values 4-147 µg/mL) [Tognolini 2007].

Effects on musculature
The essential oil had a spasmogenic effect on smooth muscle of isolated guinea-pig ileum at a concentration of $8 + 10^{-5}$ g/mL. On a preparation of isolated rat phrenic nerve diaphragm it caused contracture and inhibition of the twitch response to nerve stimulation at a concentration of 2×10^{-4} g/mL [Lis-Balchin 1997].

Fennel oil significantly and dose-dependently reduced the intensity of oxytocin-induced contractions ($p<0.01$ at 50 µg/mL) and PGE_2-induced contractions ($p<0.01$ at 10 and 20 µg/mL) of the isolated rat uterus. The oil also reduced the frequency of contractions induced by PGE_2 (but not by oxytocin) [Ostad 2001].

Local anaesthetic activityof anethole
Trans-anethole concentration-dependently reduced electrically-evoked contractions of rat phrenic nerve-hemidiaphragm, by 10.3% at 10^{-3} µg/mL, by 43.9% at 10^{-2} µg/mL, by 79.7% at 10^{-1} µg/mL and by 100% at 1 µg/mL [Ghelardini 2001].

Tumour-inhibiting activity of anethole
Anethole at a concentration below 1 mM has been shown to be a potent inhibitor of TNF-induced cellular responses, such as activation of NF-κB and other transcription factors, and also to block TNF-induced activation of the apoptotic pathway. This might explain the role of anethole in suppression of inflammation and carcinogenesis [Chainy 2000].

Cytotoxic effect
Fennel oil showed cytotoxic effects in six different cancer cell lines with IC_{50} values of 0.51-3.78 µg/mL [EL-Bastawesy 2008].

In vivo experiments

Secretolytic and expectorant effects
Anethole and fenchone vapour were given by inhalation to urethanized rabbits at doses of 1 to 243 mg/kg b.w. added to the steam vaporizer (the amount actually absorbed by the animals was estimated as not more than 1% of that added to the vaporizer). Inhalation of anethole did not affect the volume but produced a dose-dependent (1-9 mg/kg) decrease in the specific gravity of respiratory tract fluid. Inhalation of fenchone produced a dose-dependent (1-9 mg/kg) augmentation of the volume output of respiratory tract fluid and a dose-dependent (1-27 mg/kg) decline in its specific gravity [Boyd 1971].

Oestrogenic effects
Trans-anethole administered orally to immature female rats at 80 mg/kg b.w. for 3 days significantly increased uterine weight, to 2 g/kg compared to 0.5 g/kg in controls and 3 g/kg

in animals given oestradiol valerate subcutaneously at 0.1 µg/rat/day ($p<0.001$). The results confirmed that *trans*-anethole has oestrogenic activity; other experiments showed that it has no anti-oestrogenic, progestational, anti-progestational, androgenic or anti-androgenic activity [Dhar 1995].

Osteoporosis-prophylactic activity
The prophylactic effect of fennel oil was evaluated in an experi-mental osteoporosis model in 6 groups of rats (n=6 each). Group 1 were sham-operated (control) rats receiving saline, groups 2-6 were ovariectomized rats receiving fennel essential oil (500, 750 or 1000 mg/kg b.w.), estradiol valerate (5 mg/kg b.w.) or saline, i.p. daily for 30 days. The fennel oil dose dependently and significantly (at the two higher doses; $p \leq 0.05$) prevented bone density loss and increased uterine weight ($p \leq 0.05$) [Jaffary 2006].

Anti-tumour activity of anethole
In Swiss albino mice with Ehrlich ascites tumour (EAT) in the paw, anethole administered orally at 500 or 1000 mg/kg on alternate days for 60 days significantly and dose-dependently reduced tumour weight ($p<0.05$ at 500 mg/kg, $p<0.01$ at 1000 mg/kg), tumour volume ($p<0.01$ at 500 mg/kg, $p<0.001$ at 1000 mg/kg) and body weight ($p<0.05$ to 0.01) compared to EAT-bearing controls. Mean survival time increased from 54.6 days to 62.2 days (500 mg/kg) and 71.2 days (1000 mg/kg). Histopathological changes were comparable to those after treatment with cyclophosphamide (a standard cytotoxic drug). These and other results demonstrated the anticarcinogenic, cytotoxic and non-clastogenic nature of anethole [Al-Harbi 1995].

Administration of fennel oil to Ehrlich ascites carcinoma bearing mice, at 100 mg/kg b.w. for three days (on days one, five and nine), demonstrated a significant ($p<0.01$) increase of serum iron. Fennel essential oil combined with radiation showed significant improvements ($p<0.01$) in iron, TIBC and transferring levels in tumour and liver tissues [El-Bastawesy 2008].

Anti-genotoxic activity of anethole
In the mouse bone marrow micronucleus test, oral pre-treatment of mice with *trans*-anethole at 40-400 mg/kg b.w. 2 and 20 hours before i.p. injection of genotoxins led to moderate, dose-dependent protective effects against known genotoxins such as cyclophosphamide, procarbazine, N-methyl-N'-nitrosoguanidine, urethane and ethyl methane sulfonate ($p<0.05$ to $p<0.01$ at various dose levels). No significant increase in genotoxicity was observed when *trans*-anethole (40-400 mg/kg b.w.) was administered alone in this model [Abraham 2001].

Enzyme modulating effect of anethole
Experiments in which rats were injected i.p. with a mixture of *trans*-anethole (100 mg/kg b.w.) and [^{14}C] parathion (1.5 mg/kg) showed no significant effect of *trans*-anethole on metabolism and excretion of the insecticide. However, when rats were fed a diet containing 1% of *trans*-anethole for 7 days and subsequently cell fractions from the livers of these rats were incubated for 2 hours compared to the control group with [^{14}C]parathion, significantly less unchanged parathion (1.6%) was recovered compared to controls (12.5%). The data were interpreted as suggesting that feeding *trans*-anethole to rats for 7 days induced the synthesis of parathion-degrading liver enzymes [Marcus 1982].

Analgesic effect of fenchone
In the mouse tail-flick test, fenchone at dosages of 0.05-0.2 mL/kg b.w. showed a significant anti-nociceptive effect ($p \leq 0.05$) [Him 2008].

Antithrombotic effect
Daily oral administration of fennel oil and anethole to mice at 30 mg/kg b.w. for 15 days showed a significant activity ($p<0.05$) against thromboembolism induced by collagen and epinephrine as compared to control [Tognolini 2007].

Antihepatotoxic activity
In rats, i.p. administration of fennel oil at 0.3 mL/kg b.w. three times a week for seven weeks showed a hepatoprotective activity against carbon tetrachloride induced liver fibrosis. The effect was expressed as decreased levels of serum ASAT, ALAT, ALP and bilirubin. The results were confirmed by histological findings. Controls were untreated and carbon tetrachloride treated rats [Özbek 2004].

Pharmacological studies in humans
No published data available.

Clinical studies
In a randomized, placebo-controlled study involving 121 infants (2-12 weeks of age) with colic diagnosed according to Wessel 1954, the verum group (29 males, 33 females) received an oral aqueous emulsion of 0.1% fennel oil and 0.4% polysorbate 80 (minimum 5 mL, maximum 20 mL) up to 4 times a day before meals and at the onset of colic periods for 7 days. In addition, the parents were instructed to limit the consumption to 12 mL/kg b.w. per day. The placebo group (26 males, 33 females) received 0.4% polysorbate in water. Diaries of crying periods were entered for the 7 days before the trial and the 7 days of the trial. There was a significant ($p<0.01$) improvement found in the fennel treatment group. Colic was eliminated in 65% of the infants in the fennel oil emulsion group and in 23.7% of the infants in the control group, indicating a marked placebo effect [Alexandrovich 2003].

In a placebo controlled, double blind study, 50 patients (15-24 years old) with moderate to severe dysmenorrhea were given a capsule four times a day for three days from the start of their menstrual period for two consecutive cycles, containing either 30 mg of fennel oil or placebo (wheat flour). Pain intensity as assessed by a visual analogue scale was significantly ($p<0.01$) reduced in the verum group compared to the placebo group [Omidvar 2012].

In an open study, 80 female students (aged 18-23 years) with primary dysmenorrhea were randomly divided into two groups (n=40 each). For three consecutive cycles, the treatment group received a capsule containing 30 mg fennel oil every 4 hours, from about 3 days before menstruation until the end of the fifth day (8 days in total). The control group did not receive any treatment or placebo. Pain and weakness were significantly reduced ($p<0.05$) after the treatment [Ghodsi 2014].

A randomised, placebo-controlled, double-blind, double-dummy, multi-centre trial investigated the efficacy of fennel oil compared to low-dose combined oral contraceptive (OC) and placebo at inducing menstrual bleeding in women (aged 15 to 45 years) using depot medroxyprogesterone acetate, who had no menstrual bleeding for the previous 45-140 days. The three groups (n=26 each) each received one pill and two capsules per day for 21 days. Group 1: 1 OC pill and 2 placebo capsules, group 2: 1 placebo pill and 2 fennel capsules (30 mg fennel oil/capsule), group 3: 1 placebo pill and 2 placebo capsules. Significantly ($p<0.001$) more women in the fennel group (73%) and the OC group (81%) experienced menstrual bleeding compared to the placebo group (19%) [Mohebbi-Kian 2014].

Pharmacokinetic properties

Pharmacokinetics in animals
No data available for fennel oil.

In mice and rats *trans*-anethole is reported to be metabolized by O-demethylation and by oxidative transformation of the C3-side chain. After low doses (0.05 and 5 mg/kg b.w.) *O*-demethylation occurs predominantly, whereas higher doses (up to 1500 mg/kg b.w.) give rise to higher yields of oxygenated metabolites [Sangster 1984].

Pharmacokinetics in humans
No data available for fennel oil.

After oral administration of radioactively-labelled *trans*-anethole (as the *methoxy*-^{14}C compound) to 5 healthy volunteers at dose levels of 1, 50 and 250 mg on separate occasions, it was rapidly absorbed. 54-69% of the dose (detected as 14C) was eliminated in the urine and 13-17% in exhaled carbon dioxide; none was detected in the faeces. The bulk of elimination occurred within 8 hours and, irrespective of the dose level, the principal metabolite (more than 90% of urinary ^{14}C) was 4-methoxyhippuric acid [49, 50]. An earlier study with 2 healthy subjects taking 1 mg of *trans*-anethole gave similar results [Sangster 1987].

Preclinical safety data

Acute toxicity
The oral LD_{50} of bitter fennel oil in rats was determined as 4.52 mL/kg [Opdyke 1976]. Values of 3.8 g/kg [Opdyke 1974] and 3.12 g/kg [Von Skramlik 1959] have also been reported for fennel oil in rats, in a more recent study the oral LD_{50} was estimated to be 1.326 g/kg [Ostad 2001].

The oral LD_{50} for fennel oil in mice was determined as 6.15 mL/kg [Özbek 2002].

Intraperitoneal LD_{50} values for *trans*-anethole have been determined as 0.65-1.41 g/kg in mice and 0.9-2.67 g/kg in rats [Lin 1991].

Reproductive toxicity
Trans-anethole exerted dose-dependent anti-implantation activity after oral administration to adult female rats on days 1-10 of pregnancy. Compared to control animals (all of which delivered normal offspring on completion of term), *trans*-anethole administered at 50, 70 and 80 mg/kg b.w. inhibited implantation by 33%, 66% and 100% respectively. Further experiments at the 80 mg/kg dose level showed that in rats given *trans*-anethole only on days 1-2 of pregnancy normal implantation and delivery occurred; in those given anethole only on days 3-5 implantation was completely inhibited; and in those given *trans*-anethole only on days 6-10 three out of five rats failed to deliver at term. No gross malformations of offspring were observed in any of the groups. The results demonstrated that *trans*-anethole has antifertility activity. From comparison with the days 1-2 group (lack of antizygotic activity), the lower level of delivery in the days 6-10 group was interpreted as a sign of early abortifacient activity [Dhar 1995].

Fennel essential oil at a concentration of 0.93 mg/mL produced a significant ($p<0.0001$) reduction in the number of differentiated foci in isolated limb bud mesenchymal cells. The findings suggest that fennel essential oil at the studied concentrations may have toxic effects on foetal cells. There was however no evidence of teratogenicity [Ostad 2004].

Mutagenicity and carcinogenicity
Fennel oil (2.5 mg/plate) and *trans*-anethole (2 mg/plate) were mutagenic in the Ames test using *Salmonella typhimurium* strains TA 98 and TA 100, and mutagenicity was potentiated by S13 activation [Marcus 1982]. In another study, *trans*-anethole was mutagenic to *Salmonella typhimurium* TA 100 in the Ames test with S9 activation, doses of 30-120 µg/plate showing a dose-dependent increase in revertants which did not exceed twice the number of the control [Sekizawa 1982]. Other investigations with metabolic activation have confirmed that *trans*-anethole is weakly mutagenic [Lin 1991].

Sweet fennel oil gave positive results in the *Bacillus subtilis* DNA-repair test [Sekizawa 1982], but fennel oil gave a negative result in the chromosomal aberration test using a Chinese hamster fibroblast cell line [Ishidate 1984].

Estragole, a minor constituent of fennel oil, has shown mutagenic potential in a number of Ames tests and proved carcinogenic in animal models [De Vincenzi 2000, Scientific committee on food 2001].

Note on the safety of estragole
As a result of new estragole carcinogenicity data, fennel (and theoretically also fennel oil) has in some cases been reported as posing risks for humans.

It is important to note that toxicological studies with rodents almost always use high doses, the results of these subsequently extrapolated to low doses. The metabolism of estragole is not the same in rodents and humans. 1′-Hydroxyestragole is considered the putative proximate toxic metabolite, which after conversion to 1′-sulfooxyestragole leads to DNA adduct formation. In mice, urinary excretion of 1′-hydroxyestragole glucuronide is dose dependent; from 1.3-5.4% at 0.05-50 mg/kg b.w. of estragole to 11.4-13.7% at 500-1000 mg/kg b.w. of estragole. In humans, 1′-hydroxyestragole glucuronide in urine stays constant at 0.2-0.4% over a wide dose range of 0.01-50 mg/kg b.w. of estragole [Gori 2012, Raffo 2011, Uusitalo 2016].

Attempts have been made to analyse or estimate the amount of estragole present in available fennel preparations [Raffo 2011, Uusitalo 2016]. Based on these data, daily consumption of three portions of herbal tea, could yield 0.2 mg of estragole, close to the average 0.166mg reported as the FAO/WHO estimate of daily consumption of estragole from spices and flavourings by the USA population.

Furthermore, all of the animal studies used estragole as a pure compound. In the case of fennel infusions, estragole is present together with other substances that can ameliorate its effects. There are indications that flavonoids present in the infusions, including quercetin, kaempferol and nevadensin, were able to inhibit the formation of 1′-sulfooxyestragole from 1,-hydroxyestragole, without influencing the capacity to detoxify the latter via glucuronidation or oxidation [Iten 2004; Alhusainy 2010; Alhusainy 2012].

Clinical safety data
In a study of 121 infants treated orally with either an emulsion of 0.1% fennel oil or placebo for 7 days, no side effects were reported [Alexandrovich 2003].

In a placebo controlled, double-blind study of 50 patients treated with either 30mg fennel oil 4 times a day for 3 days or placebo, no side effects were reported [Omidvar 2012].

In an open study 40 patients received the same treatment without side effects [Ghodsi 2014].

In a double-blind, double-dummy study, 3 groups of patients (n=26 each) were treated with various combinations of fennel oil, low-dose oral contraceptive (CO) and placebo. In the CO group 8 patients reported nausea while 3 patients receiving fennel reported heartburn [Mohebbi-Kian 2014].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2019
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
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CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
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CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
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COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
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CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
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ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2018
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
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LINI SEMEN	Linseed	Online Series, 2017
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MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
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ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
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RHEI RADIX	Rhubarb	Online Series, 2019
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ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
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SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
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TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
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The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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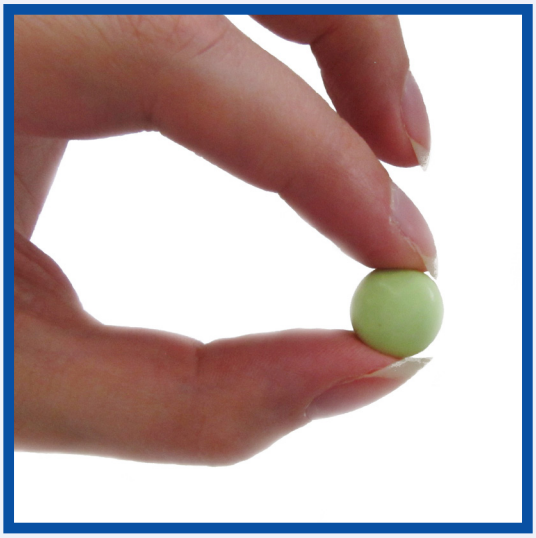

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Plant illustrated on the cover: *Foeniculum vulgare*

FOREWORD

It is a great pleasure for me, on behalf of my colleagues in ESCOP, to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

FOENICULI FRUCTUS 2019

Fennel Fruit

DEFINITIONS

Bitter fennel (Foeniculi amari fructus) consists of the dry, whole cremocarps and mericarps of *Foeniculum vulgare* Miller subsp. *vulgare* var. *vulgare*. It contains not less than 40 mL/kg of essential oil, calculated with reference to the anhydrous drug. The oil contains not less than 60.0 per cent of anethole and not less than 15.0 per cent of fenchone [Fennel, bitter].

Sweet fennel (Foeniculi dulcis fructus) consists of the dry, whole cremocarps and mericarps of *Foeniculum vulgare* Miller subsp. *vulgare* var. *dulce* (Miller) Thellung. It contains not less than 20 mL/kg of essential oil, calculated with reference to the anhydrous drug. The oil contains not less than 80.0 per cent of anethole [Fennel, sweet].

The materials comply with the monographs of the European Pharmacopoeia [Fennel bitter, Fennel sweet].

CONSTITUENTS

The characteristic constituents of bitter fennel include 3-8.5% of essential oil. The main components of the bitter fennel oil are anethole (50-75%) and fenchone (12-33%). It also contains estragole (2-5%), α-pinene, limonene, camphene, *p*-cymene, β-pinene, β-myrcene, α-phellandrene, sabinene, γ-terpinene and terpinolene [Tóth 1967; Trenkle 1972; Brand 2007; Tschiggerl 2010].The essential oil of sweet fennel contains predominantly anethole (80->90%), estragole (3-10%) and fenchone (1-10%). Other components include α-pinene and limonene [Brand 2007] as well as β-pinene, β-myrcene and *p*-cymene [Toth 1967; Trenkle 1972].

Fennel fruit also contains water-soluble glycosides of essential oil components, hydroxyfenchone glycoside, hydroxyl-*p*-menthane glycoside, 1,8-cineole glycoside and other water-soluble glycosides [Kitajima 1998a; 1998b; 1998c; Ishikawa 1998a;1998b; 1998c; 1998d]. As well as small amounts of flavonoids (e.g. kaempferol and quercetin glycosides) and the trimethoxyflavone nevadensin [Brand 2007, Alhusaini 2010].

Chemotypes with essential oil containing a high amount of estragole (79-94%) and very little trans-anethole (<1%) are identified [Miralda 1999; Miguel 2010]. These chemotypes do not comply with the monograph of the European pharmacopoeia.

CLINICAL PARTICULARS

Therapeutic indications

Dyspeptic complaints such as mild, spasmodic gastro-intestinal ailments, bloating and flatulence [Madaus 1976; Brand 2007; Bradley 2006; Stahl-Biskup 2009].
Catarrh of the upper respiratory tract [Madaus 1976; Merkes 1980; Müller-Limmroth 1980; Brand 2007; Fintelman; Weiss 2009; Stahl-Biskup 2009; Brayfield 2014].

For symptomatic treatment of discomfort associated with menstrual periods [Moslemi 2012; Nejad 2006; Jahromi 2003].

In these indications, the efficacy is plausible on the basis of human experience and longstanding use.

Posology and method of administration

Dosage

Adult daily dose: 5-7 g of crushed fruits as an infusion or equivalent aqueous preparations, i.e. instant teas [Brand 2007; Stahl-Biskup 2009].
Compound fennel tincture: 5-7.5 g [Brand 2007].
Fennel syrup or honey: 10-20 g [Brand 2007].

E/S/C/O/P MONOGRAPHS

Children, average daily dose: 0-1 year of age, 1-2 g of crushed fruits as an infusion or equivalent preparation, i.e. instant teas; *1-4 years of age*, 1.5-3 g; *4-10 years of age*, 3-5 g; *10-16 years of age*, the adult dose [Dorsch 2002].

Fennel syrup or fennel honey: *1-4 years of age*, 3-6 g; *4-10 years of age* 6-10 g; *10-16 years of age*, adult dose [Dorsch 2002].

See note on safety concerns regarding estragole under preclinical safety data.

Method of administration
For oral administration.

Duration of use
Infusions or equivalent preparations: If symptoms persist for more than two weeks, consult your doctor, especially for paediatric use.

Contra-indications
Persons with known sensitivity to anethole should avoid the use of fennel [Andersen 1978, Franks 1998].

Special warnings and special precautions for use
Persons with known sensitivity to anethole should avoid the use of fennel [Andersen 1978, Franks 1998].

Interaction with other medicaments and other forms of interaction
None reported for humans.

Pregnancy and lactation
Fennel fruit may be used during pregnancy and lactation at the recommended dosage, as infusions only [Keller 1992].

Effects on ability to drive and use machines
None known.

Undesirable effects
A single case of allergic reaction to fennel has been reported [Schwartz 1997].

Rare cases of contact dermatitis caused by anethole-containing preparations [Andersen 1978; Franks 1998].

Overdose
No toxic effects reported [Keller 1992].

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antimicrobial effects
Acetone, *n*-butanol, ethanol and ether extracts inhibited the growth of a range of bacteria including *Escherichia coli* and *Staphylococcus aureus*, and also exhibited antifungal activity against *Candida albicans* and other organisms [Maruzzella 1959].

An ethanolic (95%) dry extract inhibited the growth of *Staphylococcus aureus* and *Bacillus subtilis* with an MIC value of 1 mg/mL for both organisms [Tanira 1996].

A methanolic dry extract inhibited the growth of 15 different strains of *Helicobacter pylori* with an MIC of 50 µg/mL using the agar dilution method [Mahady 2005].

A methanolic dry extract inhibited Gram-positive and Gram-negative bacteria and *Candida albicans* with MIC values of 1.3-3.7% [Gulfraz 2008].

A water extract and an acetone extract (drug:solvent 1:1) inhibited the growth of various bacteria with MIC values of 20-80 mg/ml and 5-15 mg/mL respectively. Controls were distilled water and acetone. Bactericidal activity for the extracts was demonstrated by viable cell count studies [Kaur 2009].

Antioxidant activity
Free radical scavenging activities of 100% methanolic, 80% methanolic, 100% ethanolic and 80% ethanolic dry extracts of sweet fennel were demonstrated in the DPPH assay with IC_{50} values of 26.75 µg/mL, 24.25 µg/mL, 26.10 µg/mL and 23.61 µg/mL respectively. The value for the positive control butylated hydroxytoluene (BHT) was 19.00 µg/mL.The same extracts inhibited linoleic acid oxidation by 48.80%, 55.31%, 50.76% and 70.35% respectively. The value for BHT was 92.07% [Anwar 2009].

Aqueous and ethanolic dry extracts showed concentration dependant antioxidant activity in various assays. The extracts inhibited lipid peroxidation by 91.6% and 98.6% respectively; the results were comparable with the values for the positive controls butylated hydroxyanisole (BHA) and BHT (94.3% and 97.8% respectively [Oktay 2003].

An aqueous dry extract inhibited the CYP-mediated oxidation of paracetamol to the hepatotoxic metabolite *N*-acetyl-*p*-benzoquinoneimine. The IC_{50} value for CYP2E1 inhibition was 23±4 µg/ml [Langhammer 2014].

Secretolytic and expectorant effects
An increase of about 12% in mucociliary transport velocity was observed in isolated ciliated epithelium from the frog oesophagus 90 seconds after application of 200 µl of an infusion from bitter fennel (4.6 g per 100 mL of water) [Müller-Limmroth 1980].

Anticoagulant activity
The anticoagulant properties of different compounds isolated from fennel were evaluated using a platelet aggregometer with acetylsalicylic acid as a positive control. Aggregation of rabbit platelets was induced by collagen. IC_{50} values for fenchone, estragole and acetylsalicylic acid were determined to be 3.9, 4.7 and 34.9 µM respectively [Lee 2006].

Effects on musculature
A 30%-ethanolic extract from bitter fennel produced a concentration-dependent decrease in acetylcholine- and histamine-induced contractility of isolated guinea pig ileum at concentrations of 2.5-10.0 mL/litre; however, taking into account the effect of ethanol, only the results with histamine were significant ($p<0.005$ at 10 mL/L) [Forster 1980]. In the same test system, the extract at 2.5 and 10.0 mL/L also concentration-dependently reduced carbachol-induced contractility [Forster 1983].

Local anaesthetic activity
Trans-anethole concentration-dependently reduced electrically-evoked contractions of rat phrenic nerve-hemidiaphragm by 10.3% at 10^{-3} µg/mL, by 43.9% at 10^{-2} µg/mL, by 79.7% at 10^{-1} µg/mL and by 100% at 1 µg/mL [Ghelardini 2001].

Tumour-inhibiting activity of anethole
Anethole at a concentration below 1 mM has been shown to be a potent inhibitor of TNF-induced cellular responses, such as activation of NF-κB and other transcription factors, and also to block TNF-induced activation of the apoptotic pathway. This

might explain the role of anethole in suppression of inflammation and carcinogenesis [Chainy 2000].

In vivo experiments

Secretolytic and expectorant effects
Anethole and fenchone vapour were given by inhalation to urethanized rabbits as doses of 1 to 243 mg/kg b.w. added to the steam vaporizer (the amount actually absorbed by the animals being considerably less, estimated as not more than 1% of that added to the vaporizer). Inhalation of anethole did not affect the volume but produced a dose-dependent (1-9 mg/kg) decrease in the specific gravity of respiratory tract fluid. Inhalation of fenchone produced a dose-dependent (1-9 mg/kg) augmentation of the volume output of respiratory tract fluid and a dose-dependent (1-27 mg/kg) decline in its specific gravity [Boyd 1971].

Effects on the digestive tract
Fennel administered orally at 24 mg/kg b.w. increased spontaneous movement of the stomach in unanaesthetized rabbits and reduced the inhibition of stomach movement induced by sodium pentobarbitone [Niiho 1977].

An aqueous extract (10% w/v), perfused through the stomach of anaesthetized rats at 0.15 mL/min. and collected over periods of 20 min., significantly increased gastric acid secretion ($p<0.02$) to more than 3-fold compared to the basal secretion determined from perfusion of saline solution [Vasudevan 2000].

Addition of 0.5% of fennel to the diet of rats for 6 weeks shortened food transit time by 12% ($p<0.05$) [Platel 2001].

Anti-inflammatory effects
Oral pre-treatment of rats with a dry 80%-ethanolic extract from sweet fennel at 100 mg/kg b.w. inhibited carrageenan-induced paw oedema by 36% ($p<0.01$) compared to 45% inhibition by indometacin at 5 mg/kg [Mascolo 1987].

Oral treatment with a methanolic (80%) dry extract (200mg/kg b.w.) significantly ($p \leq 0.05$) reduced carrageenan-induced rat paw oedema compared to control (10 mg/kg 0.5% sodium carboxymethyl cellulose).The same extract significantly ($p \leq 0.05$) reduced arachidonic acid-induced mouse ear oedema [Mascolo 1987].

In the formaldehyde-induced arthritis assay, a model for subacute inflammation, daily application (200 mg/kg/day for 7 days) significantly ($p \leq 0.05$) decreased the plasma activity of alanine aminotransferase compared to control. The results were comparable with indomethacin 10 mg/kg b.w. [Choi 2004].

Pre-treatment of rats with an unspecified ethanolic dry extract at 50, 100 and 200 mg/kg b.w. i.p. significantly ($p<0.05$) reduced carrageenan-induced paw oedema. The effect of 100 mg/kg of the extract was comparable to diclofenac at 10 mg/kg b.w., while 200 mg/kg b.w. was found to be more effective than the standard drug [Elisabeth 2014].

Oestrogenic effects
Oral administration of an acetone extract to adult female ovariectomized rats at 0.5-2.5 mg/kg b.w. caused dose-dependent oestrogenic effects: induction of the oestrus phase (after 10 days, in 40% of rats at 0.5 mg/kg, in 100% at 2.5 mg/kg), increase in mammary gland weight ($p<0.05$ at 0.5 mg/kg, $p<0.01$ at 2.5 mg/kg) and increase in weights of endometrium, cervix and vagina ($p<0.01$ to $p<0.001$ at 2.5 mg/kg). Oestrogenic effects were also evident in mature male rats after treatment with the extract at 1.5 or 2.5 mg/kg/day for 15 days: no significant change in body or organ weights but, particularly at the higher dose, significant changes in protein and acid and alkaline phosphatases in the testes, vas deferens, seminal vesicles and prostate were shown [Malini 1985].

Trans-anethole administered orally to immature female rats at 80 mg/kg b.w. for 3 days significantly increased uterine weight, to 2 g/kg compared to 0.5 g/kg in controls and 3 g/kg in animals given oestradiol valerate subcutaneously at 0.1 µg/rat/day ($p<0.001$). The results confirmed that *trans*-anethole has oestrogenic activity; other experiments showed that it has no anti-oestrogenic, progestational, anti-progestational, androgenic or anti-androgenic activity [Dhar 1995].

Antifertility effect
Oral administration of an aqueous dry extract to male rats in daily doses of 250 and 500 g/kg b.w. for 60 days, caused a significant decrease ($p<0.01$ and $p<0.001$) in the weight of reproductive organs (testes, epididymis, seminal vesicle, ventral prostate and vas deferens) compared to control (distilled water). The motility of spermatozoa and their density were diminished significantly ($p<0.01$). The study also showed a significant ($p<0.001$) reduction in the number of pregnancies, fertilizations and implantations [Agarwal 2006a].

Oral administration of a hydroethanolic (50%) extract to male rats at a daily dose of 100 mg extract corresponding to 2.5 g herbal drug/kg b.w. for 60 days, showed a significantly ($p<0.01$) reduced total protein content of the reproductive organs compared to control (water). Sperm motility was decreased significantly ($p<0.0001$) and fertility was reduced to 20% [Agarwal 2006b].

Hypotensive effect
A lyophilized aqueous extract administered orally at 190 mg/kg b.w. (equivalent to crude drug at 1000 mg/kg) for 5 days significantly lowered the systolic blood pressure of spontaneously hypertensive (SH) rats ($p<0.05$), but had no effect on blood pressure of normotensive rats. The extract also significantly increased the urine output of SH rats, by 80% at day 3 ($p<0.05$), and increased renal excretion of sodium and potassium ($p<0.05$), suggesting that fennel acted mainly as a diuretic and natriuretic in the SH rats [El Bardai 2001].

Anti-tumour activity
In Swiss albino mice with Ehrlich ascites tumour (EAT) in the paw, anethole administered orally at 500 or 1000 mg/kg on alternate days for 60 days significantly and dose-dependently reduced tumour weight ($p<0.05$ at 500 mg/kg, $p<0.01$ at 1000 mg/kg), tumour volume ($p<0.01$ at 500 mg/kg, $p<0.001$ at 1000 mg/kg) and b.w. ($p<0.05$ to 0.01) compared to EAT-bearing untreated controls. Mean survival time increased from 54.6 days to 62.2 days (500 mg/kg) and 71.2 days (1000 mg/kg). Histopathological changes were comparable to those after treatment with cyclophosphamide (a standard cytotoxic drug). These and other results demonstrated the anticarcinogenic, cytotoxic and non-clastogenic nature of anethole [Al-Harbi 1995].

Fennel fruit given to mice for 15 days at concentrations of 4% and 6% in test diets significantly ($p<0.01$) reduced dimethylbenzpyrene- and croton oil-induced skin papillogenesis and benzo[a]pyrene-induced fore stomach papillogenesis, compared to controls (normal diet). A significant enhancement ($p<0.01$) in the activities of the antioxidant enzymes were also demonstrated [Singh 2008].

Anti-genotoxic activity of anethole
In the mouse bone marrow micronucleus test, oral pre-treatment

of mice with *trans*-anethole at 40-400 mg/kg b.w. 2 and 20 hours before i.p. injection of genotoxins led to moderate, dose-dependent protective effects against known genotoxins such as cyclophosphamide, pro-carbazine, N-methyl-N'-nitrosoguanidine, urethane and ethyl methane sulfonate ($p<0.05$ to $p<0.01$ at various dose levels). No significant increase in genotoxicity was observed when trans-anethole (40-400 mg/kg b.w.) was administered alone [Abraham 2001].

Enzyme modulating effect
Experiments in which rats were injected i.p. with a mixture of *trans*-anethole (100 mg/kg b.w.) and [^{14}C] parathion (1.5 mg/kg) showed no significant effect of *trans*-anethole on metabolism and excretion of the insecticide. However, when rats were fed a diet containing 1% of trans-anethole for 7 days and subsequently cell fractions from the livers of these rats were incubated for 2 hours compared to the control group with [14C]parathion, significantly less unchanged parathion (1.6%) was recovered compared to controls (12.5%). The data were interpreted as suggesting that feeding *trans*-anethole to rats for 7 days induced the synthesis of parathion-degrading liver enzymes [Marcus 1982].

Antioxidant activity
Oral administration of a methanolic dry extract to rats at 200 mg/kg b.w., significantly ($p<0.05$) increased the plasma superoxide dismutase and catalase activities and the HDL-cholesterol level [Choi 2004].

Analgesic effect
In the mouse tail-flick test i.p.administered fenchone at dosages of 0.05-0.2 mL/kg b.w. showed a significant anti-nociceptive effect ($p\leq0.05$) [Him 2008].

An unspecified fennel ethanolic dry extract at 50, 100 and 200 mg/kg b.w. i.p. significantly ($p<0.01$) decreased acetic acid-induced writhing in mice. The results for 100 and 200 mg/kg were comparable with diclofenac sodium at 10mg/kg b.w. The same dosages of the extract significantly ($p<0.001$) reduced the number of formalin induced paw lickings. The results were comparable with those of diclofenac 10mg/kg b.w. and pentazocine 5mg/kg b.w. [Elisabeth 2014].

The analgesic activity of a methanolic (80%) dry extract was demonstrated using the hot-plate test in mice at an oral dosage of 200 mg/kg b.w. The extract significantly ($p\leq0.05$) reduced the hot-plate thermal stimulation [Choi 2004].

Oral administration to mice of an ethanolic (95%) dry extract at 500 mg/kg b.w. showed significant analgesic effect after 90 min. ($p<0.05$) and 150 min. ($p<0.01$) using the hot- plate method [Tanira 1996].

Antithrombotic effect
Daily oral administration of anethole to mice at 30 mg/kg b.w. for 15 days showed a significant activity ($p<0.05$) against thromboembolism induced by collagen and epinephrine as compared to control [Tognolini 2007].

Antipyretic activity
An ethanolic (95%) dry extract given orally at 500mg/kg b.w. to yeast-fevered mice caused a significant decrease ($p<0.01$) of the rectal temperature after 30 and 90 minutes [Tanira 1996].

Diuretic activity
An ethanolic (95%) dry extract administered orally to rats at 500 mg/kg b.w. showed a significant diuretic effect after 5 hours ($p<0.01$) and after 24 hours ($p<0.05$) as compared to control. The effect was comparable to that of urea at 960 mg/kg b.w. [Tanira 1996].

Pharmacological studies in humans
No published data available.

Clinical studies
In a randomised, double-blind, placebo controlled study, 38 women with diagnosed mild to moderate idiopathic facial hirsutism were treated twice a day for 12 weeks with a cream containing either a fennel ethanolic dry extract (1:12.5) at 1% or 2%, or a placebo (vehicle cream). Hair diameters were measured during the treatment period and necessary epilations served as an indirect measurement of the hair growth. The fennel extract containing creams reduced hair diameters by 7.8% and 18.3% respectively, and a decreased frequency of epilations was observed only in the fennel groups compared to placebo [Javidnia 2003].

In a randomized, double-blind, placebo-controlled study, 2 groups of 22 women aged 15-45 years old, with mild to moderate idiopathic hirsutism limited to the face, were treated with either a gel containing 3% of an 80% hydroalcoholic dry extract of fennel or a placebo gel. After 24 weeks of treatment the average thickness of the hairs was significantly ($p<0.001$) reduced in the fennel gel group compared to baseline [Akha 2014].

In an open study, 30 women aged 15-24 years old, with moderate to severe primary dysmenorrhoea, were evaluated for three menstrual cycles. In the first cycle no medication was given, in the second cycle the patients were treated orally with mefenamic acid at 250 mg every 6 hours, and in the third cycle with 2% sweet fennel water, 25 drops orally every 4 hours, during menstruation. The patients assessed the intensity of their dysmenorrhoea for each day of menstruation by a linear analogue technique with a scale of 1-10. Both treatments significantly ($p< 0.001$) relieved pain and other symptoms compared with control. Mefenamic acid had a more potent effect than fennel, but the difference was not significant [Jahromi 2003].

In a double-blind study involving young women with primary dysmenorrhoea, two groups of patients were randomized to receive 30 drops of an undefined fennel extract (n=55) or 250 mg of mefenamic acid (n=55), every 6 hours for the first 3 days of menstruation. After treatment, 80% of the fennel group and 73% of the mefenamic acid group had no pain. Fennel extract and mefenamic acid were equally effective in reducing limitations in activity and need for rest [Nejad 2006].

In a single blind, randomized, clinical study, female students suffering from mild or acute dysmenorrhoea were randomised into three groups of 25 each. The patients received capsules containing either a fennel hydroalcoholic extract (46 mg/capsule), vitamin E (100 I.U./capsule) or placebo, at one capsule every 6 hours for 3 days from the start of menstruation for 2 consecutive menstrual periods. If the pain persisted for more than 2 h after treatment they were allowed to use the analgesics they regularly used. The intensity of pain decreased significantly ($p<0.05$) in the fennel group [Moslemi 2012].

Pharmacokinetic properties

Pharmacokinetics in animals
No data available for fennel.

In mice and rats *trans*-anethole is metabolized by *O*-demethylation and by oxidative transformation of the C3-side chain. After low doses (0.05 and 5 mg/kg b.w.) *O*-demethylation occurs predominantly, whereas higher doses (up to 1500 mg/kg b.w.) give rise to higher yields of oxygenated metabolites [Sangster 1984a; 1984b].

Pharmacokinetics in humans
No data available for fennel.

After oral administration of radioactively-labelled *trans*-anethole (as the methoxy-^{14}C compound) to 5 healthy volunteers at dose levels of 1, 50 and 250 mg on separate occasions, it was rapidly absorbed. 54-69% of the dose (detected as ^{14}C) was eliminated in the urine and 13-17% in exhaled carbon dioxide; none was detected in the faeces. The bulk of elimination occurred within 8 hours and, irrespective of the dose level, the principal metabolite (more than 90% of urinary ^{14}C) was 4-methoxyhippuric acid [Caldwell 1988; Lin 1991]. An earlier study with 2 healthy subjects taking 1 mg of *trans*-anethole gave similar results [Sangster 1987].

Preclinical safety data

Acute toxicity
Oral administration of an ethanolic extract to mice at 0.5, 1 and 3 g/kg b.w. caused no mortality and no significant difference in body and vital organ weights or in external morphological, haematological or spermatogenic parameters in comparison with the control group over a period of 24 hours [Shah 1991].

Oral administration of an ethanolic (95%) dry extract at doses of 0.5, 1 and 3 g/kg b.w. did not cause any deaths in mice. The 3g/kg dose reduced locomotor activities [Tanira 1996].

The LD_{50} in mice given a dried ether extract i.p. was determined to be 5.52 mL/kg b.w. [Özbek 2003a].

LD_{50} values for i.p. *trans*-anethole have been determined as 0.65-1.41 g/kg in mice and 0.9-2.67 g/kg in rats [Lin 1991].

Subchronic toxicity
Oral administration of an ethanolic extract to mice at 100 mg/kg for 3 months caused no significant differences in mortality or in haematological and spermatogenic parameters compared to the control group [Shah 1991].

Reproductive toxicity
Trans-anethole exerted dose-dependent anti-implantation activity after oral administration to adult female rats on days 1-10 of pregnancy. Compared to control animals (all of which delivered normal offspring on completion of term), *trans*-anethole administered at 50, 70 and 80 mg/kg b.w. inhibited implantation by 33%, 66% and 100% respectively. Further experiments at the 80 mg/kg dose level showed that in rats given *trans*-anethole only on days 1-2 of pregnancy normal implantation and delivery occurred; in those given anethole only on days 3-5 implantation was completely inhibited; and in those given *trans*-anethole only on days 6-10 three out of five rats failed to deliver at term. No gross malformations of offspring were observed in any of the groups. The results demonstrated that *trans*-anethole has antifertility activity. From comparison with the days 1-2 group (lack of antizygotic activity), the lower level of delivery in the days 6-10 group was interpreted as a sign of early abortifacient activity [Dhar 1995].

Mutagenicity and carcinogenicity
Aqueous and methanolic extracts of fennel showed negative results in the Ames test using *Salmonella typhimurium* strains TA 98 and TA 100, with or without metabolic activation [Morimoto 1982; Yamamoto 1982]. These extracts also gave negative results in the *Bacillus subtilis* rec-assay [Morimoto 1982].

Trans-anethole (2 mg/plate) was mutagenic in the Ames test using *Salmonella typhimurium* strains TA 98 and TA 100, and mutagenicity was potentiated by S13 activation [Marcus 1982]. In another study, *trans*-anethole was mutagenic to *Salmonella typhimurium* TA 100 in the Ames test with S9 activation, doses of 30-120 µg/plate showing a dose-dependent increase in revertants which did not exceed twice the number of the control [Sekizawa1982]. Further investigations with metabolic activation have confirmed that trans-anethole is weakly mutagenic [Lin 1991].

Estragole, a constituent of fennel fruit, has shown mutagenic potential in a number of Ames tests and proved carcinogenic in animal models [De Vincenzi 2000; Scientific committee on food 2001;Iten 2004; Raffo 2011].

Note on the safety of estragole
As a result of new estragole carcinogenicity data, fennel (and theoretically also fennel oil) has in some cases been reported as posing risks for humans.

It is important to note that toxicological studies with rodents almost always use high doses, the results of these subsequently extrapolated to low doses. The metabolism of estragole is not the same in rodents and humans. 1′-Hydroxyestragole is considered the putative proximate toxic metabolite, which after conversion to 1′-sulfooxyestragole leads to DNA adduct formation. In mice, urinary excretion of 1′-hydroxyestragole glucuronide is dose dependent; from 1.3-5.4% at 0.05-50 mg/kg b.w. of estragole to 11.4-13.7% at 500-1000 mg/kg b.w. of estragole. In humans, 1′-hydroxyestragole glucuronide in urine stays constant at 0.2-0.4% over a wide dose range of 0.01-50 mg/kg b.w. of estragole [Gori 2012, Raffo 2011, Uusitalo 2016]. Attempts have been made to analyse or estimate the amount of estragole present in available fennel preparations [Raffo 2011, Uusitalo 2016]. Based on these data, daily consumption of three portions of herbal tea, could yield 0.2 mg of estragole, close to the average 0.166mg reported as the FAO/WHO estimate of daily consumption of estragole from spices and flavourings by the USA population.

Furthermore, all of the animal studies used estragole as a pure compound. In the case of fennel infusions, estragole is present together with other substances that can ameliorate its effects. There are indications that flavonoids present in the infusions, including quercetin, kaempferol and nevadensin, were able to inhibit the formation of 1′-sulfooxyestragole from 1,-hydroxyestragole, without influencing the capacity to detoxify the latter via glucuronidation or oxidation [Iten 2004; Alhusainy 2010; Alhusainy 2012].

Clinical safety data
In a randomized, double-blind, placebo controlled study, 68 infants with a diagnosis of infantile colic received either a preparation containing chamomile, vervain, licorice and fennel, or placebo. No adverse effects were observed [Weizman 1993].

In a study of 93 infants treated with an undefined extract of *Matricaria chamomilla*, *Foeniculum vulgare* var. *dulce* and *Melissa officinale*, no adverse effects were observed [Savino 2005].

In a randomized, double-blind, placebo controlled study of 44 women treated topically with a gel containing 3% of a hydroethanolic fennel extract, 3 patients reported dermal irritation and 7 patients reported itching [Akha 2014].

No adverse effects were observed in a study involving 38 women treated topically (on the face) with a cream containing fennel ethanolic dry extract twice a day for 12 weeks [Javidnia 2003].

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E/S/C/O/P
MONOGRAPHS

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ABSINTHII HERBA	Wormwood	Second Edition, 2003
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ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2019
ANGELICAE RADIX	Angelica Root	Supplement 2009
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COLAE SEMEN	Cola	Online Series, 2014
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CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
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CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
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ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
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LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2019
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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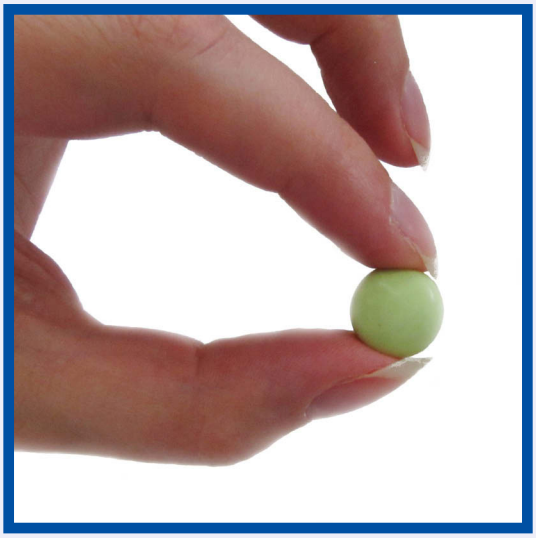
The Scientific Foundation for Herbal Medicinal Products

Frangulae cortex
Frangula Bark

2017



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Herbal Medicinal Products

FRANGULAE CORTEX
Frangula Bark

2017

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

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Plant illustrated on the cover: *Frangula alnus*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
CCl_4	carbon tetrachloride
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase

INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

FRANGULAE CORTEX 2017

Frangula Bark

DEFINITION

Frangula bark consists of the dried, whole or fragmented bark of the stems and branches of *Rhamnus frangula* L. (*Frangula alnus* Miller). It contains not less than 7.0 per cent of glucofrangulins, expressed as glucofrangulin A ($C_{27}H_{30}O_{14}$; Mr 578.5) and calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Frangulae cortex].

CONSTITUENTS

The main active constituents of the dried bark are glucofrangulins A and B (emodin-6-O-α-L-rhamnosyl-8-O-β-D-glucoside and emodin-6-O-β-D-apiosyl-8-O-β-D-glucoside respectively), frangulins A, B and C (emodin-6-O-β-L-rhamnoside, emodin-6-O-β-D-apioside and emodin-6-O-β-D-xyloside), and emodin-8-O-β-D-glucoside, together with small amounts of other anthraquinone glycosides, dianthrones and aglycones [Wagner 1969; Labadie 1970; Hörhammer 1972; Wagner 1974; Lemli 1978; Hänsel 1994; Hänsel 1999].

CLINICAL PARTICULARS

Therapeutic indications
For short term treatment of occasional constipation [Preston 1986; Sonnenberg 1989].

Posology and method of administration

Dosage
The correct individual dose is the smallest required to produce a comfortable soft-formed motion.

Adults and children over 12 years: preparations equivalent to 20-30 mg of glucofrangulins daily, calculated as glucofrangulin A [Hänsel 1994].
Elderly: dose as for adults.

Not recommended for use in children under 12 years of age.
The pharmaceutical form must allow lower dosages.

Method of administration
For oral administration.

Duration of administration
Use for more than 1 – 2 weeks requires medical supervision.
If the symptoms persist during the use of the medicinal product, a doctor or a pharmacist should be consulted.

Contraindications
Not to be used in cases of: intestinal obstruction and stenosis, atony, inflammatory bowel diseases (e.g. Crohn's disease, ulcerative colitis), appendicitis; abdominal pain of unknown origin; severe dehydration states with water and electrolyte depletion.
Not to be used in pregnancy or in children under 12 years of age [Hänsel 1994; Sweetman 2002].

Special warnings and precautions for use
As for all laxatives, frangula bark should not be given when any undiagnosed acute or persistent abdominal symptoms are present.

If laxatives are needed every day the cause of the constipation should be investigated. Long term use of laxatives should be avoided. Use for more than 2 weeks requires medical supervision. Chronic use may cause pigmentation of the colon (pseudomelanosis coli) which is harmless and reversible after drug

discontinuation. Abuse with diarrhoea and consequent fluid and electrolyte losses may cause: dependence with possible need for increased dosages; disturbance of the water and electrolyte (mainly hypokalaemia) balance; an atonic colon with impaired function. Intake of anthranoid-containing laxatives for more than a short period of time may result in aggravation of constipation. Hypokalaemia can result in cardiac and neuromuscular dysfunction, especially if cardiac glycosides, diuretics or corticosteroids are taken. Chronic use may result in albuminuria and haematuria.

In chronic constipation stimulant laxatives are not an acceptable alternative to a changed diet [Preston 1986; Müller-Lissner 1988; Sonnenberg 1989; Bingham 1989; Coenen 1990; Klauser 1990a, Klauser 1990b; Bingham 1991; Klauser 1992; Hänsel 1994; Sweetman 2002].

Note: A detailed text with advice concerning changes in dietary habits, physical activities and training for normal bowel evacuation should be included on the package leaflet. An example is given in the booklet "Médicaments à base de plantes" published by the French health authority (Paris: Agence du Médicament).

Interaction with other medicinal products and other forms of interaction
Hypokalaemia (resulting from long term laxative abuse) potentiates the action of cardiac glycosides and interacts with antiarrhythmic drugs or with drugs which induce reversion to sinus rhythm (e.g. quinidine). Concomitant use with other drugs inducing hypokalaemia (e.g. thiazide diuretics, adrenocorticosteroids and liquorice root) may aggravate electrolyte imbalance [Hänsel 1994].

Pregnancy and lactation

Pregnancy
Not recommended during pregnancy.

There are no reports of undesirable or damaging effects during pregnancy or on the foetus when used in accordance with the recommended dosage schedule. However, experimental data concerning a genotoxic risk from several anthranoids (e.g. emodin and physcion) and frangula bark extract are not counterbalanced by sufficient studies to eliminate a possible risk [Schmidt 1955; Brown 1976; Liberman 1980; Tikkanen 1983; Bruggeman 1984; Westendorf 1990; Westendorf 1993].

Lactation
Breastfeeding is not recommended as there are insufficient data on the excretion of metabolites in breast milk.

Excretion of active principles in breast milk has not been investigated. However, small amounts of active metabolites (e.g. rhein) from other anthranoids are known to be excreted in breast milk. A laxative effect in breast-fed babies has not been reported [Faber 1988; Sweetman 2002].

Effects on ability to drive and use machines
None known.

Undesirable effects
Abdominal spasms and pain, in particular in patients with irritable colon; yellow or red-brown (pH dependent) discolouration of urine by metabolites, which is not clinically significant [Cooke 1977; Tedesco 1985; Ewe 1986; Hänsel 1994; Sweetman 2002].

Overdose
The major symptoms are griping and severe diarrhoea with consequent losses of fluid and electrolyte, which should be replaced.

Treatment should be supportive with generous amounts of fluid. Electrolytes, particularly potassium, should be monitored; this is especially important in the elderly and the young [Hänsel 1994].

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

1,8-dihydroxyanthracene derivatives possess a laxative effect. For the greater part the glycosides (frangulins and glucofrangulins) are not absorbed in the upper gut; they are converted by the bacteria of the large intestine into the active metabolite (emodin-anthrone). There are two different mechanisms of action:

(i) an influence on the motility of the large intestine (stimulation of peristaltic contractions and inhibition of local contractions) resulting in accelerated colonic transit, thus reducing fluid absorption,

(ii) an influence on secretion processes (stimulation of mucus and active chloride secretion) resulting in enhanced fluid secretion [Casparis 1925; Schultz 1952;Ferguson 1956; Cresseri 1966; Fairbairn 1970; van Os 1976; Fairbairn 1976; Lemmens 1976; Longo 1980; de Witte 1993].

Defecation takes place after a delay of 8-12 hours due to the time taken for transport to the colon and metabolization into the active compound.

In vitro experiments
Emodin inhibited the aggregation of rabbit platelets induced by arachidonic acid and collagen, without affecting that induced by ADP or PAF. Frangulin B selectively and dose-dependently inhibited collagen-induced aggregation and ATP release in rabbit platelets, without affecting those induced by arachidonic acid, ADP, PAF and thrombin. Frangulin B also inhibited the platelet aggregation induced by trimucytin, a collagen receptor agonist [Teng 1993].

Frangulin B showed potent inhibitory activity against tumour necrosis factor-α (TNF-α) formation in lipopolysaccharide/γ-interferon-stimulated murine microglial cell line N9 (IC_{50}: 42.6 µM, $p<0.01$) [Wei 2001].

Anti-proliferative properties
Emodin displays antiproliferative properties which seem to be related to its role in the regulation of redox status, inhibition of kinases, through activation of microsomal enzymes, activation of repair mechanisms in various cellular systems generating ROS (reactive oxygen species) and increasing the susceptibility of tumour cells to standard cytotoxic therapeutic agents [Shieh 2004, Srinivas 2007].

Other effects
A methanolic extract (undefined) of frangula bark containing anthraquinones and flavonoids was active against various fungi *in vitro* [e.g. *Trichoderma viride, Doratomyces stemonitis, Aspergillus niger, Penicillium verrucosum, Alternaria alternate, Aueobasidium pullulans* and *Mucor mucedo*], and was in most cases superior to isolated emodin [Manjlovic 2005].

Clinical studies
In a randomized, controlled clinical trial, 45 post-colostomy surgery patients received either a standardized postsurgical laxative protocol (n = 19; intervention group) or laxative

treatments as preferred by the surgical team (n = 26; comparison group). The standardized protocol consisted of first line treatment with either a combination of sterculia and frangula bark or liquid paraffin, and second line treatment with an iso-osmotic polyethylene glycol. The effects of the treatments on constipation were compared, as measured by faecal loading on plain abdominal film, stomal therapy nurse activity, patient comfort and length of hospital stay. The presence of faecal loading (constipation) was lower (p=0.05) in the intervention group (1 episode) compared to the comparison group (7 episodes). Stomal therapy nurse activity, in terms of the number of empty bag changes, was significantly higher in the comparison group (p=0.03). No statistically significant difference was found regarding the other 2 parameters. The findings of the study support the benefits of this standardized laxative protocol for prevention of constipation for post-colostomy surgery patients [Stott 2012].

Pharmacokinetic properties

It is generally assumed (by analogy with sennosides from senna) that the glycosides (frangulins and glucofrangulins) are largely not split by human digestive enzymes in the upper gut and therefore not absorbed to a large extent. They are converted by the bacteria of the large intestine into the active metabolite (emodin-anthrone). Mainly anthraquinone aglycones are absorbed and transformed into their corresponding glucuronide and sulphate derivatives. After oral administration of frangula bark extract, rhein, emodin and traces of chrysophanol are found in human urine [Longo 1980; de Witte 1993].

Oral administration of emodin to rabbits at 10 mg/kg b.w. resulted in a very low serum concentration (approximately 2.5 µg/ml). Emodin was found to be highly bound (99.6%) to serum protein [Liang 1995].

Preclinical safety data

No studies are available on single dose toxicity, repeated dose toxicity, reproductive toxicity or on *in vivo* carcinogenicity of frangula bark or frangula bark preparations.

Embryonic / Developmental toxicity

Mouse embryos at the blastocyst stage administered *in vitro* with 25-75µM emodin exhibited significantly increased apoptosis and a corresponding decrease in total cell number. The implantation success rate of blastocysts pretreated with emodin was lower than that of control. Moreover, *in vitro* treatment was associated with increased resorption of post-implantation embryos and decreased fetal weight. In an *in vivo* mouse model, the application of emodin in drinking water led to apoptosis and decreased cell proliferation, and inhibited early embryonic development to the blastocyst stage [Chang 2012a].

In an *in vivo* mouse model, application of drinking water containing 20-40µM emodin led to decreased oocyte maturation and *in vitro f*ertilization. Pretreatment with a caspase-3-specific inhibitor prevented these effects, suggesting that the impairment of embryo development occurs via a caspase-dependent apoptotic process [Chang 2012b].

The effects of emodin on embryo/fetal growth, viability and morphological development were studied in rats and mice. Ingested dose was 0, 31, 57 and 80-144 mg emodin/kg/day in rats and 0, 94, 391 and 1005 mg emodin/kg/day in mice. The rat maternal lowest observed adverse effect level (LOAEL) was 1700 ppm; the no adverse effect level (NOAEL) was 850 ppm. The rat developmental toxicity NOAEL was ≤1700 ppm; LOAEL not established. In mice, maternal toxicity NOAEL was 2500 ppm (LOAEL: 6000 ppm). The developmental toxicity LOAEL was 6000 ppm and the NOAEL was 2500 ppm [Jahnke 2004].

Genotoxicity/Cancerogenicity

Various frangula bark extracts have been shown to be genotoxic in several *in vitro* bacterial mutation tests. Emodin, the main laxative principle of frangula bark, was mutagenic in the Ames test but gave inconsistent results in gene mutation assays (V79 HGPRT), positive results in the UDS test with primary rat hepatocytes but negative results in the SCE assay. Other anthraquinone constituents also gave positive results in limited experiments [Brown 1976; Liberman 1980; Tikkanen 1983; Bruggeman 1984; Westendorf 1990; Müller 1996].

In a 2-year study, male and female F344/N rats were exposed to 280, 830 or 2500 ppm of emodin in the diet, corresponding to an average daily dose of emodin of 110, 320 or 1000 mg/kg b.w. in male rats and 120, 370 or 1100 mg/kg b.w. in female rats. No evidence of carcinogenic activity of emodin was observed in male rats. A marginal increase in the incidence of Zymbal's gland carcinoma occurred in female rats treated with the high dosage but was interpreted as questionable.

In a further 2-year study, on B6C3F1 mice, males were exposed to 160, 312 or 625 ppm of emodin (corresponding to an average daily dose of 15, 35 or 70 mg/kg b.w.) and females to 312, 625 or 1250 ppm of emodin (corresponding to an average daily dose of 30, 60 or 120 mg/kg b.w.). There was no evidence of carcinogenic activity in female mice. A low incidence of renal tubule neoplasms in exposed males was not considered relevant [NTP Technical Report 1999].

Clinical safety data

Despite a lack of formal preclinical data on frangula bark, epidemiological studies suggest that there is no carcinogenic risk in humans from the use of anthranoid laxatives [Loew 1994; Loew 1997; van Gorkom 1999].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
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ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Supplement 2009
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
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HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
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HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
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MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
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MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
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MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
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PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
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PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
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RATANHIAE RADIX	Rhatany Root	Online Series, 2017
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SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
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TARAXACI RADIX	Dandelion Root	Second Edition, 2003
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URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
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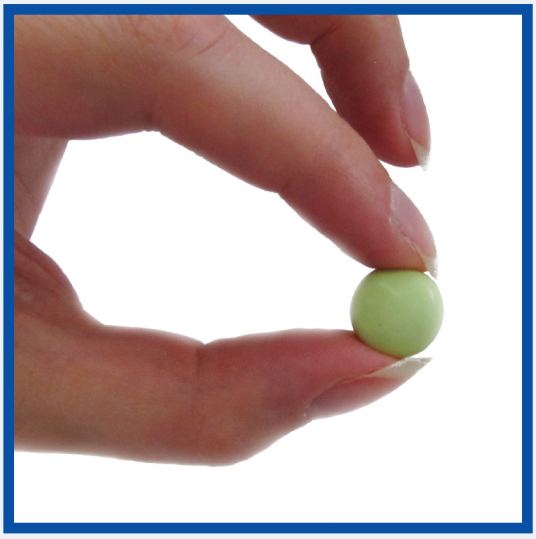

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

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Members of ESCOP
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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
CCl_4	carbon tetrachloride
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase

INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

FUMARIAE HERBA

2018

Fumitory

DEFINITION

Fumitory consists of the whole or fragmented, dried aerial parts of *Fumaria officinalis* L. harvested in full bloom. It contains not less than 0.4 per cent of total alkaloids, expressed as protopine ($C_{20}H_{19}NO_5$; M_r 353.4) and calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Fumitory].

CONSTITUENTS

The main characteristic constituents are isoquinoline alkaloids (0.3-1.3%) of the protopine, spirobenzylisoquinoline, protoberberine, benzophenanthridine and indenbenzazepine types, the principal ones being protopine and fumarophycine together with sinactine, sanguinarine, fumarofine and others. Over 20 alkaloids have been identified [Manske 1938; MacLean 1969; Hermansson 1973; Murav'eva 1975; Forgacs 1982, 1986; Mardirossian 1983; Sener 1985; Sousek 1999; Suau 2002; Sturm 2006; Paltinean 2016; Gorecki 2010; Blaschek 2016].

Other constituents include flavonol glycosides such as quercitrin, isoquercitrin, rutin, quercetin 3,7-diglucoside, quercetin 3-arabinoglucoside and their aglycones [Massa 1971; Torck 1971; Păltinean 2017], aliphatic acids (fumaric and malic acids), several hydroxycinnamoylmalic acids (a total of 1.3%), and hydroxycinnamic acids including caffeic, coumaric, sinapic and ferulic acids [Boegge 1995; Sousek 1999, Ivanov 2014].

CLINICAL PARTICULARS

Therapeutic indications
Digestive complaints (e.g. stomach ache, nausea, vomiting, feeling of fullness, flatulence) due to hepatobiliary disturbance [Fablet 1963; Colson 1967; Roux 1967; Salembier 1967; Warembourg 1967; Dornier 1968; Devin 1969; Heully 1969; Fiegel 1971; Roux 1977; Zacharewicz 1979; Bradley 1992; Gorecki 2010; Blaschek 2016].

Posology and method of administration

Dosage

Adult daily dose: 4-6 g of the drug as an aqueous dry extract [Fablet 1963; Colson 1967; Roux 1967, 1977; Salembier 1967; Warembourg 1967; Dornier 1968; Devin 1969; Heully 1969; Fiegel 1971; Zacharewicz 1979] or infusion [Bradley 1992; Gorecki 2010; Barnes 2007; Blaschek 2016]; other equivalent preparations, e.g. liquid extract (1:1, ethanol 25% V/V) and tincture (1:5, ethanol 45% V/V) [Bradley 1992; Barnes 2007].

Method of administration
For oral administration.

Duration of administration
No restriction. If symptoms persist or worsen, medical advice should be sought.

Contra-indications
Biliary obstruction.

Special warnings and special precautions for use
In cases of gallstones, fumitory should not be used without medical advice.

Interaction with other medicaments and other forms of interaction
None reported.

E/S/C/O/P
MONOGRAPHS

Pregnancy and lactation
No data available.

In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None known.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Effects on smooth muscle
An alkaloid-rich dry extract (not further specified) had a biphasic effect on isolated muscle, increasing resting tone at low concentrations (10^{-5} to 5×10^{-5} g/mL), and reducing the amplitude of spontaneous contractions at concentrations above 5×10^{-4} g/mL in isolated rabbit jejunum and 10^{-3} g/mL in isolated rat duodenum. The extract had a concentration-dependent smooth muscle-relaxing effect on barium chloride-induced contractions of isolated rat duodenum (EC_{50}: 10^{-4} g/mL), with approximately 5% of the effect of papaverine. At 10^{-5} to 10^{-4} g/mL the extract induced muscle contraction in isolated rat uterus, the effect being less pronounced at higher concentrations. In isolated dog saphenous vein it antagonized noradrenaline-induced contractions (EC_{50}: 8.5×10^{-6} g/mL), as did papaverine and protopine (EC_{50}: 2.9×10^{-5} and 2.3×10^{-5} g/mL respectively). In contrast, the extract increased spontaneous venous contraction within a concentration range of 10^{-6} to 10^{-4} g/mL [Reynier 1977].

An aqueous dry extract (not further specified) exhibited cholecystokinetic activity on isolated bile duct and Oddi's sphincter from pigs, as shown by contraction of the bile duct and relaxation of its Oddi's sphincter from a concentration of 10^{-4} g/mL. The extract at 5×10^{-4} g/mL inhibited morphine-induced contraction of Oddi's sphincter. In contrast, pure protopine at 10^{-5} to 10^{-7} g/mL had a strong contractile effect on Oddi's sphincter [Kimura 1972].

Neuroprotective effects
An alkaloid–rich extract (ethyl acetate; not further specified) was tested for the inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). The extract exhibited inhibitory activities against both enzymes with IC_{50} values of 39.23 ± 1.96 µg/mL and 40.32 ± 1.08 µg/mL respectively. The isolated alkaloids parfumidine and sinactine did not show any inhibitory activity on these enzymes but inhibited propyl oligopeptidase activity with IC_{50} values of 99 ± 5 µM and 53 ± 2 µM respectively [Chlebek 2016].

In another study, an alkaloid-rich extract (ethanol; not further specified) exhibited AChE inhibition with an IC_{50} value of 0.26 ± 0.01 mg/mL as compared to galanthamin with an IC_{50} value of 0.31 ± 0.05 mg/mL [Vrancheva 2016].

Other effects
Ethanolic extracts (not further specified) showed radical-scavenging activities in various assays such as DPPH and ABTS, as well as antioxidant capacity in CUPRAC, FRAP, lipid peroxidation and ferric reduction [Ivanov 2014; Khamtache-Abderrahim 2016].

An aqueous extract (not further specified) was tested in an *Agrobacterium tumefaciens*–induced potato disk tumour assay. The activity was similar to the positive control camptothecin (both 100%), compared to water as the negative control [Pehlivan Karakas 2012].

Protopine and allocryptopine induced a concentration-dependent increase in CYP1A1 mRNA in HepG2 cells after 24 h treatment. Both alkaloids induced a concentration-dependent increase of CYP1A1 and CYP1A2 mRNA in human hepatocytes, whereas the activation of the aryl hydrocarbon receptor in the induction of CYP1A mRNA levels by either protopine or allocryptopine was mild or negligible. Neither protopine nor allocryptopine caused an increase in CYP1A protein and activity levels in both cell types [Vrba 2011].

In vivo experiments

Hepatobiliary effects
No significant variations in bile flow were observed after intravenous administration of an aqueous dry extract (not further specified) to rats at 25, 50 and 100 mg/kg in the bile fistula model [Boucard 1966a, 1966b]. When the extract (50 mg/kg) was administered intravenously 30 minutes before or at the same time as sodium dehydrocholate (25 mg/kg), increases in bile flow 1 hour later were 45% and 28% respectively, compared to 66% when the choleretic agent was given alone [Boucard 1966b]. Under similar conditions, the increase in bile flow of 77% after sodium dehydrocholate (25 mg/kg) was reduced by simultaneous administration of the fumitory dry extract, to 55% (25 mg/kg of sodium dehydrocholate + 25 mg/kg of extract), 43% (25 mg/kg of sodium dehydrocholate + 50 mg/kg of extract) and 35% (25 mg/kg of sodium dehydrocholate + 100 mg/kg of extract) [Boucard 1966a]. A reduction in bile secretion induced by oral administration of 10 mg/kg of sodium azide was almost completely antagonized by simultaneous oral administration of the extract at 100 mg/kg [Boucard 1966b].

The same extract was administered intravenously to bile duct-cannulated dogs at 20 or 50 mg/kg. In dogs with low biliary output (0.09-0.20 mL/hour/kg) increases in bile flow of 29-218% were observed, while those with a high biliary output (0.63-1.38 mL/hour/kg) were characterized by reductions of 27-41%. Similar effects were observed with the extract in the presence of the choleretic agent sodium dehydrocholate, administered intravenously [Giroux 1966].

The choleretic activity of an aqueous dry extract (not further specified) intravenously administered at 40 mg/kg was confirmed in both normal rats and sodium azide-induced hypocholeretic rats in the bile fistula model. On the other hand, increased bile secretion induced by sodium hydrocholate was not reduced by intravenous administration of the extract at 40 mg/kg [Kimura 1972].

Intraduodenal administration of an alkaloid-rich dry extract (not further specified) to bile duct-cannulated rats at 200 mg/kg produced, not only an increase of 23% in bile flow, but also increases in biliary-excreted bilirubin and cholesterol of 33% and 20% respectively, in comparison to control. Under the same conditions protopine at 6 mg/kg produced comparable effects, while sodium dehydrocholate at 25 mg/kg had an effect only on the excreted volume [Reynier 1977].

The effect of an aqueous dry extract (not further specified) on

experimental cholelithiasis, induced by a diet supplemented with cholesterol-cholic acid, was evaluated in mice at two dosages, 0.2% and 0.4%, as an addition to their lithogenic diet. After 6 weeks of treatment the mean numbers of gallstones per mouse were significantly reduced ($p<0.05$), from 1.61 in the control group to 1.00 in the 0.2% group and 0.64 in the 0.4% group [Lagrange 1973].

Hepatoprotective effects
An aqueous dry extract (not further specified) was administered intravenously at 50 and 100 mg/kg b.w. to normal rats and to rats intoxicated with a single i.p. dose of carbon tetrachloride (CCl_4). Compared to controls 48 hours after administration of CCl_4, alkaline phosphatase activity was unchanged in excreted bile and serum, while in the liver it was significantly higher ($p<0.01$) in the group of CCl_4-intoxicated rats that received 50 mg/kg of extract and in each of the groups treated with 100 mg/kg of extract, indicating stimulation of hepatocytes [Guesnier 1974].

Cardiac effects of fumitory alkaloids
An extract comprising the total alkaloids (TAF, extracted with sulphuric acid and purified by ion exchange), administered intravenously to mice at 1-1.5 mg/kg, slowed the heart rate by 14-22% for 90 minutes. Cardiac fibrillation in mice induced by a normally lethal intravenous dose of calcium chloride was inhibited by TAF at 5 mg/kg i.v., enabling 50% of the animals to survive and delaying deaths of the other animals longer than in the control group ($p<0.001$); protopine at the same dose level did not influence survival, but 15% of animals survived after cryptopine at 10 mg/kg. TAF administered intravenously to rabbits prior to an arrhythmogenic dose of adrenaline prevented arrhythmia in 50% of the animals at 0.5 mg/kg and 90% at 1 mg/kg. Under the same conditions protopine was ineffective at 0.5 mg/kg but prevented arrhythmia in 40% of animals at 1 mg/kg, while cryptopine was ineffective at both dose levels [Gorbunov 1977].

A study in dogs demonstrated that TAF at 1-2 mg/kg i.v. prevented or substantially reduced ischaemic shifts caused by experimental occlusion of the coronary artery, while 5.5 mg/kg prevented arrhythmia induced by ligation of the intraventricular branch of the left coronary artery [Gorbunov 1980].

Diuretic activities
An ethanolic extract (not further specified) produced a significant ($p<0.05$) increase in urine volume of rats, 24 h after oral administration of a single dose of 250 mg/kg b.w. The extract also significantly ($p<0.05$) increased the urinary excretion of sodium and potassium [Paltinean 2017].

Neuropharmacological activities
An ethanolic extract (not further specified) administered i.p. to mice at doses of 200 and 500 mg/kg b.w. significantly ($p<0.01$) decreased the number of movements in the actophotometer assay, and also significantly ($p<0.01$) increased the time spent in the open arm and the number of entrances into the open arms in the elevated plus-maze test [Sharma 2014].

Pharmacological studies in humans

Amphocholeretic effect
In a study carried out 7-10 days after choledochostomy (establishment of external drainage from the bile duct) in 25 patients, bile flow was measured before and after a single oral dose of 1500 mg of an aqueous dry extract (not further specified). The extract had an amphocholeretic effect, promoting bile secretion if it was below the normal threshold and inhibiting bile secretion if it was excessive [Salembier 1967]. In a subsequent study of this type, daily oral administration of 4 × 250 mg of the same extract to 20 post-choledochostomy patients for an average of 12 days produced similar effects on bile secretion, increasing weak bile flow and reducing elevated bile flow; antispasmodic and biliary analgesic effects were also observed [Devin 1969].

In an open study, the effect on choleresis of a single dose of an aqueous dry extract (not further specified) was investigated in 20 healthy volunteers. They received either 500 mg of the extract in physiological serum (12 subjects) or placebo (8 subjects) through an intraduodenal probe. Monitoring of bile secretion for 30 minutes confirmed that the extract facilitated normalization of bile flow by increasing or decreasing the flow in relation to the baseline secretion [Heully 1969].

Clinical studies
In a randomized, double-blind, cross-over study 30 patients with various biliary disorders including dyskinesia, cholecystitis and post-cholecystectomy syndrome, which had previously been treated for 2 years without success, were treated with 6 × 250 mg of an aqueous dry extract (not further specified) or placebo daily for 14 days and consecutively vice versa in a second 14-day phase. The group taking the extract during the first phase experienced a significant reduction ($p<0.01$) in the intensity of symptoms (right upper abdominal pains, postprandial pains, flatulence, nausea, vomiting, stomach rumblings, impaired sleep and headaches) and this lasted throughout the subsequent placebo phase. In the other group the intensity of symptoms increased during the second week of placebo treatment and then declined significantly ($p<0.01$) during treatment with the extract in the second phase [Zacharewicz 1979].

In an open study 45 patients, previously treated for 2 years without success for dyskinesia of the biliary tract, post-cholecystectomy syndrome, chronic cholecystitis or cholangitis, received 4 × 250 mg of an aqueous dry extract (not further specified) daily for 16 days and were given a follow-up examination 14 days later. The overall improvement in symptoms (right upper abdominal pains, postprandial pains, flatulence, nausea, vomiting, stomach rumblings, impaired sleep and headaches) was very good in 32 (71%) and moderate in 7 patients, while no effect was apparent in the remaining 6 [Zacharewicz 1979].

Daily treatment of 105 patients with biliary disorders of various origin (dyskinesia, hepatomegaly, gallstone complaints and post-cholecystectomy symptoms) with 6 × 250 mg of an aqueous dry extract (not further specified) for 2 weeks to 6 months led to substantial improvements in, or complete absence of, symptoms (right upper abdominal pains, nausea, retching, occasional vomiting and poor tolerance of food) in more than 80% of patients [Fiegel 1971].

The efficacy of an aqueous dry extract (not further specified) was evaluated in five observational studies involving a total of 286 patients, treated in most cases with 750-1500 mg/day for 1-3 months. The patients could be classified into six groups: biliary dyskinesia (n=90), biliary lithiasis (n=23), migraine (frequently associated with concomitant nausea or vomiting; n=106), hepatobiliary insufficiency (n=29), post-cholecystectomy symptoms (n=29) and jaundice following viral hepatitis (n=9). Global efficacy assessed by the physicians was excellent or good in 75%, 100%, 83%, 75%, 72% and 66% of patients in the respective groups [Fablet 1963; Colson 1967; Roux 1967; Warembourg 1967; Dornier 1968].

Thirty-one patients suffering from diarrhoea or chronic constipation of biliary origin were given 1000 or 1500 mg of an aqueous dry extract (not further specified) respectively.

Improvement was excellent or good in 71% of cases after treatment periods of at least 15 days [Roux 1977].

Pharmacokinetic properties
No data available.

Preclinical safety data

Single dose toxicity
The acute intraperitoneal LD_{50} of an aqueous dry extract (not further specified) was 1.91 g/kg b.w. in mice and 1.88 g/kg in rats [Cahen 1964].

Repeated dose toxicity in rats
No delayed growth, changes in vital organs or haematological abnormalities were evident in rats following 3 months of oral treatment with an aqueous dry extract (not further specified) at 2.4 g/kg/day [Cahen 1964].

Inhibition of hERG Channel
An extract (dichlormethane/ethanol; not further specified) did not show inhibitory activity in a hERG (human Ether-a-go-go Related Gene) screening based on semi-automated voltage-clamp system using *Xenopus* oocytes [Kratz 2016].

Clinical safety data
Over 500 individuals have participated in open and controlled studies with fumitory aqueous dry extracts (not further specified), taking 750-1500 mg/day for up to 6 months. The tolerability of the treatments was very good [Salembier 1967; Devin 1969; Heully 1969; Zacharewicz 1979; Fiegel 1971; Fablet 1963; Colson 1967; Dornier 1968; Roux 1967, 1977; Warembourg 1967]. Minor adverse events occurred in a very few cases involving gastrointestinal discomfort and/or an allergic reaction with pruritis [Fablet 1963; Roux 1967].

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E/S/C/O/P MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Supplement 2009
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003

LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

E/S/C/O/P Monographs

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The second edition of ESCOP Monographs, published as a hardback book in 2003 with a Supplement in 2009, has been widely acclaimed for its authoritative information on the therapeutic uses of herbal medicines. Monographs covering a total of 107 herbal substances include extensive summaries of pharmacological, clinical and toxicological data, and copious references to scientific literature form an important part of each text.

Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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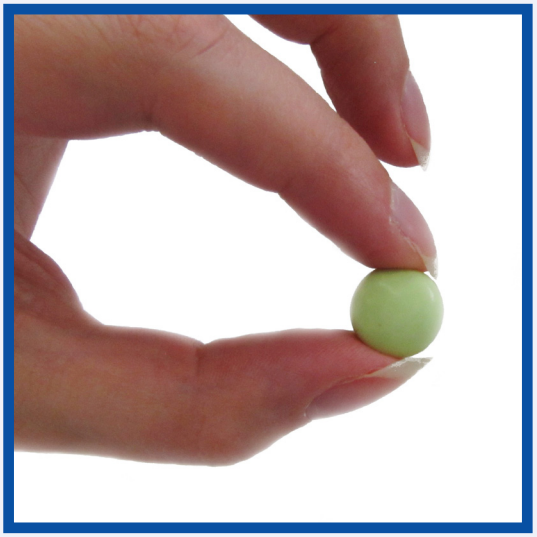
The Scientific Foundation for Herbal Medicinal Products

Gentianae radix

Gentian Root

2014



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Plant illustrated on the cover: *Gentiana lutea*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

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Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

GENTIANAE RADIX

2014

Gentian Root

DEFINITION

Gentian root consists of the dried, fragmented underground organs of *Gentiana lutea* L.

The material complies with the monograph of the European Pharmacopoeia [Gentian root].

CONSTITUENTS

Secoiridoids including gentiopicroside (1-4%) and amarogentin (0.025-0.4%); lignans, triterpenoids, oligosaccharides including gentianose and gentiobiose; xanthones (ca. 0.1%), mainly gentisin, isogentisin and gentioside; and traces of essential oil [Atkinson 1969; Bricout 1974; Franz 1975, 1978; Quercia 1980; Sticher 1980; Takino 1980; Chialva 1986; Nikolaeva 1983; Rossetti 1984; Hayashi 1988; Buffa 1991; Krupinska 1991; Kakuda 2003; Toriumi 2003; Ando 2007; Blaschek 2009].

CLINICAL PARTICULARS

Therapeutic indications

As a bitter in loss of appetite e.g. after illness; dyspeptic complaints [Gentiana BHP 1983; Wegener 1998; Meier 2007; Schilcher 2010]

Posology and method of administration

Dosage

Adults: 0.1-2 g of drug in 150 mL of water in infusion, decoction or maceration, up to 3 times daily. Tincture (1:5, ethanol 45-70 % V/V): average single dose of 1 ml, up to 3 times daily. Hydroethanolic extracts of equivalent bitterness value [Wegener 1998; Meier 2007].
Children, average daily dose: 4-10 years of age, 1-2 g of drug; 10-16 years, 2-4 g; in ethanol-free dosage forms [Dorsch 2002].
The dosage may be adjusted according to the bitterness sensitivity of the individual [Henschler 1966; Meier 2007].

Method of administration
For oral use in liquid preparations; for loss of appetite, a single dose administered half to one hour before a meal; in dyspeptic complaints, a single dose after the meal [Wichtl 1999; Meier 2007].

Duration of administration
No restriction. If symptoms persist, consult your doctor.

Contraindications
Gastric or duodenal ulcers; hyperacidity [Fintelmann 2009; Meier 2007; Schilcher 2010].

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
In accordance with general medical practice, the product should not be used during pregnancy without medical advice [Duke 1985].

Effects on ability to drive and to use machines
None known.

E/S/C/O/P MONOGRAPHS

Undesirable effects
Occasional headaches may occur [Meier 2007; Schilcher 2010].

Overdose
Overdose may lead to nausea or vomiting [Meier 2007].

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments
An aqueous extract from gentian root directly stimulated acid secretion in cultured cells from rat gastric mucosa, causing a concentration-dependent 1.7-fold acid increase at 100 µg/mL ($p<0.01$) [Gebhardt 1997].

Selective antifungal activity of gentian root extracts has been reported [Meier 2007].

Gentian root extract was reported to stimulate phagocytic activity of human leukocytes indicating possible immunostimulatory activity [Schmolz 1992].

A methanolic dry extract (2:1) was found to have significant stimulatory effects on mitotic activity of cultured chicken embryonic fibroblast cells ($p<0.10$). The extract increased the number of polygonal fibroblasts. The number of collagen granules in the embryonic fibroblasts was significantly increased ($p<0.01$); the percentage of vacuole-containing cells and round cells was significantly decreased ($p<0.005$) [Öztürk 2006].

Ethanol (yield 32%) and water (yield 18%) extracts moderately inhibited rat lens aldose reductase and human recombinant aldose reductase, while methanol (yield 38%) and ether (yield 5%) extracts demonstrated greater inhibitory activity with IC_{50} values of 112±15, 79±12 µg/mL (rat) and 23±5, 36±5 µg/mL (human), respectively [Akileshwari 2012].

Accumulation of sorbitol in red blood cells under high glucose load was assessed. An inhibition of 35% was achieved by 100 µg/mL of the aqueous extract and by 50 µg/mL of the methanol extract. The ether extract showed 52% inhibition at 50 µg/mL and 63% inhbition at 100 µg/mL ($p<0.05$) [Akileshwari 2012].

Among the methanol, hydroethanolic (96%, 75%, 50% and 25% v/v) and aqueous extracts investigated the 50% hydroethanolic extract showed the greatest activity, inhibiting 71% of human neutrophil myeloperoxidase after 10 min, and inducing almost 100% inhibition of myeloperoxidase after 15 min of exposure. Of the constituents tested, gentiopicroside showed the highest level of inhibition (IC_{50}=0.8±0.1 µg/mL), followed by amarogentin (IC_{50}=2.4±0.1 µg/mL) [Nastasijevic 2012].

In the DPPH test a 50% hydroethanolic extract showed anti-radical activity with an IC_{50} value of 20.6 µg/mL [Nastasijevic 2012].

In an ex vivo study nine healthy volunteers (three males and six females, age 35-50 years) received a single oral dose of 15 g of a 30% ethanolic extract of *Gentiana lutea*. Sera and peripheral blood mononuclear cells (PBMC) were extracted from blood samples taken before, and 2 h after the consumption of the extract. The PBMC were re-incubated in the serum they were extracted with for another hour and subsequently exposed to X-ray radiation (0 Gy, 6 Gy and 8 Gy). Radioresistance of the PBMC was increased in most subjects when isolated from the serum after Gentian extract consumption [Menkovic 2010].

In vivo experiments

Gastric and choleretic activity
In general, the bitter principles of gentian root stimulate secretion of gastric juice and bile and thereby enhance appetite and digestion [Schmid 1966; Paris 1971].

Gentian tincture administered orally to dogs, using a method which prevented the tincture reaching the stomach, increased secretion of gastric juice by 30% compared to untreated animals [Meier 2007].

Gentian root extract (equivalent in bitterness value to 200 Ph. Helv. VI units/g), administered intragastrically to anaesthetized rats, increased gastric secretion dose-dependently (12%, 27% and 37% above controls). Moreover, pH did not change at concentrations of 0.25% and 1% but increased from 4.25 to 4.85 at 4%. A dose of 0.5 mL/kg did not affect the incidence of gastric ulceration in rats with pyloric ligation [Leslie 1978]. In pylorus-ligated rats, duodenal administration of the methanolic extract, as well as the ethylacetate and butanol fractions, suppressed gastric juice secretion and total acid output in a dose-dependent manner, but not the total pepsin output. Administration of the extract and fractions resulted in a dose-dependent protective effect against gastric ulcers induced by acetylsalicylic acid plus pyloric ligation (ID_{50} values: 892, 126 and 137 mg/kg respectively). The protective effects of the ethylacetate fraction at 250 mg/kg and butanol fraction at 500 mg/kg were almost equivalent to 60 mg/kg cimetidine. Oral administration of the fractions as well as of amarogentin, gentiopicroside, amaroswerin and swertiamarin exerted dose-dependent protective effects against gastric ulcers induced by immersion stress (ID_{50} values for amarogentin, amaroswerin and swertiamarin: 5.76, 2.58 and 167 mg/kg respectively).The protective effect of gentiopicroside at 250 mg/kg equivalent to 1000 mg/kg of butanol fraction was 26.5%. Oral administration of the fractions, as well as amarogentin, gentiopicroside, amaroswerin and swertiamarin, showed protective effects against gastric ulcers induced by ethanol. 125 mg/kg of the ethylacetate fraction, 5.0 mg/kg of amarogentin, 5.0 mg/kg of amaroswerin and 20 mg/kg of swertiamarin decreased the ulcer index by 24.2%, 33.7%, 45.4% and 30.8%, respectively [Niiho 2006].

An ethanolic extract was administered intraduodenally to rats at 500 mg/kg b.w. daily, for 2 days prior to and 1 day after i.p. administration of carbon tetrachloride. Bile flow impaired by CCl_4 significantly increased in comparison with untreated control rats treated with CCl_4 only ($p<0.005$) and also in comparison with those which received neither CCl4 nor the extract ($p<0.01$), suggesting that the extract had choleretic activity [Öztürk 1998].

Other effects
Bronchosecretion in rabbits was elevated in comparison with control animals after administration of extract directly to the stomach by gavage for 3 days (at the equivalent of 12.6 mg/kg/day of dried root) [Chibanguza 1984].

A methanolic extract (2:1) was investigated for effects on the central nervous system of mice. Both 250 mg/kg ($p<0.05$) and 500 mg/kg ($p<0.10$) i.p. administration led to a significant increase in the swimming time of mice compared to control. Both doses also significantly increased ($p<0.05$) the latency of tail clip responses compared to control. Whereas latency in the tail immersion test decreased significantly in mice given 250 mg/kg ($p<0.005$) and 500 mg/kg ($p<0.01$) [Öztürk 2002].

Gentiopicroside significantly inhibited the production of TNF in models of hepatic injury in mice after i.p. administration at

30 mg/kg b.w./day ($p<0.05$) or 60 mg/kg b.w./day ($p<0.01$) for 5 days [Kondo 1994].

Gentiopicroside was administrated intragastrically to mice at 50, 100 and 200 mg/kg b.w. twice daily for 3 days. Doses of 100 and 200 mg/kg significantly ($p<0.05$ and $p<0.01$ respectively) reduced mechanical allodynia caused by complete Freund's Adjuvant (CFA)-induced inflammation in the left hind-paw. Further investigations (including recordings at the anterior cingulate cortex (ACC), confirmed that NMDA NR2B receptors mediated this gentiopicroside inhibition of peripheral inflammation, responding in a dose-dependent manner, Western blot analysis of total ACC homogenates showed that NR2B, but not NR2A, subunit expression was significantly reduced in CFA-injected mice treated with gentiopicroside (100 mg/kg) (182.1±32.5%; $p<0.05$ compared to CFA-injected mice). Moreover, gentiopicroside decreased cAMP levels in the ACC of mice by 20.3±2.8 pmol/mg, 16.5±2.6 pmol/mg, and 14.7±2.1 pmol/mg at doses of 50, 100 and 200 mg/kg respectively ($p<0.05$ compared to CFA-injected mice) or *in vitro* (slice incubation with 10 mM for 4-6 h) acting as an adenyl cyclase inhibitor. Gentiopicroside had no effect on the basal excitatory or inhibitory synaptic transmission [Chen 2008].

Pharmacological studies in humans

Patients with inflammatory conditions of the gastrointestinal tract, ulcerative colitis (n = 8), Crohn's disease (n = 2) and non-specific inflammatory disorders (n = 9), who had elevated secretory immunoglobulin (sIgA) levels (20-200 mg/dL) in their saliva, were treated with gentian root tincture (3 × 20 drops/day) for 8 days. A group of 8 healthy individuals (sIgA levels of 3-25 mg/dL) was treated in the same way. Apart from two patients in whom sIgA increased, sIgA levels steadily declined in both groups [Zimmermann 1986].

Secretion of gastric juice in 10 healthy individuals was stimulated after one oral dose of alcoholic gentian root extract equivalent to 0.2 g of dried root. In the same experiment emptying of the gall bladder, observed using X-ray contrast, was increased and prolonged; this was interpreted as a cholagogic effect [Glatzel 1967].

Clinical studies

In an open study, 205 patients with various dyspeptic symptoms (heartburn, vomiting, stomach aches, nausea, loss of appetite, constipation, flatulence) were treated with a dry hydroethanolic extract (5:1) at a dosage of 240 mg twice or three times daily (average daily dose 576 mg of extract, equivalent to 2.9 g of dried root) for about 15 days. Improvements in symptoms were evident after 5 days in most cases and by the end of the study the average level of improvement was 68%. The efficacy of the preparation was assessed by the doctors as excellent (symptoms eliminated) in 31% of patients, good in 55%, moderate in 9% and inadequate in 5% of cases [Wegener 1998].

Pharmacokinetic properties

Metabolic conversion of gentiopicroside was demonstrated *in vitro*, using human intestinal bacteria. Gentiopicroside anaerobically incubated with *Veillonella parvula* ssp. *parvula*, produced five metabolites which were identified as erythrocentaurin, gentiopicral, 5-hydroxymethylisochroman-1-one, 5-hydroxymethyl-isochromen-1-one and *trans*-5,6-dihydro-5-hydroxymethyl-6-methyl-1*H*,3*H*-pyrano[3,4-c] pyran-1-one [El-Sedawy 1989].

The pharmacokinetics and bioavailability of gentiopicroside from gentian decoctions and gentiopicroside alone were compared after oral administration to rats. The doses were adjusted to deliver equivalent amounts of gentiopicroside (150 mg/kg b.w.). The bioavailability of gentiopicroside from the decoction was markedly higher than gentiopicroside alone (AUC of 70.0±13.9 µg h/mL compared to 32.7±12.9 µg h/mL). Changes in plasma levels of gentiopicroside following oral administration of gentiopicroside alone or from the decoction showed elimination half times ($T_{(1/2)Ke}$) of 3.35 ± 0.76 h and 6.21 ± 3.07 h, respectively. The mean maximum concentrations of gentiopicroside at 0.75-1.6 h after the administration of gentiopicroside or decoction were 5.78 and 10.53 µg/mL respectively. The relative bioavailability of gentiopicroside from the decoction was 214% in comparison to gentiopicroside alone [Wang 2007].

After 60 min reperfusion following liver ischaemia, rats received a single i.v. dose of gentiopicroside (5 mg/kg b.w.) to investigate the influence of liver ischemia/reperfusion on the pharmacokinetics of gentiopicroside. The control group without liver ischemia received the same dose of gentiopicroside. Compared to control, a significant increase ($p<0.05$) of the AUC was found (564.7±95.1 min µg/mL and 801.2±87.4 min µg/mL, control and ischemia/reperfusion group respectively). However, the clearance (Cl) was significantly ($p<0.05$) decreased in the liver of ischemia/reperfusion group. Furthermore, to determine hepatic CYP enzyme involvement in the metabolism of gentiopicroside in rats, the rats were pretreated with a non-specific hepatic CYP inhibitor (SKF-525A). Gentiopicroside levels were increased and the clearance was decreased in the group pretreated with SKF-525A compared to the group of gentiopicroside alone (5 mg/kg i.v.), AUC was found as 564.7±95.1 min µg/mL in the group of gentiopicroside alone and 1148.6±254.2 min µg/mL ($p<0.05$) in the group pretreated with SKF-525A. The rats were given a single dose of drug (5 mg/kg i.v. and 100mg/kg p.o.) to determine the bioavailability of gentiopicroside. The average AUC values were 564.7 and 1162.9 min µg/mL in the i.v. and the oral groups respectively. Based on the equation of oral bioavailability, bioavailability of gentiopicroside was found to be approximately 10.3% [Chang-Lio 2012].

Preclinical safety data

Acute toxicity

In mice, an acute oral LD_{50} of 25 mL/kg was determined for two gentian extracts (30% or 37% ethanol, bitterness value of 200 Ph. Helv. units/g) [Leslie 1979].

Repeated dose toxicity

Rabbits treated with 12.6 mg/day of gentian extract for 3 days did not exhibit symptoms of toxicity or abnormal clinical serum parameters with the exception of a slightly reduced number of erythrocytes in the treatment group compared to a control group [Chibanguza 1984].

Subchronic toxicity

No treatment-related adverse effects were observed in rats treated orally for 13 weeks with 1.6 mL/kg of a combination product containing alcoholic extracts of gentian root, chamomile and liquorice. No effect was observed on reproduction, fertility or mating performance in female rats, and no teratogenic effect was observed in rabbits [Leslie 1979].

Mutagenicity

Gentian root extracts and isolated minor constituents showed weak mutagenicity in the Ames test with *Salmonella typhimurium* strains TA 97, 98, 100 and 2637 activated with S9 enzyme mixture [Morimoto 1983; Göggelmann 1986; Matsushima 1985; Meier 2007]. Mutagenic activity of the extract was attributed to low levels of the xanthones gentisin and isogentisin. Isogentisin and other xanthones in gentian root have a striking structural resemblance to quercetin, a well known mutagen in food, which

is reported to be non-carcinogenic and considered to be safe [Fintelmann 1988; Bertram 1989; Meier 2007].

Clinical safety data
From a total of 205 patients who received on average 576 mg of a dry hydroethanolic extract (equivalent to 2.9 g of dried gentian root), for 15 days, only 5 (2.4%) reported mild adverse effects such as flatulence, stomach cramp, nausea or headache [Wegener 1998].

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MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Second Edition, 2003
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Second Edition, 2003
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Second Edition, 2003
RATANHIAE RADIX	Rhatany Root	Supplement 2009
RHAMNI PURSHIANI CORTEX	Cascara	Second Edition, 2003
RHEI RADIX	Rhubarb	Second Edition, 2003
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Second Edition, 2003
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Second Edition, 2003
SALICIS CORTEX	Willow Bark	Second Edition, 2003
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Second Edition, 2003
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Second Edition, 2003
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VIOLAE HERBA CUM FLORE	Wild Pansy	Supplement 2009
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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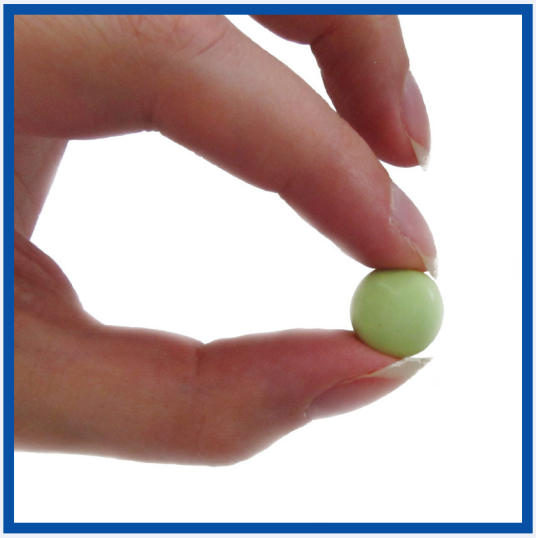
The Scientific Foundation for Herbal Medicinal Products

Ginseng radix

Ginseng

2023



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MONOGRAPHS

***The* Scientific Foundation for**
Herbal Medicinal Products

GINSENG RADIX
Ginseng

2023

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FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

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Abbreviations
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Information on the member organizations and people involved in the production of ESCOP monographs and other activities can be found on the website (www.escop.com):

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ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

GINSENG RADIX

2023

Ginseng

DEFINITION

Whole or cut dried root, designated white ginseng; treated with steam and then dried, designated red ginseng, of *Panax ginseng* C. A. Mey. It contains not less than 0.40 per cent for the sum of ginsenosides Rg1 ($C_{42}H_{72}O_{14}.2H_2O$; Mr 837) and Rb1 ($C_{54}H_{92}O_{23}.3H_2O$; M_r 1163) (dried drug).

The material complies with the monograph of the European Pharmacopoeia [Ginseng].

CONSTITUENTS

Triterpene saponins, mainly tetracyclic dammarane-type. Examples of protopanaxadiol saponins are: ginsenosides Ra1, Ra2 and Ra3; ginsenosides Rb1, Rb2 and Rb3; ginsenosides Rc and Rd; and malonyl-ginsenosides Rb1, Rb2, Rc and Rd. Examples of protopanaxatriol saponins are: ginsenosides Re and Rf; 20-gluco-ginsenoside Rf; ginsenosides Rg1, Rg2 and Rh1) and pentacyclic oleanane-type (ginsenoside Ro). The ginsenosides considered the most important are ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf, Rg1 and Rg2, with Rb1, Rb2, Re and Rg1 being the most abundant. The total ginsenoside content of a 6-year-old main root varies between 0.7 and 3%. The lateral roots can contain two to three times more saponins than the main root while the slender roots can contain up to 10 times more [Soldati 1979; Sticher 1979; Shibata 1985; Sprecher 1987; Tang 1992; Schweins 2018; Bruneton 1995; Cui 1995; Corthout 1999; Ma 2005; Richter 2005; Bauer 2016; Chung 2017; Ali 2017; Xiong 2017; Li 2019].

Other constituents include peptidoglycans called panaxans, acetylenic compounds such as panaxynol, pyrazine derivatives such as 3-*sec*-butyl-2-methoxy-5-pyrazine, oligo- and polysaccharides, phenolic compounds such as vanillic acid and salicylates, and traces of essential oil containing sesquiterpenes such as eremophilene [Sprecher 1987; Tang 1992; Cho 2015; Lee SM 2015].

In general, for white ginseng the protopanaxadiol type ginsenosides (Rb2, Rb3, Rc, Rd, Re1, F2, Ro) as well as protopanaxatriol type ginsenosides (Re, Rf, Rg1, Noto-R1) are relatively more abundant. Malonyl ginsenosides mRa1/Ra2, mRa3, mRb1, mRb2, mRb3, mRc, mRd, mRe and mRg1 are considered to be characteristic components that are decreased by processing. Conversely, ginsenosides (20S- and 20R-) Rg3, Rh1, and Rh2 are considered to be characteristic components of red ginseng. Higher levels of protopanaxadiol type ginsenosides (Ra1, Ra2 and Ra3, Rb1) and protopanaxatriol type ginsenosides (Rg4) are also found in red ginseng [Shibata 2001, Wu 2015, Zhang 2012, He 2018].

CLINICAL PARTICULARS

Therapeutic indications

Decreased mental and physical capacities such as weakness, exhaustion, tiredness and loss of concentration, as well as during convalescence [Forgo 1981a, 1983, 1985; Hallstrom 1982; D'Angelo 1986; Van Schepdael 1993; Kim 2006; Schott 2006; Lee N 2016].

Posology and method of administration

Dosage

Adult daily dose: 0.5 g up to a maximum of 2 g of dried root; equivalent preparations [Forgo 1981a; Hallstrom 1982; Van Schepdael 1993; Kim 2006; Schott 2006; Lee N 2016, Schweins 2018].

Method of administration

For oral administration.

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Duration of administration
If symptoms persist or worsen after one month, medical advice should be sought.

Contraindications
None known.

Special warnings and precautions for use
Do not exceed the recommended dose.

Diabetics should consult a physician prior to taking ginseng root [Sotaniemi 1995].

Interaction with other medicaments and other forms of interaction
Ginseng intake may slightly reduce blood glucose levels [Sotaniemi 1995, Buettner 2006]. Interaction of ginseng with warfarin is possible [Janetzki 1997, Dong 2017].

Pregnancy and lactation
In accordance with general medical practice, ginseng should not be used during pregnancy or lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
Rare cases of allergic reactions, hypoglycaemia [Adusumilli 2002; Wiwanitkit 2004; Lee 2006, Kim 2008].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Cell proliferation
Red ginseng extract (0.52 mg/g ginsenoside Rg1 and 4.03 mg/g ginsenoside Rb1) at concentrations of 200 or 300 µg/mL caused significant ($p<0.05$) suppression of PMA-induced cell motility and invasion in SW480 human colon cancer cells. The extract also inhibited the PMA-induced matrix metallopeptidase-2 (MMP-2) and MMP-9 activity, as well as their protein and mRNA expression, in a dose-dependent manner in the SW480 cells [Seo 2011].

An extract (not further specified) facilitated the early differentiation of human embryonic stem cells into mesendoderm lineage cells [Kim 2011].

An aqueous extract (not further specified) of Korean red ginseng exerted a dose-dependent inhibitory effect on the invasion and motility, but not adhesion, of highly metastatic SK-Hep1 cells without causing cytotoxicity. Zymographic analyses revealed downregulating effects on MMP-2, MMP-9 and uPA activities in SK-Hep1 cells. Western blot analyses also showed dose-dependent decreases in MMP-2 and MMP-9 protein expressions, increased levels of tissue inhibitor of matrix metalloproteases 1, tissue inhibitor of matrix metalloproteases 2 and type-1 plasminogen activator inhibitor [Ho 2012].

Cytotoxicity of a ginseng extract (not further specified) was measured by MTT assay after exposure of MDA-MB-231, MCF-10A and MCF-7 breast cancer cells to concentrations of 0.25, 0.5, 1, 1.5, 2 and 2.5 mg/well. The treatment resulted in inhibition of cell proliferation in a dose- and time-dependent manner. The p53, p21WAF1 and p16INK4A expression levels were up-regulated in ginseng-treated MDA-MB-231 and MCF-7 cancer cells compared to untreated controls and in MCF-10A cells [Al Shabanah 2016].

An extract (not further specified) activated caspase-3 in human renal carcinoma cell lines and promoted apoptosis by cleaving poly(ADP-ribose) polymerase which abolished the expression of B-cell lymphoma 2, B-cell lymphoma-extra large, survivin, inhibitors of apoptosis proteins-1/2, cyclooxygenase-2, cyclin D1, MMP-9, and vascular endothelial growth factor as well as upregulating pro-apoptotic gene products [Kim C 2017].

The effects of an ethanolic extract (70%, not further specified) on cell proliferation, apoptosis and hair growth were observed using cultured outer root sheath (ORS) keratinocytes and human hair follicles with or without Dickkopf-1 application. The extract significantly ($p<0.05$) stimulated proliferation and inhibited apoptosis in ORS keratinocytes. The extract antagonized the effect of Dickkopf-1-induced catagen-like changes of hair follicles [Lee Y 2017].

The major metabolite of Rb1, 20-O-β-D-glucopyranosyl-20(S)-protopanaxadiol (GPD), was tested using SK-MEL-28 cells. Cells were treated with or without 20 µM, 40 µM, 60 µM of GPD or 60 µM of Rb1. GPD significantly inhibited cell growth at 40 µM ($p<0.0001$) and 60 µM ($p<0.001$). Incubation with 40 µM GPD induced the autophagic markers LC3-II, p62/sQSTM1 and Beclin-1. This dose also significantly ($p<0.005$) induced apoptosis, increased DNA fragmentation and caused higher expression of the cleaved forms of PARP, caspase-3, caspase-7 and caspase-9 [Kang 2014].

Cultured human dermal fibroblasts were treated with 0, 1, 10 and 100 ng/mL and 1 and 10 µg/mL of ginsenoside Rb1. At 10 ng/mL to 1 µg/mL cell proliferation increased significantly ($p<0.05$). Collagen synthesis was significantly ($p<0.05$) augmented at 1 ng/mL to 1 µg/mL [Lee GY 2016].

Effects on angiogenesis
In human umbilical vein endothelial cells (HUVECs), ACE activity was reduced after 10 min incubation with an aqueous extract (4% ginsenosides) at concentrations of 5.0 and 10.0 mg/mL. This effect was additive with the ACE inhibitor enalaprilat. No effect was seen on NO production. Angiotensin I-induced contraction of bovine mesentheric arteries was attenuated by 0.1 and 0.5 mg/mL of the extract, while no endothelium-dependent or -independent relaxation was seen [Persson 2006].

An ethanolic extract (yield 4.76%; 82.65% ginsenosides) stimulated tube formation at 10 µg/mL in HUVECs, while higher concentrations increased tube formation in a dose-dependent manner. Treatment of HUVECs with the extract resulted in dose- and time-dependent increases in ERK1/2 phosphorylation as well as time-dependent increases in Akt and eNOS phosphorylation. NO production was increased in HUVECs treated with the extract when compared with control, an effect abolished by the addition of N(G)-monomethyl-L-arginine. The extract-mediated increase in ERK phosphorylation was abolished by treatment with wortmannin (phosphatidylinositol 3-kinase inhibitor) and PD98059 (MEK inhibitor) but was only partially suppressed by the addition of N(G)-monomethyl-L-arginine [Kim 2007].

Neuroprotection
Treatment with 500 µg/mL of an aqueous extract (not further specified) protected neuronal cells from apoptosis mediated by PCB 52, attenuated lipid peroxidation, generation of ROS and DNA fragmentation, and reduced the PCB 52-induced

proteolytic cleavage of β-catenin and poly(ADP-Ribose)-polymerase [Lee JY 2004].

An aqueous extract (not further specified) increased cell viability of primary rat astrocytes in a stress model generated by H_2O_2. Exposure of astrocytes to H_2O_2 decreased the activities of CAT, SOD, glutathione peroxidases (GPx) and glutathione reductase (GR), and increased ROS formation. The extract reversed almost all of these effects in the H_2O_2-injured primary cultures [Naval 2007].

An extract (4.37% ginsenosides) inhibited kainic acid-induced excitotoxicity in a dose-dependent manner. The extract also inhibited the intracellular ROS and $[Ca^{2+}]i$ elevation in hippocampal cells in a concentration-dependent manner [Han 2012].

An aqueous extract (not further specified) of Korean red ginseng inhibited neuronal damage and generation of intracellular ROS induced by excitatory amino acids, such as glutamate and N-methyl-D-aspartate (NMDA), or by Aβ (25-35) in primary cultured rat cortical cells. The extract increased phosphorylation of Bad at Ser 112 and inhibited Bax expression and caspase 3 activity. It also inhibited DNA fragmentation induced by NMDA or Aβ (25-35) and β-secretase activity [Kim 2014].

A fraction from an 80% ethanolic extract at 100 µg/mL prevented serum deprivation-induced PC12 cell apoptosis through the PKA/CREB pathway [Lin 2009].

Activation of biosynthesis
A standardized extract (4% ginsenosides) stimulated D-glucose transport in Ehrlich ascites tumour cells and D-glucose uptake in rabbit cerebral cortical tissue [Hassan Samira 1985; Yamasaki 1993].

After addition of a 70% methanolic extract (yield 37-43%) to rat primary hepatocytes, upregulation of CYP7A1 mRNA and protein levels was observed [Kawase 2013]. The extract at 100 and 500 µg/mL resulted in upregulation of CYP8B1 and MRP2 mRNA. Ginsenoside Re enhanced the mRNA level of CYP8B1, whereas ginsenosides Rb2 and Rg2 enhanced MRP2 mRNA levels [Kawase 2014].

Immunomodulation
The mitogenic activity and anticomplement activity of a standardized extract (4% ginsenosides) and several fractions were tested in mice spleen cell cultures. The extract possessed anticomplement and mitogenic activities while the strongest anticomplement activity was observed with a crude polysaccharide fraction consisting of arabinose, galactose and glucose with small amounts of galacturonic acid, glucuronic acid and rhamnose; its molecular weight was estimated to be 3.68×10^5 kD [Yamada 1995].

Peripheral blood mononuclear cells from healthy volunteers (n=20) or from patients with chronic fatigue syndrome (n=20) or AIDS (n=20) were tested, in the presence or absence of varying concentrations of an extract (not further specified), for natural killer cell activity against K562 cells and for antibody-dependent cellular cytotoxicity against human herpes virus 6-infected H9 cells. The extract at concentrations of 1, 10 and 100 µg/mL significantly ($p<0.05$ to $p<0.001$) enhanced the cellular immune function of peripheral blood mononuclear cells from all groups [See 1997].

An extract (not further specified; 1-62 µg/mL) demonstrated a considerable increase in IL-12 production in human serum, whereas IL-10 production was unchanged for all concentrations. An IL-12 peak was observed at around 4 µg/mL, giving increases of 6- to 146-fold. Concentrations above 1 µg/mL resulted in higher IL-12 production [Larsen 2004].

The phagocytic capacity of polymorphonuclear cells and monocytes, but not peripheral blood mononuclear cells, was enhanced by an extract standardized to ginsenosides [Kang 2008].

In a cell proliferation assay, an extract (not further specified) enhanced the proliferation of RAW 264.7 macrophages and protected against cell regression in macrophages treated with methotrexate. The extract induced the production of NO and the expression of inducible NOS and COX-2 mRNA. Furthermore, it enhanced the production of cytokines including IL-1α, IL-1β, IL-6, TNF-α and granulocyte-macrophage colony-stimulating factor as well as chemokines such as macrophage chemotactic protein-1. No changes were seen in normal T-cells, regardless of methotrexate co-administration [Jang 2010].

An extract (not further specified) partially protected human epithelial cells from respiratory syncytial virus (RSV)-induced cell death and viral replication. It also inhibited the production of RSV-induced pro-inflammatory cytokine (TNF-α) in murine dendritic and macrophage-like cells [Lee JS 2015].

An aqueous extract (not further specified) increased IgA production by LPS-stimulated B cells. Ginsenoside Rg1 and 20(S)-Rg3 induced IgA production and expression of GLTα transcripts by LPS-stimulated B cells [Park 2015].

Ex vivo experiments

Antioxidant properties
In experiments with perfused rabbit lungs, an extract (not further specified) inhibited vasoconstriction induced by the thromboxane analogue U46619 or by acetylcholine after exposure to free radicals generated by electrolysis. The extract was superior to pure ginsenosides [Rimar 1996; Gillis 1997].

Two groups of 5 rats were given an extract (not further specified) at 10 mg/mL in drinking water (corresponding to 1.6 g/kg/day) for one week, then one group was exposed to hyperbaric 100% oxygen (HBO) for 6 hours. Control groups received water only and water only with exposure to HBO, respectively. Isolated perfused hearts from the rats were subjected to mild ischaemia and then reperfused. The extract prevented myocardial ischaemia/reperfusion damage and the impairment of endothelial functionality induced by ROS arising from HBO exposure [Maffei Facino 1999].

Immunomodulation
After oral administration of a standardized extract (not further specified) to mice at 10 mg/day for 4 consecutive days, the effects on immune response were investigated. The extract enhanced the antibody plaque forming cell response and circulating antibody titre against sheep erythrocytes [Singh 1984].

The above finding was confirmed by experiments involving oral administration to mice of an aqueous extract with a defined ginsenoside content (0.58% ginsenosides). Administration at 10, 50 or 250 mg/kg b.w. daily for 5-6 days resulted in enhanced immune responses in a battery of six *ex vivo* tests which included primary and secondary immune response against sheep red cells, natural killing activity, mitogen-induced proliferation, interferon production and T-cell mediated cytotoxicity [Jie 1984].

Other effects
An extract (not further specified; 1-40 mg/mL) showed a dose-

dependent relaxation of isolated corpus cavernosum tissues of New Zealand white rabbits precontracted by phenylephrine [Choi 1998].

An extract (4% ginsenosides) reduced maximal proliferation of rat smooth muscle cells to 24% of control values and antagonized norepinephrine-induced maximal vasoconstriction of rat aorta by 21% and 44% at concentrations of 1.44 mg/mL and 2.88 mg/mL of the extract respectively *in vitro*. In rats treated orally with either 2 mg/kg or 200 mg/kg of the extract for 7 days prior to a carotid artery balloon injury, the neointima-to-lumen area ratio was significantly ($p<0.01$) reduced compared to control [Wu 2001].

The protective effect of an extract (26.1% ginsenosides) against ischaemia/reperfusion injury in isolated guinea pig hearts was evaluated. The animals were pre-treated with 250 or 500 mg/kg of extract p.o. for 14 days prior to excision. Ischaemia was induced in the excised hearts for 60 min, followed by 120 min reperfusion. The extract increased haemodynamic parameters such as aortic flow, coronary flow and cardiac output as well as left ventricular systolic pressure, the maximal rate of contraction and maximal rate of relaxation. Moreover, the extract improved electrocardiographic indices such as the QRS, QT and RR intervals, suppressed LDH, creatine kinase-MB fraction and cardiac troponin I and ameliorated MDA and glutathione [Lim 2013].

In vivo experiments

Hormonal effects
Estradiol valerate was used to induce polycystic ovaries in female Sprague-Dawley rats. Sixty days following induction, the rats were left untreated (control) or administered orally for 60 days with either saline or an extract of Korean ginseng (not further specified; 200 mg/kg b.w). The numbers of corpora lutea and corpora albicantia were higher in the treatment group than in the control group, while the number of cystic follicles was lower. Nerve growth factor (NGF) is considered a factor in the pathogenesis of PCOS: the induced increase in NGF-mRNA was significantly ($p<0.05$) attenuated in the treatment group compared to control. Likewise, the concentration of NGF protein was significantly ($p<0.05$) lower in the treatment group [Jung JH 2011].

Intraperitoneal administration of ginseng saponin fractions to rats stimulated adrenocorticotrophic hormone (ACTH) secretion from the hypothalamus/hypophysis, leading to increased corticosterone synthesis and excretion [Hiai 1979a, 1979b].

Effects on performance
After oral administration of an extract (not further specified), rats were subjected to learning and memory retention tests, and ^{14}C-phenylalanine transport across the blood-brain barrier was also determined. After i.p. injection with the extract, the brain stem and brain cortex were analyzed for concentrations of monoamines and 3',5'-cyclic adenosine monophosphate (AMP), and for the activity of phosphodiesterase and adenylate cyclase. The following results were obtained [Petkov 1978]:

- Improved learning and memory retention (5 of 7 tests) after oral administration at 20 mg/kg b.w. for 3 days, but unchanged or even decreased learning and memory retention (4 of 7 tests) after 100 mg/kg for 3 days.
- Increased ^{14}C-phenylalanine transport across the blood-brain barrier after 30 mg/kg p.o. for 5 days.
- Unchanged phosphodiesterase activity in brain stem and cortex after 50 mg/kg i.p. for 5 days.
- Decreased adenylate cyclase activity (with or without NaF-activation) after 30 mg and 200 mg/kg i.p. for 5 days, except after 30 mg without NaF-activation when adenylate cyclase activity was increased.
- Decreased 3',5'-cyclic AMP concentration in brain stem and cortex after 200 mg/kg i.p. for 5 days.
- Increased dopamine and noradrenaline concentrations in brain stem, whereas the serotonin concentration was decreased in brain stem and increased in brain cortex, after 50 mg/kg i.p. for 5 days [Petkov 1978].

The effects of a hydroalcoholic extract with a standardised level of saponins (not further specified) on resistance to stress and exhaustion were measured using a cold stress test in normal and adrenalectomized rats and a forced swimming test in mice, with tests comparing acute and subacute dosing and oral and i.p. administration. Physiological and biochemical tests (body weight increase, food and water consumption, urine analysis for sodium, potassium and chloride, liver proteins, total cholesterol, total lipids and triglycerides, adrenal total cholesterol, blood and serum glucose, triglyceride and cholesterol values) were also carried out. Significant ($p<0.05$) effects seen in this study were [Bombardelli 1980]:

- Time to exhaustion was significantly prolonged in mice following a single i.p. administration of the extract at doses equivalent to 7.5 and 37.5 mg/kg ginseng total saponins, and in mice administered 37.5 mg/kg/day p.o. for 15 days.
- A single i.p. dose of extract (equivalent to 3.75 mg/kg ginseng total saponins) significantly attenuated the body temperature decline in cold stressed normal rats but not adrenalectomized rats as compared to controls (saline). Hydrocortisone (10 mg/kg i.p.) significantly attenuated the cold stress-induced temperature decline in adrenalectomized rats but not normal rats.
- Body temperature decline under cold stress in rats was significantly attenuated after i.p. administration of the extract (single dose, 3.75 mg/kg ginseng total saponins) compared to controls receiving saline solution. Hydrocortisone (10 mg/kg) did not show this activity. In adrenalectomized rats, the extract did not have cold-protective activity whereas hydrocortisone did.
- In weanling rats, the fresh weight of thymus was dose-dependently reduced after i.p. administration of the extract for 4 days at doses corresponding to 1.5-30 mg/kg of ginseng total saponins.

Liver protein, total cholesterol and total lipids were reduced in male and female rats after 15 days of i.p. administration of the extract at doses corresponding to 3 or 30 mg/kg/day ginseng total saponins (some but not all results were significant, $p<0.05$). Such changes were not seen after oral administration of extract doses corresponding to 1.87 or 37.5 mg/kg/day ginseng total saponins except for a reduction of liver protein in female rats receiving the higher oral dose. Adrenal total cholesterol was markedly reduced only in orally treated animals. Furthermore, body weight was monitored weekly in a prolongation of the same experiment comparing 5 weeks oral administration with 4 weeks i.p. administration. Growth retardation was seen in male rats after both oral and i.p. treatment but was stronger and dose-dependent after i.p. treatment. In female rats weak and non-dose-dependent growth retardation was only seen after i.p. treatment [Bombardelli 1980].

Male Swiss mice (7 weeks old) received, as their only liquid for up to 96 days, either distilled water or an infusion (not further

specified) from ginseng corresponding to 33 mg dry root/mL. The average consumption per mouse corresponded to 274 mg root daily. No significant differences between treatments were seen in weight gain or in the cold swimming test after 35, 46 or 96 days [Lewis 1983].

Pre-treatment of rats with a 1% methanolic extract (not further specified; 20 mg/kg b.w. i.p. twice with a 24-hour interval) led to significant differences in energy metabolism after exercise (swimming for 30 or 60 minutes) as measured by plasma concentrations of relevant metabolites. Glucose was higher ($p<0.01$) and free fatty acids were lower ($p<0.01$) after 30 minutes, whereas pyruvic acid ($p<0.05$) and lactic acid ($p<0.01$) were significantly lower after 60 minutes of exercise as compared to saline-treated controls [Avakian 1984].

The effects of an extract (not further specified) administered i.p. to rabbits were investigated using electrocorticograms. The extract stimulated metabolic activity of cerebral tissue. Incubation of sliced rabbit brain tissue with the same extract (23 and 46 µg/mL) led to highly significant metabolic changes ($p<0.0005$ compared to control values): glucose uptake increased, while lactate and pyruvate production and the lactate/pyruvate ratio decreased [Hassan Samira 1985].

Male Wistar rats received an extract (standardised to 4% ginsenosides) at a dose of 50 mg/kg p.o. or control (saline) for 12 weeks, with or without exercise training. The muscles of the hindlimb were then extracted after 24 hours of inactivity. The activity of citrate synthase (CS) increased with treadmill exercise but not by the extract. There were no significant changes in lactic dehydrogenase levels, from either the exercise or the extract. The extract did increase the capillary density and the mitochondrial content of the red gastrocnemius muscle. No potentiation was seen when exercise and the extract were combined [Ferrando 1999].

An extract (4% ginsenosides) at doses of 3, 10, 100 or 500 mg/kg or saline (as control) were administered orally for three months to male Wistar rats. The rats were then further split into resting or acute exercise groups (n=8/group). Mitochondrial function was significantly ($p<0.05$) reduced after exercise, this effect was attenuated by 100 mg/kg of the extract, demonstrating a membrane stabilizing capacity. Glutathione status did not show significant changes after exercise or treatment. Lipid peroxidation was significantly ($p<0.05$) higher in all muscles after exercise, but was significantly ($p<0.05$) reduced by about 74% by the extract [Voces 2004].

Treatment of male Wistar rats with an extract (4% ginsenosides) at 100 mg/kg/day p.o. for three months protected their muscles from exercise injuries. The extract significantly ($p<0.05$) reduced the eccentric exercise-induced increases in nitrate concentration in vastus and rectus muscles and carbonyl content in all muscles tested as compared to controls (saline only). The extract also preserved mitochondrial membrane integrity [Cabral de Oliveira 2005].

Male ICR mice received 50, 100, 200 or 400 mg/kg of an extract (not further specified) orally for 1, 3 or 7 days. Administration of 100 mg/kg for 3 or 7 days significantly ($p<0.05$) increased locomotor activity and suppressed the production of plasma corticosterone. This dose increased the time spent in the open arms of the elevated plus maze, and significantly increased rearing frequency ($p<0.05$) as well as endurance time ($p<0.01$-0.05), and decreased falling frequency on the rotating rod after 3 or 7 days. A single dose of 200 mg/kg significantly ($p<0.01$) prolonged endurance time. The same dose for 1 or 3 days significantly ($p<0.05$) increased swimming time in a cold pool, whereas at 100 mg/kg, swimming times were increased after 3 or 7 days of treatment [Choi 2011].

Seven week old ICR mice (n=42) received an extract (19.6% ginsenosides; 1 g/kg/day p.o.) during two weeks of endurance training. Mice were randomized to a training only group (control) and a training with extract group. Glycogen was measured in muscles and liver before, immediately after, and one hour after the exercise. Fat oxidation during the initial 20 min of exercise increased in the treatment group compared to control. The glycogen concentration in white and red gastrocnemius muscle did not differ between the groups immediately after the exercise [Hwang 2014].

The effect of a ginseng extract (4% ginsenosides; 25 mg/kg/day p.o.) on exercise stress was examined in 6-week old male BALB/c mice, with attention on the expression of AMPK, PGC-lα and SIRTI genes, as a coordinated system that controls energy intake. Animals were divided into 4 groups (n=10 each): control group, control extract group, untreated swimming group and extract-treated swimming group. Acute exercise stress (single 60 m swim session) increased the expression of AMPK and PGC-1α, while prolonged exercise stress (daily 60 m swim for 2 weeks) decreased their expression in untreated animals. In contrast, the extract demonstrated no effect on gene expression after acute exercise, but after prolonged exercise AMPK and PGC-1α expression were increased when compared to control mice and untreated swim group mice. Expression of SIRT1 was significantly increased in treated animals after prolonged exercise as compared to the control mice but not the untreated swim group mice [Pannacci 2016].

An ethanolic extract (not further specified) at doses of 5 or 25 mg/kg/day p.o. for four weeks increased grip strength and endurance swimming time, decreased levels of serum lactate, ammonia, creatine kinase and blood urea nitrogen, and economized glucose levels after acute exercise in ICR mice. Glycogen in the gastrocnemius muscle was also increased [Ma 2017].

Crude saponins from 7 different types of ginseng were administered orally to rats at 50 mg/kg b.w. 48 hours and 1 hour before a warm water swimming test. Swimming time until drowning, plasma levels of lactic acid, glucose, insulin and glucagon, and liver glycogen were measured in both resting and drowned animals. The only significant difference ($p<0.05$) was an increased plasma level of glucose in drowned rats that had received saponins from one of the *Panax ginseng* samples tested, when compared to placebo and American ginseng. Plasma glucose levels were lower in most of the resting rats that had received saponins compared to those receiving placebo, but the differences were within normal biological variability [Martinez 1984].

Immunomodulatory and anti-tumour activities

Daily subcutaneous administration to thymectomized rats of an extract (not further specified) corresponding to 25 mg ginseng/kg b.w. for 10 days gave significant protection ($p<0.04$ to $p<0.004$) against intratracheal challenge with alginate-embedded *Pseudomonas aeruginosa* [Song 1997].

BALB/c mice injected with CT-26 mouse colon cancer cells received various doses (0, 75, 150 or 300 mg/kg b.w. p.o.) of an extract (0.519 mg/g ginsenoside Rg1 and 4.028 mg/g ginsenoside Rb1) for 3 weeks. Numbers of pulmonary nodules and plasma MMP-2 and MMP-9 activity were significantly ($p<0.05$) decreased [Seo 2011].

In a mouse model, injection with an ethanolic extract (yield 4.76%; 82.65% ginsenosides; 1 mg/mL) resulted in a significantly

($p<0.01$) higher number of newly formed blood vessels compared to control. After treatment of rat aortic rings with the extract, an approximately 13-fold increase in microvessel sprouting at the cut edge was observed as compared with control (0.23±37 vs. 3.31±.68). The angiogenic activity of the extract at 500 mg/mL was comparable to that induced by VEGF at 10 ng/mL [Kim 2007].

Mice were administered with an extract (20.37% saponins) at 25, 50 or 100 mg/kg p.o. for 15 days, and then infected with a lethal *S. pneumoniae strain*. Survival rate, body weight and colonization were determined. Mice pre-treated with the extract at 100 mg/kg had higher survival rates and body weights as well as lower bacterial number and morbidity than controls. The highest dose decreased cytokine levels including TNF-α ($p<0.05$) and IL-1β ($p=0.051$), NO ($p<0.05$) and neutrophil infiltration 48 h post-infection. In pneumococcal infection, extract pre-treatment downregulated toll-like receptor (TLR) 4 and TNF-α expressions in RAW 264.7 macrophage cells and increased cell survival by activating phosphoinositide 3-kinase (PI3K)/AKT signaling [Nguyen 2015].

In mice, intranasal pretreatment with an extract (not further specified) prevented loss of body weight after infection with respiratory syncytial virus (RSV). The extract improved lung viral clearance and enhanced the production of interferon (IFN-γ) in bronchoalveolar lavage cells upon RSV infection. Analysis of cellular phenotypes in bronchoalveolar lavage fluids showed that the treatment increased the populations of CD8+ T cells and CD11c+ dendritic cells upon RSV infection [Lee JS 2015].

Lactating female mice were intramammarily inoculated with an extract (27% Rg1) at 50 mg/mL or placebo, and then challenged with *Staphylococcus aureus*, while another group was inoculated with *S. aureus* alone. The number of bacteria recovered from mammary glands was lower in the extract treated *S. aureus*-infected mice (group I) compared with placebo-treated *S. aureus*-infected mice (group II) and *S. aureus*-infected mice (group III). The mRNA expression of TLR2, TLR4, IL-1α and TNF-α was influenced by the treatment; the transcript levels for all genes were higher in group I compared with groups II and III. Activation of NF-κB and the number of monocytes-macrophages in mammary gland tissue was significantly ($p<0.05$) increased in group I compared with groups II and III [Silvestrini 2017].

Water soluble ginseng oligosaccharides (1, 12.5 or 25 mg/kg p.o. for 10 days) inhibited tumour growth in H22 ascites inoculated ICR male mice with inhibitory ratios of 36.3%, 58.2% and 41.9% respectively. The relative spleen and thymus weights after 12.5 and 25 mg/kg significantly ($p<0.05$) increased compared with control (saline). All doses significantly ($p<0.01$-0.05) increased TNF-α production, splenocyte proliferation and Con-A-/LPS-induced splenocyte proliferation compared with control. The 12.5 and 25 mg/kg doses significantly ($p<0.01$) enhanced NK cell activity in YAC-1 cell lysis at the E:T cells ratio used compared to control. The two higher doses also significantly ($p<0.01$) improved macrophage phagocytosis and promoted NO generation compared to control [Jiao 2014].

Anti-ulcer effects

Oral administration to rats of 50, 200 or 500 mg/kg b.w. of a 70%-methanol extract dose-dependently inhibited gastric ulceration induced by pyloric ligation, serotonin or endotoxin, but did not inhibit stress-induced ulceration. Serotonin- or endotoxin-induced effects on gastric mucosal tissue blood flow were reduced. Most effects observed after doses of 200 mg/kg or 500 mg/kg were significant ($p<0.05$ or $p<0.01$) [Matsuda 1984].

One week after infection with *Helicobacter pylori*, male mongolian gerbils (5 weeks old) were fed with either a standard diet or a diet containing 200 mg of an aqueous ginseng extract (7% saponins) for 6 weeks. The extract had no effect on the amount of viable *H. pylori* in the stomach. However gastric mucosal lesions showed less inflammatory cell infiltration, hyperplasia and intestinal metaplasia than those of the controls. *H. pylori*-induced chronic inflammation was reduced, neutrophil infiltration in the gastric mucosal lesions was inhibited and increases in myeloperoxidase and lipid peroxide were significantly ($p<0.05$) suppressed. mRNA expression of KC, IL-1α, and iNOS was significantly ($p<0.05$) lower in the treatment group [Bae 2014].

Effect on allergic rhinitis

In a study examining the anti-allergic activity of an extract (not further specified) in a mouse model of allergic rhinitis, female Balb/c mice (n=40) were divided into four groups: saline only (G1); ovalbumin (G2; OVA, 25 μg i.p.); OVA+extract (G3; 2 g/kg p.o.) and OVA+dexamethasone (G4). Serum IgE levels were significantly ($p=0.0006$) lower in G3 than in G2. In the nasal lavage fluid, IL-4 and IL-5 levels were significantly ($p<0.05$) lower in G3 than in G2. Eosinophil count, IL-4, IL-5 and Mucin 5AC-positive cells were significantly ($p<0.05$) lower in G3 and G4 than G2 [Jung 2013].

Antidiabetic effects

Sprague Dawley rats (six weeks old) were divided into four groups: control; streptozotocin (STZ)-induced diabetic group; and STZ-induced groups treated with 100 or 250 mg/kg p.o. of an extract (not further specified) for 28 days. The extract prevented renal hypertrophy in a dose-dependent manner. Elevated plasma and urine parameters induced by STZ were significantly ($p<0.05$-0.001) improved by the extract. The gene and protein expression levels of N-(carboxymethyl) lysine and receptors for advanced glycation end products in treated rats were significantly ($p<0.05$-0.001) reduced compared to the STZ only group. Mitogen-activated protein kinases, the phosphorylated forms of Erk and Jun, as well as the gene and protein expression levels of iNOS and COX-2, were significantly ($p<0.05$-0.001) suppressed with the extract [Quan 2013].

Hepatoprotective effects

Intraperitoneal administration of a single dose of an extract (not further specified) to rats at 50 mg/kg b.w. did not protect the animals against CCl_4-induced hepatotoxicity [Kumazawa 1990].

In rats injected with dexamethasone (0.5 mg and 1.0 mg/day) for 7 days (days 1 to 7), the addition of i.p. administration of an extract (corresponding to 100 mg of root/day on days 5 to 9) significantly ($p<0.05$) reduced the dexamethasone-elevated levels of alanine amino transferase (ALAT) and aspartate amino transferase (ASAT) down to normal [Lin 1995].

Liver fibrosis was induced in mice by CCl_4 injection i.p. three times per week for four weeks. After administration of CCl_4 alone or CCl_4 with an extract (not further specified; 30, 100 or 300 mg/kg p.o. 30 min. prior to each CCl_4 injection) the mice were sacrificed on day 28. Concomitant treatment with the extract at all doses significantly ($p<0.01$) decreased the levels of plasma aminotransferases increased by CCl_4 and the extent of the liver fibrosis was significantly ($p<0.01$) reduced. Treatment with 300 mg/kg distinctly inhibited the scores of collagen deposition in the lateral lobes. The extract prevented the elevation of transforming growth factor β1 and plasminogen activator inhibitor mRNA levels in the liver [Ki 2013].

The effects of an extract (5 mg/g ginsenosides Rb1 and Rg1) on obesity and lipid metabolism were examined in castrated C57BL/6J mice. Mice were divided into three groups (n=8 each)

and given for 8 weeks either a low-fat diet (LFD; group 1), a high fat diet (HFD; group 2) or an HFD supplemented with 5% extract (group 3). Compared with the HFD mice, mice in group 3 showed decreased body weight, adipose tissue mass and adipocyte size without effects on food intake. Serum levels of triglycerides and total cholesterol were lowered in group 3 as compared to group 2. The extract also markedly reduced HFD-induced hepatic lipid accumulation. Concomitantly, the extract decreased mRNA expression of adipogenesis-related genes (SREBP-1C, PPARγ, FAS, SCD1, and ACC1) in visceral adipose tissues compared with the HFD alone [Shin 2018].

Neuroprotective effects
Rodents received a standardised ginseng extract at various oral doses and time intervals prior to and/or following exposure to the Parkinsonism-inducing neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (mice) or its toxic metabolite, 1-methyl-4-phenylpyridinium (rats). The extract blocked tyrosine hydroxylase-positive cell loss in the substantia nigra and reduced the appearance of locomotor dysfunction [Van Kampen 2003].

An ethanolic extract (not further specified) administered 0 and 90 min after induction of ischaemia, at an optimal i.p. dose of 200 mg/kg, protected CA1 (hippocampal) neurons against transient forebrain ischaemia as demonstrated by the density of neuronal cells in the four-vessel occlusion rat model. The extract also decreased the level of MDA and increased the expression of GPx and SOD [Kim 2009].

In a study investigating the neuroprotective activity of red ginseng extract against kainic acid-induced excitotoxicity, the elevated malondialdehyde levels following injection with kainic acid (30 mg/kg i.p.) were significantly lowered in male mice pre-treated with an extract (4.37% saponins; 30 and 200 mg/kg i.p.) for 10 days ($p<0.01$ and $p<0.001$ respectively) [Han 2012].

An extract (not further specified; 360 mg/kg i.p.) was administered to mice for 2 weeks before occlusion of the middle cerebral artery for 1 hour, followed by reperfusion for 4 and 24 hours (ischaemia/reperfusion I/R). Pretreatment with the extract resulted in a reduction of oxidized hydroethidine signals in ischaemic areas. At 4 and 24 hours after I/R, the number of 8-hydroxyguanosine- and apoptosis signal-regulating kinase 1 (ASK1)-positive cells decreased in the ischaemic penumbra. The extract efficiently attenuated the protein levels of activated ASK1 in the ischaemic penumbra. DNA fragmentation and the infarct volume were reduced by pretreatment 24 hours after I/R. The extract also resulted in better performance in the rotarod test after I/R [Cheon 2013].

Oral administration of an extract (not further specified; 100 mg/kg/day) reduced dopaminergic cell loss, microgliosis and accumulation of α-synuclein aggregates in a rodent model of Parkinson's disease. The extract fully prevented the reduction of locomotor activity and coordination [Van Kampen 2014].

To explore the role of ginseng on neural degeneration, an advanced glycation end product (AGE) model of experimentally inducing Alzheimers was used. AGEs were injected bilaterally into the CA3 region of brains of adult male Sprague-Dawley rats. Subjects were divided into groups including no treatment, and injections of Bovine serum albumin (BSA), AGE (500 µg) and antibodies against receptors for AGE (RAGE - 50 µg). The following day rats began oral treatment for 30 consecutive days with either an aqueous extract (yield 10.4%, 3.31% specified saponins; 0.25, 0.5 or 1 g/kg) or donepezil hydrochloride (2 mg/kg as a positive control) dissolved in saline, or saline alone. The Morris water maze test and step-down type passive avoidance test were performed to evaluate memory and cognitive abilities. The oxidation indexes in the hippocampus were detected. Immunohistochemistry was conducted to visualize the receptors for advanced glycation end products (RAGEs) and nuclear factor-kappa-light-chain-enhancer of activated B cell (NF-B). The AGEs-induced increase in escape latency in the Morris water maze test was shortened by all doses of ginseng from day 2 to 4 ($p<0.01$ and $p<0.05$). Extract treatment at 1 g/kg and 0.5 g/kg also significantly decreased the number of errors ($p<0.01$ and $p<0.05$) and prolonged the latency ($p<0.05$ and $p<0.01$) in the step-down passive avoidance test. The AGEs-induced alterations of MDA, SOD and glutathione levels in the hippocampus were significantly ($p<0.05$ to 0.01) improved by the extract and comparable with the activity of donepezil and anti-RAGE antibody. The increased expressions of RAGE and NF-κB in the cortex and hippocampus in AGEs-induced Alzheimer´s disease were downregulated by the extract in a dose-dependent manner [Tan 2015].

Mice were given either saline, morphine (10 mg/kg i.p.) or both morphine and an extract (not further specified; 250 and 500 mg/kg p.o., 30 min prior to morphine) daily for 7 days. Rats received the morphine with or without 20(S)-ginsenoside Rg3, 20(S)-ginsenoside Rh or the metabolite compound K, with the substances being directly pumped into the right lateral ventricle of the brain for 7 days. Naloxone hydrochloride (5-10 mg/kg i.p.) was injected 6 h after the final morphine injection or cessation of the morphine infusion to induce morphine withdrawal in the mice and rats respectively. The extract at both doses significantly inhibited the number of naloxone-precipitated jumps ($p<0.01$), reduced conditioned place preference score ($p<0.01$) and restored the level of glutathione ($p<0.05$) in mice compared to control. Ginsenosides Rh2, Rg3 and compound K attenuated to varying degrees the morphine-dependent behavioural patterns such as teeth chattering, grooming, wet-dog shake and escape behaviour in rats. Activated N-methyl-D-aspartate acid receptor subunit 1 and extracellular signal-regulated kinase in the frontal cortex of rats and cultured cortical neurons from mice were downregulated by ginsenosides Rh2 and Rg3, and compound K, to varying degrees [Yayeh 2016].

A study examined the effects of an extract (4% ginsenosides) on ethanol-induced depression in mice. For 8 days, mice were administered single oral doses of either water, amitriptyline or various doses (100, 200, 400 and 800 mg/kg) of the extract one hour prior to i.p. injection with water or ethanol. On day 9, in the forced swimming test, 100, 200 and 800 mg/kg of the extract showed antidepressant effects by a significant decrease of immobility time compared to -water-treated controls ($p<0.001$, $p<0.01$, and $p<0.0001$ respectively) while only the highest dose significantly ($p<0.001$) increased swimming time compared to the water control group. In the ethanol-treated mice, compared to those administered ethanol alone, all doses of the extract significantly ($p<0.01$ to $p<0.0001$) reduced immobility time while only the lowest and highest doses significantly ($p<0.0001$) increased swimming time. In ethanol-treated mice, those also receiving the extract demonstrated significant ($p<0.05$ to $p<0.0001$) increases of brain-derived neurotrophic factor in the hippocampus (200, 400 and 800 mg/kg) and prefrontal cortex (200 and 800 mg/kg) compared to ethanol alone [Boonlert 2017].

Mice were treated with an extract (not further specified; 100 and 500 mg/kg/day p.o.) for 12 days following an intrahippocampal β-amyloid (AβO; 10 µM) injection. The extract reduced memory impairment by inhibiting hippocampal cell death caused by AβO. In addition, it restored the reduced synaptophysin and choline acetyltransferase intensity and the lower levels of ionized calcium-binding adaptor molecule 1 in the hippocampus compared with vehicle-treated controls [Choi 2017].

Ginseng total saponins (GTS) were administered i.p. to rats twice a day for 1 week after an induced traumatic brain injury (TBI). GTS (5-80 mg/kg) improved the recovery of neurological functions, including learning and memory, and reduced cell loss in the hippocampal area. The higher doses were more effective than 5 and 10 mg/kg. GTS treatment (20 mg/kg) increased the expression of Neural Growth Factor, Glial cell line-Derived Neurotrophic Factor and Neural Cell Adhesion Molecule, inhibited the expression of Nogo proteins and tenascin-C, and increased the number of BrdU/nestin-positive neural stem cells in the hippocampal area. It also alleviated the secondary brain injury and ameliorated the neurological functions at doses of 5-80 mg/kg [Hu 2014].

Antiviral effect
Female mice (3-4 weeks old) were fed a diet containing 50 mg/kg of an aqueous extract (19.64 mg/g ginsenosides) for up to 80 days prior to intranasal challenge with a lethal dose of H5N1 virus. The survival rate increased depending on the duration of extract administration. Lung tissue from the extract-fed mice showed signs of mild pneumonia with some lymphocyte infiltration while the tissue from virus-challenged controls exhibited severe interstitial pneumonia with heavy lymphocyte infiltration and some mucus in the bronchioles. The induction of TNF-α and IL-4 was similar between controls and extract-treated mice after virus challenge. Induction of IFN-α and IFN-β was significantly ($p<0.05$) higher in the treatment group [Park EH 2014].

Cardiovascular effects
Male Wistar rats (7 weeks old) received a normal or high cholesterol diet with or without oral administration of a 70% methanolic ginseng extract (yield 37-43%) at a dose of 500 mg/kg b.w. Disruption of the membrane localization of multidrug resistance-associated protein (MRP2) was suppressed by the extract in hypercholesterolaemic rats [Kawase 2014]. In an earlier study the same extract decreased levels of LDL and HDL [Kawase 2013].

To evaluate the cardiac effects of an ethanol extract (4% ginsenosides), Wistar-Albino rats were randomly assigned to control group, acute low-dose group (ALD: 100 mg/kg extract for 1 day), subacute low-dose group (SALD: 100 mg/kg extract for 28 days), acute high-dose group (AHD: 500 mg/kg extract for 1 day) and subacute high-dose group (SAHD: 500 mg/kg extract for 28 days). Mean blood pressures were significantly ($p<0.05$) lower in all extract treated groups compared with control. Among various haemodynamic, biochemical, echocardiographic, genetic and immunohistopathologic parameters, troponin I and myoglobin levels were increased in the SALD, AHD and SAHD groups. Mitral E-wave velocity was reduced in all extract groups. Acidophilic cytoplasm and pyknotic nuclei in myocardial fibres were observed in AHD and SAHD groups. Cu/Zn-SOD1 gene expressions were significantly ($p<0.05$) higher in the extract-treated groups whereas caveolin 1 and VEGF-A gene expressions were not changed [Parlakpinar 2019].

Hypertensive rats given a dried aqueous extract (50 g dried cut root /500 mL water) at either 100 or 200 mg/kg b.w. p.o. for 8 weeks showed significantly ($p<0.05$) reduced systolic blood pressure at 4 and 6 weeks compared to control (untreated hypertensive and normotensive rats) [Moon 2019].

Other effects
Adult male Sprague-Dawley rats were administered i.p. methanolic (80%) extracts of wild ginseng (50, 100 or 200 mg/kg; yield 11.6% w/w) or cultivated ginseng (500 mg/kg; yield 20.6% w/w), 30 min. before twice daily morphine injection (40 mg/kg, s.c.) for 5 days. Only the 200 mg/kg dose of extract significantly ($p<0.05$) attenuated the anxiety and depression-like behavioural responses due to morphine withdrawal 72 h after the last morphine injection as compared to the morphine only control group [Lee B 2011].

The behavioural despair model and chronic unpredictable mild stress (CUMS) model were used in mice and rats respectively. Forced swimming test (FST), tail suspension test (TST) and locomotor activity were performed in mice, while the open-field test, food consumption and sucrose preference were assessed in rats. Intragastric administration of compound K, an intestinal metabolite of panaxadiol ginsenosides, at 10 or 30 mg/kg for 14 days significantly shortened the immobility time in FST ($p<0.01$) and TST ($p<0.05$). For CUMS rats, compound K alleviated the depressant-like behaviours, including decreased food consumption, spontaneous locomotor activity and lower sucrose preference, while WAY-100635, a 5-HT1A receptor antagonist, attenuated this effect. In addition, compound K increased the levels of 5-HT, DA and their metabolites in the prefrontal cortex and hippocampus of CUMS rats and reversed overexpression of MAO-B in the prefrontal cortex and hippocampus. Compound K also increased the total glutathione (GSH) and GPx activity in the hippocampus and prefrontal cortex. Expression of brain derived neurotrophic factor and nerve growth factor was increased in treated rats [Song W 2018].

Pharmacological studies in humans

Effects on exercise performance
Twenty top class male athletes received 200 mg of a standardized extract daily for 9 weeks. In a bicycle ergometer exercise lasting 8 minutes, post-treatment values were higher for maximal oxygen absorption and lower for blood lactate and heart rate during exercise compared to pretreatment values. The differences were significant ($p<0.001$) [Forgo 1981a].

In a double-blind study, athletes were given either 200 mg of ginseng extract standardized to 7% ginsenosides (n=10) or 200 mg of ginseng extract standardized to 4% ginsenosides + 400 mg of vitamin E (n=10) or placebo (n=10) daily for 9 weeks. In the same bicycle ergometer test, significant differences were observed in favour of both ginseng preparations compared to placebo with respect to heart rate ($p<0.05$), blood lactate ($p<0.01$) and maximal oxygen absorption ($p<0.01$) after exercise. Differences between the two ginseng preparations were not significant. Levels of testosterone and luteinising hormone in plasma and of free cortisol in urine were unchanged after all treatments [Forgo 1981a, 1983].

A double-blind, placebo-controlled study involving 28 trained male athletes examined the persistence of effects of a 9-week treatment with 200 mg of ginseng extract (4% ginsenosides) or placebo beyond the treatment period. Compared to placebo, the extract produced significant improvements in maximal oxygen uptake during exercise ($p<0.01$), heart rate at maximal exercise ($p<0.001$), forced expiratory volume ($p<0.01$), forced vital lung capacity ($p<0.05$) and visual reaction time ($p<0.01$). These positive effects lasted for at least 3 weeks after treatment [Forgo 1985].

In a study of the blood oxygenation status of 8 male and 2 female middle-aged subjects (average age 50 years), a significant ($p<0.05$) increase in resting arterial pO_2 was observed after 4 weeks of daily oral treatment with 200 mg of a standardized extract; the resting arterial pO_2 increased by 4.5 mm Hg. In synergy with oxygen treatment the increase was 10.1 mm Hg. Venous pO_2 decreased (4.3 mm Hg) [Von Ardenne 1987].

In a double-blind, placebo-controlled, crossover study, 43 top triathletes received either 200 mg of a standardized extract or placebo daily for periods of 10 weeks. Significant differences ($p<0.05$) in various endurance parameters were seen only after the second treatment phase [Van Schepdael 1993].

The effects of a standardized extract on psychological mood states and perceptual response to submaximal and maximal exercise stress were evaluated in 19 young adult females who received either 200 mg of the extract (n=10) or placebo (n=9) daily. The results did not show an enhancement in psychological function characteristics either at rest or during exercise stress [Smith 1995].

The effects of a standardized extract (300 mg/day) were compared to placebo in 41 healthy male students (aged 19-26 years), either untrained or undergoing regular bicycle ergometer training, in an 8-week randomized, double-blind study. Administration of the extract produced significant ($p<0.05$) effects comparable to training on VO2 max and various aerobic fitness parameters, as well as on anaerobic power and leg muscle strength, when compared to the untrained placebo group. However, no clear synergistic effect on these fitness variables was noted when administration of the extract was combined with the exercise training [Cherdrungsi 1995].

In a double-blind, placebo-controlled, crossover study in 8 healthy volunteers (mean age 25 years) regularly practising physical activities, 30 days of oral treatment with 400 mg of a standardized extract did not improve performance during supramaximal exercise, nor did it influence blood lactate or blood testosterone levels [Collomp 1996].

In a randomized, double-blind, placebo-controlled study, healthy female volunteers received 200 mg of a standardised extract (equivalent to 1000 mg ginseng root; n=10) or placebo (n=9) daily for 8 weeks. Assessment before and after treatment with a standard, graded maximal exercise protocol found the extract had no effect on maximal work performance or on resting, exercise and recovery oxygen uptake, respiratory exchange ratio, minute ventilation, heart rate or blood lactate [Engels 1996].

In another randomized, double-blind, placebo-controlled study, healthy male volunteers received either a standardised extract at 200 mg (equivalent to 1000 mg ginseng root; n=11) or 400 mg (equivalent to 2000 mg ginseng root; n=10) or placebo (n=10), daily for 8 weeks. Assessment before and after treatment with a standard, graded maximal exercise protocol found the extract had no effect on oxygen consumption, respiratory exchange ratio, minute ventilation, blood lactic acid concentration, heart rate or perceived exertion at either dose [Engels 1997].

In a randomized, double-blind, placebo-controlled study, 24 healthy, active women were given either a ginseng extract (4% ginsenosides; 400 mg/day; n=12) or placebo (n=12) for 8 weeks. Before and at the end of the trial, each participant performed an all-out-effort, 30-second leg cycle ergometry test (Wingate protocol) followed by a controlled recovery under constant laboratory conditions. Analysis of variance using pre-test to post-test change scores revealed no significant difference between the ginseng and placebo groups for peak anaerobic power output, mean anaerobic power output, rate of fatigue and immediate post-exercise recovery heart rates [Engels 2001].

In a randomized, double-blind study, 38 active healthy adults (26-28 years) took either a standardized extract (400 mg/day, equivalent to 2 g root) or placebo for 8 weeks. Pre-treatment, the secretory immunoglobulin A (SigA) secretion rate, SigA:protein ratio and saliva flow rate were significantly ($p<0.05$) lower after the maximal exercise test (consecutive Wingate tests with recovery periods) than beforehand. Both peak and mean mechanical power output significantly ($p<0.05$) declined across consecutive Wingate tests. There were no significant differences between the extract and placebo groups in any of the post-treatment scores, before or after the exercise test, including salivary parameters, exercise performance and heart rate recovery. The study concluded that the extract did not improve physical performance and heart rate recovery of individuals undergoing repeated bouts of exhausting exercise [Engels 2003].

The effect of an extract (8% ginsenosides; 1200 mg/day), in addition to a strength training programme (60 minutes per week), was examined in a randomized, placebo-controlled, double-blind study involving 24 men and 46 women (64.7 ±5.5 years). Subjects were assigned to one of 4 groups and treated for 12 weeks with either: the extract alone, strength training alone, extract & strength training or placebo. The study revealed significant ($p<0.01$) reductions in heart rate and body fat percentage in the extract-treated groups compared to placebo and strength values were also higher after 12 weeks of treatment [Schott 2006].

Sixty physically active men (aged 17-22 years) were randomized to receive either ginseng (3 g/day of powdered root; n=30) or placebo (n=30) for 8 weeks in a double-blind study. Blood lactic acid levels for determination of lactate threshold (LT) were measured during an incremental cycle ergometer test. LT exercise performance and heart rate (HR) responses to exercise were determined at baseline and after 8 weeks of treatment. Substrate oxidation rates during steady state exercise were assessed upon study completion. There were no significant differences between the extract and placebo groups in terms of LT before and after treatment, exercise heart rate, total exercise time, peak power output and oxidation rates of fat or carbohydrate [Kulaputana 2007].

Eighteen male students (aged 19-22 years) were randomly assigned to receive either an extract (n=9) or placebo (n=9). All performed a high-intensity uphill treadmill running task. The extract group received 20 g of Korean red ginseng extract (not further specified, mixed with 200 mL of water) three times a day for 7 days prior to the test and for three days after the test, while the placebo group ingested 200 mL of water infused with Agastachis Herba (10 g/500 ml water) using the same schedule. The exercise induced a significant elevation in plasma creatine kinase (CK) in both groups. However, the CK level was significantly ($p<0.05$) lower in the extract group compared to placebo 72 h post-exercise, and IL-6 levels were significantly ($p<0.05$) decreased at the 2 h and 3 h recovery periods compared to placebo. Plasma glucose and insulin levels were significantly ($p<0.05$) reduced following the extract compared to placebo, at 60 min and 90 min post-exercise respectively [Jung HL 2011].

In a randomised, double-blind, placebo-controlled, crossover study, healthy, active women (38.7±7.8 years; n=10) and men (41.2±9.7 years; n=9) participated in three 14 day treatment cycles consisting of supplementation with a high dose (960 mg/day) of a ginseng preparation (compound K), a low dose (160 mg/day) of the ginseng and placebo. After 14 days of supplementation participants performed an acute resistance exercise trial (5 sets of 12 repetitions of the leg press at 70% of one-repetition-maximum). Ratings of perceived exertion were assessed after each set. Muscle pain/soreness was assessed before exercise and 24 hours post exercise. Psychomotor performance and peak power were measured before exercise, immediately post exercise and 24 hours after exercise. Each treatment cycle was separated by a minimum one-week washout period. The high

dose significantly reduced perceived exertion during exercise compared with the low dose (p=0.004) and placebo (p=0.038). The high dose (p=0.014) and low dose (p=0.011) showed significantly lower muscle soreness at 24 hours post exercise compared to placebo. Analysis of peak power demonstrated the presence of responders (n=13) and non-responders (n=6). Responders showed a significantly (p<0.05) greater effect of the high dose on maintenance of neuromuscular function (peak power) when compared to low dose and placebo. Blood was taken at rest and immediately, 30 min, 60 min and 24 h after exercise. Cortisol, superoxide dismutase, total glutathione, nonspecific antioxidant activity, total antioxidant power and creatine kinase were measured. Ginseng attenuated exercise stress-induced elevated levels of circulating cortisol for up to an hour after exercise at both doses in women and the highest dose in men. Twenty-four hours after exercise, the high dose significantly (p<0.05) reduced muscle damage (reduction in creatinine kinase) compared to the placebo and low dose. There was a significant (p<0.05) increase in various enzymatic and antioxidant activities at the higher dose as compared to placebo and the low dose. The ginseng demonstrated a dose dependent inhibitory effect on the hypothalamus-pituitary-adrenal axis responses to physical stress in humans [Caldwell 2018; Flanagan 2018].

In a 12-week randomized, double-blind, placebo-controlled trial, a ginsenoside complex (10% protopanaxadiol ginsenosides: Rg3, Rg5, Rk1) was evaluated in sedentary healthy adults (n=117: 85 women, 32 men, aged: 22-60) assigned into one of three groups: low-dose ginsenoside (100 mg/d, n=39), high-dose ginsenoside (500 mg/d, n=39) or placebo (n=39). All participants underwent a supervised 12-week aerobic and resistance exercise training course. The effects on physical performance were assessed by maximal oxygen consumption (VO_2 max), anaerobic threshold (AT), lactic acid and muscle strength of the dominant knee at baseline, at every visit and after the training programme. VO_2 max of the high- and low-dose groups significantly (p<0.001 and p=0.005) improved during exercise compared to baseline. There were no definite changes in AT and lactic acid levels over time. After exercise significant differences were seen in the VO_2 max (p=0.029) and AT (p=0.038) between the high-dose ginsenoside and placebo groups. No significant difference in VO_2 max between the low-dose ginsenoside and placebo groups was observed as well as no differences in muscular strength between the three groups [Lee 2018].

Effects on cognition, mental performance and the stress response
In a double-blind, placebo-controlled, crossover study, 12 nurses working night shifts (3-4 consecutive nights followed by 3 days of rest) were given 1.2 g of ginseng (not further specified) or placebo for three consecutive nights of night work and tested on the morning after the third night. Crossover medication was given after an interval of at least 2 weeks. A third test series was carried out during normal daytime working, after no medication and a good night's sleep (GNS). The subjects assessed their mood, physical well-being and degree of lethargy with linear self-rating scales; two psychophysiological performance tests and haematological tests were also carried out. The detrimental effects of night shifts on mood and performance were clearly seen. A constant trend in favour of ginseng compared to placebo was noted. Ginseng ratings were favorable for mood criteria, but less favourable for physical well-being criteria. Ginseng restored blood glucose levels raised by night shift stress to the GNS level [Hallstrom 1982].

Various tests of psychomotor performance were carried out in a group of 16 healthy male volunteers given a standardized ginseng extract (2 × 100 mg daily for 12 weeks) and in a similar group given placebo in a double-blind, randomized study. A favourable effect of ginseng relative to baseline performance was observed in attention (cancellation test), processing (mental arithmetic, logical deduction), integrated sensory-motor function (choice reaction time) and auditory reaction time. End performance of the ginseng group was superior (p<0.05) to placebo only in mental arithmetic. No differences were found in tests of pure motor function, recognition and visual reaction time [D'Angelo 1986].

The effects of 400 mg/day of a standardized extract for 8-9 weeks on a variety of cognitive functions were compared with placebo in a randomized, double-blind study involving 112 healthy volunteers aged more than 40 years. The extract group (n=55) showed a tendency to faster simple reactions and significantly (p=0.02) better abstract thinking than controls (n=57). No significant difference between groups in concentration, memory or subjective experience was observed [Sørensen 1996].

In a double-blind, placebo-controlled, crossover trial, 27 healthy young adults completed a 10 minute "cognitive demand" test battery at baseline followed by intake of either an extract (4% ginsenosides) or placebo and 30 minutes later consumed a drink containing glucose or placebo. A further 30 minutes later they completed the "cognitive demand" battery six times in immediate succession. Depending on their allocation on the particular day, participants received either 0 mg extract/0 mg glucose (placebo); 200 mg extract/0 mg glucose (ginseng); 0 mg extract/25 g glucose (glucose) or 200 mg extract/25 g glucose (ginseng/glucose combination). The test battery comprised a Serial Threes subtraction task (2 min); a Serial Sevens subtraction task (2 min); a Rapid Visual Information Processing task (5 min); and a "mental fatigue" visual analogue scale. Blood glucose levels were measured prior to the day's treatment, and before and after the post-dose completions of the battery. Both the extract and glucose enhanced performance of the mental arithmetic task and ameliorated the increase in subjective feelings of mental fatigue experienced by participants during the later stages of the sustained, cognitively demanding task performance. There was no evidence of a synergistic relationship between the extract and exogenous glucose ingestion on any cognitive outcome measure. The extract caused a reduction in blood glucose levels 1 hour following consumption when ingested without glucose [Reay 2006].

A randomized, double-blind, placebo-controlled, crossover trial was performed in 30 volunteers (mean age 22.87 years) receiving 200 mg extract (4% saponins), 400 mg of extract and placebo for 8 days, in a counter balanced order, with a 6-day wash-out period. Subjective mood and working memory were assessed on days 1 and 8 of each treatment period, at pre-dose, 1, 2.5 and 4 h post-dose. Dose-dependent treatment effects (p<0.05) were observed. The 200 mg dose slowed the decrease in mood at 2.5 and 4 h on day 1 and at 1 and 4 h on day 8, and slowed responding on a mental arithmetic task across day 1 and at 1 and 2.5 h on day 8. The 400 mg dose improved calmness (restricted to 2.5 and 4 h on day 1) and improved mental arithmetic across days 1 and 8 [Reay 2010].

In a randomized, double-blind, placebo-controlled study, 52 healthy volunteers were randomly allocated to receive either enzyme-modified ginseng (2 g/day) or placebo for 4 weeks. Fatigue scores using the Visual Analogue Fatigue Scale (VAFS) and Revised Piper Fatigue Scale (RPFS) were considered as the primary outcome measure. Quality of life scores were investigated using the Short-Form Health Survey (SF-36). The VAFS score decreased more in the treatment group than in the placebo group (p=0.033). There was no difference in the RPFS and SF-36 scores between the two groups [Lee N 2016].

In a single-blind, randomized study involving 65 healthy students (mean age 23±4 years), 35 received ginseng (500 mg/day, not further specified) and 30 placebo. Data were obtained at baseline and after one month. Psychological stress was induced with a psychomotor performance task and visual working memory accuracy testing and assessed using the Perceived Stress Scale (PSS) and by serum malondialdehyde (MDA) levels. There were significant ($p<0.0001$) improvements in reaction times in the psychomotor performance task and in visual working memory accuracy in the ginseng group after treatment compared to baseline; there was no significant difference in the placebo group. The control group showed a significant ($p=0.0004$) increase in serum MDA associated with a significant ($p<0.0001$) increase in the PSS score. In contrast, in the ginseng group a much smaller but still significant ($p=0.02$) increase in the PSS score was associated with a significant ($p<0.0001$) reduction in serum MDA [Al-Kuraishy 2017].

Subjects (mean age 40.4±1.5 years) working in stressful occupations and with high scores on the stress response inventory (≥81) and Beck Depression Inventory (≥10 ≤30), were randomized to receive either red ginseng (2 g/day; n=32) or placebo (n=31) for 6 weeks in a double-blind study. In assessing parameters associated by the authors with adaptogenic activity, all participants underwent a comprehensive psychological evaluation using the Perceived Stress Scale, the Profile of Mood States questionnaire and the Sheehan Disability Scale, cognitive evaluation involving the continuous performance test (CPT), as well as biological evaluation including blood levels of lipids, glucose, catecholamines and inflammatory markers and heart rate variability. After 6 weeks of treatment with ginseng, there were no significant effects on psychological or cognitive function, although there was a non-signficant improvement in the correct response time in the CPT. In the ginseng group however, triglyceride levels were significantly ($p=0.048$) increased but still within the normal range and the level of epinephrine was significantly ($p=0.043$) decreased, with a significant ($p=0.041$) negative correlation between these two effects, this being consistent with one hypothesis for mediating stress [Baek 2019].

Immunumodulatory activity

The effects of ginseng on various immune parameters were studied in a randomized, double-blind study in healthy volunteers of both sexes (18 to 50 years), treated orally with either 2 × 100 mg of a standardized extract (n=20) or 2 × 100 mg of a dry aqueous extract (n=20) or placebo (n=20) daily for 8 weeks. The standardized extract increased the chemotaxis of circulating polymorphonuclear leucocytes ($p<0.05$ at week 4 and $p<0.001$ at week 8), the phagocytosis index and phagocytosis fraction ($p<0.001$ at weeks 4 and 8), total lymphocytes (T3) ($p<0.05$ at week 4 and $p<0.001$ at week 8), the T-helper subset (T4) ($p<0.05$ at week 4 and $p<0.001$ at week 8), the helper/suppressor (T4/T8) ratio ($p<0.05$ at weeks 4 and 8) and enhanced induction of blastogenesis in circulating lymphocytes ($p<0.05$ at weeks 4 and 8 after induction by cocanavalin A and pokeweed mitogen; $p<0.001$ at weeks 4 and 8 after induction by lipopolysaccharide) and natural killer cell activity ($p<0.05$ at week 4 and $p<0.001$ at week 8). The dry aqueous extract increased the chemotaxis of circulating polymorphonuclear leucocytes ($p<0.05$ at weeks 4 and 8), the phagocytosis index and phagocytosis fraction ($p<0.05$ at week 8), T3 ($p<0.05$ at week 4 and $p<0.001$ at week 8), T4 ($p<0.05$ at week 8) and enhanced induction of blastogenesis in circulating lymphocytes ($p<0.05$ at week 8 after induction by cocanavalin A and pokeweed mitogen) and natural killer cell activity ($p<0.05$ at week 8). With placebo, only enhancement of natural killer cell activity was significant ($p<0.05$) after 8 weeks [Soldati 1988; Scaglione 1990].

Healthy volunteers were treated with 100 mg of a standardized ginseng extract (n=114) or placebo (n=113) daily for 12 weeks in a multicentre, randomized, double-blind, placebo-controlled study investigating potential effects of ginseng on resistance to influenza and common cold. All participants received an anti-influenza polyvalent vaccine at week 4. There was a highly significant difference ($p<0.001$) between the extract and placebo groups with regard to the frequency of influenza or colds observed between weeks 4 and 12 (15 cases in the verum group versus 42 cases in the placebo group). Antibody titres at week 8 were also significantly ($p<0.0001$) higher after verum treatment (272 units versus 171 units after placebo) and natural killer cell activity was almost twice as high in the verum group as in the placebo group at weeks 8 and 12 ($p<0.0001$) [Scaglione 1996].

A study in two groups of 10 healthy young males evaluated the effects of 300 mg of a standardized extract daily for 8 weeks in comparison with placebo on peripheral blood leukocytes and lymphocyte subsets. No significant differences were observed [Srisurapanon 1997].

Antioxidant activity

In a randomized, double-blind, placebo-controlled trial, 82 postmenopausal women (aged 45-60 years) received either 3 g/day of red ginseng or placebo for 12 weeks. Antioxidant enzyme activity (superoxide dismutase, glutathione peroxidase) and oxidative stress markers (malondialdehyde, 8-hydroxydeoxyguanosine) were assessed, and the homeostatic model assessment of insulin resistance index was calculated, at baseline and at the end of the trial. A total of 71 women completed the study. Serum superoxide dismutase activity was significantly increased with ginseng compared to baseline ($p<0.001$) and placebo ($p=0.004$). Serum malondialdehyde levels showed a signficant decrease compared to baseline ($p=0.001$), but a non-significant decrease compared with placebo ($p=0.064$). No significant changes were noted in serum glutathione peroxidase and 8-hydroxydeoxyguanosine levels. Ginseng showed no significant effects on fasting glucose, fasting insulin, insulin resistance, inflammatory markers and liver enzymes [Seo 2014].

Clinical Studies

Effects on fatigue and quality of life

In a randomized, double-blind, placebo-controlled trial, 53 cancer patients (15 men, 38 women) received either ginseng (not further specified; 3 g/day; n=32) or placebo (n=21) for 12 weeks. Results from the quality of life questionnaires showed significant improvements in the ginseng group compared to placebo for the General Health Questionnaire-12 total score ($p<0.01$) and the 'psychological domain' scores of the WHO Quality of Life Assessment-Bref (WHOQOL-BREF; $p=0.02$). There was a tendency towards improvement in the WHOQOL-BREF 'physical health' ($p=0.06$) and 'environment' ($p=0.07$) domain scores in the ginseng group when compared with placebo [Kim 2006].

In a randomized, double-blind, placebo-controlled trial, 90 patients (21 men and 69 women; aged 20 to 60 years) with idiopathic chronic fatigue received either an extract (20% ethanol; 1 or 2 g per day) or placebo for 4 weeks. Fatigue severity, was assessed at 0, 2 and 4 weeks using a self-rating numeric scale (NRS) and a visual analogue scale (VAS), with change in fatigue severity considered a primary endpoint. Glutathione reductase (GSH-Rd) activity and serum levels of ROS, MDA and GSH were determined at 0 and 4 weeks. All groups demonstrated a time-dependent decrease in the total NRS score; although the decrease was greater in the extract groups compared to placebo, the difference was not significant. In the

sub-analysis, physical symptoms did not significantly improve but the mental fatigue symptoms did significantly ($p<0.01$) improve in the extract groups compared to placebo. Only the higher dose significantly ($p<0.05$) reduced the VAS score compared to placebo. ROS and MDA levels were significantly ($p<0.05$ to $p<0.01$) lowered in both extract groups compared to placebo. The lower dose significantly ($p<0.05$) increased total serum GSH concentration and GSH-Rd activity compared to placebo [Kim 2013].

In a randomized, double-blind, placebo-controlled study, 60 female multiple sclerosis patients received either 250 mg ginseng (not further specified) or placebo twice daily for 3 months. Outcome was measured by the Modified Fatigue Impact Scale (MFIS) and the Iranian version of the Multiple Sclerosis Quality Of Life Questionnaire (MSQOL-54), and 52 patients (86%) completed the study. Ginseng demonstrated significantly greater improvements compared to placebo with regards to MFIS ($p=0.046$) and MSQOL ($p\leq0.0001$) scores [Etemadifar 2013].

In a randomized, double-blind, placebo-controlled clinical trial, 180 participants (aged 18 to 60 years) with a Traditional Chinese Medicine (TCM) diagnosis of 'deficiency syndrome' were allocated to receive capsules containing either placebo or red ginseng (3.5-4.8 g/100g of total saponins) at 1.8 or 3.6 g per day for 4 weeks; 174 patients completed the study. The primary outcomes related to safety evaluation, assessed using a 'fire-heat symptom scale' to measure changes in "fireness", as well as other safety parameters including adverse event reports, various laboratory tests and electrocardiogram. The secondary outcomes related to efficacy, assessed using a TCM fatigue symptom scale and a self-assessment fatigue scale. After 4 weeks of treatment the placebo and ginseng groups showed a significant ($p<0.05$) decrease in 'fireness' compared to their baseline values, but there were no significant differences found between the groups. Ginseng time- and dose-dependently improved fatigue compared to placebo in the self-assessment scale, with the higher dose demonstrating a signficantly greater effect from week 1 and the lower dose from week 2 ($p<0.05$ to 0.001). Ginseng also significantly ($p<0.05$ to $p<0.01$) improved the total TCM symptom score compared to placebo in a time- and dose-dependent manner. The total effective rate (number of patients exhibiting ≥ 30% total symptom score improvement) was much higher in the low and high dose ginseng groups compared to placebo, at 75%, 84.5% and 30.4% of cases respectively [Zhang 2019].

Effects on cognitive and psychological function
A Cochrane review included 9 randomized, double-blind, placebo-controlled trials. Results of the analysis suggested improvement of some aspects of cognitive function, behavior and quality of life. The evaluation showed a lack of evidence with regard to a cognitive enhancing effect of ginseng in healthy participants and no high-quality evidence about its efficacy in patients with dementia [Geng 2010].

In a double-blind, placebo-controlled study involving 50 ambulant patients suffering from asthenia, depressive syndrome or neurovegetative disorders, the effects of 8 weeks of daily treatment with 200 mg of a standardized extract on performance were evaluated by two psychometric tests and a comprehensive psychological questionnaire (Sandoz Clinical Assessment Geriatric). Significant improvement ($p<0.05$ and $p<0.01$) was seen in most of the parameters [Rosenfeld 1989].

In a 12-week randomized study, 61 patients (24 males and 37 females; aged 50-80 years) with Alzheimer's disease were assigned to receive red ginseng powder capsules at a low-dose (4.5 g/day, n=15) or a high-dose (9 g/day, n=15) or no treatment (control; n=31). The patients at the high dose showed significant improvement on the Alzheimer's Disease Assessment Scale ($p=0.032$) and Clinical Dementia Rating ($p=0.006$) after 12 weeks as compared to control [Heo 2008].

An open study assessed the effects on cognitive function of a standardised ginsenoside complex (high temperature treated ginseng). Alzheimer's patients (n=40; aged 50-90 years) were randomized to one of four treatment groups: 1.5 g/day (n=10), 3 g/day (n=10), 4.5 g/day (n=10) and control (n=10; untreated) for 24 weeks. Patients given 4.5 g/day showed significant ($p<0.05$) improvement on the Mini-Mental State Examination and The Alzheimer's Disease Assessment at 12 and 24 weeks compared to baseline [Heo 2012].

In randomized, double-blind, placebo-controlled clinical trial, 90 subjects with mild cognitive impairment received either 3 g of ginseng powder or placebo for 6 months. The verum group showed significant improvement in visual learning ($p=0.041$) and visual recall ($p=0.040$) after 6 months when compared to placebo [Park 2013].

In an open, 8-week prospective study, 35 female outpatients (aged 18-65 years), in remission from major depression but with some residual symptoms, were given an extract (21.08 mg/g ginsenosides) at an initial dose of 2 g/day and increasing to 3 g/day by week four and continuing at 3 g/day for the following 4 weeks. Symptoms were evaluated using the Depression Residual Symptom Scale, Montgomery-Åsberg Depression Rating Scale (MADRS), the Clinical Global Impressions Scale for Severity (CGI-S) and the Depression and Somatic Symptom Scale (DSSS). Residual symptoms improved from baseline to week four, still being apparent at week eight ($p<0.01$). The mean MADRS score decreased from 6.7 ± 2.7 at baseline to 5.1 ± 3.1 at week four and to 4.4 ± 3.1 at week eight ($p<0.001$). The general severity of symptoms was significantly ($p<0.001$) attenuated on the CGI-S from baseline (16.7 ± 11.1) to week four (13.1 ± 9.6) and week eight (14.1 ± 9.6; $p<0.001$). DSSS scores as reported by patients also significantly ($p<0.01$) improved from 16.7 ± 11.1 at baseline to 13.1 ± 9.6 at week four and 14.1 ± 9.6 at week eight [Jeong 2014].

Effects on respiratory disease
In a controlled single-blind study with a standardized extract (200 mg/day) in 40 patients suffering from chronic bronchitis, the extract significantly ($p<0.001$) improved alveolar macrophage activity compared to baseline [Scaglione 1994].

In a pilot study involving 15 patients with severe chronic respiratory diseases, a standardized extract was administered orally at 200 mg/day for 3 months and respiratory parameters such as vital capacity, expiratory volume and flow, ventilation volume and walking distance were evaluated. The extract improved pulmonary function and oxygenation capacity, which seemed to be the reason for improved walking capacity [Gross 1995].

In a nonblinded, randomised, comparative pilot trial the effects of an extract (4% ginsenosides) on the bacterial count [number of colony forming units (CFU)] in the bronchial systems of 75 patients with acute bacterial attacks of chronic bronchitis were investigated. All patients received 875 mg amoxicillin and 125 mg clavulanic acid twice daily for 9 days. They were then further randomised into two groups, one (n = 37) receiving only the antibacterial treatment, the second (n = 38) also receiving 100 mg of the extract. Beneficial effect of the extract on the reduction of bacterial counts were seen in the bronchial systems of patients. Significant group and time effects were found after analysis of the evolution of bacterial counts. Statistically

significant differences between treatment groups were observed on days 4, 5, 6 and 7 (p = 0.0049, p = 0.0104, p = 0.0175, p = 0.0182, respectively), while a borderline trend (p = 0.0554) was seen on day 8 [Scaglione 2001].

Effect on dry eye syndrome
In a randomized, double-blind, placebo-controlled study involving 49 glaucoma patients treated with antiglaucoma eye drops, the effects of ginseng on dry eye syndrome were evaluated. Patients received either 3 g of extract (16.46 mg/g ginsenosides; n=24) or placebo (n=25) daily for 8 weeks. The extract significantly (p<0.01) improved the tear film stability and total Ocular Surface Disease Index score as compared to placebo [Bae 2015].

Antidiabetic effects
In a systematic review and meta-analysis of randomized controlled trials in diabetic patients and healthy individuals, 16 trials were included. Ginseng significantly reduced fasting blood glucose compared to control (MD = 20.31 mmol/L [95% CI: 20.59 to 20.03], p=0.03) with no significant effect on fasting plasma insulin, glycated haemoglobin or homeostasis model assessment of insulin resistance. Subgroup analyses did show significant reductions in glycated haemoglobin (MD = 0.22% [95%CI: 0.06 to 0.37], p=0.01) in parallel compared to crossover trials [Shishtar 2014a].

In a randomized, double-blind, placebo-controlled, crossover study, 20 patients with type 2 diabetes (mean age 51.5±1.9; mean HbA1c 7.4 ±0.4%) took ginseng (not further specified; 2 x 369 mg capsules three times daily; n=10) or placebo (n=10) for 4 weeks. After treatment with ginseng, a significant (p<0.05) decrease in insulin resistance by 45% was shown when compared to a 12% decrease with placebo [Ma 2008].

In a randomized, double-blind, placebo-controlled study, 15 overweight or obese subjects (aged 46±3 years) with impaired glucose tolerance or newly diagnosed type 2 diabetes were given either an extract (not further specified; 3-8 g/day; n=5), ginsenoside Re (250-500 mg/day; n=5) or placebo (n=5) for 30 days. Neither the extract nor ginsenoside Re improved beta-cell function or insulin sensitivity [Reeds 2011].

In a double-blind, randomized, placebo-controlled study, 72 diabetic patients (older than 18 years) received 1.5, 2 or 3 g/day of a vinegar ginseng extract (yielding high levels of ginsenoside Rg3) or placebo for 8 weeks. The extract significantly (p<0.05) reduced the HbA1c level compared to baseline: by 0.56 ± 0.25% in the 1.5 g group, by 0.31 ± 0.12% in the 2 g group and by 0.29 ± 0.11% in the 3 g group; with the 1.5 g group also exhibiting a significant (p=0.021) decrease compared to placebo. Patients' fasting plasma glucose concentration decreased by 21.40, 14.27 and 6.76 mg/dL for the 1.5, 2 and 3 g doses respectively, and by 2.25 mg/dL for placebo; the lowest extract dose alone demonstrated a significant decrease compared to both baseline and placebo (p<0.05). The percentage of patients whose HbA1c decreased by >0.5% was significantly (p<0.01) greater than placebo in the 1.5 and 2 g groups. Interestingly, improved glucose homeostasis in this study was associated with decreased concentrations of pro-inflammatory cytokines, TNF-α and IL-6, both implicated in the pathogenesis of diabetes. [Yoon 2012].

In a randomized, double-blind, placebo-controlled trial involving 42 patients (aged 20-75 years) with either type 2 diabetes or fasting glucose impairment, one group was given placebo and the other fermented red ginseng (2.7 g/day) for four weeks. A significant reduction in postprandial glucose levels (p=0.008) and increase in postprandial insulin levels (p=0.04) compared to placebo were observed. Fasting blood glucose in the ginseng group was significantly (p=0.04) lower at week 4 compared to baseline but not compared to placebo. Fasting plasma insulin and lipid profiles were not significantly different from placebo [Oh 2014].

In a randomized, double-blind, placebo-controlled trial, 41 individuals (aged 20-70 years) received either 5 g/day of red ginseng (n=21) or placebo (n=20) for 12 weeks. Subjects were either newly diagnosed with type 2 diabetes or had a fasting blood glucose level between 100 to 125 mg/dL. A 2-h glucose tolerance test was taken at baseline and after 12 weeks of treatment. At 30 minutes, significant decreases in serum glucose (-22.24 ± 10.77 mg/dL; p=0.016) and whole blood glucose levels (-17.52 ± 5.22 mg/dL; p=0.002) were found when compared to baseline. There were no significant changes in blood glucose-related indices in the placebo group [Bang 2014].

The antidiabetic effects of a fermented ginseng extract were evaluated in an 8-week, randomized, double-blind, placebo-controlled study. Participants with impaired fasting glucose levels, without any diagnosed disease, received either the extract at 960 mg/day (total saponins 16.18 mg/g; n=12) or placebo (n=11). Impaired fasting glucose and postprandial glucose levels significantly (p<0.05) decreased in the verum group compared to placebo [Park SH 2014].

Effects on erectile dysfunction
A meta-analysis of 7 randomized, clinical trials involving 349 individuals showed a significant effect on erectile dysfunction (p<0.00001). Subgroup analyses (n=135) demonstrated beneficial effects of red ginseng in psychogenic erectile dysfunction [Jang 2008].

In a double-blind, placebo-controlled, crossover study, 45 patients (mean age 54 years) were treated with 900 mg ginseng (not further specified) or placebo three times a day for 8 weeks, with a 2-week washout period between treatments. The mean International Index of Erectile Function (IIEF) scores were significantly (p<0.01) higher after treatment with ginseng when compared to placebo [Hong 2002].

In a randomized, double-blind, placebo-controlled study, 60 patients with mild to moderate erectile dysfunction received either 1 g of red ginseng (not further specified; n=30) or placebo (n=30) 3 times daily. The International Index of Erectile Function (IIEF-5) score was significantly improved after verum as compared to baseline (from 16.4 ± 2.9 to 21.0 ± 6.3, p<0.0001). In contrast, no difference was observed in the placebo group (from 17.0 ± 3.1 to 17.7 ± 5.6, p>0.05). The Global Assessment question found that 20 patients in the ginseng group reported significantly improved erection (p<0.01); with no improvement reported in the placebo group. Scores on questions 2 (rigidity), 3 (penetration), 4 and 5 (maintenance) were significantly (p<0.01) higher for verum compared to placebo after 12 weeks. For the verum, there was a significant improvement in total score (IIEF-5 score) in questions 3 and 5 (p<0.001 and p<0.0001 respectively). The levels of serum testosterone, prolactin and cholesterol were not significantly different between verum and placebo after treatment (p>0.05) [De Andrade 2007].

In an 8-week randomised, placebo-controlled trial, 69 patients with mild to moderate erectile dysfunction received either placebo (n=34) or an extract (not further specified; n=35). Erectile function was significantly (p<0.05) improved in the verum group compared with placebo after 8 weeks of treatment. Sexual desire was significantly (p<0.001) increased in the ginseng group compared to baseline [Ham 2009].

Table 1: Clinical studies with ginseng preparations

First Author Year	Study Design	Type of patient No. of patients (test/control)	Preparation type Daily dose Duration of treatment	Studied parameters	Outcome
METABOLIC CONDITIONS					
Hosseini 2017	R / D-B / P-C	Men and women with type 2 diabetes 48 (24/24)	Standardized ginseng extract 3 x 100 mg 42 days	Effect on chemerin, apelin and glycaemic biomarkers	Improved fasting glucose ($p<0.01$) and fasting insulin ($p<0.05$), no effect on chemerin or apelin.
Hosseini 2016	R / D-B / P-C	12 Men and 28 women with type 2 diabetes 40 (20/20)	Standardized ginseng extract 100 mg 56 days	Blood sugar control and inflammatory markers	Reduced fasting blood glucose, IL-6 ($p<0.05$) and high sensitive C-reactive protein (hsCRP) ($p<0.01$).
Bang 2014	R / D-B / P-C	28 Men and 13 women with type 2 diabetes 41 (21/20)	Korean red ginseng 5 g 84 days	Antidiabetic effect	Significant decrease in serum insulin and C-peptide concentrations ($p<0.01$) at 30 minutes.
Park SH 2014	R / D-B / P-C	Patients with impaired fasting glucose 23 (12/11)	Hydrolyzed ginseng extract 2 x 480 mg 56 days	Antidiabetic effect	Decreased fasting plasma glucose ($p<0.05$) and postprandial glucose ($p<0.05$) at 60 minutes.
Shishtar 2014b	R / D-B / P-C/ crossover	12 Men and 13 women with well-controlled type 2 diabetes 25 (23/2)	Korean white ginseng (dried, powdered whole root) Single dosing on 5 separate occasions, in random order, with 1 g, 3 g or 6 g of ginseng or 3 g wheat bran (administered twice).	Effect on vascular and glycaemic parameters	Beneficial effect on augmentation index (at 3 g; indicator of arterial stiffness) ($p=0.04$). No effect on postprandial, glycaemic or other vascular parameters.
Delui 2013	R / D-B / P-C	Hyperlipidaemic patients 40 (20/20)	Ginseng extract (7 mg of ginsenosides and 2-3% ginsenosides Rb1, Rb2, Rb3, Rc, Rd, Re, Rg1) 500 mg 56 days	Hyperlipidaemia	No significant effects on lipid profile, hsCRP level and pro-oxidant/anti-oxidant balance.
Lee KJ 2013	R / D-B / P-C	Postmenopausal healthy females 93 (49/44)	Fermented red ginseng 2.1 g 14 days	Diabetes-ameliorating effects	Significantly increased levels of dehydroepiandrosterone sulfate ($p<0.05$), growth hormone and estradiol ($p<0.1$). Decreased levels of glycosylated haemoglobin ($p<0.05$), insulin ($p<0.05$), and homeostatic model assessment of insulin resistance ($p<0.1$).
Oh 2014	R / D-B / P-C	Women and men with impaired fasting glucose or type 2 diabetes 42 (21/21)	Fermented red ginseng extract 2.7 g 28 days	Postprandial glucose-lowering effect	Significant reduction in postprandial glucose levels ($p<0.01$) and increase in postprandial insulin level ($p<0.05$). No effect on fasting glucose, insulin, and lipid profiles.

Hosseini 2014	R / D-B / P-C	28 Women and 12 men with type 2 diabetes 40 (20/20)	Standardized ginseng extract 300 mg 56 days	Biomarkers of Systemic Low-Grade inflammation	Reductions in inflammatory makers IL-6 ($p<0.05$) and hsCRP ($p<0.01$).
Cho 2013	R / D-B / P-C	Healthy overweight/ obese participants 68 (34/34)	Korean red ginseng 6 g 84 days	Effect on insulin sensitivity	No improvement in insulin sensitivity.
Kwon 2012	R / D-B / P-C	Obese women 50 (24/26)	Korean red ginseng 3 x 6 g 56 days	Obesity	Reduction in body mass index. Improved quality of life.
Yoon 2012	R / D-B / P-C	Men with type 2 diabetes 72 (64/18)	Vinegar extract 1.5, 2, 3 g 56 dayS	Diabetes type-2	Moderate improvement of HbA1c level ($p<0,05$).
FATIGUE					
Yennurajalingam 2017	R / D-B / P-C	Cancer patients with an average intensity of ≥ 4/10 on the ESAS (scale, 0-10) 112 (56/56)	Standardized ginseng extract 400 mg 28 days	Cancer-related fatigue	Improvement in cancer-related fatigue, not superior to placebo.
Jiang 2017	R / D-B / P-C	18 Women and 42 men with advanced non-small cell lung cancer 60 (34/26)	Fermented red ginseng extract 3 g 60 days	Fatigue in patients with advanced non-small cell lung cancer	Significant improvement of Fatigue Symptom Inventory score ($p<0.01$), Chinese medicine symptoms score ($p<0.01$), psychological status ($p<0.01$), physical conditions ($p<0.01$), quality of life ($p<0.01$); reduced chemotherapy toxicity ($p<0.01$).
CARDIOVASCULAR CONDITIONS					
Barari 2017	R / controlled	Healthy, unathletic women (32)	Ginseng extract 5 mg/kg 42 days	Effect on serum vascular endothelial growth factor and platelet-derived growth factor	Influence on the levels of angiogenic factors (only on platelet-derived growth factor).
Zaheri 2016	R / P-C	Healthy male athletes 24 (12/12)	Standardized ginseng extract 200 mg 30 days	Heart rate, systolic and diastolic blood pressure	Improved blood circulation during exercise, decreased peripheral vascular resistance, increased oxygen delivery to actively contracting muscles.
Cha 2016	R / D-B / P-C	Non-obese, non-diabetic, prehypertensive patients (female 12, male 50) 62 (31/31)	Korean red ginseng 5 g 84 days	Blood pressure lowering effect	Reduced systolic and diastolic blood pressure ($p<0.01$), increased dihydrobiopterin levels ($p<0.05$) and decreased palmitic amide ($p<0.05$) and lysophosphatidylcholines ($p<0.01$).
Rhee 2014	R / D-B / P-C	47 Women and 43 men with systolic blood pressure (120-159) and diastolic (80-99) 90 (60/30)	Ginsenoside protopanaxatriol 100, 300 mg 56 days	Blood pressure lowering effect	Significantly reduced systolic and diastolic blood pressure ($p<0.05$) at 4th and 8th week.
Jovanovski 2014	R / D-B / P-C/ crossover	Healthy women (7) and men (9) 16 (12/4)	Korean red ginseng 3 g 12 days	Endothelial dysfunction	Significantly improved flow-mediated vasodilatation ($p<0.05$). Brachial blood pressure remained unchanged ($p=0.45$).

First Author Year	Study Design	Type of patient No. of patients (test/control)	Preparation type Daily dose Duration of treatment	Studied parameters	Outcome
Kang 2013	R / D-B / P-C	Healthy women (23) and men (17) 40 (20/20)	Korean red ginseng 1.5 g 56 days	Blood flow	Improved blood circulation.
Chen IJ 2012	Prospective study (observation phase and active treatment phase)	25 Women and 15 men with intradialytic hypotension 38 (18 patients BP ≥140/90 mmHg /20 patients BP <140/90 mmHg)	Korean red ginseng 3.5 g per session 56 days	Intradialytic hypotension	Significant reduction in degree of blood pressure drop during hemodialysis ($p<0.05$). More activation of vasoconstrictors (endothelin-1 and angiotensin II) during hemodialysis was found. The postdialytic levels of endothelin-1 and angiotensin II increased significantly ($p<0.01$).
Park 2012	R / D-B / P-C	21 Women and 39 men with metabolic syndrome 60 (29/31)	Korean red ginseng 4.5 g 84 days	Cardiovascular risks in metabolic syndrome	No effect on blood pressure, lipid profile, oxidized LDL, fasting blood glucose or arterial stiffness.
CNS DISORDERS					
Teik 2016	R/ D-B / P-C / crossover	28 women, 20 men 48 (32/16)	Panax ginseng extract 500, 1000 mg 3 days	Cognitive performance	No effect.
Jeong 2015	R / P-C	Healthy women (39) and men (12) 51 (26/25)	Korean red ginseng 56 days	White matter microstructure and cognitive functions	Significantly reduced intrusion errors ($p=0.005$), increased fractional anisotropy ($p=0.04$), improved response inhibition and white matter microstructure integrity in the prefrontal cortex.
Lee KJ 2014	R / D-B/ P-C	Postmenopausal women without hypertension or diabetes 93 (49/44)	Fermented red ginseng (FRG) 2.1 g 14 days	Depression	The effect of FRG consumption on cognitive depression (CD) occurred via the energy factor, which is mainly attributable to cholesterol. The structural regression path of the energy factor on CD showed a significant ($p<0.05$) difference between the FRG group and the placebo group.
Ko 2014	R / D-B / P-C	26 girls and 44 boys (6-15 years old) with ADHD 70 (33/37)	Korean red ginseng extract 1 g 56 days	Hyperactivity/ impulsivity	Significantly decreased inattention/ hyperactivity scores ($p<0.05$), significantly decreased quantitative electroencephalography theta/beta ratio ($p=0.001$).
Lee MH 2014	R /crossover	Healthy men 25 (13/12)	Red ginseng 32.1 mg 14 days	Effect on alcohol consumption and hangover symptoms	Relief of symptoms of alcohol hangover.

Park 2013	R / D-B / P-C	Participants with mild cognitive impairment 90 (45/45)	Korean ginseng 3 g 180 days	Cognitive impairment	Significant improvement in visual learning ($p<0.05$) and visual recall ($p<0.05$).
Han 2013	Not randomized Overnight polysomno-graphic study	Healthy men 15	Red ginseng extract 3 x 1.5 g 7 days	Sleeping behaviours	Total wake time was significantly reduced ($p=0.05$), sleep efficacy was significantly increased ($p=0.05$). Improved quality of sleep.
Heo 2012	R / P-C	30 Women and 10 men with Alzheimer's disease (30/10)	Heat-processed ginseng-SG-135 (standardized extract) 1.5, 3, 4.5 g 168 days	Alzheimer's disease	Significant improvement with 4.5 g SG treatment on the Alzheimer's Disease Assessment Scale ($p<0.05$) and Mini-Mental State Examination score ($p<0.05$).
Yeo 2012	R / D-B / P-C	Healthy men 15 (8/ 7)	Korean red ginseng 4.5 g 14 days	Cognitive and motor function	Improved cognitive function.
Chen EYH 2012	R/ D-B / P-C	51 Women and 13 men with schizophrenia 64 (32/32)	Ginsenosides (Rb1 and Rg1) 100 mg 28 days	Schizophrenia	Improved working memory.
SEXUAL FUNCTION AND INFERTILITY					
Park 2016	R / D-B / P-C	Infertile men with varicocele 80 (40/40)	Korean red ginseng 1.5 g 84 days	Infertility	Significant improvement in sperm concentration ($p<0.05$), motility ($p<0.01$), morphology ($p<0.01$) and viability ($p<0.05$).
Momoi 2015	R / D-B / P-C	Healthy men with partial impotence 45 (30/15)	Korean ginseng 200, 600 mg 12 days	Sexual function	Increased International Index of Erectile Function score ($p<0.05$).
Chung 2015	R / D-B / P-C/ crossover	Premenopausal women 23 (12/11)	Korean red ginseng 3 x 1 g 56 days	Sexual function	Improved sexual desire, arousal, orgasm and satisfaction domains.
Kim SY 2012	R / D-B / P-C	Postmenopausal women 72 (36/36)	Red ginseng + ginsenosides 3 g + 60 mg 84 days	Menopausal symptoms	Decreased menopausal symptoms. Significant improvements in Kupperman index ($p=0.032$) and in menopause rating scale($p=0.035$). Significantly decreased total cholesterol ($p=0.009$) and low-density lipoprotein cholesterol ($p=0.015$).
RESPIRATORY DISEASE					
Lee CS 2012	R / D-B / P-C	Healthy women (62) and men (38) 100 (50/50)	Korean red ginseng extract 3 x 1 g 84 days	Acute respiratory illness	Protection from contracting acute respiratory illness and may have the tendency to decrease the duration and scores of acute respiratory illness symptoms.
Wu 2014	R / D-B / P-C	9 Women and 1 man with Chronic Obstructive Pulmonary Disease (COPD) stage II-IV 10 (5/5)	Standardized ginseng extract 2-200 mg 70 days	COPD	No outcome because there were no exacerbations.

First Author Year	Study Design	Type of patient No. of patients (test/control)	Preparation type Daily dose Duration of treatment	Studied parameters	Outcome
IMPROVEMENT OF QUALITY OF LIFE IN CANCER PATIENTS					
Kim HS 2017	R / D-B / P-C	Patients with epithelial ovarian cancer 30 (15/15)	Red ginseng 3 g 90 days	Quality of life	Improved emotional functioning (p=0.027) and decreased symptoms of fatigue (p=0.012), nausea, vomiting (p=0.004) and dyspnea (p=0.021), reduced anxiety (p=0.015) and interference affecting life (p=0.035) and improved daytime somnolence (p=0.043).
Zhang 2018	R / D-B/ P-C multi-centre	Patients with stage 3, 4 non-small cell lung cancer 414 (199/215)	Ginsenoside Rg3 366 days	Median survival time	Adding ginseng to chemotherapy improved patients' symptoms and reduced chemotherapy-induced myelosuppression (p<0.05).
Kang 2018	R/ D-B / P-C	Patients underwent surgery for adenocarcinoma of the colon and received FOLFOX (20 women, 22 men) 42 (21/21)	Korean red ginseng 3 g 21 days	Protective effect in oxaliplatin-mediated splenomegaly in colon cancer	Beneficial effects against oxaliplatin-induced splenomegaly. The rate of splenomegaly was significantly lower (p=0.03).
Ma 2014	R / P-C	33 Women and 63 men with non-small cell lung cancer 96 (48 /48)	Ginseng polysaccharides with dendritic cells 0.5 mg/kg 30 days	Effect on the balance of Th1/ Th2 T helper cells	Increased expression of Th1 cytokines (IN-γ, IL-2) (p<0.01) and ratio of Th1/ Th2 (INF-γ/IL-4, IL-2/IL-5) (p<0.05), decreased FACT-L scores and expression of Th2 cytokines (IL-4, IL-5) (p<0.05).
OTHER EFFECTS					
Cho 2018	R / D-B / P-C/ crossover	Women with rheumatoid arthritis 70 (32/38)	Korean red ginseng 2 g 56 days	Rheumatoid arthritis	No significant effect.
Flanagan 2018	D-B / P-C	Healthy women (10) and men (9)	Hydrolyzed Korean Ginseng extract 960, 160 mg 14 days	Hypo-pituitary-adrenal and oxidative activity induced by intense work stress	Significantly reduced muscle damage (p<0.05) and hypo-pituitary-adrenal responses to physical stress (p<0.05).
Rocha 2018	R/ D-B / prospective	21 Women and 3 men with irritable bowel syndrome 24 (12 extract/12 trimebutine 600 mg/d)	Dry extract 300 mg 60 days	Improvement in abdominal pain	Decrease in abdominal pain.
Choi 2016	R / S-B / controlled	11 Women and 27 men with chronic Hepatitis B 38 (19/19)	Korean red ginseng + antiviral 3 g Single dose	Chronic Hepatitis B	Significant differences in hyaluronic acid (p=0.032) and transforming growth factor-β (p=0.008).
Jung 2016	R / D-B / P-C	Men with metabolic syndrome 62 (32/30)	Red ginseng 3 g 28 days	Effect on peripheral blood mitochondrial DNA copy number and hormones	Favorable effect on mitochondrial function and hormones. Increase in total testosterone level. Significantly decreased diastolic blood pressure (p<0.05) and serum cortisol (p<0.05).

Kim 2015	R / open-label	35 Women and 24 men with chronic tinnitus 59 (19: 1.5 g/day/ 20: 3 g/day/ 20 placebo)	Korean red ginseng 1.5, 3 g 28 days	Symptoms and Quality of Life in chronic tinnitus	Significant improvement of emotional and mental health scores ($p<0.05$) at 3 g/day.
Bae 2015	R / D-B / P-C	29 Women and 25 men with with glaucoma 54 (27/27)	Korean red ginseng 3 g 56 days	Effect on dry eye syndrome	Significant improvement of tear film stability and total Ocular Surface Disease Index score ($p<0.01$).
Doosti 2014	R / P-C / D-B	Men exposed to continuous noise 48 (16 NAC/16 ginseng /16 placebo)	Korean red ginseng 200 mg/day or N-acetyl-cysteine (NAC) 1200 mg/day 14 days	Noise induced hearing loss	Reduced noise-induced temporary threshold shift.
Park KS 2014	R / D-B / P-C	Women with cold hypersensitivity in the hands and feet 80 (40/40)	Korean red ginseng 2 x 3 g 56 days	Cold hypersensitivity (hands and feet)	Significantly higher skin temperature of hands ($p=0.027$) and feet ($p=0.004$), lower visual analogue scale scores ($p<0.05$), higher recovered temperature of the right 5th finger and less parasympathetic activity.
Park SY 2014	R / P-C	44 Women and 27 men with idiopathic sudden sensorineural hearing loss 73 (37 steroid group/ 36 steroid+KRG group)	Korean red ginseng with steroid 3 g 60 days	Sudden sensorineural hearing loss	No effect.
Cho 2014	R / D-B / P-C	Healthy women (70) and men (2)	Korean ginseng extract 6 g 98 days	Natural killer cell activity	Enhanced natural killer cell cytotoxic activity.
Lee JK 2013	R / D-B / P-C / multi-centre	18 Women and 10 men with biliary colic 28 (14/14)	Korean red ginseng 3 x 2.5 g 168 days	Adjuvant to the standard regimen of bile acids for gallstones	No significant reduction in stone burden ($p=0.09$).
Braz 2013	R / D-B / P-C	Women with fibromyalgia 38 (13 amytriptiline/ 13 placebo/ 12 ginseng)	Ginseng extract (27% ginsenosides) 100 mg 84 days	Fibromyalgia	Reduced number of tender points and improved quality of life.
Kim YH 2012	R / P-C	Pregnant women due to have a caesarean section 41 (21/20)	Red ginseng 4.5 g 24 days	Effect on lipid peroxide levels, protein carbonyl levels and antioxidative ability	Decreased protein carbonyl formation and prevented the reduction of antioxidative ability in maternal blood during the postpartum period after caesarean section.
Kim JY 2012	R / D-B / P-C	Healthy women (34) and men (23) 57 (19 low dose / 19 high dose/ 19 placebo)	Korean red ginseng 3, 6 g 56 days	Lymphocyte DNA damage, antioxidant enzyme activity, LDL oxidation	Decreased lymphocyte DNA damage and LDL oxidation by upregulating antioxidant enzyme activity.

R = Randomized; D-B = Double-blind; S-B = Single-blind; P-C = Placebo-controlled

In a randomised, double-blind, placebo-controlled trial, 45 healthy men with partial impotence (aged 35-70 years) received 200 or 600 mg of ginseng (not further specified) or placebo, daily for 12 weeks. Ginseng did not significantly improve male sexual function when compared to placebo, but did significantly ($p<0.05$) increase the IIEF scores when compared to baseline in both the 200 and 600 mg groups [Momoi 2015].

Pharmacokinetic properties

Pharmacokinetics in animals
The pharmacokinetics of ginsenoside Rg1 were studied in rats, comparing oral and intravenous administration. Rapid absorption of 1.9-20.0% of orally administered Rg1 (t_{max} 30 minutes) and rapid excretion of intravenous Rg1 (almost 60% of the dose in bile within 4 hours and 24% in urine within 12 hours) were detected by TLC [Odani 1983a]. Orally administered ginsenoside Rb1 was very poorly absorbed (approx. 0.1%) from rat intestine. Excretion of Rb1 after intravenous administration was biphasic with a half-life of 11.6 minutes for the α-phase and 14.6 hours for the β-phase. Excretion was mainly in the urine (44% within 120 hours) and minimal in bile (0.8% within 24 hours) [Odani 1983b].

The pattern of decomposition products of ginsenosides Rb1 and Rg1 in rat stomach was similar to hydrolysis products under mild acidic conditions except for one compound. Decomposition products in the large intestine were due to the activitiy of enteric bacteria and an enteric enzyme [Odani 1983c].

A study in mini pigs following intravenous administration of Rb1 and Rg1 confirmed findings in rats and rabbits. Rb1 was excreted biexponentially (two-compartment open model) with a half-life of 16 hours in the β-phase (20 minutes in the α-phase). Rg1 pharmacokinetics are best explained by a one-compartment model, the elimination half-life being 27 minutes [Jenny 1985].

A study in mice involving intravenous or oral administration of tritiated [^{3}H] ginsenoside Rg1 indicated rapid absorption after oral administration (approx. 30% after 1 hour). Relatively high concentrations were found in blood, liver, bile, subcutis, conjunctiva and epithelia of oral and nasal cavities and oesophagus, whereas the concentration in muscle and endocrine organs was low. Addition of total ginseng extract or of a purified ginsenoside fraction to oral administration of tritiated Rg1 did not change the distribution pattern of Rg1. Excretion of intact Rg1 in faeces and urine was low, whereas excretion of metabolites was high [Strömbom 1985].

After oral administration of ginsenoside Rb2 to rats, six decomposition products were found in the large intestine. Five decomposition products resulted from stepwise cleavage of sugar moieties [Karikura 1990].

The decomposition of ginsenoside Rb2 in rat stomach was compared to its decomposition in 0.1N HCl. There was little decomposition in the stomach with only small amounts of 24- and 25-hydroxy- and -hydroperoxy-derivatives. Decomposition in 0.1 N HCl solution yielded two main derivatives due to cleavage of a sugar moiety [Karikura 1991a].

A further study investigated the decomposition of ginsenosides Rb1 and Rb2 in rat stomach and large intestine, in 0.1 N HCl and in crude hesperidinase solution. The results confirmed a quantitatively small decomposition to hydroxy- and hydroperoxy-derivatives in rat stomach, whereas decomposition in 0.1 N HCl yielded derivatives arising from cleavage of sugar moieties. The decomposition of Rb1 and Rb2 by cleavage of sugar moieties in rat large intestine was partly due to bacteria and partly due to enteric enzymes such as β-glucosidase. Different decomposition products were found in rat large intestine compared to those found after treatment with hesperidinase [Karikura 1991b].

After oral administration of total saponins at a dose of 400 mg/kg, the protopanaxadiol type saponins exhibited relatively long t_{max} values, while the protopanaxatriol type saponins had varying tmax values, $t_{1/2}$ of 20(S)-epimers were longer than those of 20(R)-epimers [Ma 2015].

A ginsenoside-Rg2 solution was applied to rat tail vein at a dose of 25 mg/kg of 20 (R,S)-ginsenoside-Rg2, corresponding to 2 mg/kg of 20 (R)-ginsenoside-Rg2 and 23 mg/kg of 20 (S)-ginsenoside-Rg2. Blood samples were collected from the arteria carotis communis immediately before and at 1, 3, 5, 10, 20, 30, 45 and 60 min after the administration. AUC was 197.7 ± 5.2 and 1092.5 ± 84.0 for 20 (R)-ginsenoside-Rg2 and 20 (S)-ginsenoside-Rg2 respectively [Gui 2007].

Permeability of compound K (an intestinal metabolite of panaxadiol ginsenosides) through Caco-2 cell layers was intermediate as compared to metoprolol (28 x 106 cm/s) and atenolol (0.25 x 106 cm/s). After i.v. administration of 1, 2 and 10 mg/kg to rats, no significant dose dependency was found in Cl (17.3-31.3 mL/min/kg), Vss (1677-2744 mL/kg), dose-normalized AUC (41.8-57.8 μg.min/mL based on 1 mg/kg) and $t_{1/2}$. Urinary excretion was minimal for both i.v. and oral doses. The amount of compound K recovered from the entire gastrointestinal tract at 24 h was 24.4%-26.2% for i.v. doses and 54.3%-81.7% for oral doses. Following oral administration (doses of 5-20 mg/kg), dose-normalized AUC (based on 5 mg/kg) was increased at the 20 mg/kg dose (85.3 μg.min/mL) compared with those at lower doses (4.50-10.5 μg.min/mL). Subsequently, the absolute oral bioavailability was increased from 1.8%-4.3% at the lower doses to 35.0% at the 20 mg/kg dose [Paek 2006].

Pharmacokinetics in humans
Dose-dependent urinary excretion of 20(S)-protopanaxatriol glycosides (1.5% of the dose) was observed in 4 healthy volunteers after oral ingestion of ginseng powder and ginseng extract preparations corresponding to 6.2-27.6 mg of ginsenosides per day [Cui 1997].

Using selective ion-monitoring GC-MS, ginsenoside aglycones were quantified in urine from athletes who claimed to have consumed ginseng preparations for at least 10 days before urine collection. Aglycone concentrations of 2-35 ng/ml were found in 60 out of 65 urine samples [Cui 1996].

Cleavage of sugar moieties from ginsenosides Rb1, Rb2, Rc and Rd by intestinal bacteria isolated from human faecal samples has been demonstrated. *Prevotella oris* was identified as the major bacterial species with this ability [Hasegawa 1997].

The bioavailability of the protopanaxadiol group (ginsenosides Ra3, Rb1, Rd, Rg3 and Rh2) and of the protopanaxatriol group (ginsenosides Rg1, Re, Rh1 and R1) was found to be less than 5% after oral administration. Approximately 0.2%-1.2% of ginsenosides were excreted in human urine [Qi 2011]

A standardized extract (4% ginsenosides; 700 mg daily) was taken orally as a single dose on an empty stomach by two healthy volunteers. Blood and urine samples were taken 30 min before drug administration. Overall, 25 blood samples were obtained from each subject. Urine samples were collected in four fractions: 0 to 3, 3 to 6, 6 to 12 and 12 to 24 h after administration. The same two hydrolysis products of the protopanaxatriol ginsenosides, G-Rh1 and G-F1, were

identified in plasma and urine in both volunteers. In addition, compound K, the main intestinal bacterial metabolite of the protopanaxadiol ginsenosides, was detected in plasma and urine. [Tawab 2003].

There was no effect on CYP3A4, CYP1A2, CYP2E1, and CYP2D6 in healthy volunteers [Gurley 2002]. In another study, ginseng had no effect on a number of CYP isoforms, including CYP3A4, CYP1A2, CYP2E1 and CYP2D6 [Gurley 2005, Liu 2006]. However, CYP2D was slightly inhibited in elderly humans [Liu 2006].

Compound K (C-K) was absorbed into the blood 24h after oral administration of ginseng to 32 male participants, with average values for t_{max}, C_{max} and AUC of 10.76+/-2.07 h, 27.89+/-24.46 (ng/mL) and 221.98+/-221.42 (µg x h/mL) respectively. There was a correlation between the C-K transforming activity of G-Rb1 and the C-K transforming activity of ginseng extract by intestinal microflora [Lee J 2009].

The pharmacokinetics of ginsenoside Rd were assessed in healthy Chinese volunteers. In the single-dose study, a randomized, open-label, 3-way crossover design was used. Participants were assigned to 10, 45 or 75 mg Rd by intravenous infusion, with a 2-week washout period between the administrations. Plasma levels of Rd were proportional to dose, with the mean C_{max} and AUC(0-infinity) ranging from 2.8 to 19.3 mg/L and 27.9 to 212.5 mg x h/L over the dose range studied. Rd was slowly cleared from plasma ($t_{1/2}$ = 17.7-19.3 h). In a multiple-dose study, 10 mg Rd was administered once daily for 6 days. Slight drug accumulation was noted. The mean steady-state C_{max}, AUC(0-infinity) and AUC(ss) were 4.0 mg/L, 51.7 mg x h/L, and 26.4 mg x h/L, respectively. The $t_{1/2}$ was 20.5 hours, which was similar to the single-dose value [Zeng 2010].

Preclinical safety data
A 1984 review [Soldati 1984] summarized the results of several toxicity studies of a standardized ginseng root extract in animals:

Single dose toxicity
- The oral LD_{50} was determined as > 5 g/kg b.w. in the rat, > 2 g/kg in the guinea pig and > 1 g/kg in mice.
- The intraperitoneal LD_{50} was > 1 g/kg in rats and mice.
- No noticeable changes were observed in cardiovascular parameters including ECG, pulse, blood pressure, cardiac output and stroke volume in mini-pigs adminstered oral doses of the extract at 0.25, 0.5 or 2.0 g/kg b.w.

Repeated dose toxicity
- No haematological or histological abnormalities were observed in rats after oral administration of the extract at 4.0 g/kg/day for 20 days.
- No treatment-related haematological or histopathological findings were observed in beagle dogs after oral administration of the extract at 1.5, 5.0 and 15 mg/kg/day for 90 days.
- An additional study showed no changes in haematological parameters in rats following subcutaneous injection of an extract (corresponding to 100 mg of root) daily for 5 days [Lewis 1983].

Reproductive toxicity
- No decrease in growth rate or reproduction and no treatment-related haematological or histopathological findings were observed in rats during a 33-week two-generation study with an extract administered orally at 1.5, 5.0 or 15 mg/kg b.w./day.

Embryo, foetal and perinatal toxicity
- No abnormalities of foetal development were found after oral administration of an extract to rats at 40 mg/kg/day during days 1-15 after mating or to rabbits at 20 mg/kg/day during days 7-15 after mating.

Genotoxicity
- In the hepatocyte-DNA repair test no genotoxicity was observed for an extract at concentrations of 0.1-10 mg/mL, with or without ginsenosides, or for ginsenoside Rg1 at 1-50 µg/ml.

In a study involving intraperitoneal administration of ginsenosides Rb1 and Rg1 to mice at 25, 50 or 100 mg/kg b.w., neither single nor repeated large doses of 100 mg/kg caused any toxic symptoms or adverse behavioural effects such as ataxia or sedation [Kim 1998].

Further toxicity studies were summarized in Chan 2007:

Table 2: LD_{50} (mg/kg) values for ginseng [NLM 1998a,1998b]

Samples	Animals	Route	LD_{50} (mg/kg)
Panax ginseng	Rat	oral	750
	Mouse	oral	200
	Mouse	i.p.	54
Ginseng root extract	Mouse	i.p.	545
Ginsenoside 3	Mouse	i.p.	910
Ginseng, saponin extract	Mouse	i.p.	637

Genotoxicity
An extract was not mutagenic in two independent bacterial mutagenicity assays, each conducted with or without exogenous metabolic activation enzymes (S9 mix from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) in the Ames-Test. Bacterial strains tested included *Salmonella typhimurium* strains TA 97, TA98, TA100, TA102, TA104, and TA1535, as well as *E. coli* strain WP2 uvrA/pKM101. Furthermore, no significant increases were seen in the frequencies of micronucleated erythrocytes in the peripheral blood of male or female B6C3F1 mice exposed to 1 to 5 g/kg ginseng via gavage for 3 months [National Toxicology Program 2011]

Carcinogenicity
There was no evidence of any carcinogenic activity of an extract in male or female F344/N rats or B6C3F1 mice administered at 1.25, 2.5 or 5 g/kg for two years. The incidence of mammary gland fibroadenoma was significantly decreased in the 5 g/kg group of female rats [Chan 2011].

The safety profile of extract G115 was evaluated in a recent review. It was summarized as having no adverse effects on reproductive parameters or treatment-related effects on animal behaviour, physical appearance or food consumption [Bilia 2020].

Clinical safety data
A Cochrane review including 9 randomized, double-blind, placebo-controlled trials found no serious adverse events associated with ginseng [Geng 2010].

In a double-blind, placebo-controlled study involving 60 females and 60 males, daily oral administration of 200 mg of a standardized extract for 12 weeks did not cause any significant differences in blood levels of sex hormones (luteinizing

hormone, follicle-stimulating hormone, testosterone, oestradiol) in comparison with placebo groups [Forgo 1981b].

In an open study of 49 menopausal women, regular speculum examinations and cytological smears from the cervix and vaginal wall did not reveal any changes during 3 months of oral treatment with 200 mg of a standardized ginseng extract per day [Reinhold 1990].

In a randomized, double-blind, placebo-controlled study involving 60 physically active men (aged 17- 22 years), oral administration of 3 g/day of powdered root for 8 weeks did not lead to any abnormal changes in markers of liver or renal function [Kulaputana 2007].

No severe adverse events were observed in a randomized, double-blind study involving 72 type 2 diabetic patients given 1500 to 3000 mg of a vinegar extract of ginseng or placebo for 8 weeks [Yoon 2012].

There were no serious adverse events reported in a randomised, double-blind, placebo-controlled trial involving 90 subjects with mild cognitive impairment given 3 g/day of ginseng powder or placebo for 6 months (Park 2013).

There were no serious adverse events reported in a randomized, double-blind, placebo-controlled study involving 28 patients given 7.5 g/day steamed ginseng root or placebo for 24 weeks, in addition to a standard bile acid regimen for dissolution of gallstones (Lee JK 2013).

In a double-blind, placebo-controlled study, 60 female multiple sclerosis patients received 250 mg ginseng (not further specified) twice daily for 3 months. No serious adverse events were observed during follow-up [Etemadifar 2013].

There were no serious adverse events reported in a randomized, double-blind, placebo-controlled trial involving 80 female participants with cold hypersensitivity in the hands and feet adminstered either 3 g/day of ginseng powder or placebo for 8 weeks (Park KS 2014).

In a randomized, double-blind, placebo-controlled clinical trial involving 180 participants, there were no signficant adverse effects demonstrated in any of the safety parameters measured [Zhang 2019].

Case reports of possible allergic response

- A 20 year old male patient experienced a respiratory allergy, with skin and blood pressure effects, after ingestion of an Asian ginseng syrup [Wiwanitkit 2004].
- A 29 year old female patient exposed to airborne Korean ginseng dust showed a positive bronchial provocation and positive responses to a skin-prick test [Lee JY 2006].
- A 34 year old woman developed recurrent dyspnoea, wheezing, and nasal symptoms associated with Korean ginseng at work. Allergen bronchial provocation testing to Korean ginseng extract showed a typical immediate response, and skin prick testing resulted in a strong positive response [Kim 2008].
- A 44-year-old man developed anaphylaxis after oral intake of fresh ginseng with rhinorrhea and nasal stiffness, followed by respiratory difficulty with wheeze and abdominal pain 10 minutes after ingestion [Lee JY 2012].

Case reports with causal relationship not established

For the following case reports, a causal relationship between the intake of ginseng and the described reaction could not be established:

- Two cases of vaginal bleeding were reported in a study on sexual dysfunction in menopausal females [Oh 2010].
- One case of hypoglycaemia was reported in a study on glucose metabolism in healthy subjects [Oh 2014].

Table 3: Clinical safety studies

First Author Year	Study Design	Type of patient No. of patients (test/control)	Preparation type Daily dose Duration of treatment	Studied parameters	Outcome
Seong 2018	R / open label	Healthy participants (male 15) (15/0)	Red ginseng extract 0.8 g, 1.5 g 15 days	Drug Interaction	Poses minimal risks for clinically relevant CYP- or OATP-mediated drug interactions, well tolerated.
Song SW 2018	R / D-B / P-C / multi-centre	Healthy participants (female 724, male 268) 992 (490/502)	Korean red ginseng 2 g 168 days	Safety and tolerability	No significant abnormal changes in anthropometric, laboratory, and vital sign measurements.
Cho 2014	R / D-B / P-C	Healthy participants (female 70, male 2) 72 (36/36)	Korean ginseng extract 6 g 98 days	Efficacy and safety	No severe adverse events or alterations in complete blood cell count or blood chemistry.
Lee NH 2012	R / D-B / P-C	Healthy participants (female 129, male 41) 170 (113/57)	Ginseng extract 1 g or 2 g 28 days	Safety and tolerability	Safe, tolerable, no toxic effect with mild adverse events, such as dyspepsia, hot flash, insomnia, and constipation.

R = Randomized; D-B = Double-blind; S-B = Single-blind; P-C = Placebo-controlled

- After consuming daily ginseng drinks for 3 months, a 26-year-old man with chronic myelogenous leukaemia taking imatinib at 400 mg/day was diagnosed with hepatotoxicity [Bilgi 2010].

- After taking a ginseng preparation for cold for 1 month, an 83 year old woman with chronic kidney disease presented to an emergency department with symptoms of dizziness and general weakness for 1 day accompanied with unexplained bradyarrhythmia [Liao 2010].

- A 12 year old boy was diagnosed with gynaecomastia after 1 month of consuming red ginseng extract daily to enhance his performance [Kakisaka 2012].

- A 64 year old man complained of the temporary loss of vision for less than one hour on 2 occasions spaced 2 days apart after using a preparation containing 500 mg Korean Ginseng daily over a period of 13 days for asthenia [Martínez-Mir 2004].

- A 56 year old woman with previous affective disorder presented in a manic episode while taking an extract. Symptoms disappeared rapidly with low doses of neuroleptics and benzodiazepines [Vazquez 2002].

- A 26 year old healthy individual with no history of psychiatric illness reported manic symptoms after taking Chinese ginseng [Engelberg 2001].

- A 39 year old female patient experienced menometrorrhagia following oral and topical use of ginseng [Kabalak 2004].

- A 48 year old woman had uterine bleeding after using a combination product containing ginseng [Palop-Larrea 2000].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Online Series, 2019
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Online Series, 2021
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Online Series, 2019
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Online Series, 2019
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHAMOMILLAE ROMANAE FLOS	Roman Chamomile Flower	Online Series, 2019
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Online Series, 2021
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Online Series, 2021
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Online Series, 2023
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HELICHRYSI FLOS	Sandy Everlastin Flower	Online Series, 2019
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Online Series, 2018

JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Online Series, 2020
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Online Series, 2021
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Online Series, 2020
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Online Series, 2021
PRUNI AFRICANAE CORTEX	Pygeum Bark	Online Series, 2020
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Online Series, 2021
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TILIAE FLOS	Lime Flower	Online Series, 2022
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Online Series, 2020
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Online Series, 2020
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

E/S/C/O/P Monographs

Online Series

The Scientific Foundation for Herbal Medicinal Products

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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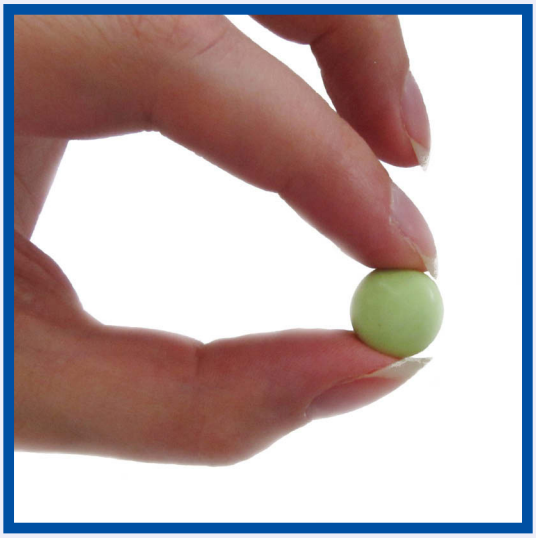
Online Series

The Scientific Foundation for Herbal Medicinal Products

Graminis rhizoma
Couch Grass Rhizome

2016



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MONOGRAPHS

***The* Scientific Foundation for**
Herbal Medicinal Products

GRAMINIS RHIZOMA
Couch Grass Rhizome

2016

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Second Edition, Supplement 2009

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

GRAMINIS RHIZOMA 2016

Couch Grass Rhizome

DEFINITION

Couch grass rhizome consists of the whole or cut, washed and dried rhizome of *Agropyron repens* (L.) Beauv. (*Elymus repens* (L.) Gould, *Triticum repens* L.); the adventitious roots are removed.

The material complies with the monograph of the European Pharmacopoeia [Graminis rhizoma].

CONSTITUENTS

The characteristic constituents are polysaccharides, principally the branched fructosan triticin (3.5 -12%) [Arni 1951, Berger 1960] together with about 10% of unidentified mucilaginous polysaccharides [Bradley 2006, Gorecki 2007]; flavonoids, especially quercetin and luteolin glycosides [Stanic 2000, Weston 1987]; phenolic glucosides (ca. 1.7%), mainly the 5-glucosides of 5-hydroxyindole-3-acetic acid and 5-hydroxytryptophan [Hagin 1989, Hagin 1991, Whitehead 1983]; phenolic acids [Whitehead 1983], and a small amount (ca. 0.02%) of essential oil containing carvacrol (10.8%), *trans*-anethole (6.8%), carvone (5.5%), thymol (4.3%), menthol (3.5%) and other terpenes [Boesel 1989].

Other constituents include phenylpropanoid esters [Koetter 1993, Koetter 1994], fatty acids [Boesel 1989], phytosterols [Boesel 1991, Koetter 1993] and sugar alcohols (mannitol and inositol) [Steinegger & Hänsel 1992].

CLINICAL PARTICULARS

Therapeutic indications
Irritable bladder and other urinary tract disorders [Hautmann 2000, Brayfield 2014]. Efficacy in these indications is plausible on the basis of human experience and long-standing use [Robert 1986, Schilcher 1989, Pieroni 2002, Pieroni 2005].

Posology and method of administration

Dosage

Adults and children over 12 years
5-10g of the drug daily as an infusion [Wichtl 2009, Blumenthal 1998, BHP 1983]; fluid extract (1:1, 20-25% ethanol V/V): 2-4 ml three times daily [BHP 1983, Hautmann 2000]; tincture (1:5, 40% ethanol V/V): 5-15 ml three times daily [BHP 1983].

Method of administration
For oral administration.

Duration of use
No restriction. If symptoms persist or worsen, medical advice should be sought.

Contraindications
None known.

Special warnings and special precautions for use
None required

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available.
In accordance with general medical practice, the product should not be used during pregnancy without medical advice.

Effects on ability to drive and use machines
None known.

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Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antiadhesive activity
A hydroethanolic (50%V/V) lyophilized extract of couch grass rhizome decreased adhesion of uropathogenic *Escherichia coli b*acteria to a cell line derived from human urinary bladder carcinoma by interacting with bacterial outer membrane proteins with an IC_{25} of 630 µg/mL ($p<0.05$) [Rafsanjani 2013].

In vivo experiments

Diuretic activity
Oral administration of an aqueous extract from couch grass rhizome (prepared as a 1% maceration) to rats at 50 ml/kg b.w. increased diuresis with a diuretic index of 1.42 compared to control animals receiving a corresponding amount of water only. Intraperitoneal administration to rats of an equivalent dose, in the form of an aqueous solution of a dry hydroalcoholic extract corresponding to 10% of dried rhizome at 5 ml/kg, increased diuresis with a diuretic index of 1.62 [Racz-Kotilla 1971].

In rats given an infusion of couch grass rhizome (3 g/litre) for 7 days instead of tap water, in combination with a standard diet, diuresis increased significantly compared to controls ($p<0.005$), as did the calcium content of the urine ($p<0.005$), while the magnesium content of the urine decreased ($p<0.005$). In a similar experiment, except with the rats on a high protein diet, no significant differences were observed in diuresis (the control value was much higher than for rats on the standard diet) or urinary calcium, but the decrease in urinary magnesium and an increase in urinary phosphate were highly significant (both $p<0.0005$), while the urinary pH decreased to 5.9 compared to 6.5 in controls ($p<0.01$) [Grases 1995].

Sedative effect
A 10% infusion of couch grass rhizome was administered orally to mice in doses equivalent to 40 and 80 mg of crude drug, and intraperitoneally in doses equivalent to 10, 15, 20, 40 and 80 mg of crude drug, per 20 g mouse. Sedative effects were measured by several methods including motility testing. Compared to the motility of control animals receiving only water (orally) or physiological saline (intraperitoneally), motility decreased to 95.9 and 36.1% respectively in groups receiving the infusion orally, and to 73.9, 51.4, 22.8, 18.9 and 2.4% respectively in groups receiving it intraperitoneally [Kiesewetter 1958].

Anti-inflammatory activity
A hydroethanolic (80% V/V) dry extract of couch grass rhizome, administered orally at 100 mg/kg b.w., inhibited carrageenan-induced rat paw oedema only weakly, by 14% compared to 45% by indometacin at 5 mg/kg. The oedema was produced by injecting 0.1 ml of a 1% carrageenan suspension into the plantar surface of the rat hind-paw [Mascolo 1987].

Effects on lipid metabolism
The effects of a lyophilized aqueous extract from couch grass rhizome on plasma cholesterol and triglyceride levels and also b.w. were evaluated in normal and streptozotocin-induced diabetic rats after a single oral dose of 20 mg/kg, and during repeated oral administration at the same dose level once a day for 2 weeks. Distilled water was used as a control and oral sodium vanadate (a known triglyceride- and cholesterol-lowering agent) at 0.8 mg/kg b.w. as a reference drug.

In normal rats
- a single dose of the extract or sodium vanadate did not cause significant changes in cholesterol or triglyceride concentrations.
- repeated administration of the extract produced a significant decrease in cholesterol after 7 days ($p<0.05$) and in triglycerides after 4 days and 7 days ($p<0.05$), although these effects were no longer evident on day 15. Sodium vanadate treatment led to significant reductions in cholesterol ($p<0.001$) and triglycerides ($p<0.01$) by day 15.
- repeated administration of the extract caused a slight increase in b.w. at day 15 ($p<0.01$), whereas with sodium vanadate b.w. significantly and continuously decreased over the treatment period ($p<0.001$ at day 15).

In streptozotocin-induced diabetic rats
- a single dose of the extract produced a significant decrease in both cholesterol ($p<0.001$) and triglyceride ($p<0.01$) concentrations at 6 hours after treatment; sodium vanadate had similar effects (cholesterol, $p<0.01$; triglycerides, $p<0.01$).
- repeated administration of the extract caused a significant decrease in both cholesterol and triglyceride concentrations at days 4 ($p<0.05$), 7 and 15 ($p<0.001$). Sodium vanadate produced comparable effects ($p<0.001$ for both cholesterol and triglycerides at day 15).
- both treatments led to a significant decrease in b.w. by day 15 ($p<0.01$ for the extract; $p<0.001$ for sodium vanadate).

The authors concluded that repeated oral administration of the couch grass rhizome aqueous extract had a lipid lowering effect and reduced b.w. in severely hyperglycaemic rats [Maghrani 2004].

Hypoglycaemic activity
In a further study by the same research group, the effects of a lyophilized couch grass rhizome aqueous extract on plasma glucose levels in normal and streptozotocin-induced diabetic rats were evaluated using the same dosage regimen, i.e. a single oral dose of the extract at 20 mg/kg or repeated oral administration at that dose level once a day for 2 weeks. Again, distilled water was used as a control and oral sodium vanadate at 0.8 mg/kg b.w. as a reference drug.

In normal rats
- a single dose of the extract caused a significant decrease in plasma glucose levels I hour ($p<0.001$) and 4 hours ($p<0.001$) after administration, with less significance after 6 hours ($p<0.05$). Sodium vanadate treatment produced a more modest decrease in plasma glucose after 2 hours ($p<0.01$) and 4 hours ($p<0.05$).
- after repeated daily administration of the extract, a significant reduction in plasma glucose was observed on day 2 ($p<0.01$), day 4 ($p<0.001$) and day 7 ($p<0.001$), returning to normal by day 15. No significant changes in plasma glucose were observed in rats treated with sodium vanadate.

In streptozotocin-induced diabetic rats
- a single oral dose of the extract caused a significant decrease in plasma glucose 4 and 6 hours after administration ($p<0.001$).
- after repeated daily administration of the extract a significant decrease in plasma glucose was observed from day 2 to day 15 ($p<0.001$); sodium vanadate had a similar effect.
- in contrast, plasma glucose levels in control rats (treated with distilled water only) gradually increased to a maximum on day 4.

Basal plasma insulin levels were not affected by the extract or sodium vanadate. It was concluded that the couch grass rhizome aqueous extract had a potent hypoglycaemic effect in streptozotocin-induced diabetic rats [Eddouks 2005].

Clinical studies
In a surveillance study (open, uncontrolled, multicentre) 313 patients, two-thirds of them female, with diagnoses of irritable bladder (34%), cystitis (29%), prostatitis (19%) and/or urethritis (5%) were treated orally with a hydroethanolic fluid extract (1:1) of couch grass rhizome; the average dosage was 50-60 drops three times daily and the average duration 12 days. More than one complaint was diagnosed in 13% of the patients and a third of the patients were treated concomitantly with other drugs, mostly antibiotics. After treatment (for 12 days on average, with a minimum of 2 and maximum of 52 days; 7-14 days in 86% of patients), between 32% and 50% of the patients were free from initially documented symptoms (e.g. nycturia, frequent or painful micturition), and between 69% and 91% reported some relief of symptoms during the course of therapy [Hautmann 2000].

In an open, uncontrolled study 99 patients with diagnoses of prostatitic adenoma (48 male patients), chronic or acute prostatitis (38 male patients) or cystitis (12 female patients) were treated orally with a fluid extract (1:1, 20% V/V ethanol) of couch grass rhizome. The dosage was 60 drops three times daily and the duration of treatment 28-31 days. The treatment was evaluated from changes in various urinary parameters associated with infection and inflammation, and by subjective feeling of discomfort. For each of the parameters the results showed improvements of 40-100%. In the two groups of male patients the treatment was assessed as good to very good by 77.1 and 78.9% of the patients respectively, while 58.3% of the female patients considered the treatment very good [Barsom 1981].

Pharmacokinetic properties
No data available.

Preclinical safety data

Mutagenicity and carcinogenicity
A couch grass rhizome liquid extract (1:1 in 20% ethanol) showed no mutagenic activity in the Ames mutagenicity test in *Salmonella typhimurium* strains TA 98 and TA 100 [Schimmer 1994].

Clinical safety data
No adverse drug reactions were reported in an open surveillance study after oral intake by 313 patients of a hydroethanolic fluid extract (1:1) of couch grass rhizome, the majority taking 50-60 drops three times daily for about 12 days [Hautmann 2000].

In an open study involving 99 patients treated orally with a hydroethanolic fluid extract of couch grass rhizome, at 60 drops three times daily for an average of 30 days, the treatment was well tolerated by 96% of the patients. One patient withdrew due to gastrointestinal symptoms [Barsom 1981].

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MONOGRAPHS

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ALOE CAPENSIS	Cape Aloes	Online Series, 2014
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The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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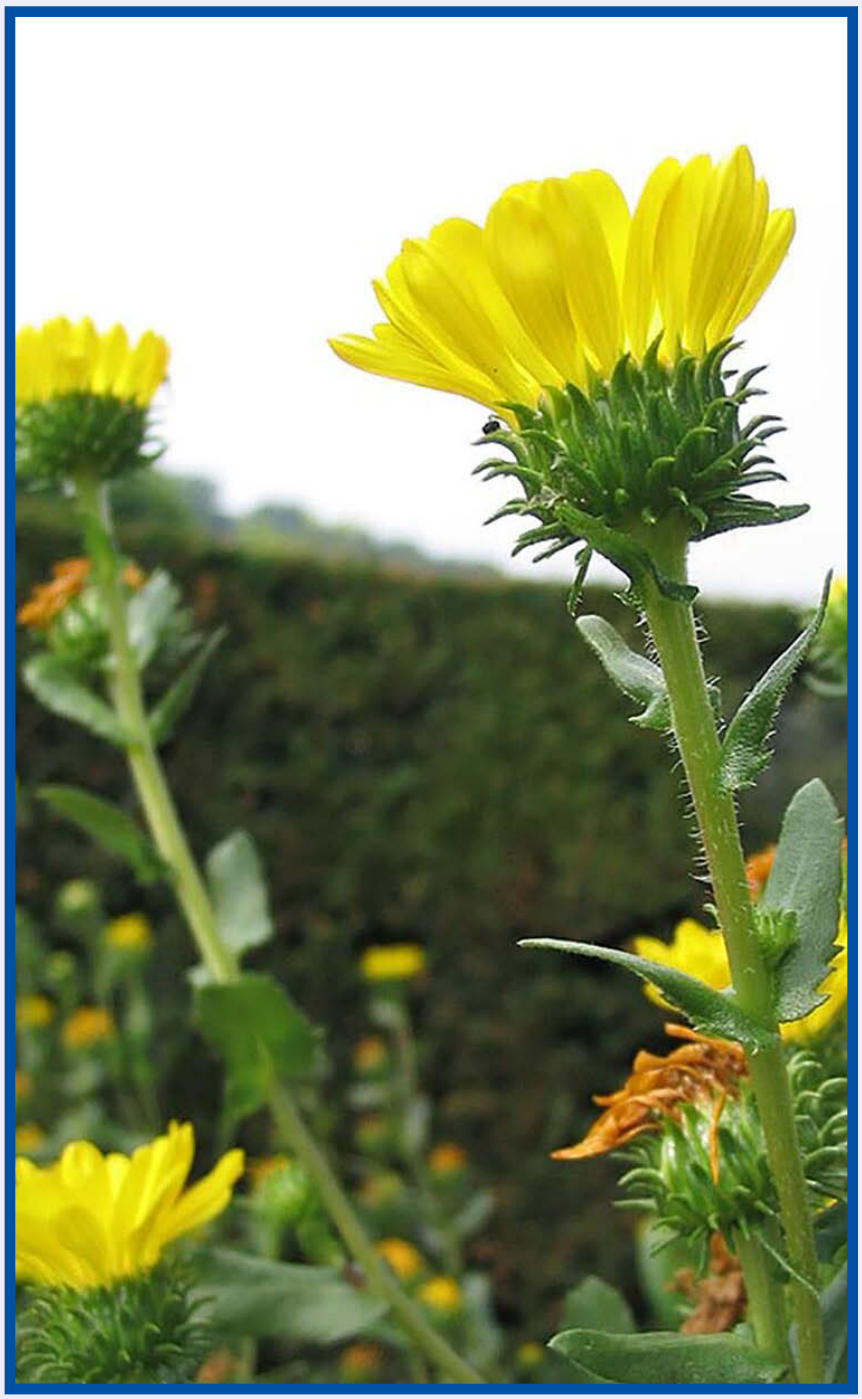

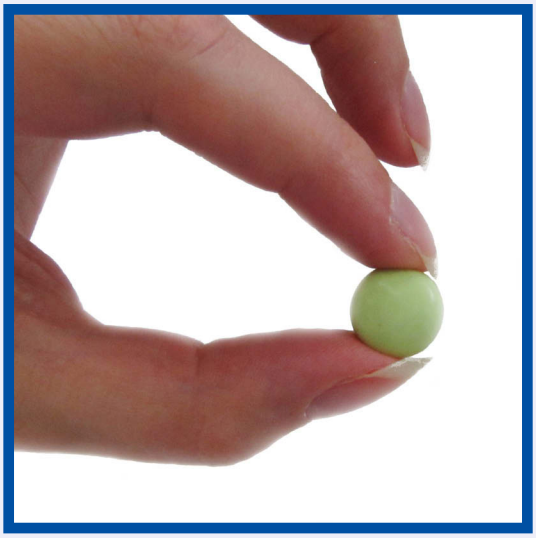

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

GRINDELIAE HERBA

2015

Grindelia

DEFINITION

Grindelia consists of the dried flowering tops of *Grindelia robusta* Nutt., *Grindelia squarrosa* Dunal, *Grindelia humilis* Hook. et Arn. or *Grindelia camporum* Greene.

The material complies with the monograph of the Pharmacopée Française [Grindélia].

CONSTITUENTS

The characteristic constituents are resin (5-20% depending on the species) consisting mainly of diterpenic acids such as grindelic acid, 7,8-epoxygrindelic acid, 17-acetoxygrindelic acid [Didry 1982; Timmermann 1987; Stahl-Biskup 1998; Bradley 2006] and borneol [Fraternale 2007]; acetylenic compounds such as matricarianol and matricarianol acetate [Schulte 1964; Bohlmann 1965; Stahl-Biskup 1998; Bradley 2006]; flavonoids such as kaempferol 3-methyl ether and 3,7-dimethyl ether and various quercetin methyl ethers [Pinkas 1978; Didry 1982; Timmermann 1994; Stahl- Biskup 1998; Bradley 2006, Krenn 2009]; and triterpenoid saponins with grindelia sapogenin D, bayogenin and oleanolic acid as the sapogenins [Kreutzer 1990, 1995; Stahl-Biskup 1998; Bradley 2006;].

Other constituents include phenolic acids such as chlorogenic, *p*-hydroxybenzoic and *p*-coumaric acids [Pinkas 1978; Didry 1982; Stahl- Biskup 1998; Gehrmann 2002; Bradley 2006], approx. 5% of tannins [Stahl- Biskup 1998] and approx. 0.2% of essential oil consisting mainly of mono- and sesquiterpenes [Kaltenbach 1991; Schäfer 1993; Stahl-Biskup 1998; El-Shamy 2000; Bradley 2006; Fraternale 2007].

CLINICAL PARTICULARS

Therapeutic indications
Productive cough; catarrh of the upper respiratory tract [Grindelia 1983; Schimmer 1988; Stahl-Biskup 1998; Gehrmann 2002; Vanaclocha 2003; Bradley 2006; Schilcher 2010].

Efficacy in these indications is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Adult daily dose: 4-6 g of the drug as a 10% infusion [Herba Grindeliae 1953; Stahl-Biskup 1998; Gehrmann 2002; Vanaclocha 2003; Schilcher 2010]; 3-6 g of fluid extract (1:1, Erg-B 6) [Extractum Grindeliae fluidum 1953; Stahl-Biskup 1998; Vanaclocha 2003; Schilcher 2010]; 1.5-3 mL of tincture (1:10 or 1:5, ethanol 60-80% V/V) [Stahl-Biskup 1998; Gehrmann 2002; Vanaclocha 2003; Bradley 2006; Schilcher 2010]; other corresponding preparations.

Method of administration
For oral administration.

Duration of administration
No restriction. If symptoms persist or worsen, medical advice should be sought.

Contra-indications
Known hypersensitivity to plants of the Compositae.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

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Pregnancy and lactation
In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
In sensitive persons, irritation of the gastric mucosa might occur [Bradley 2006; Schilcher 2010].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antibacterial activity
Several ethanolic extracts [Pinkas 1978; Didry 1982; Schimmer 1988; Kreutzer 1990], resin fractions [Pinkas 1978; Didry 1982; Kreutzer 1990], a polyphenolic fraction [Pinkas 1978] and phenolic acids from grindelia [Didry 1982] inhibited the growth of various bacteria, including *Staphylococcus aureus* and *Bacillus subtilis*. No antibacterial activity was exhibited by isolated flavonoids [Schimmer 1988] or a saponin fraction [Kreutzer 1990].

Antifungal activity
In the plate diffusion test concentrations of up to 10 mg/plate of a mixture of bisdesmosidic grindelia saponins dose-dependently inhibited growth of the fungi *Candida tropicalis*, *Mucor mucedo*, *Trichoderma viride* and *Botrytis cinerea*. In another experiment, the same mixture inhibited the growth of three of these species with MIC values between 0.31 and 5.0 mg/plate (comparable to aescin) compared to 0.6 µg per plate for clotrimazole [Kreutzer 1995].

The essential oil of grindelia inhibited the growth of *Penicillium expansum*, *Aspergillus flavus*, *Trichoderma viride*, *Phomopsis* spp, *Monilia fructigena* and *Fusarium culmorum* by 9-83% in comparison with the control (econazole, 100%) when applied for 6 days at 2 µl per plate and by 71-83% at 30 µl per plate [Menghini 1995].

A methanolic extract at a concentration of 2 mg/mL agar, inhibited the growth of *Fusarium oxysporum*, *F. verticillioides*, *Penicillium expansum*, *P. brevicompactum*, *Aspergillus flavus* and *A. fumigatus* by 33-73% [Zabka 2012].

Antispasmodic activity
A fluid extract (1:1, ethanol 75%) and a polyphenolic fraction from grindelia exhibited mild antispasmodic activity on contractions of isolated guinea pig ileum. ED_{50} values for acetylcholine-, histamine-, serotonin- and bradykinin-induced contractions were respectively 150-200 µg/mL, 40 µg/mL, 10-50 µg/mL and 10-40 µg/mL for the fluid extract, and 150-200 µg/mL, 10-20 µg/mL, 10 µg/mL and 10 µg/mL for the polyphenolic fraction [Pinkas 1978]. However, this effect could not be confirmed in later research using a 50% V/V methanolic extract of grindelia; no antispasmodic activity was observed on spontaneous or acetylcholine- or barium chloride-induced contractions of isolated guinea pig ileum at concentrations up to 800 µg/mL [Izzo 1996].

Anti-inflammatory activity
Grindelia extracts of varying polarity have been tested in a neutrophil elastase assay. As observed earlier with various phenolic compounds [Melzig 2001], the extracts had a remarkable inhibitory effect (greater than 50%) at 1 mg dried extract/0.5 mL, indicating anti-inflammatory potential [Gehrmann 2002, 2003]. In the thrombin activity test the IC_{50} values of an acetone extract and a carbon dioxide extract were 330 and 500 µg/mL respectively [Gehrmann 2004].

In a murine macrophage model the inflammatory effects of *E.coli* lipopolysaccharide (LPS) were significantly mitigated by a methanolic extract of *Grindelia robusta*, including by reducing the expression of inducible NO synthase (iNOS), NO itself, TNF-α and IL-12 production (all at $p<0.05$), and IL-1β ($p<0.01$) [Verma 2010]. The extract also prevented LPS-mediated nuclear translocation of NF-κB [Verma 2010; La 2010].

Quercetin-3-methylether and 6-hydroxy-kaempferol-3,6-dimethylether inhibited the activity of neutrophil elastase with IC_{50} of 19 µM and 194 µM compared to a synthetic inhibitor (0.1 µM) [Krenn 2009].

In vivo experiments

Anti-inflammatory activity
A *Grindelia robusta* dry extract (80% ethanol) orally administered at 100 and 200 mg/kg b.w. dose-dependently inhibited carrageenan-induced rat paw oedema by 41% ($p<0.01$) and 63% ($p<0.001$) respectively, compared to 45% inhibition by indometacin at 5 mg/kg [Mascolo 1987].

The anti-inflammatory activity of a grindelia fluid extract (1:1, ethanol 75%) and a polyphenolic fraction was demonstrated in similar experiments. When administered intraperitoneally at 65 mg/kg 30 minutes before carrageenan injection, the extract inhibited rat paw oedema by 53% compared to 24% by lysine acetylsalicylate at 100 mg/kg and 30% by rutin at 100 mg/kg [Pinkas 1978].

Expectorant activity
In urethanized cats, rabbits and guinea pigs, respiratory tract fluid (RTF) was collected from the trachea 3 hours before and 4 hours after gastric administration of a grindelia fluid extract conforming to NF 7 (1942) at doses from 0.1 to 10 mL/kg body weight. The RTF output 2 hours after administration increased by 79% in cats while no effect was observed in rabbits or guinea pigs. Controls showed increases or decreases in RTF output of up to 30% over 4 hours [Boyd 1946].

Antispasmodic activity
A grindelia fluid extract (1:1, ethanol 75%) and a polyphenolic fraction showed no antispasmodic activity against histamine- or serotonin-induced bronchospasms after i.p. administration to guinea pigs. Only slight activity against acetylcholine- and bradykinin-induced bronchospasms was seen at higher doses (close to the toxic dose) [Pinkas 1978].

Clinical data
Grindelia preparations were used in earlier times to treat exanthema caused by intoxication with *Rhus toxicodendron* (poison oak) [Weiss 1966]. In a recent case study involving a woman suffering from poison oak dermatitis, application of a tincture (85% ethanol) from fresh flower buds of *Grindelia spp.* had an immediate effect, diminishing pruritus and decreasing transudation. The tincture mixed into a calendula cream base produced further relief [Canavan 2005].

Pharmacokinetic properties
No data available.

Preclinical safety data
No mortality occurred and no toxic effects were apparent in rats after a single oral dose of a *Grindelia robusta* dry extract (80% ethanol) at 2.5 g/kg b.w. [Mascolo 1987].

Intraperitoneal LD_{50} values in mice for a fluid extract and a polyphenolic fraction from grindelia were determined as 250 mg/kg and > 500 mg/kg b.w. respectively [Pincas 1978].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Second Edition, 2003
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Supplement 2009
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Second Edition, 2003
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Second Edition, 2003
FUMARIAE HERBA	Fumitory	Supplement 2009
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Supplement 2009
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Second Edition, 2003
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Second Edition, 2003

LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Supplement 2009
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Second Edition, 2003
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Second Edition, 2003
RATANHIAE RADIX	Rhatany Root	Supplement 2009
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Second Edition, 2003
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Second Edition, 2003
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Second Edition, 2003
SALICIS CORTEX	Willow Bark	Second Edition, 2003
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Second Edition, 2003
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Supplement 2009
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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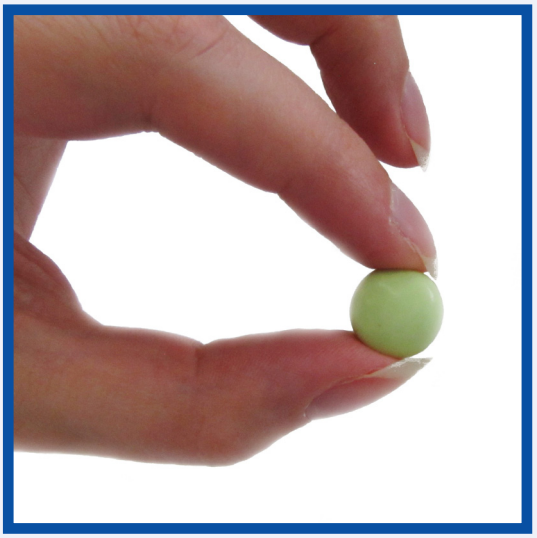
The Scientific Foundation for Herbal Medicinal Products

Hamamelidis aqua
Hamamelis Water

2012



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MONOGRAPHS

***The* Scientific Foundation for**
Herbal Medicinal Products

HAMAMELIDIS AQUA
Hamamelis Water

2012

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Hamamelidis aqua - Hamamelis Water

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

Edited by Simon Mills and Roberta Hutchins
Cover photograph by Simon Mills (*Hamamelis virginiana*)
Cover and text design by Martin Willoughby
Typeset in Optima by Roberta Hutchins

Plant illustrated on the cover: *Hamamelis virginiana*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

HAMAMELIDIS AQUA 2012

Hamamelis Water

DEFINITION

Hamamelis water is a clear, colourless distillate prepared from recently cut and partially dried dormant twigs of *Hamamelis virginiana* L.

The material complies with the monograph of the United States Pharmacopeia [Witch hazel].

CONSTITUENTS

From 1 kg of partially dried dormant twigs the USP method of preparation yields approximately 1 litre (980 g) of hamamelis water containing 14-15% of ethanol (added after distillation) [Witch hazel]. Since it is obtained by a distillation process, the constituents are those of the volatile fraction, devoid of tannins [Hoffmann-Bohm 1993; Bradley 2006].

Distillation of fresh twigs yielded 0.09% of volatile fraction on the dry weight basis, consisting of aliphatic hydrocarbons (45.4%, predominantly alkanes), terpenes (approx. 30%; mainly sesquiterpenes such as α-ylangene and monoterpenes such as linalool), phenylpropanoids (7.5%) such as *trans*-anethole and eugenol, and aliphatic aldehydes (6.1%) and alcohols (5.3%); over 160 compounds were detected [Hartisch 1996; Engel 1998; Bradley 2006].

Distillation of fresh leaves yielded 0.13% of volatile fraction on the dry weight basis consisting of aliphatic hydrocarbons (62.8%, predominantly alkanes), terpenes (21.1% including 3.7% of linalool and 9.8% of the acyclic diterpene *trans*-phytol), aliphatic aldehydes (3.8%) and, fatty acids and fatty acid esters (3.6%); over 170 compounds were detected [Hartisch 1996; Engel 1998; Bradley 2006].

CLINICAL PARTICULARS

Therapeutic indications

External use
Treatment of bruises, skin irritations, sunburn, insect bites, external haemorrhoids [Sorkin 1980; Pfister 1981; Witch Hazel 1982; Swoboda 1991; Hoffmann-Bohm 1993; Korting 1993; Korting 1995; Hughes-Formella 1998; Falch 1999; Hughes-Formella 2002; Welzel 2005; Wolff 2007; Bradley 2006; Bühring 2008; Welzel 2009; Schilcher 2010,]. Minor inflammatory conditions of the skin and mucosa [Sorkin 1980; Witch Hazel 1982; Hoffmann-Bohm 1993; Korting 1993; Hughes-Formella 1998; Bradley 2006; Wolff 2007; Bühring 2008; Schilcher 2010].

Efficacy in these indications is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

External use
For compresses on the skin: Hamamelis water undiluted or diluted 1:3 with water; in semi-solid preparations, 20-30% [Sorkin 1980; Pfister 1981; Moore 1989; Swoboda 1991; Hoffmann-Bohm 1993; Korting 1993; Korting 1995; Hughes-Formella 1998; Bradley 2006; Schilcher 2010]. Apply as often as required [Witch hazel 1982].
For mucosa: Hamamelis water undiluted or diluted with water, several times daily [Hoffmann-Bohm 1993; Bradley 2006; Schilcher 2010].

For children (0 to 16 years of age), application of the distillate diluted with water (1:4) is recommended [Bühring 2008].

Cream or ointment containing 5.35 or 6.25 % distillate (1:1-2; ethanol 6%), to be applied several times daily onto the respective areas [Welzel 2005; Wolff 2007; Welzel 2009]. The ointment is also suitable for children (from 4 weeks of age onwards) [Wolff 2007].

Method of administration
For local application.

Duration of administration
No restriction.

Contraindications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available.

Effects on ability to drive and use machines
None known.

Undesirable effects
Although the content of volatile fraction is very low, allergic reactions of the skin may occur in very rare cases [Witch Hazel 1982; Hoffmann-Bohm 1993; Granlund 1994].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In the following text, material prepared by distillation of partially dried dormant twigs of *Hamamelis virginiana* and complying with the monograph for Witch Hazel USP [Witch hazel] (or Hamamelis Water BPC 1973 [Hamamelis water], which is essentially similar) is described as 'hamamelis water'. Material prepared by distillation of fresh leaves and twigs of *Hamamelis virginiana* is described as 'hamamelis distillate'.

In vitro studies

Antimicrobial activity
In the agar diffusion test, a lotion consisting of 90 % hamamelis distillate and 5 % urea caused a weak inhibition of the growth of *Enterobacter aerogenes, Klebsiella pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Staphylococcus xylosis, Candida albicans, Candida krusei, Candida tropicalis, Mallassezia furfur, Saccharomyces cerevisiae* and *Torulopsis glabrata*. The lotion without hamamelis failed to demonstrate antimicrobial activity [Gloor 2002].

Pharmacological studies in humans

Anti-inflammatory effect
The activity of hamamelis distillate against erythema was evaluated in creams with two concentrations of the drug (containing 0.64 mg or 2.56 mg per 100 g of carbonyl compounds, sometimes called "hamamelis ketones") and two different vehicles: oil-in-water emulsions, with or without phosphatidylcholine (PC). The effects were compared with those of hydrocortisone 1% cream, four base preparations and an untreated control area in two randomized, double-blind studies, each involving 24 healthy volunteers; in one study erythema was induced by UV irradiation, in the other by repeated stripping of the horny layer with adhesive tape. 24 hours after UV-irradiation noteworthy reductions in erythema were observed only after use of low dose hamamelis-PC cream and hydrocortisone 1% cream, more pronounced from the latter. Erythema 4-8 hours after stripping of the horny layer was significantly suppressed by hydrocortisone cream, while less pronounced but noteworthy reductions were observed with low and high dose hamamelis-PC creams. The results demonstrated some anti-inflammatory activity of hamamelis distillate in a PC-containing vehicle. A four-fold increase of drug concentration did not, however, increase activity [Korting 1993].

The anti-inflammatory effects of an aftersun lotion containing 10% of hamamelis water, in comparison with the corresponding vehicle, were tested in 30 healthy volunteers using a UV-B erythema test at four different UV-B intensities. Chromametry was used to compare the degrees of erythema in treated areas of skin with those in irradiated but untreated control areas 7, 24 and 48 hours after irradiation. Erythema suppression ranged from approx. 20% at 7 hours to 27% after 48 hours in the hamamelis water-treated areas, and from 10% at 7 hours to 12% after 48 hours in areas treated with the vehicle. Hamamelis water led to a highly significant reduction in erythema compared to the vehicle ($p = 0.00001$) and untreated, irradiated skin ($p = 0.00001$) [Hughes-Formella 1998].

Hamamelis distillate ointment applied to the skin of 22 healthy volunteers, and also 5 patients suffering from atopic neurodermitis and psoriasis, had a mild anti-inflammatory effect, causing a decrease in blood circulation as indicated by measurements of the thermal conductivity of the skin [Sorkin 1980].

Three different lotions containing 10% hamamelis distillate (no further details available), two vehicles (distillate replaced by ethanol 15% or by water, respectively), dimethindene maleate 0.1 % gel, hydrocortisone 1 % cream and 0.25 % lotion were tested in 40 light-skinned volunteers undergoing a modified UV erythema test with UV dosages of 1.2, 1.4 and 1.7 MED. The test preparations were applied occlusively over a 48-hour period following irradiation. Differences in chromametric observations after 24, 48 and 72 hours were calculated as delta a* values. In erythema suppression, the hydrocortisone preparations were most effective (delta a* between 0.3 and 7.3). An anti-inflammatory effect was seen for all hamamelis preparations (delta a* between 1.8 and 8.2) as well as for the vehicle (delta a* between 1.7 and 8.6), compared to the irradiated untreated control (delta a* between 2.6 and 8.3). Compared to the erythema in the control group, the percentage suppression by the hamamelis lotions ranged from 1-6 % at 24 hours to 23-30% at 72 hours [Hughes-Formella 2002].

Antimicrobial effect
The antimicrobial activity of a lotion consisting of 90 % hamamelis distillate and 5 % urea was examined in an occlusion test (test areas of 4cm x 4cm on the subjects back) in 15 healthy volunteers. This test was followed by an expanded flora test in order to determine the same effect for a higher level of bacterial colonization. Bacteriological testing was performed 24 or 48 hours after starting the test, respectively. The lotion showed significant antimicrobial activity on aerobic bacteria in both tests as compared to untreated subjects ($p<0.001$), and appeared to be more effective than the lotion without hamamelis

distillate (significant p< 0.01 in the expanded flora test).No significant differences were found against anaerobic bacteria [Gloor 2002].

Clinical studies

Analgesic effect
In a randomised, open study involving 300 postnatal mothers, three topical agents were evaluated for their efficacy in achieving analgesia for episiotomy pain following instrumental (forceps) vaginal delivery: hamamelis water, or ice or a foam containing hydrocortisone acetate 1% and pramoxine hydrochloride 1%. Oral analgesics were permitted and taken to the same extent in all three groups. According to data collected from 266 women, the three topical agents were equally effective in achieving analgesia, with no significant differences on day 1, although from subjective and professional assessment about one-third of all mothers derived little benefit from any agent. On days 3 and 5 ice tended to be better. 126 mothers were further assessed after 6 weeks; no differences were found between the three groups in terms of healing, pain and intercourse patterns [Moore 1989].

Dermatological conditions
In a randomized, double-blind study, 22 patients suffering from atopic dermatitis were treated on one forearm with an ointment containing hamamelis distillate (250 mg/g) and on the other forearm with bufexamac ointment (50 mg/g) three times daily for 3 weeks. From assessment of symptoms such as reddening, scaling, lichenification, itching and infiltration, no statistical difference was observed between the two treatments; both forearms showed clear improvements [Swoboda 1991].

In a randomized, double-blind, paired trial, 72 patients suffering from moderately severe atopic eczema were treated for 2 weeks with a cream containing hamamelis distillate (5.35 g/100g), or the corresponding vehicle-only cream, or hydrocortisone 0.5% cream. The reduction in total scores for three basic criteria, itching, erythema and scaling, was significantly greater ($p<0.0001$) following application of hydrocortisone in comparison with hamamelis distillate, while the score for the hamamelis preparation did not differ from that of the vehicle [Korting 1995].

In another double-blind study, 116 patients with eczema of different aetiologies were treated with either ointment A containing hamamelis distillate (25 g/100g) or hamamelis preparation B (not defined, but presumed to contain distillate) as a control, or both preparations (applied to different hands), several times daily for 4-6 weeks. Improvements in the symptoms of itching, burning sensations, infiltration, reddening and scaling were observed in the majority of cases with both preparations; ointment A gave superior results with endogenous eczema but not with toxic-degenerative eczema [Pfister 1981].

A preparation containing 90% hamamelis water USP and 5 % urea was given as a compress twice daily to 39 patients suffering from seborrhoeic dermatitis. Scaling and erythema decreased, and in 80 % of the patients symptoms disappeared completely or almost completely after 8 weeks. Similar preparations with different lipid contents were applied as the only treatment to 40 otherwise pre-treated patients with dry and itching skin conditions. Symptoms disappeared or improved after 8 weeks in 65 % of the patients. Only 4 patients had to continue with steroids [Falch 1999].

In an open-label study 89 patients, of minimum 55 years of age, suffering from dry skin (lipid content less than 6 µg sebum/cm^2 skin, humidity ≤55 arbitrary units) received an ointment containing 6.25 % hamamelis distillate (1:1-2 distilled with ethanol 6% m/m) which was applied to the dry areas twice daily for 4 weeks. After 4 weeks the mean lipid content increased significantly from 2.4 to 59.8 µg sebum/cm^2 ($p<0.0001$), the mean skin humidity from 47.6 to 55.6 arbitrary units ($p<0.0001$). Scaling and fissures measured by visual scores were also reduced significantly ($p<0.0001$), and symptoms like skin tension, roughness and itching also improved ($p<0.0001$) [Welzel 2005].

In a similar open study, 35 patients suffering from various drug-induced skin symptoms such as dry skin, scaling, fissures, roughness and itching received the same preparation twice daily for 4 weeks. After this period, the mean lipid content increased significantly from 0.69 to 60.91 µg sebum/cm^2 ($p<0.001$), the mean skin humidity from 43.53 to 45.12 arbitrary units. Scaling and fissures measured by visual scores were also reduced ($p<0.001$), and symptoms like skin tension, roughness and itching improved ($p<0.001$) [Welzel 2009].

In a multicentre, prospective study in 309 children (between 27 days and 11 years of age) suffering from superficial skin injuries, local inflammations of the skin or diaper dermatitis, 231 children received an ointment containing 6.25 % hamamelis distillate (1:1-2 distilled with ethanol 6% m/m) and 78 children a dexpanthenol ointment, each at an individual dose for 7 to 10 days. For all diagnoses a significant ($p<0.0001$) improvement was seen between the beginning and the end of therapy. 83.5% of the physicians and 83.1 % of the parents assessed the efficacy of Hamamelis ointment as very good or good, the efficacy being comparable to dexpanthenol [Wolff 2007].

Pharmacokinetic properties
No data available.

Preclinical safety data
Based on studies performed in F344 rats and B6C3F1 mice which were topically administered 50 or 100% hamamelis water within the National Cancer Institute's (NCI) National Toxicology Program (NTP), hamamelis water is interpreted as showing no carcinogenic effects [Hasemann 1984].

Clinical safety data
In an open-label study in 89 patients as well as in a similar open study in 35 patients with various symptoms related to dry skin who received an ointment containing 6.25 % hamamelis distillate (1:1-2 distilled with ethanol 6% m/m) twice daily for 4 weeks, no serious adverse events were reported that might have had a causal relationship with the medication [Welzel 2005, Welzel 2009].

In a multicentre, prospective study in 231 children (between 27 days and 11 years of age) who received an ointment containing 6.25 g hamamelis distillate (1:1-2 distilled with ethanol 6% m/m) at an individual dose for 7 to 10 days, 99.1% of the physicians and 98.2 % of the parents assessed the tolerability of Hamamelis ointment as very good or good [Wolff 2007].

A preparation containing 90% hamamelis water USP and 5 % urea was given as a compress twice daily to 50 patients suffering from peri-anal dermatitis, and 50 patients suffering from eczematoid dermatitis. No allergic or other intolerance reactions were found [Falch 1999].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
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ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
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TARAXACI RADIX	Dandelion Root	Second Edition, 2003
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URTICAE RADIX	Nettle Root	Second Edition, 2003
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VALERIANAE RADIX	Valerian Root	Supplement 2009
VIOLAE HERBA CUM FLORE	Wild Pansy	Supplement 2009
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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Hamamelidis cortex

Hamamelis Bark

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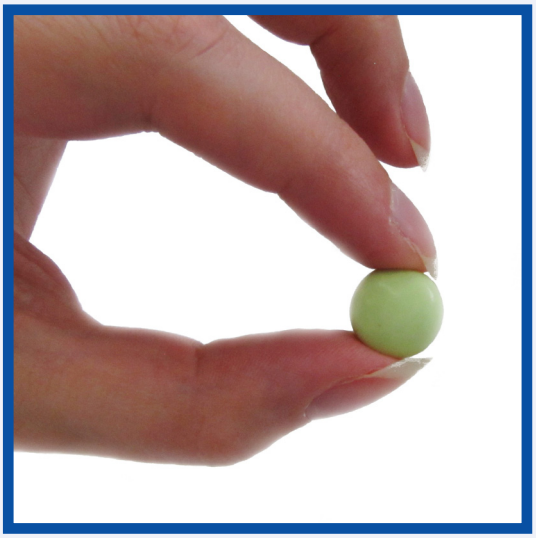

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Edited by Simon Mills and Roberta Hutchins
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Plant illustrated on the cover: *Hamamelis virginiana*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

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Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

HAMAMELIDIS CORTEX 2012

Hamamelis Bark

DEFINITION

Hamamelis bark consists of the dried bark from stems and branches of *Hamamelis virginiana* L., collected in spring. It contains not less than 4.0% of hide powder-precipitable tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) and calculated with reference to the dried drug.

The material complies with the monograph of the Deutscher Arzneimittel-Codex [Hamamelisrinde] or the British Herbal Pharmacopoeia [Hamamelis Bark].

CONSTITUENTS

The main characteristic constituent is hamamelitannin, a mixture of the α- and β-forms of 2',5-di-*O*-galloylhamamelose [Mayer 1965; Friedrich 1974; Vennat 1988; Hoffmann-Bohm 1993; Haberland 1994; Hartisch 1996; Wang 2003; Bradley 2006]. Proanthocyanidins are also present including: procyanidin dimers such as catechin-(4α→8)-catechin, 3-*O*-galloyl-epicatechin-(4β→8)-catechin and epicatechin-(4β→8)-catechin-3-*O*-(4-hydroxy)benzoate [Friedrich 1974; Vennat 1988; Hartisch 1996a,b]; prodelphinidins such as epigallocatechin-(4β→8)-catechin, 3-*O*-galloyl epigallocatechin-(4β→8)-catechin and 3-*O*-galloyl epigallocatechin-(4β→8)-gallocatechin [Hartisch 1996a,b; Bradley 2006]; and proanthocyanidin oligomers consisting of 4-9 catechin/gallocatechin units, some of which are 3-*O*-galloylated [Hartisch 1996b; Hartisch 1997; Dauer 1998; Dauer 2003b; Bradley 2006].

Other constituents include flavan-3-ols such as (+)-catechin, (+)-gallocatechin, (–)-epicatechin-3-*O*-gallate, and (–)-epigallocatechin-3-*O*-gallate [Friedrich 1974; Hartisch 1996a; Wang 2003; Bradley 2006]; di-and tri-*O*-galloyl-hamameloses and related 4-hydroxybenzoates [Haberland 1994; Hartisch 1996a,b], pentagalloyl glucose [Friedrich 1974], gallic acid [Friedrich 1974; Vennat 1988; Wang 2003; Bradley 2006] and about 0.1% of volatile oil [Hoffmann-Bohm 1993; Bradley 2006].

CLINICAL PARTICULARS

Therapeutic indications

Internal use
Inflammation of mucous membranes of the oral cavity [Hoffmann-Bohm 1993; Laux 1993; Hiller 2009].
Short-term symptomatic treatment of diarrhoea [Hamamelis Bark; Laux 1993; Bradley 2006].

External use
Haemorrhoids [Reynolds 1982; Van Hellemont 1988; Hoffmann-Bohm 1993; Laux 1993; Bradley 2006, Schilcher 2007; Hiller 2009], minor injuries and local inflammations of the skin [Reynolds 1982; Hoffmann-Bohm 1993; Laux 1993; Hörmann 1994; Millikan 2003; Bradley 2006; Bühring 2008; Hiller 2009; Schilcher 2010].
Symptomatic treatment of problems related to varicose veins, such as painful and heavy legs [Van Hellemont 1988; Hoffmann-Bohm 1993; Bradley 2006; Hiller 2009; Schilcher 2010].

Efficacy in these indications is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Internal use
2-10 g of the drug daily as a decoction, used as a mouthwash [Van Hellemont 1988; Hoffmann-Bohm 1993; Bradley 2006; Hiller 2009; Schilcher 2010], or

2-3 g daily as a tea [Bradley 2006; Hiller 2009].
2-4 ml of tincture, used diluted as a mouthwash 3 times daily [Hamameils Bark; Bradley 2006].
Other preparations: the equivalent of 0.1-1 g of the drug, 1-3 times daily [Hoffmann-Bohm 1993; Bradley 2006; Hiller 2009; Schilcher 2010].

External use
5-10 g of the drug as a decoction in 250 ml of water [Hoffmann-Bohm 1993; Bradley 2006; Bühring 2008].
Use of the decoction (e.g. for compresses and baths) is also recommended in children [Bühring 2008].
Extracts in semi-solid or liquid preparations corresponding to 20-30% of the drug [Hoffmann-Bohm 1993].

Method of administration
For oral administration or local application.

Duration of administration
No restriction. Medical advice should be sought if diarrhoea persists for more than 3 days.

Contraindications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used internally during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
In sensitive persons, stomach irritation may occasionally occur after intake of hamamelis bark preparations [Hoffmann-Bohm 1993]. Chamomile-sensitive persons might also react to hamamelis preparations after topical application [Paulsen 2008].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro studies

Astringent effect
The astringent effect of a tincture (1:3; 62% ethanol) prepared from fresh hamamelis bark was demonstrated with hide powder [Gracza 1987].

Antibacterial activity
Hamamelitannin did not affect growth of *Staphylococcus aureus* and *Staphylococcus epidermidis* even at high concentrations, but significantly ($p<0.05$) down-regulated RNAIII production in reporter cells at a dose of 50 µg in both strains. Furthermore, it inhibited bacterial cell attachment of both strains in a concentration-dependent manner and reduced delta-haemolysin production in a Western blotting test. [Kiran 2008].

Cytotoxic activity
After 4 days of incubation, polyphenols isolated from hamamelis bark showed moderate cytotoxicity to GLC_4 lung carcinoma and COLO 320 cells. The 3-*O*-galloyl compounds were more effective than other compounds. IC_{50} values of galloyl compounds were between 38 µM and 110 µM for GLC_4 and between 18.3 µM and 90.8 µM for COLO 320 cells; almost complete inhibition of growth was observed at 200 µM [Hartisch 1996a].

Polymeric proanthocyanidins from Hamamelis bark, in contrast to polysaccharides thereof, significantly ($p<0.1$, $p<0.05$) increased proliferation of human keratinocytes after 9 days of incubation at doses of 1 and 10µg/ml, respectively. There was no influence on the differentiation towards cornified cells [Deters 2001].

Polyphenolic fractions from Hamamelis bark (containing catechins, proanthocyanidins and gallotannins) inhibited cell proliferation in HT29 and HCT116 human colon cancer cell lines with average IC_{50} values of 28 and 29 µg/ml, respectively; the highly galloylated fractions being the most effective. In the same way, these fractions were able to arrest different phases of the cell cycle and to induce early apoptosis in HT29 cancer cells [Lizzárraga 2008].

Fractions rich in pyrogallol-containing polyphenols (proanthocyanidins, gallotannins, gallates) protected red blood cells from free radical-induced haemolysis in a dose-dependent manner with IC_{50} values between 21.5 and 24.5 µg/ml. They were mildly cytotoxic to HaCat keratinocytes (IC_{50} between 38 and 68 µg/ml) and 3T3 fibroblasts (IC_{50} between 33 and 51 µg/ml), and also inhibited the proliferation of tumoural SK-Mel 28 melanoma cells (IC_{50} between 26 and 39 µg/ml) [Touriño 2008].

Cell and DNA-protective effects
Catechin, hamamelitannin and two proanthocyanidin fractions prepared from Hamamelis bark were investigated in human Hep G2 cell lines using single cell gel electrophoresis (SCGE) for the detection of DNA-damage. Catechin and a low-molecular weight proanthocyandin fraction (W_M) caused only slight increases in DNA-migration up to concentrations of 166 µg/ml whereas hamamelitannin and the proanthocyandin fraction with higher molecular weight (W_A) dose-independently led to a two-fold enhancement of DNA-migration at the same concentrations. Treatment of the cells with the test compounds in a dose-range of 2–166 µg/ml prior to the exposure to 10 µM (2.5 µg/ml) benzo(a)pyrene (B(a)P) led to a significant reduction of induced DNA damage. The inhibitory effects of proanthocyanidins were stronger than those of catechin and hamamelitannin; the lowest effective concentrations were about 2 µg/ml [Dauer 2003a].

Hamamelitannin at concentrations of 1 to 100 µM inhibited TNF-mediated cell death and DNA fragmentation in a dose-dependent manner with a 100% protection at concentrations higher than 10µM. The protective effect was comparable to that of epigallocatechin gallate, and higher than gallic acid with less than 40% protection. However, hamamelitannin at concentrations of 1 to 100 µM did not alter the TNF-induced upregulation of endothelial adhesiveness [Habtemariam 2002].

Anti-inflammatory effects
In the lyso-PAF:acetyl-CoA acetyltransferase assay, hamamelitannin proved to be ineffective [Hartisch 1997], but in the same assay a proanthocyanidin oligomer isolated from hamamelis bark showed inhibitory potential [Hartisch 1996a, 1997]. A range of compounds from hamamelis bark had an inhibitory effect on 5-lipoxygenase (from a cytosol fraction of RBL-1 cells), galloyl compounds showing greater potency than

other substances; hamamelitannin had the strongest effect with an IC_{50} of 1.0 µM [Hartisch 1996a, 1997].

Anti-inflammatory effects of polyphenols isolated from hamamelis stem and twig bark were evaluated in human polymorphonucleocytes (PMNs) and human macrophages. With the exception of hamamelitannin, all the tested substances inhibited the synthesis of platelet activating factor (PAF) in human PMNs. Dimeric galloylated proanthocyanidins showed the strongest effects with IC_{50} values of 7.8 and 6.4 µM. The synthesis of leukotriene B_4 (LTB_4) in PMNs was inhibited by the tested substances. Oligomeric proanthocyanidins had stronger activity (IC_{50}: 1.5 µM) than hamamelitannin, which had the weakest effect (IC_{50}: 12.5 µM). The polyphenols were shown to inhibit zymosan-induced luminol-dependent chemiluminescence in human macrophages, with galloylated proanthocyanidins having stronger effects (IC_{50}: 2.3 and 2.0 µM) than hamamelitannin (IC_{50}: 10.5 µM) [Hartisch 1996a].

Antiviral activity
Hamamelitannin and fractions obtained by ultrafiltration from a hydroethanolic extract of hamamelis bark exhibited antiviral activity against *Herpes simplex* virus type 1 in monkey kidney cells. After 2-3 days the ED_{50} of hamamelitannin for antiviral activity was 26 µg/ml, compared to 6.3 µg/ml for a fraction consisting mainly of oligomeric to polymeric proanthocyanidins and 0.42 µmol/ml for acyclovir as a positive control [Erdelmeier 1996].

Radical-scavenging effects
A dry 50%-ethanolic extract from hamamelis bark exhibited active-oxygen scavenging activity, determined by an electron spin resonance (ESR) spin-trapping technique, with IC_{50} values of 0.17 µg/ml for superoxide anions, 7.79 µg/ml for hydroxyl radicals and 44.08 µg/ml for singlet oxygens, compared to 4.10, 3.30 and 21.18 µg/ml respectively for ascorbic acid. The extract at 50 µg/ml also protected murine dermal fibroblasts from cell damage induced by active-oxygen, increasing the survival rate to 69.0% ($p<0.01$) compared to about 15% for the control [Masaki 1995].

A suppressive effect of hamamelitannin against depolymerization of hyaluronic acid (induced by a xanthine/xanthine oxidase system) was demonstrated by measuring the viscosity of a 0.9 mg/ml solution; the inhibitory rate was 73.8% for hamamelitannin compared to 24.7% for ascorbic acid and 84.4% for superoxide dismutase [Masaki 1993].

Polyphenolic fractions from Hamamelis bark induced a dose-dependent protection against DNA damage in the hydroxyl radical system at doses from 10-100 µM, and showed oxygen radical scavenging activity as detected by (ESR) spectroscopy, being most effective at a dose of 50µM [Lizzárraga 2008].

The radical scavenging properties of hamamelitannin and gallic acid were evaluated in further experiments using ESR spin-trapping. For superoxide anion scavenging, the IC_{50} values were 1.31 µM for hamamelitannin and 1.01 µM for gallic acid, compared to 23.31 µM for ascorbic acid [Masaki 1993, 1994, 1995]. In hydroxyl radical scavenging, hamamelitannin gave the lowest IC_{50} of 5.46 µM, compared to 78.04 µM for gallic acid and 86.46 µM for propyl gallate (a well-known antioxidant). In singlet oxygen scavenging, the IC_{50} values of hamamelitannin and gallic acid were 45.51 µM and 69.81 µM respectively, compared to 66.66 µM for propyl gallate [Masaki 1994].

Fractions rich in pyrogallol-containing polyphenols (proanthocyanidins, gallotannins, gallates) were strong free radical scavengers against ABTS (6mmol Trolox equiv/g), DPPH (ED_{50} between 26.1 and 58.8 µg fraction/µmol radical) and tris-(2,4,6-trichloro-3,5-dinitrophenyl)-methyl HNTTM radical (ED_{50} between 38.2 and 86.2 µg/µmol) [Touriño 2008]. In a similar experiment, fractions of polyphenols with different degrees of galloylation and polymerization demonstrated protection against erythrocyte lipid peroxidation, haemolysis and 3T3 cytotoxicity caused by H_2O_2 at concentrations of 25, 50 and 75 µg/mL [Mitjans 2011].

The ability of polyphenolic fractions from Hamamelis bark to reduce the α-tocopheroxyl radical was investigated in a homogenous hexane system and a phospholipid-like system based on sodium dodecyl sulfate (SDS) micelles. Tocopheroxyl radicals were monitored and quantified by ESR spectroscopy in the absence and presence of phenolic substances. Polyphenolic fractions from hamamelis reduced 80% of α-tocopheroxyl radicals in the hexane system and approximately 90% in SDS micelles [Pazos 2009].

Hamamelitannin was also found to have antioxidative and scavenging activities against organic radicals such as 1,1-diphenyl-2-picrylhydrazyl (DPPH). Expressed as an index number (the number of mol required to scavenge one mol of DPPH), hamamelitannin and gallic acid gave results of 9.4 and 8.8 respectively, compared to 2.2 for DL-α-tocopherol and 2.0 for ascorbic acid [Masaki 1994].

The protective activities of hamamelitannin and gallic acid on cell damage induced by superoxide anion radicals were evaluated in a cell-culture system using murine fibroblasts. Hamamelitannin and gallic acid showed significant protective activity against superoxide radicals at minimum concentrations of 50 µM and 100 µM respectively ($p<0.01$) [Masaki 1994, 1995]; at 50 µM, hamamelitannin enhanced the survival of fibroblasts to 52.4% compared to 36.9% for the control [Masaki 1995]. Pre-treatment of fibroblasts with hamamelitannin at 200 µM for 24 hours at 37°C before exposure to superoxide anions increased cell survival to 63.8%, compared to 25.4% for gallic acid and 19.0% for the control. Further observations confirmed that hamamelitannin is superior to gallic acid in protecting against cell damage induced by superoxide anions and suggested that the high affinity of hamamelitannin for cells or membranes may be an important factor for protecting cells against active oxygen species [Masaki 1995].

In contrast, against cell damage induced in murine fibroblasts by hydroxyl radicals, hamamelitannin showed protective activity at a minimum concentration of 500 µM whereas gallic acid was effective at 50 µM. Against cell damage induced by singlet oxygens hamamelitannin at 100 µM enhanced survival to 80.6% ($p<0.01$), while gallic acid had no significant effect at 100 µM and required 500 µM to enhance survival to 98.6% ($p<0.01$) compared to 60.4% survival for controls [Masaki 1994].

Hamamelitannin and a fraction of molecular weight < 3 kDa obtained by ultrafiltration from a hydroethanolic hamamelis bark extract were found to have greater radical scavenging activity (ED_{50} values of 29 and 80 ng/ml respectively) than a higher molecular weight procyanidin fraction (≥ 3 kDa; ED_{50} 160 ng/ml) as quantified by the emission of chemiluminescence during autoxidation of mouse brain lipids [Erdelmeier 1996].

An extract from Hamamelis leaf and bark (ethanol 60%) showed antioxidant activity in the ABTS assay in a dilution of 1:10000 with an activity equivalent to 40 µM of trolox [Pereira da Silva A 2000].

Hamamelitannin showed strong peroxynitrite (ONOO-)-scavenging properties at a concentration of 5.0 µg/ml in an

experiment using dihydrorhodamine 123 as a substrate for oxidation [Choi 2002].

Antimutagenic activity
In the Ames mutagenicity test, a tincture (1:5) and a methanolic extract (1:5) of hamamelis bark dose-dependently inhibited 2-nitrofluorene-induced mutagenicity in *Salmonella typhimurium* TA98, by 60% and 54% respectively at 100 µl/plate. It was demonstrated that the antimutagenic effect increased with increasing degree of polymerisation of proanthocyanidins, the most active fraction consisting of catechin and gallocatechin oligomers with an average degree of polymerization of 9.2 [Dauer 1998].

In vivo studies

Anti-inflammatory effect
A hydroethanolic extract of hamamelis bark showed a significant anti-inflammatory effect (43% inhibition of oedema; $p<0.05$) in the croton oil ear oedema test in mice when applied topically at 250 µg per ear. After ultrafiltration of the crude extract, this effect was shown to be mainly due to proanthocyanidins of molecular weight ≥ 3 kDa (69% inhibition at 250 µg per ear; $p<0.05$); proanthocyanidins of lower molecular weight had no effect and hamamelitannin produced only 7% inhibition [Erdelmeier 1996].

Anti-bacterial effect
Preincubating grafts contaminated with *Staphylococcus aureus* and *Staphylococcus epidermidis* with hamamelitannin for 7 days prevented infections in male Wistar rats (n=10). No bacteria were found after preincubation with >20µg oh hamamelitannin, while the bacterial load in the untreated control group was 10^7 CFU/ml. In a parallel experiment, grafts soaked with increasing hamamelitannin concentrations were implanted into the animals and bacteria injected onto the graft. Hamamelitannin caused a significant ($p<0.05$) decrease in bacterial load, whereas in the untreated control group 10^7 CFU/ml were found. No bacterial load was found in grafts soaked with 30 mg/l hamamelitannin [Kiran 2008].

Pharmacological studies in humans
The irritant sodium lauryl sulphate was applied at 0.5% twice daily for 3 days under patch occlusion to seven healthy volunteers who also received a semi-solid preparation containing 1 % of hamamelis procyanidin or placebo. Treatment with the preparation reduced increased transepidermal water loss by 53% as compared to placebo (45%). The treatment also increased the clinical scoring of dermal inflammation better than placebo and reduced erythema formation [Deters 2001].

Pharmacokinetic properties
No data available.

Preclinical safety data
No data available.

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SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
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TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Second Edition, 2003
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VIOLAE HERBA CUM FLORE	Wild Pansy	Supplement 2009
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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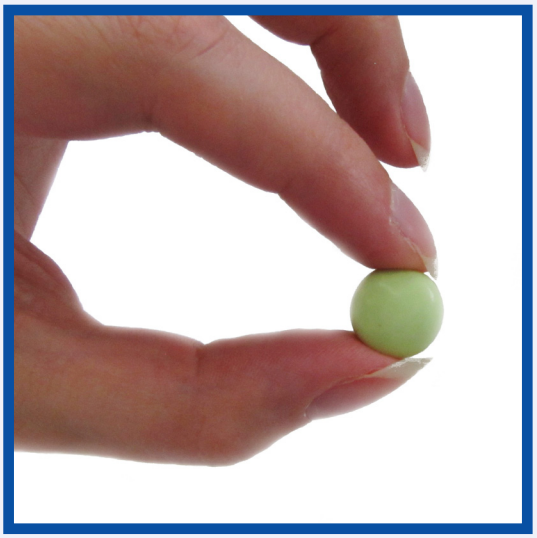
Hamamelis Leaf

2012



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***The* Scientific Foundation for**
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Hamamelis Leaf

2012

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

Edited by Simon Mills and Roberta Hutchins
Cover photograph by Simon Mills (*Hamamelis virginiana*)
Cover and text design by Martin Willoughby
Typeset in Optima by Roberta Hutchins

Plant illustrated on the cover: *Hamamelis virginiana*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
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Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
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Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

HAMAMELIDIS FOLIUM 2012

Hamamelis Leaf

DEFINITION

Hamamelis leaf consists of the whole or cut, dried leaf of *Hamamelis virginiana* L. It contains not less than 3 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) and calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Hamamelis Leaf].

CONSTITUENTS

The main characteristic constituents are tannins (5-10%) [Egerer 2005; Bradley 2006; Hiller 2009], including condensed tannins (mainly proanthocyanidin oligomers with catechin and/or gallocatechin units) and hydrolysable gallotannins, notably a small amount of hamamelitannin [Vennat 1988, 1992; Scholz 1994; Wang 2003; Bradley 2006; Hiller 2009, Duckstein 2011]. (+)-Catechin, (+)-gallocatechin, (–)-epicatechingallate and (–)-epigallocatechingallate are also present [Friedrich 1974; Bradley 2006, Duckstein 2011].

Other constituents include flavonoids such as kaempferol, quercetin, quercitrin, isoquercitrin and myricetin; phenolic acids such as caffeic acid and gallic acid [Bernard 1977; Vennat 1992; Hoffmann-Bohm 1993; Laux 1993; Bradley 2006; Hiller 2009, Duckstein 2011]; and a volatile fraction, 0.04-0.14% [Messerschmidt 1971; Bradley 2006; Hiller 2009], containing aliphatic hydrocarbons (63%), mono- and sesquiterpenes (11%) and aldehydes and ketones (4.6%) among over 170 compounds detected [Engel 1998; Bradley 2006].

CLINICAL PARTICULARS

Therapeutic indications

Internal use
Symptomatic treatment of complaints related to varicose veins, such as painful and heavy legs, and of haemorrhoids [Bernard 1977; Van Hellemont 1988; Hoffmann-Bohm 1993; Laux 1993; Hörmann 1994; MacKay 2001; Egerer 2005; Hiller 2009; Schilcher 2010].

External use
Bruises, sprains and minor injuries of the skin [Hoffmann-Bohm 1993; Laux 1993; Bradley 2006; Bühring 2008; Hiller 2009].
Local inflammations of the skin and mucosa [Bernard 1977; Hoffmann-Bohm 1993; Laux 1993; Millikan 2003; Bradley 2006; Bühring 2008; Hiller 2009; Schilcher 2010].
Haemorrhoids [Hoffmann-Bohm 1993; Laux 1993; Hamamelis Leaf (BHP) 1983; Bradley 2006; Hiller 2009; Schilcher 2010].
Relief of the symptoms of neurodermitis atopica [Wokalek 1993; Bradley 2006] and feeling of heavy legs [Hoffmann-Bohm 1993].

Efficacy in these indications is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Internal use
Adults: 2-3 g of drug as infusion [Hoffmann-Bohm 1993; Bradley 2006; Hiller 2009] or 2-4 ml of liquid extract (1:1, 45% ethanol), three times daily [Hamamelis Leaf (BHP) 1983; Bradley 2006].

External use
Extracts in semisolid or liquid preparations, containing 5-10% of drug [Hoffmann-Bohm 1993; Bradley 2006; Schilcher 2010].

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Decoctions, 5-10 g of drug per 250 ml water for compresses or washes [Hoffmann-Bohm 1993; Bradley 2006; Hiller 2009; Schilcher 2010].
Suppositories containing 200 mg of dried extract, 1-2 per day [Bradley 2006; Schilcher 2010].
Ointment containing 10% of liquid extract [Bradley 2006; Schilcher 2010].
The use of the decoction (e.g. for compresses or bathes) is also recommended in children [Bühring 2008]

Method of administration
For oral administration and topical application.

Duration of administration
If symptoms persist or worsen, medical advice should be sought.

Contraindications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, the drug should not be used internally during pregnancy without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
In sensitive patients there is a possibility of stomach upsets after taking hamamelis leaf preparations [Hoffmann-Bohm 1993]. Chamomile-sensitive persons might also react to hamamelis preparations after topical application [Paulsen 2008].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments
A lyophilized 10% decoction and a methanolic dry extract of Hamamelis leaf both exerted antibacterial activity on various periodonthopathic bacteria such as *Eikenella corrodens*, *Actinomyces odontolyticus*, *Porphyromonas gingivalis* and *Prevotella* spp.; the methanolic extract being more potent with MICs from 32 µg/mL onwards [Iauk 2003].

An extract from Hamamelis leaf and bark (ethanol 60%) showed antioxidant activity in the ABTS assay in a dilution of 1:10000 with an activity equivalent to 40 µM of trolox [Pereira da Silva A 2000].

In vivo experiments
Venotonic activity was demonstrated in experiments where the dried residue (300 mg) from aqueous or various hydroethanolic extracts of hamamelis leaf was added to one litre of an isotonic dextran/water (60 g/litre) solution. This was perfused at constant pressure into the arteries of the hind quarters of rabbits (45-100 drops/min). Venoconstriction, measured in terms of output on the venous side, was reduced by up to 60-70% depending on the type of extract [Bernard 1972].

A dry 70%-ethanolic extract of hamamelis leaf, administered orally to rats at 200 mg/kg daily for 19 days, significantly inhibited paw swelling ($p<0.05$) in the chronic phase of adjuvant-induced arthritis but was not active against the acute phase of oedema [Duwiejua 1994].

Pharmacological studies in humans
In a study conducted on 30 human volunteers, topical application of a hydroglycolic extract of hamamelis leaf produced a significant reduction in skin temperature ($p<0.001$ after 5 min; $p<0.03$ after 60 min), which was interpreted as a vasoconstrictor effect [Diemunsch 1987].

Clinical studies
In a pilot study involving cases of neurodermitis atopica, a cream containing hamamelis leaf extract was applied twice daily for 2 weeks in six groups of patients:
- Group I consisted of 7 children aged from 6 to 14 years with atopic neurodermitis on the feet (chilblains). After treatment the condition had considerably improved in all the patients.
- Group II consisted of 5 children with eczema in the flexure of the joints in subacute and chronic forms. After treatment 3 children were completely cured; in 2 children a considerable reduction of the inflamed condition and a clear reduction of the itch was noted.
- In Group III, which consisted of 10 adults with eczema in the flexure of the joints, 7 cases showed a good response and in three cases there was reasonable improvement.
- Group IV consisted of 5 adults with eczema of the neck and throat. 3 cases were completely cured and a remarkable reduction in symptoms was noted in the remaining 2 cases.
- Group V consisted of 3 adults with atopic eczema of the trunk. In one patient there was extensive healing and in the others a noticeable reduction of symptoms.
- Group VI consisted of 2 cases of atopic xerodermia. Following twice daily application of the cream, there was an improvement of the skin barrier situation and a clear diminution of desquamating pruritus in both cases [Wokalek 1993].

Pharmacokinetic properties
No data available.

Preclinical safety data

Carcinogenicity
NIH black rats (15 male and 15 female) were injected subcutaneously with a lyophilised aqueous leaf extract at a dosage of 10 mg (dissolved in 0.5 ml of saline solution) weekly for a period of 78 weeks; 30 animals were injected with saline solution only. The animals were observed for a period of 90 weeks. After about 73 weeks, three of the male rats developed malignant mesenchymoma, while in the control animals no tumours developed. The tumour rate was not considered to be significant [Hoffmann-Bohm 1993].

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MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Supplement 2009
ALOE CAPENSIS	Cape Aloes	Second Edition, 2003
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Second Edition, 2003
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
BALLOTAE NIGRAE HERBA	Black Horehound	Supplement 2009
BETULAE FOLIUM	Birch Leaf	Second Edition, 2003
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Second Edition, 2003
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
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CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Supplement 2009
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Second Edition, 2003
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Second Edition, 2003
FUMARIAE HERBA	Fumitory	Supplement 2009
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Supplement 2009
GRINDELIAE HERBA	Grindelia	Supplement 2009
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Second Edition, 2003
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Second Edition, 2003
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003

LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Supplement 2009
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Second Edition, 2003
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Second Edition, 2003
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Second Edition, 2003
RATANHIAE RADIX	Rhatany Root	Supplement 2009
RHAMNI PURSHIANI CORTEX	Cascara	Second Edition, 2003
RHEI RADIX	Rhubarb	Second Edition, 2003
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Second Edition, 2003
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Second Edition, 2003
SALICIS CORTEX	Willow Bark	Second Edition, 2003
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Second Edition, 2003
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VIOLAE HERBA CUM FLORE	Wild Pansy	Supplement 2009
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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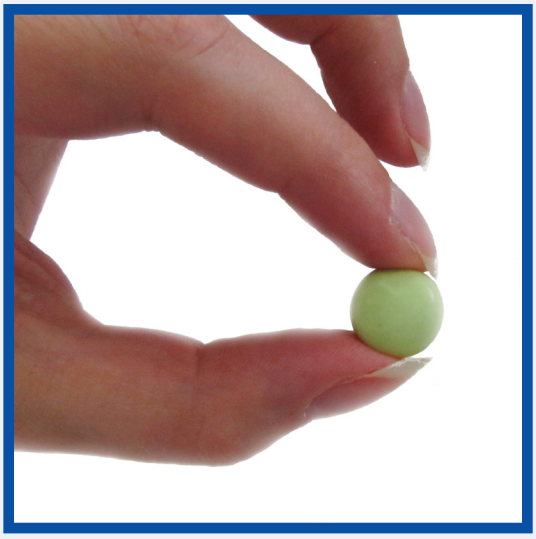
The Scientific Foundation for Herbal Medicinal Products

Helichrysi flos
Sandy Everlasting Flower

2019



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HELICHRYSI FLOS
Sandy Everlasting Flower

2019

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Plant illustrated on the cover: *Helichrysum arenarium*

FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

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Information on the member organizations and people involved in the production of ESCOP monographs and other activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

HELICHRYSI FLOS

2019

Sandy Everlasting Flower

DEFINITION

Sandy everlasting flower consists of the whole or cut, dried, fully opened flowers of *Helichrysum arenarium* (L.) Moench. It contains not less than 0.6 percent of flavonoids, calculated as hyperoside ($C_{21}H_{20}O_{12}$, Mr. 467.4) with reference to dried drug [DAC/NRF] or calculated as quercetin ($C_{15}H_{10}O_{7}$, M_r 302.24) with reference to dried drug [Farmakopea Polska].

The material complies with the monograph of the Farmakopea Polska or DAC/NRF.

CONSTITUENTS

The main constituents are the flavonoids naringenin, luteolin, quercetin, kaempferol, apigenin and their glucosides, isosalipuroside [Mericli 1986, Jarzycka 2013, Czinner 2000], hispidulin [Babotă 2018], essential oil containing mainly sesquiterpene hydrocarbons, oxygenated monoterpenes, δ-cadinene trans-caryophyllene and carvacrol [Lemberkovics 2001, Radušiene 2008], coumarins such as umbelliferone, scopoletin, helichrysin A, helichrysin B, aesculetin [Derkach 1986], phenolic acids such as caffeic acid, shikimic acid [Dombrowicz 1992, Czinner 2000], dicaffeoylquinic acid derivatives, chlorogenic acid, crypto-chlorogenic acid, neochlorogenic acid [Jarzycka 2013], everlastosides [Morikawa 2009a], arenariumosides [Morikawa 2009b], tumoroside and arenariumosides in the form of dimeric dihydrochalcone glycosides [Morikawa 2015].

CLINICAL PARTICULARS

Therapeutic indications
Symptomatic treatment of digestive disorders [Ożarowski 1987; Teuscher 2016].

In these indications, the efficacy is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Adults:
1.5 g of comminuted herbal substance in 150-250 mL of water as a decoction for 10 minutes, 2 - 3 times daily, or 3 g of comminuted herbal substance, in 150-200 mL of boiling water, as an infusion, 1 - 3 times daily [Ożarowski 1986, Teuscher 2016].

Method of administration
For oral administration.

Duration of use
No restriction.

Contra-indications
Known sensitivity to members of the Asteraceae (Compositae).

Special warnings and precautions for use
None required.

Interaction with other medicinal products and other forms of interaction
None reported.

Pregnancy and lactation
No data available.

In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

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Effects on ability to drive and use machines
Not relevant.

Undesirable effects
None reported.

Overdose
Not applicable.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antibacterial activity
The inhibition of *Mycobacterium tuberculosis* with various degrees of drug resistance was demonstrated by an ethanolic extract of dried flowers (not further specified) [Skvortsova 2015].

The effect of a methanolic extract (total phenolic content 160.17 mg/g) on lower respiratory tract pathogens was investigated. *Staphylococcus aureus* ATCC 25923 was more susceptible to the extract than *Streptococcus pneumoniae* ATCC49619 (MIC= 0.62 and 1.25 mg/mL respectively). The extract showed an MIC of 2.5 mg/mL against methicillin-resistent *S. aureus* and penicillin-resistent *S. pneumoniae*. The activity was higher against ampicillin-resistent *Moraxella catarrhalis* isolate (MIC=0.15 mg/mL) [Gradinaru 2014].

Antioxidant and radical-scavenging effects
Lyophilised aqueous extracts from two different samples (total polyphenol content: 10.6±0.6 g% and 17.6±0.7 g% respectively; total flavonoid content: 0.57±0.04 g% and 2.0±0.2 g% respectively) were investigated for their effect on microsomal lipid peroxidation in rat liver microsomes. NADPH+Fe^{3+}-induced lipid peroxidation in liver microsomes was dose-dependently decreased by the extracts to a significantly (P_1=0.003, P_2=0.005) greater extent than found for silibinin. The extracts also concentration-dependently stimulated NADPH cytochrome P450 reductase activity; although both extracts had a significantly (P_1= 0.017, P_2=0.018) greater effect than silibinin, the extract with a higher content of polyphenols and flavonoids demonstrated greater activity [Czinner 2001].

The antioxidant properties of two different lyophilised aqueous extracts (total flavonoid content: 0.57±0.06 g% (1) and 2.0±0.2 g% (2); total polyphenol content: 10.7±0.6 g% (1) and 17.6±0.7% (2)) were compared to silibinin using DPPH in a hydrogen-donating ability assay, and also measured by reducing power compared to ascorbic acid. Total scavenger capacity was detected with a chemiluminometric method, the H_2O_2/OH• luminol-microperoxidase system. Lyophilisate 2 proved to be more effective (IC_{50}=0.14±0.015 mg) at scavenging DPPH radicals compared to lyophilisate 1 (IC_{50}=0.36±0.015 mg) and silibinin standard dose (IC_{50}=0.65±0.08 mg). Lyophilisate 2 showed greater reducing power (1049.0 ± 2.86 ascorbic acid equivalents (ASE/mg)) compared with silibinin (447.7±5.16 ASE/mg) and lyophilisate 1 (379.7±4.21 ASE/mg). The total scavenging activity of lyophilisate 2 (IC_{90}=0.154µg) was much greater than silibinin (IC_{90}=194µg) 2 and lyophilisate 1 (IC_{90}=230µg) [Czinner 2000].

UV-protecting effects
The UVA/UVB protecting effects of an ethyl acetate fraction of an extract rich in chlorogenic acid (265.3±7.5 mg of gallic acid equivalent per g of extract) and flavonoids (84.0±3.2 mg of quercetin equivalent per g of extract) were studied. The fraction showed protective effects (PF-UVA $_{\text{in vitro}}$ =7.0 (±0.2) and UVA/UVB=1.1) [Jarzycka 2013].

Anti-inflammatory effects
The effect of the flavonoids narirutin, naringenin, astragalin and kaempferol on the proliferation of vascular smooth muscle A7r5 cells for 24 h at concentrations of 10, 50 and 100 µM was measured. Astragalin and kaempferol inhibited the proliferation of A7r5 cells (50 µM). Kaempferol (25 and 50 µM) inhibited the expression of CRP, VEGF and JNK2 and decreased NO synthesis (20 µM) [Mao 2017].

An antimutagenic activity of an extract (not further specified) at doses of 100 and 200 mg/kg was found in erythrocytes of white mice pre-treated with cyclophosphamide as a mutagen [Durnova 2015].

In vivo experiments
A single dose of a hydroethanolic extract (ethanol 70% V/V; not further specified) was tested for diuretic activity in male and female rats (50 mg/kg b.w. p.o.). Urine was collected at 4 and 24 hours after administration of the extract and tested for sodium, potassium and creatinine levels. The volume of urine was significantly ($p<0.001$) increased compared to control (water-alcohol load) after 4 and 24 hours. Sodium concentration was significantly increased after 4 hours ($p<0.001$) and 24 hours ($p<0.05$), compared to control. Potassium concentration was significantly ($p<0.05$) increased compared to control after both 4 and 24 hours. Creatinine levels were significantly greater than control after 4 hours ($p<0.05$) and 24 hours ($p<0.001$) [Kurkin 2015].

A methanolic extract (5:1) inhibited the increase in blood glucose in sucrose-loaded mice at 500 mg/kg. The extract also inhibited the enzymatic activity of dipeptidyl peptidase-IV (DPP-IV, IC_{50}=42.1 µg/ml), but not of intestinal α-glucosidase [Morikawa 2015].

The choleretic, diuretic, spasmolytic and hypotensive effects of an ethanolic extract (A; not further specified) and three different fractions thereof, obtained with diethyl ether (B) and ethyl acetate (C) as well as the aqueous fraction (D) were studied. The pure compounds 3-kaempferol glucoside, 5-naringenin glucoside and apigenin, as well as a decoction and an infusion, were also studied. In 171 male rats, a choleretic activity was found for the pure flavonoids and preparations A, B and C when administered i.v. or intraduodenally. The infusion and decoction were active only after i.v. administration. No diuretic effect was found in dogs and rats. The spasmolytic effect was tested on gallbladder from hamster and rabbits and on smooth muscle isolated from rats and rabbits. Only fraction D induced muscle contraction while all other preparations had no effect. In dogs, hypotensive effects of the preparations were seen after i.v. administration only [Szadowska 1962].

Clinical studies
No published clinical data currently available.

Pharmacokinetic properties
No data available.

Preclinical safety data
No data available.

Clinical safety data
No data available.

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Online Series, 2019
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Online Series, 2019
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Online Series, 2019
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHAMOMILLAE ROMANAE FLOS	Roman Chamomile Flower	Online Series, 2019
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HELICHRYSI FLOS	Sandy Everlastin Flower	Online Series, 2019
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013

HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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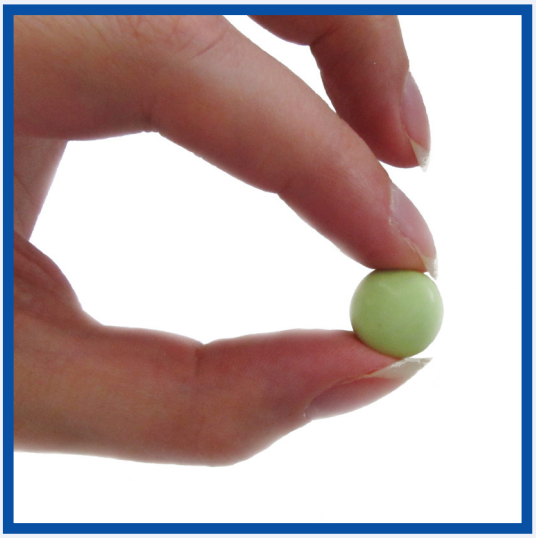
Hydrastis rhizoma
Goldenseal rhizome

2013



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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

Edited by Simon Mills and Roberta Hutchins
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Plant illustrated on the cover: *Hydrastis canadensis*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

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Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

HYDRASTIS RHIZOMA 2013

Goldenseal rhizome

DEFINITION

Goldenseal rhizome consists of the whole or cut, dried rhizome and root of *Hydrastis canadensis* L. It contains not less than 2.5 per cent of hydrastine ($C_{21}H_{21}NO_6$; M_r 383.4) (dried drug) and not less than 3.0 per cent of berberine ($C_{20}H_{19}NO_5$; M_r 353.4) (dried drug).

The material complies with the monograph of the European Pharmacopoeia [Goldenseal].

CONSTITUENTS

Isoquinoline alkaloids (2.4 – 7%) [Blaschek 2007; Barnes 2007; Upton 2001; Galeffi 1997; Bradley 1992]:

- protoberberine alkaloids: berberine (2.5 – 4.5%), berberastine (2 – 3%), canadine (0.5 – 1%), corypalmine and isocorypalmine.
- phthalylisoquinoline alkaloids: hydrastine (1.5 – 4%), hydrastidine and isohydrastidine.
- benzylisoquinoline alkaloids: candaline and canadinic acid.

Quinic acid derivatives (up to 2.5%), mainly 5-O-(4′-[β-D-glucopyranosyl] trans-feruloyl)quinic acid [McNamara 2004] and methylated luteolin 7-methyl ethers [Hwang 2003].

CLINICAL PARTICULARS

Therapeutic indications

Digestive disorders such as dyspeptic complaints and gastritis; as an adjuvant in menorrhagia and dysmenorrhoea [Blaschek 2007; Barnes 2007; Mills 2013; Bradley 1992].

Efficacy in these indications is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

0.5-1 g of the drug as a decoction three times daily; 0.3-1 mL of a liquid extract (1:1, ethanol 60%) three times daily [Bradley 1992; Barnes 2007]. Preparations accordingly.

Method of administration
For oral administration.

Duration of administration
No restriction. If symptoms persist or worsen, medical advice should be sought.

Contraindications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
Investigations in healthy volunteers with goldenseal rhizome revealed significant interactions with drugs that are metabolized by cytochrome P450 3A4/5 (e.g. midazolam) and 2D6 (e.g. debrisoquin) [Gurley 2005; Gurley 2008a; Gurley 2008b].

Pregnancy and lactation
The product should not be used during pregnancy or lactation [Blaschek 2007; Barnes 2007; Upton 2001; Mills 2013; Bradley 1992].

E/S/C/O/P
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Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
Although no case reports are available, exaggerated reflexes, depression, delirium, vomiting and cyanosis have been mentioned [Blaschek 2007; Barnes 2007].

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

The pharmacodynamics of the key constituents berberine and hydrastine have been studied thoroughly. The following pharmacological activities have been demonstrated [Simeon 1989; Mills 2013; Upton 2001]:

For berberine:
- antibacterial, antifungal, antiparasitic
- antidiarrhoeal, intestinal antisecretory
- antiarrhythmic, positive inotropic
- cytotoxic, antitumoral
- cholagogue, choleretic

For hydrastine:
- choleretic
- sedative
- antibacterial
- vasoconstrictive

In vitro experiments

Antimicrobial and antiviral activity
A 95% ethanolic extract of goldenseal rhizome exhibited antimicrobial activity against *Staphylococcus aureus, Klebsiella pneumoniae, Mycobacterium smegmatis* and *Candida albicans* with an MIC of 1 mg/mL [Gentry 1998].

A 70% ethanolic extract of goldenseal rhizome (standardized to 10 mg/mL berberine) showed antimicrobial activity against *Staphylococcus aureus* and *Streptococcus sanguis* with MICs of 0.12 mg/mL and 0.5 mg/mL (expressed as berberine) respectively. The bactericidal activity was evaluated in a contact test: the extract at 10 mg/mL was bactericidal within 4 to 30 min against the test strains compared to 0.5 - 4 min for hydrogen peroxide at 30 mg/mL [Scazzocchio 2001; Villinski 2003].

A 95% methanolic extract of goldenseal rhizome inhibited the growth of 15 strains of *Helicobacter pylori* with an MIC_{50} range of 12.5-50 µg/mL [Mahady 2003].

In a screening of herbal extracts for antibacterial activity against *H. pylori* and *Campylobacter jejuni* a goldenseal rhizome extract (ethanol 45%; DER 1:3) showed inhibition of both organisms [Cwikla 2010].

A methanolic (100%) extract of goldenseal rhizome showed antimicrobial activity against *Streptococcus mutans* and *Fusobacterium nucleatum* with MICs of 250 µg/mL and 62.5 µg/mL respectively. A bioguided fractionation of the extract led to the isolation of berberine (MIC: 125 µg/mL and 15.6 µg/mL, respectively) and 6-C-methylluteolin 7-methyl ether (MIC: 250 µg/mL and 375 µg/mL, respectively) as active compounds [Hwang 2003].

A 50% ethanolic extract of goldenseal rhizome completely inhibited influenza A growth in RAW 264.7 cells from a concentration of 2.5 µM of berberine (IC_{50} = 0.22 µM) compared to pure berberine (IC_{50} 0.01 µM) and amantadine (IC_{50} 27 µM) [Cecil 2011].

Smooth muscle relaxant activity
A 70% ethanolic extract of goldenseal rhizome dose-dependently inhibited the contractions induced by adrenaline on rabbit aorta strips (IC_{50} 0.88 µM, calculated as berberine). The activity of the isolated alkaloids berberine, canadine and canadaline was less pronounced (IC_{50} 2.6 – 5.2 µM) [Palmery 1996].

An extract of goldenseal rhizome providing berberine at 2.45 µM significantly ($p<0.01$) inhibited the contractions of rabbit prostate strips induced by noradrenaline or phenylephrine [Baldazzi 1998].

A 70% ethanolic extract of goldenseal rhizome induced relaxation in bladder detrusor muscle strips comparable to isoproterenol (88% of the isoproterenol-evoked response). Propranolol reduced this response by 69% suggesting that β-adrenergic stimulation was not the only mechanism [Bolle 1998].

A 70% ethanolic extract of goldenseal rhizome dose-dependently inhibited spontaneous contractions of uterine strips of non-pregnant rats (IC_{50} 10 µg/mL) and contractions induced by serotonin (IC_{50} 19.9 µg/mL), oxytocin (IC_{50} 10.5 µg/mL) and acetylcholine (IC_{50} 10 µg/mL). The extract also relaxed carbachol precontracted guinea-pig trachea (EC_{50} 1.6 µg/mL). The effect was partially antagonized by timolol, suggesting again that β-adrenergic stimulation was not the only mechanism [Cometa 1998].

In another study using the same guinea-pig trachea model the relaxant effect of a 70% ethanolic extract of goldenseal rhizome was confirmed (EC_{50} 1.5 µg/mL). The EC_{50} value of isolated alkaloids was also determined: hydrastine (72.8 µg/mL), berberine (34.2 µg/mL), canadine (11.9 µg/mL) and canadaline (2.4 µg/mL). Timolol antagonized the effect of canadine and canadaline but not that of berberine and hydrastine, while an adenosine receptor antagonist (xanthine amine congener) antagonized the effect of canadaline and hydrastine, but not of berberine and canadine. Extract concentrations from 0.01 to 0.1 µg/mL significantly ($p<0.01$) potentiated the relaxant effect of isoprenaline [Abdel-Haq 2000].

Antioxidant activity
A 70% ethanolic extract of goldenseal rhizome showed an antioxidant activity in the ABTS assay in a dilution of 1:1000. The effect was comparable to 40 µM trolox [Pereira da Silva 2000].

Immunomodulating activity
A 50% ethanolic extract of goldenseal rhizome reduced the production of TNF-α and the interleukins 6, 10 and 12 in lipopolysaccharide-stimulated macrophages in a dose-dependant manner [Clement-Kruzel 2008].

A 50% ethanolic extract of goldenseal rhizome significantly ($p<0.05$) inhibited the production of TNF-α and PGE_2 in influenza A infected RAW 264.7 cells at a concentration corresponding to 25 µM of berberine [Cecil 2011].

Influence on cytochrome P450
Serial dilutions (from 100% to 1.56%) of 21 commercial ethanolic herbal extracts and tinctures were analyzed for their ability to inhibit CYP3A4 via a fluorometric microtiter plate assay. An ethanolic extract of goldenseal rhizome gave the strongest inhibition with a calculated IC_{50} of 0.03% [Budzinski

2000]. In the same assay an aqueous extract of two commercial products (product 1 containing 450 mg goldenseal rhizome powder per capsule; product 2 containing 250 mg extract, standardised to 10% alkaloids, and 65 mg rhizome powder per capsule) gave an IC_{50} of 3.03 and 3.23 mg/mL, respectively [Budzinski 2008].

A commercial extract of goldenseal rhizome (containing approximately 17 mM berberine and hydrastine) inhibited CYP2C9 (diclofenac 4-hydroxylation), CYP2D6 (bufuralol 1'-hydroxylation) and CYP3A4 (testosterone 6β-hydroxylation) in human hepatic microsomes. The inhibition of CYP3A4 was non-competitive with an apparent Ki (= dissociation constant of inhibitor) of 0.1% extract [Chatterjee 2003].

An aqueous and ethanolic extract of goldenseal rhizome (equivalent to 20 µM berberine plus hydrastine) exerted more than 50% inhibition of the activity of CYP2C8 (paclitaxel 6α-hydroxylation), CYP2D6 (dextromethorphan O-demethylation) and CYP3A4 (midazolam 1-hydroxylation and testosterone 6β-hydroxylation) in human hepatic microsomes. CYP2E1 activity (p-nitrophenol hydroxylation) was inhibited by 20 µM berberine, but was not affected by either the extracts or hydrastine (20 µM). The extracts, as well as berberine and hydrastine, stimulated human P-glycoprotein (Pgp) ATPase activity at about 50% of the activity of verapamil (20 µM) [Etheridge 2007]. Goldenseal rhizome tea (containing 0.2% hydrastine and 1.0% berberine) had a greater stimulatory effect on Pgp ATPase than verapamil (20 µM) [Budzinski 2008].

In contrast to the previous study, CYP2E1 activity (p-nitrophenol hydroxylation) was inhibited by berberine (4 µM, 11% decrease), hydrastine (4 µM, 64% decrease) and by a 50% ethanolic extract of goldenseal rhizome (corresponding to 1.7 µM berberine and 1.2 µM hydrastine, 55% decrease). Inhibition of CYP2E1 appeared to be competitive with a Ki of 18 µM, 2.8 µM and 0.1%, respectively [Raner 2007].

In vivo experiments

Antidiabetic activity
Goldenseal rhizome, incorporated into the diet at 6.25% for 9 days, significantly ($p<0.05$) reduced hyperphagia and polydipsia in streptozotocin-diabetic mice [Swanston-Flat 1989].

Immunomodulating activity
Goldenseal rhizome extract (not further specified) was administered to rats for 6 weeks (1 g/l of drinking water). At days 0, 14 and 28 the antigen KLH (keyhole limpet haemocyanin) was injected. The treated group showed a significant ($p<0.05$) increase in the primary IgM response during the first 2 weeks, whereas the IgG levels in treated and control rats were identical [Rehman 1999].

Lipid-lowering activity
Berberine (1.8 mg/animal) or goldenseal rhizome extract (125 µL/animal, equivalent to 0.9 mg berberine) was administered intraperitoneally to hyperlipidaemic hamsters once per day for 24 days. Goldenseal significantly ($p<0.001$) reduced total plasma cholesterol by 31%, LDL-cholesterol by 25% and triglycerides by 33% compared to control. The activity of berberine was almost identical [Abidi 2006].

Anti-carcinogenic activity
Oral administration of a 90% ethanolic extract (not further specified; 0.06 mL three times daily for 4 months) to mice chronically fed p-dimethylaminoazobenzene (0.06% in the diet) and phenobarbital (0.05% in the diet) markedly reduced the liver tumour incidence and the elevation of serum phosphatases and transferases. This was confirmed histopathologically by a decrease of necrosis, vascular congestion, fibrosis and damage to intracellular organelles in livers of the treated mice [Karmakar 2010].

Pharmacological studies in humans

In a randomized, cross-over study 12 healthy volunteers received a goldenseal rhizome extract (900 mg, 3 times daily) for 28 days. Probe drug cocktails (midazolam and caffeine, chlorzoxazone and debrisoquin) were administered before and at the end of supplementation. Determination of cytochrome P450 activity 1A2, 2D6, 2E1 and 3A4/5 revealed a significant ($p<0.05$) inhibition (approximately 40%) of CYP2D6 and CYP3A4/5 [Gurley 2005].

In a cross-over study, the pharmacokinetics of indinavir were characterized in 10 healthy volunteers before and after treatment with goldenseal rhizome (1140 mg, twice daily for 28 days). No significant differences in peak concentration or oral clearance were observed, suggesting that interactions with drugs metabolized by CYP3A4 are unlikely [Sandhu 2003]. However indinavir is less suitable as a probe for assessing changes in CYP3A4/5 activity due to its high oral bioavailability [Gurley 2005].

In a randomised, cross-over study the effect of treatment with a standardized goldenseal rhizome extract (1070 mg, containing 24.1 mg isoquinoline alkaloids, 3 times daily for 14 days) on digoxin pharmacokinetics was determined in 20 healthy volunteers. No clinically relevant effects on digoxin pharmacokinetics were observed. When compared to rifampin (= rifampicin) and clarithromycin, goldenseal rhizome does not seem to be a potent modulator of P-glycoprotein [Gurley 2007].

Sixteen healthy volunteers received a goldenseal rhizome extract, corresponding to 132 mg hydrastine and 77 mg berberine per day, for 14 days. Midazolam (8 mg, per os) was admistered 1 day before and on the last day of treatment. Comparison of pre- and post-treatment midazolam pharmacokinetic parameters revealed significant inhibition of CYP3A: AUC(0-∞): 108 vs. 175 ng*h/mL ($p<0.001$); apparent oral clearance normalised to body weight: 1.3 vs. 0.8 l/h/kg ($p<0.001$); $T_{1/2}$ elimination: 2.0 vs. 3.2 h ($p<0.001$) and C_{max}: 51 vs. 71 ng/mL ($p<0.05$)[Gurley 2008b]. In a comparable study, debrisoquin (5 mg, orally) was administered before and at the end of goldenseal treatment (same product, same dose). Comparison of pre- and post-treatment urinary recovery ratios of debrisoquin revealed significant ($p<0.05$) inhibition of CYP2D6: 0.71 vs. 0.37 [Gurley 2008a].

Clinical studies
No data available.

Pharmacokinetic properties
No data available for goldenseal rhizome.

Pharmacokinetics in animals
After oral administration of berberine sulphate to rats at doses of up to 1 g/kg body weight only very small amounts of berberine were detected in a few tissues. As anticipated for a quaternary alkaloid, examination of the intestines revealed that berberine was neither well absorbed nor destroyed [De Smet 1992].

After oral administration of berberine to rats at a lower dose of 40 mg/kg, berberine, four metabolites and their glucuronide conjugates were found in plasma, bile and urine. Berberine reached its peak plasma concentration (10 µg/l) after 2 hours and was eliminated within 12 hours (AUC(0-∞): 37 ng*h/mL). However, the peak plasma concentration and AUC(0-∞) values

of the metabolites were much higher: e.g. AUC(0-∞) of 1880 ng*h/mL for the main metabolite berberrubine. About 34% of the oral dose was absorbed from the gastrointestinal tract within 1 hour. [Zuo 2006].

Intravenous administration of berberine to rats at 10 mg/kg resulted in $T_{1/2}$ elimination of 0.3 h and AUC0-∞ of 265 µg*min/mL (plasma)/1470 µg*min/mL (bile). Only a small amount of berberine was detected in urine. Berberine was rapidly transported from blood into liver and bile (via P-glycoprotein) and metabolised with phase I demethylation (CYP450) and phase II glucuronidation [Tsai 2004].

The cumulative urinary and biliary excretion of berberine after i.v. administration of 2 mg/kg to rabbits was 4.9% and 0.5% of the administered dose respectively (AUC: 0.84 µg*h/mL) [Chen 1995].

Berberine was given orally to dogs in a single dose of 280 mg/kg body weight and resulted in a C_{max} of 15.5 µg/mL, T_{max} of 3.7 h and AUC0-∞ of 777 µg*h/mL. At this high dose vomiting occurred within 1 h [Upton 2001].

Pharmacokinetics in humans
In an uncontrolled study, 56 patients with congestive heart failure received 1.2 g berberine per day orally for 2 weeks. Peak plasma levels were reached in about 2.4 h ranging from 0.07 to 0.19 mg/l [Zeng 1999].

After oral administration of berberine to 5 volunteers at a dose of 900 mg/day for 3 days, 3 sulphate-conjugated metabolites were isolated and identified in the urine [Pan 2002].

An oral dose of 300 mg berberine was given 3 times daily for 2 days to 12 healthy volunteers. In their urine 7 metabolites could be identified: phase I metabolites formed by cleavage of the dioxymethylene ring or by demethylation, most of them were conjugated with glucuronic or sulphuric acid (phase II metabolites) [Qiu 2008].

Preclinical safety data

Acute and repeated dose toxicity
The oral LD_{50} of an extract of goldenseal rhizome in mice was 1620 mg/kg body weight [Blaschek 2007; Mills 2013].

The oral LD_{50} of berberine in mice was 329 mg/kg body weight. Oral doses of up to 100 mg/kg of berberine sulphate have been well tolerated in animal studies without permanent effects. However, prolonged administration caused organ damage and death after 8 to 10 days [De Smet 1992; Mills 2013].

The LD_{50} values of hydrastine in rats were 1000 mg/kg body weight (oral), 1270 mg/kg (subcutaneous) and 104 mg/kg (intraperitoneal) [Blaschek 2007].

Chronic toxicity
In a two-year toxicity study goldenseal rhizome powder (3.9% berberine, 2.8 hydrastine and 0.2% canadine) was administered to male and female rats and mice at up to 25% of the feed. The primary finding was an increase in liver tumours in rats and mice at the highest dose (25%) [Dunnick 2011].

Reproductive toxicity
In a preliminary experiment, non-pregnant, female rats were dosed by oral gavage with a liquid extract of goldenseal rhizome (45% ethanol; 333 mg/mL goldenseal; standardized to 9.3 mg/mL berberine and 8.4 mg/mL hydrastine) at incremental doses for 8 days. The maximum dose of 1.86 g/kg body weight/day was non-maternotoxic. This dose was administered daily to female rats on either gestation days (GD) 1-8 or GD 8-15. Controls received an equivalent dose of ethanol. On GD 20, foetuses were weighed and examined for signs of external, internal, or skeletal malformations: differences between the treated and control group could not be demonstrated. When rat embryos were explanted and cultured with the extract at concentrations from 0.5 to 6 µL/mL, growth retardation and embryotoxicity were demonstrated in a dose-dependent manner. A possible poor absorption of orally administered goldenseal could explain the discrepancy between the in vivo and in vitro results [Yao 2005].

Berberine was administered in the feed and by gavage to pregnant rats on GD 6-20 and to pregnant mice on GD 6-17. A mortality of 33% of the mice exposed to 792 mg/kg/day berberine by gavage was observed. The following NOAEL (no observed adverse effect level) and LOAEL (lowest observed adverse effect level) values were determined [Jahnke 2006]:

	LOAEL (mg/kg/day)	NOAEL (mg/kg/day)
Rat maternal toxicity	420	223
Rat developmental toxicity	>792	792
Mouse maternal toxicity	666	450
Mouse developmental toxicity	792	666

Clinical safety data

In an 11 year old girl with diabetic ketoacidosis, the degree of hypernatraemia and hyperosmolality was enhanced after intake of 500 mg goldenseal (not further specified) 2 to 3 times daily for 2 weeks, but a causal relationship could not be demonstrated [Bhowmick 2007].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Supplement 2009
ALOE CAPENSIS	Cape Aloes	Second Edition, 2003
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Second Edition, 2003
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
BALLOTAE NIGRAE HERBA	Black Horehound	Supplement 2009
BETULAE FOLIUM	Birch Leaf	Second Edition, 2003
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Second Edition, 2003
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Supplement 2009
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Second Edition, 2003
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Second Edition, 2003
FUMARIAE HERBA	Fumitory	Supplement 2009
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Supplement 2009
GRINDELIAE HERBA	Grindelia	Supplement 2009
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Second Edition, 2003
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Second Edition, 2003
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003

LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Supplement 2009
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Second Edition, 2003
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Second Edition, 2003
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Second Edition, 2003
RATANHIAE RADIX	Rhatany Root	Supplement 2009
RHAMNI PURSHIANI CORTEX	Cascara	Second Edition, 2003
RHEI RADIX	Rhubarb	Second Edition, 2003
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Second Edition, 2003
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Second Edition, 2003
SALICIS CORTEX	Willow Bark	Second Edition, 2003
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Second Edition, 2003
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VIOLAE HERBA CUM FLORE	Wild Pansy	Supplement 2009
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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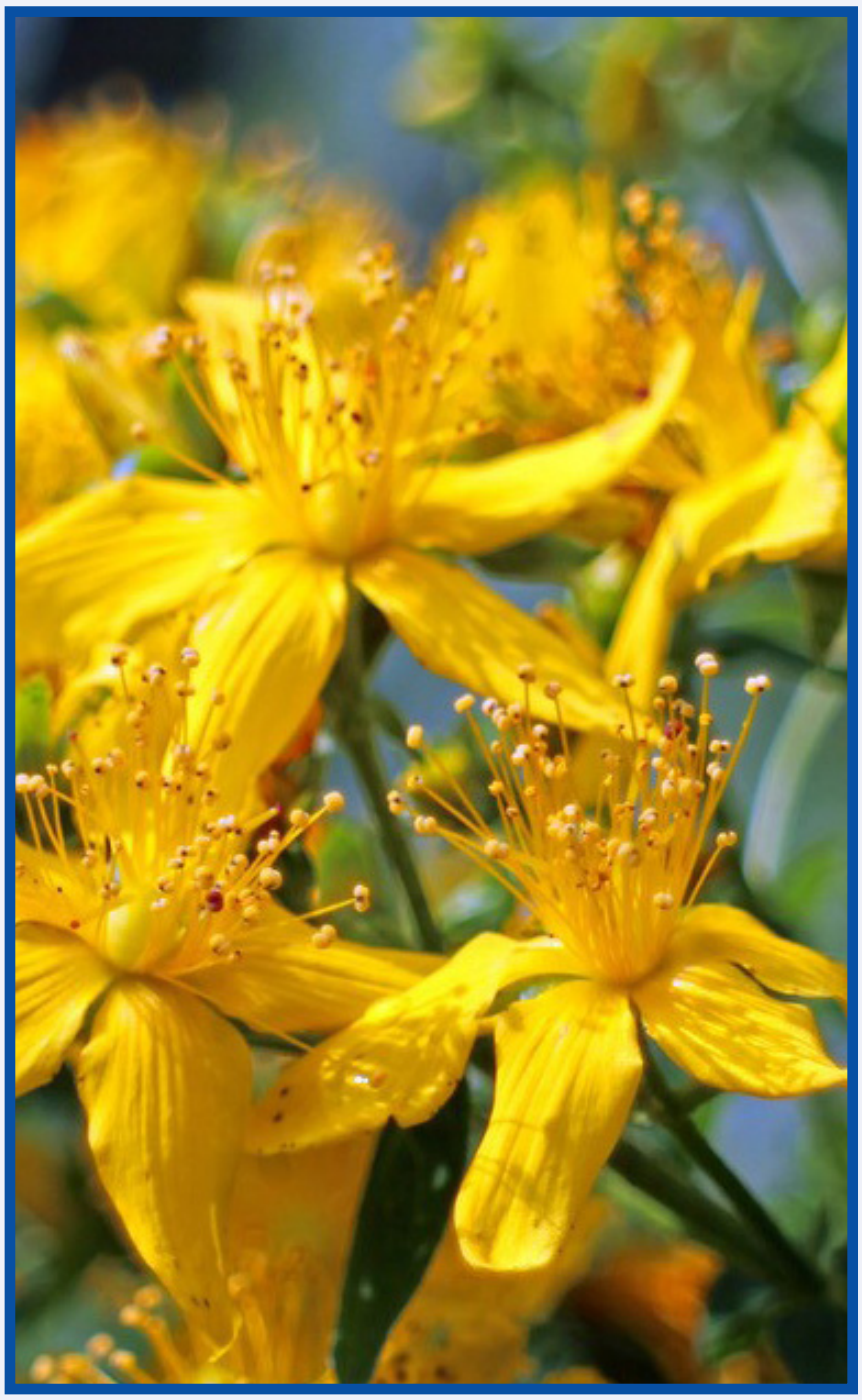
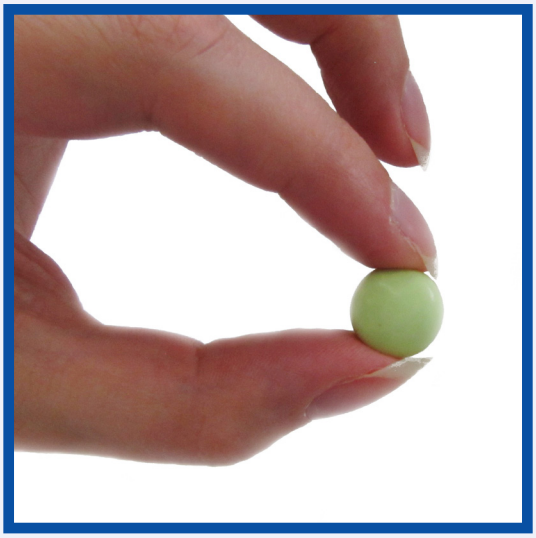
The Scientific Foundation for Herbal Medicinal Products

Hyperici herba

St. John's Wort

2018





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MONOGRAPHS

***The* Scientific Foundation for**
Herbal Medicinal Products

HYPERICI HERBA
St. John's Wort

2018

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Second Edition, completely revised and expanded

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

Edited Roberta Hutchins and Simon Mills
Cover and text design by Martin Willoughby
Typeset in Optima by Roberta Hutchins

Plant illustrated on the cover: *Hypericum perforatum*

FOREWORD

It is a great pleasure for me, on behalf of my colleagues in ESCOP, to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
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The monograph text
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Information on the member organizations and people involved in the production of ESCOP monographs and other activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
CCl_4	carbon tetrachloride
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase

INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

TABLE OF CONTENTS: PHARMACOLOGICAL PROPERTIES

HYPERICI HERBA

2018

St. John's Wort

DEFINITION

St. John's wort consists of the whole or cut, dried flowering tops of *Hypericum perforatum* L., harvested during flowering time. It contains not less than 0.08 per cent of total hypericins, expressed as hypericin ($C_{30}H_{16}O_8$; M_r 504.4) and calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia 2013 [St. John's wort].

CONSTITUENTS

The characteristic constituents are naphthodianthrones and phloroglucinols. Naphthodianthrones (0.05-0.3%), consisting mainly of hypericin and pseudohypericin, accumulate primarily in the flowers and buds [Nahrstedt 1997]. Other naphthodianthrones are the biosynthetic precursors protohypericin and protopseudohypericin (which are transformed into hypericin and pseudohypericin respectively on exposure to light), and a small amount of cyclopseudohypericin [Häberlein 1992; Krämer 1992; Schütt 1994].

The principal phloroglucinols are hyperforin (2-4.5%) and adhyperforin (0.2-1.8%) [Bergonzi 2001; Greeson 2001; Maisenbacher 1992]. Both compounds have limited stability [Bilia 2001; Fuzzati 2001; Orth 1999a; Orth 1999b; Trifunovic 1998] and their oxidated derivatives are also present [Bergonzi 2001].

Quercetin glycosides (2-4%), including hyperoside, quercitrin, isoquercitrin and rutin are the main flavonoids [Hölzl 1987; Nahrstedt 1997; Tekel'ová 2000]; a small amount of free quercetin [Tekel'ová 2000]. Biflavonoids such as I3,II8-biapigenin (0.1-0.5%) and I3',II8-biapigenin (amentoflavone, 0.01-0.05%) occur exclusively in the flowers [Berghöfer 1987; Nahrstedt 1997; Tekel'ová 2000].

Other constituents include phenylpropanoids, such as chlorogenic acid and other caffeoylquinic and *p*-coumaroylquinic esters [Jürgenliemk 2002; Nahrstedt 1997]; polymeric and oligomeric proanthocyanidins [Brantner 1994; Nahrstedt 1997] including procyanidins A2, B1, B2, B3, B5, B7 and C1 [Melzer 1991; Ploss 2001], together with catechin and epicatechin monomers [Nahrstedt 1997; Ploss 2001]; trace amounts of xanthones such as 1,3,6,7-tetrahydroxyxanthone (0.0004% in the leaves and stems) [Nahrstedt 1997; Sparenberg 1993]; and essential oil (0.1-0.25%), containing mainly higher *n*-alkanes and monoterpenes [Nahrstedt 1997].

CLINICAL PARTICULARS

Therapeutic indications

Preparations based on hydroalcoholic extracts (50-68% ethanol, 80% ethanol or 80% methanol)

Preparations based on dry extract, extraction solvent **ethanol (50-68% V/V)**:

Episodes of mild depressive disorders [Häring 1996; Harrer 1999; Hoffmann 1979; Hübner 1994; Kalb 2001; Kugler 1990; Laakmann 1998; Lenoir 1999; Philipp 1999; Quandt 1993; Schlich 1987; Sommer 1994; Witte 1995; Woelk 2000a].

Preparations based on dry extract, extraction solvent **methanol (80% V/V)**, and dry extract, extraction **solvent ethanol (80% V/V or 50% V/V)**:

Mild to moderate depressive episodes in accordance with ICD-10 categories F32.0, F32.1, F33.0 and F33.1 (see the boxes below) [Anghelescu 2006; Bjerkenstedt 2005; Brenner 2000; Fava 2005; Gastpar 2005; Gastpar 2006; Halama 1991; Hänsgen 1994; Häring 1996; Harrer 1994; Harrer 1991; Holsboer-Trachsler 1999; Hübner 2000; Hübner 1994; Kaehler 1999; Kasper 2006; Kasper

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2007a; Kasper 2007b; Kasper 2008; Lecrubier 2002; Lehrl 1993; Mannel 2010; Meier 1997; Montgomery 2000; Sarris 2012; Schmidt 1989; Schrader 2000; Schrader 1998; Shelton 2001; Singer 2011; Sommer 1994; Szegedi 2005; Uebelhack 2004; Vorbach 1997; Vorbach 1994; Wheatley 1997; Woelk 1994].

Other Preparations

Comminuted and powdered herbal substance as well as the following preparations:

- Dry extract (DER 4-7:1), extraction solvent ethanol 38% (m/m)
- Liquid extract (DER 1:13), extraction solvent maize oil or other suitable vegetable oil
- Tincture (DER 1:10), extraction solvent ethanol 45-50% (V/V)
- Tincture (DER 1:5), extraction solvent ethanol 50% (V/V)
- Liquid extract (DER 1:2), extraction solvent ethanol 50% (V/V)
- Liquid extract (DER 1:5-7), extraction solvent ethanol 50% (V/V)
- Expressed juice from the fresh herb:

Relief of temporary mental exhaustion [Behnke 2002; Engesser 1996; Harrer 1991; Hoffmann 1979; Kugler 1990; Lenoir 1999; Quandt 1993; Rapaport 2011; Schlich 1987; Schmidt 1989; Warnecke 1986; Werth 1989].

Liquid extract (DER 1:4-20), extraction solvent vegetable oil; tincture (DER 1:10), extraction solvent ethanol 45-50% (V/V); and tincture (DER 1:5), extraction solvent ethanol 50% (V/V):

Symptomatic treatment of minor inflammations of the skin (such as sunburn) and as an aid in healing of minor wounds [Gerlach 2008; Samadi 2010; WHO 2002; Wichtl 2004].

Comminuted herbal substance:

Symptomatic relief of mild gastrointestinal discomfort: [Grünwald 2004; Hänsel 1993; Wichtl 2004].

Posology and method of administration

Dosage

Preparations based on hydroalcoholic extracts (50-68% ethanol, 80% ethanol or 80% methanol)

Adults and children from 12 years:

450-1050 mg daily of hydroalcoholic dry extracts with DERs of 2.5-5:1, 4-7:1 or 5-7:1 [Anghelescu 2006; Bjerkenstedt 2005; Brenner 2000; Fava 2005; Gastpar 2005; Gastpar 2006; Halama 1991; Hänsgen 1994; Häring 1996; Harrer 1994; Harrer 1999; Holsboer-Trachsler 1999; Hübner 2000; Hübner 1994; Kaehler 1999; Kalb 2001; Kasper 2006; Kasper 2008; Laakmann 1998; Lecrubier 2002; Lehrl 1993; Mannel 2010; Martinez 1994; Meier 1997; Philipp 1999; Rapaport 2011; Rychlik 2001; Schrader 2000; Schrader 1998; Shelton 2001; Sommer 1994; Szegedi 2005; Uebelhack 2004; Vorbach 1994; Wheatley 1997; Woelk 2000a; Woelk 1994].

1200 – 1800 mg daily of hydroalcoholic dry extracts with DERs of 2.5-5:1, 4-7:1 or 5-7:1 [Anghelescu 2006; Davidson 2002; Kasper 2006; Rychlik 2001; Shelton 2001; Szegedi 2005; Vorbach 1997].

Children from 6 to 12 years under medical supervision only.

Other Preparations

Comminuted and powdered herbal substance as well as the following preparations:

- Dry extract (DER 4-7:1), extraction solvent ethanol 38% (m/m)
- Liquid extract (DER 1:13), extraction solvent maize oil or other suitable vegetable oil
- Tincture (DER 1:10), extraction solvent ethanol 45-50% (V/V)
- Tincture (DER 1:5), extraction solvent ethanol 50% (V/V)

Classification of severity of depression based on ICD-10 criteria [WHO 2010]

Mild depressive episode [F32.0/F33.0]	Mild first manifestation or mild recurrence of at least two target signs and at least two associated symptoms
Moderate depressive episode: [F32.1/F33.1]	Two or three target signs and at least three or four associated symptoms (first manifestation or recurrent episode)
Severe depressive episode: [F32.2/F33.2/F32.3/F33.3]	All three target signs and at least four associated symptoms, some particularly pronounced (first manifestation or recurrent, without or with psychotic symptoms)

Diagnostic features of depressive disorders according to ICD-10 Chapter V Primary Care Version [WHO 2010]

Target signs	***Associated symptoms***
Low or sad mood	Disturbed sleep
Loss of interest or pleasure	Feelings of guilt and unworthiness
Fatigue or loss of energy	Reduced self-esteem and self-confidence
	Poor concentration
	Disturbed appetite
	Decreased libido
	Suicidal thoughts or acts
	Agitation or slowing of movement or speech
	Weight loss

Symptoms of anxiety or nervousness are also frequently present

- Liquid extract (DER 1:2), extraction solvent ethanol 50% (V/V)
- Liquid extract (DER 1:5-7), extraction solvent ethanol 50% (V/V)
- Expressed juice from the fresh herb:

Single oral dose:
60 – 200 mg extract, liquid preparation 1-20 mL, comminuted herbal substance (tea) 1.5-2 g, or powdered herbal substance 300-500 mg.

Daily oral dose:
180-360 mg extract, liquid preparation 6-30 mL, comminuted herbal substance (tea) 3-6 g, or powdered herbal substance 900-1000 mg.

[Harrer 1991; Hoffmann 1979; Kugler 1990; Quandt 1993; Schlich 1987; Schmidt 1989; Warnecke 1986; Werth 1989; Engesser 1996].

Undiluted preparations for cutaneous administration: liquid extract (DER 1:4-20), extraction solvent vegetable oil; tincture (DER 1:10), extraction solvent ethanol 45-50% (V/V) or tincture (DER 1:5), extraction solvent ethanol 50% (V/V) [Gerlach 2008; Samadi 2010; WHO 2002; Wichtl 2004].

Comminuted herbal substance for tea preparation: single dose 2 g and daily dose 4 g [Grünwald 2004; Hänsel 1993; Wichtl 2004]

Elderly: dose as for adults.

Method of administration
For oral administration and topical application.

Duration of administration

- Mild to moderate depressive episodes: no restriction. If symptoms persist for more than 6 weeks or worsen, medical advice should be sought.

- Relief of temporary mental exhaustion: if symptoms persist for more than 1 week or worsen, medical advice should be sought.

- Symptomatic treatment of minor inflammations of the skin (such as sunburn), as an aid in healing of minor wounds and symptomatic relief of mild gastrointestinal discomfort: if symptoms persist for more than 2 weeks or worsen, medical advice should be sought.

Contra-indications
Known hypersensitivity to the active substance.

Oral administration of preparations with a daily dose of more than 1000 mg drug equivalent or more than 1 mg hyperforin:
Concomitant use with certain immunosuppressants (ciclosporin, tacrolimus or sirolimus for systemic use), certain anti-retroviral HIV-drugs from the group of non-nucleoside reverse transcriptase inhibitors (e.g. nevirapine), protease inhibitors (e.g. indinavir, amprenavir), certain cytostatics (e.g. imatinib, irinotecan), and oral anti-coagulants of the coumarin type (e.g. warfarin). See also section on Interactions.

Special warnings and special precautions for use
Very rarely, and especially in fair-skinned persons, unwanted reactions of the skin (sunburn-like redness) may occur following ingestion of St. John's wort preparations and subsequent exposure to sun light. Intense UV-exposure should be avoided during treatment.

Oral administration of preparations with a daily dose of more than 1000 mg drug equivalent or more than 1 mg hyperforin:
Caution is indicated at the beginning and end of treatment with concomitant medications, as well as when making dosage changes of St. John's wort products.

Treatment with St. John's wort extract should be assessed with respect to interactions with co-medication at least 5 days before elective surgery, and again upon discharge from the hospital.

Interaction with other medication and other forms of interaction

Oral administration of preparations with a daily dose of more than 1000 mg drug equivalent or more than 1 mg hyperforin:
Administration of St. John´s wort extracts and some of its components can lead to an induction of several enzymes of the cytochrome P450 enzyme family (CYP3A, CYP2B6, CYP2C9 and CYP2C19), and the P-glycoprotein drug efflux transporter. For this reason, interactions can occur with drugs including amitryptiline, fexofenadine, benzodiazepines, methadone, simvastatin, finasteride, digoxin. The concomitant use of cyclosporine, tacrolimus for systemic use, amprenavir, indinavir and other protease inhibitors, irinotecan and anticoagulants (such as warfarin or phenprocoumon) is contraindicated. Women using oral hormonal contraceptives should be informed about possible mid-cycle bleeding as a result of interactions, and counselled on the use of additional contraceptive measures due to a possible reduction in contraceptive efficacy [Johne 1999, 2002; Sugimoto 2001; Gurley 2002; Dresser 2003; Eich-Hochli 2003; Mai 2004; Müller 2004; Murphy 2005; Lundahl 2009].

Several cases of serotonergic effects following concomitant use of St. John's wort preparations with certain antidepressants have been reported [Gordon 1998; Lantz 1999; Prost 2000; Dannawi 2002; Mannel 2004; Bonetto 2007]. The attribution of these cases to St. John's wort remains unclear.

Patients taking other medicines on prescription should consult a physician or pharmacist before taking St. John's wort.

Pregnancy and lactation
Studies generally do not show an impact of St. John's wort on pregnancy and lactation, although some animal studies showed equivocal results [Borges 2005; Dugoua 2006]. Several of these studies offer exposure data in humans [Demling 2004; Klier 2006; Lee 2003; Moretti 2009].
In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
Clinical studies indicate no negative influence on general performance or the ability to drive [Friede 1998; Herberg 1994; Schmidt 1991; Schmidt 1993].

Undesirable effects
At therapeutic dose levels (up to the equivalent of 6 g of drug), occasional mild gastrointestinal disturbances, nausea, restlessness, fatigue, headache, insomnia or allergic reactions have been reported.

Onset of mania and hypomania were reported in several patients with latent bipolar disorders [Fahmi 2002; Moses 2000; Nierenberg 1999].

Phototoxicity
Very rarely, and especially in fair-skinned people, reactions of the skin (sunburn-like redness) may occur after ingestion of St. John's wort preparations and subsequent exposure to sun light. Intense UV-exposure should be avoided during treatment [He 2004; Schempp 2003b].

Overdose
A 16-year old girl experienced seizures and confusion after ingestion of up to 4.5 g of an unspecified St. John's wort extract per day in the preceding 2 weeks and an additional 15 g before admission to the hospital [Karalapillai 2007]. On day 6 EEG was normalised and the patient discharged.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Effects on enzyme activity
A potent irreversible inhibition of monoamine oxidase type A and B (MAO-A and MAO-B) was found for hypericin with IC_{50} values of 6.8×10^{-4} M and 4.2×10^{-4} M respectively [Suzuki 1984]. As other studies did not confirm the MAO inhibitory effects of hypericin [Cott 1997; Demisch 1989; Sparenberg 1993; Thiede 1994], the MAO inhibition was explained as being due to a purity of only 80% of the tested compound with a possible 20% flavonoid content, for which MAO inhibition has been confirmed.

An extract (not further specified) as well as flavone, xanthone and kaempferol (IC_{50} of 1 to 3 µM) led to >90% inhibition of MAO-A [Demisch 1989].

Hyperforin (35 µmol/L) showed a 30.3% inhibition of MAO-A. A significant inhibition of MAO-A activity (66 – 96.2%), and to a lesser extent MAO-B activity (2.2 – 29.9%), was demonstrated for quercetin, quercitrin, luteolin and kaempferol at concentrations of 39 – 65 µmol/L [Sparenberg 1993].

Inhibition of MAO-A in rat brain homogenates was only shown at non-physiological concentrations of 1-10 mM of a methanolic (80% V/V) extract or a flavonoid-rich fraction thereof. Hypericin did not inhibit MAO-A. Additional *ex vivo* experiments did not show a relevant inhibition of MAO-A or MAO-B after i.p. administration of 100 mg/kg b.w. of 1,3-dihydroxyxanthone, quercitrin or the extract [Bladt 1994].

An undefined extract of fresh flowers and buds (containing 0.1% hypericin), at a concentration of 5 µg/mL, inhibited MAO-A by 93% and MAO-B by 49% [Cott 1997].

A weak inhibitory effect (IC_{50} = 120 and 370 µg/mL for MAO-A and MAO-B respectively) was obtained with a methanolic (80% V/V) extract containing 0.3% hypericin and 1.5% hyperforin [Müller 1997]. A hyperforin-enriched (38%) CO2 extract (>500 µg/mL) demonstrated no inhibition [Müller 1998].

Hyperforin at doses of up to 50 µg/mL did not inhibit MAO-A or MAO-B activities [Chatterjee 1998a].

On fractionation of an extract, an inhibition of catechu-*O*-methyl-transferase (COMT) was found for the hydrophilic fractions containing mainly flavonoids, as well as hypericins and xanthones. Hypericin did not inhibit COMT from pork liver at concentrations up to 1 mM (500 mg/L) [Thiede 1994].

Several studies have shown an inhibition of dopamine-β-hydroxylase by ethanolic extracts, hypericin and pseudohypericin [Kleber 1999].

Effects on receptor binding and regulation
Depression has been attributed to reduced availability of serotonin (5-HT), norepinephrine or dopamine, disturbances of the opioid receptor family, the GABA system, modulation of oestrogen, *N*-methyl-D-aspartate (NMDA) and glutamate receptor binding and the corticosteroidal system in the CNS. Consequently, antidepressants increase the levels of neurotransmitters, e.g. by reuptake inhibition or by inhibiting the degradation. NMDA receptor-mediated processes or binding to opioid receptors have also been considered to be involved in the mechanism of action of conventional antidepressants.

Adrenergic receptors
Methanolic extracts, hypericin and kaempferol (at 100 µg/mL) showed either no or only weak inhibition of the adrenergic α_1, α_2 and β_1-receptors [Müller 1996; Raffa 1998; Rolli 1995].

Hypericin potently inhibited binding to rat β_1 and β_2 receptors, whereas pseudohypericin had no effect on either receptor. Rutin and miquelianin showed significant binding inhibition at human α_{2C} adrenergic receptors (52.81% and 59.60% respectively) [Butterweck 2002b].

Cholinergic receptors
Hypericin at 1 µM displayed weak affinity (12%) to rat cortex nicotinic acetylcholine (Ach) receptors, and moderate affinity to muscarinic Ach receptors (49%) [Raffa 1998].

At human muscarinic Ach receptors M_1-M_5, hypericin (10 µM) was a weak inhibitor of M_2 receptor binding. Inhibition of binding to human muscarinic receptor subtypes was observed for miquelianin (M_2: 53.75%; M_5: 55.20%) and rutin (M_5: 53.82%). Affinity of rutin to M_5 receptors was K_i = 35.13 ± 13 nM (reference: pirenzepine, K_i = 20.59 ± 2.4 nM) [Butterweck 2002b].

Corticosteroid receptors
Corticotropin-releasing hormone (CRH) is a family of neuro-humoral factors which act on receptors including CRH_1. The corticosteroidal system seems to be particularly important in the pathogenesis of depression by changes in the regulation of the hypothalamic-pituitary-adrenal axis. Elevated levels of CRH have been found in the cerebrospinal fluid of depressed patients [Arborelius 1999], and antidepressant drugs are considered to normalise CRH hypersecretion [Stout 2002].

Bisanthraquinone glycosides isolated from an ethanolic (50%) extract inhibited binding of [^{125}I]-sauvagine to CRH_1-receptors with IC_{50} values of 1 to 4 µM. The IC_{50} values of hyperforin and hypericin were 10 and 6 µM [Wirz 2000].

A methanolic extract (0.2% hypericin and 5% hyperforin) weakly inhibited [^{125}I]-astressin binding to the human CRH_1 receptor with an IC_{50} of 50 µg/mL whereas hypericin (IC_{50} approximately 300 nM) and pseudohypericin (IC_{50} of 2 µM) were more active. Hyperforin did not show any inhibition up to 10 µM [Simmen 2001].

The effect of hypericin, pseudohypericin and hyperforin on corticotropin-releasing factor (CRF)-stimulated cAMP formation was studied in recombinant Chinese hamster ovary cells. Only pseudohypericin selectively antagonised CRF (K_B 0.76 µM) [Simmen 2003].

Dopamine (DA) receptors and DA transporters
Hypericin and pseudohypericin (10 µM) markedly inhibited binding to rat D_3- and D_4- receptors (approximately 70-80% inhibition), with pseudohypericin being more potent on D_4-receptors. Inhibition by hyperforin (10 µM) was 57% for the binding to D_1- and 63% to D_5-receptors. Amentoflavone (10 µM) showed a complete inhibition of binding to the D_3-receptor and a 70% inhibition at the DA transporter [Butterweck 2002b].

Weak interaction with the rat DA transporter system was shown for a methanolic extract (IC_{50} 24.5 µg/mL) and a CO_2 extract (IC_{50} of 12.9 µg/mL) [Gobbi 2001].

GABA receptors
An ethanolic extract inhibited binding to human $GABA_A$-receptors with an IC_{50} value of 1 to 3 µg/mL [Simmen 1999; Simmen 2001]. A hydromethanolic extract (4.5% hyperforin, 0.5% hypericin and 0.4% biapigenin) showed an inhibition of binding to $GABA_A$ and $GABA_B$ receptors with IC_{50} values of 5.5 and 8.0 µg/mL respectively [Gobbi 2001; Gobbi 1999].

A hydromethanolic (80%) extract inhibited radioligand binding of muscimol to the $GABA_A$-receptor with an IC_{50} of 1.6 ± 0.5 µg/mL [Wonnemann 1997].

A flower extract, obtained by extraction with methanol followed by acetone, inhibited binding of flumazenil to benzodiazepine receptors in rat brain with an IC_{50} of 6.8 µg/mL. Leaf extracts up to 200 µg/mL showed 25% inhibition of flumazenil binding. Of the single constituents tested, amentoflavone had an IC_{50} of 14.9 nM, whereas hypericin, flavones and glycosylated flavonoids did not inhibit binding [Baureithel 1997]. Amentoflavone binding activity at the benzodiazepine receptor with an IC_{50} of 6 nM was comparable to the affinity of diazepam [Nielsen 1988].

Various undefined extracts were investigated for their effect on neurotransmitter re-uptake. Dopamine re-uptake was studied in synaptosomes from male Wistar rats, all other uptake mechanisms (5-HT, norepinephrine (NE), GABA and L-glutamates) in those from female NMRI mice. Adhyperforin, like hyperforin, showed a strong inhibition in all systems. Hyperforin-free preparations were able to inhibit the uptake in a weak to moderate manner. Moreover, a weak to moderate inhibition was found for a fraction containing oligomeric procyanidins [Wonnemann 2001].

Amentoflavone (10 µM) inhibited binding to rat GABA receptors by 98%. Quercitrin, isoquercitrin, hyperoside and miquelianin exhibited only a weak effect. The affinity of amentoflavone to the rat benzodiazepine receptor was K_i = 6.17 ± 1.2 nM (reference: diazepam, K_i = 10.6 ± 2.2 nM) [Butterweck 2002b].

Examination of the binding sites of amentoflavone suggested a complex mechanism of interaction at the $GABA_A$ receptor. Amentoflavone appeared to act as an antagonist at the classical benzodiazepine site, whereas the functional effects at $GABA_A$ receptors were most likely mediated by mechanisms distinct from the benzodiazepine and lorecazole binding sites [Hansen 2005].

In a screening of an ethanolic extract, binding of radiolabelled ligands was strongly inhibited at the $GABA_A$ receptor (IC_{50} 1 µg/mL) [Simmen 1999].

A hydromethanolic extract demonstrated reduced expression of serotonin receptors in a neuroblastoma cell line model [Müller 1993].

NMDA and glutamate receptors
Binding of the selective ligand Ro 63-6599 to the human metabotropic glutamate receptor 2 (mGlu2) was not inhibited by an ethanolic (50%) extract or a methanolic/acetonic extract containing 0.2% hypericin and 5% hyperforin [Simmen 2001].

This was supported by a study finding that an unspecified extract did not inhibit binding to NMDA receptors. However, hypericin did bind to NMDA receptors with moderate affinity (K_i approximately 1 µg/mL) [Cott 1997].

No inhibition of binding to rat NMDA receptors was observed for the constituents hypericin, hyperforin, pseudohypericin, amentoflavone, quercitrin, isoquercitrin, hyperoside and miquelianin [Butterweck 2002b].

In contrast, a hydromethanolic (80%) extract inhibited radioligand binding (CGP 39653) to NMDA receptors with an IC_{50} of 7.0 ± 2.7 µg/mL [Wonnemann 1997].

Hyperforin (at 10 µM) inhibited NMDA-induced calcium influx in rat cortical neurons demonstrating binding and antagonistic activity at NMDA receptors. In hippocampal preparations from male Wistar rats, hyperforin inhibited NMDA-receptor-mediated release of choline from phospholipid membrane [Kumar 2006].

Oestrogen receptors
Binding of oestradiol to the oestrogen receptor (ERα) was weakly inhibited (IC_{50} 200 µg/mL) by an ethanolic (50%) extract. Oestrogen binding to ERα receptor was inhibited by biapigenin (IC_{50} 1 µM) [Simmen 1999]. Other flavonoids did not significantly inhibit binding to ER-α (IC_{50} > 10 µM) [Simmen 1999; Simmen 2001].

Opioid receptors
An affinity of 1 µM hypericin with more than 40% inhibition was shown for non-selective muscarinic cholinergic receptors and for non-selective σ-opioid receptors [Raffa 1998].

Binding of [^{3}H]-naloxone to human recombinant µ- and κ-opioid receptors was inhibited by an ethanolic (50%) extract with IC_{50}-values of 25 – 40 µg/mL and 90 – 200 µg/mL respectively. The extract was approximately 10 times less active than a methanolic extract with approximately 0.2% hypericin and 5% hyperforin. Corresponding activity was observed for the hexane fraction of the ethanolic extract containing phloroglucinols, and the pure compounds hyperforin and adhyperforin. Hypericin and pseudohypericin inhibited binding to opioid receptors at 1-4 µM. No activity was observed with procyanidins, rutin, hyperoside, isoquercitrin, quercitrin, quercetin and I3,II8-biapigenin at concentrations of 10 µmol/mL [Simmen 1999; Simmen 2001; Simmen 1998].

In a screening of an ethanolic extract, binding of radiolabelled ligands was strongly inhibited at the opioid receptors µ (IC_{50} 4 µg/mL), κ (IC_{50} 3 µg/mL) and δ (IC_{50} 5 µg/mL) [Simmen 1999].

A hydromethanolic extract (0.5% hypericin, 4.5% hyperforin and 0.4% biapigenin) and a CO_2 extract (0.1% hypericin, 24% hyperforin and trace amounts of biapigenin) only weakly inhibited ligand binding at rat brain cortex σ-receptors. Hypericin showed an inhibition IC_{50} of 2.8-7.3 nM. The effect was light-dependent, with increasing IC_{50}-values under exclusion of light. Hyperforin did not inhibit ligand binding at rat brain cortex σ-receptors [Gobbi 2001].

In contrast, a methanolic extract with approximately 0.2% hypericin and 5% hyperforin inhibited binding to opioid receptor sub-types µ, κ and δ at low micromolar ranges. Corresponding activity was also observed for hyperforin and adhyperforin [Simmen 2001].

At a concentration of 10 µM neither hypericin nor pseudohypericin inhibited binding to the human opioid δ receptor. A slight inhibition was found for hypericin at the µ-receptor, and for pseudohypericin at the κ-receptor. No binding at opioid receptors was determined for quercitrin, isoquercitrin, rutin, hyperoside and miquelianin. Binding to opioid receptor δ was inhibited by amentoflavone (75%, K_i of 36.5 ± 14 nM) [Butterweck 2002b].

Serotonin receptors
After fractionation of an ethanolic (50%) extract the most distinct inhibition of binding was found at the 5-HT_6 (IC_{50} 1 µg/mL) and 5-HT_7 (IC_{50} 4 µg/mL) receptors for the hexane fraction containing hyperforin-related compounds, whereas the CH_2Cl_2 (pigments) and methanolic fractions (flavonoids and hypericins) were less active. Hypericin inhibited binding with an IC_{50} of 6 µM at 5-HT_6, and an IC_{50} > 10 µM at 5 HT_7. Hyperforin inhibited binding to 5-HT_6 (IC_{50} of 2 µM) and 5-HT_7 (IC_{50} of 3 µM). Flavonoids and I3,II8-biapigenin did not exhibit any effect on ligand binding to the serotonin receptors 5-HT_6 and 5-HT_7 at concentrations up to 10 µM [Simmen 1999; Simmen 2001]

A methanolic (80%) extract inhibited binding to 5-HT_{1A}-receptors with an IC_{50} of approximately 50 µg/mL [Rolli 1995].

A methanolic (80%) extract had a slight effect on binding to mouse brain 5-HT_2 receptors at a concentration of 100 µg/mL [Müller 1996].

An unspecified extract from fresh flowers and buds (containing approximately 0.1% of hypericin) showed 54% inhibition of binding (K_i = 25 µg/mL) to the 5-HT_1 receptor [Cott 1997].

A hydromethanolic extract (0.5% hypericin, 4.5% hyperforin and 0.4% biapigenin) and a CO_2 extract (0.1% hypericin, 24% hyperforin and trace amounts of biapigenin) did not inhibit ligand binding at recombinant rat 5-HT_6 and 5-HT_7 receptors [Gobbi 2001].

Amentoflavone (10 µmol/L) inhibited binding to the 5-HT_{1D} receptor (85%). Inhibition at the rat 5-HT_{2C} receptor was weaker (46%) [Butterweck 2002b].

Other receptors
A hydromethanolic extract (0.5% hypericin, 4.5% hyperforin and 0.4% biapigenin) and a CO_2 extract (0.1% hypericin, 24% hyperforin and trace amounts of biapigenin) did not inhibit ligand binding at neuropeptide NPY_1 and NPY_2 receptors from rat brain cortex and rat brain hippocampus respectively [Gobbi 2001].

Either no or weak inhibition of binding to histamine H_1 receptors was found for an ethanolic (50%) extract, hypericin at 1 µM, hyperforin at 10 µM, and the flavonoids amentoflavone, quercitrin, isoquercitrin, hyperoside and miquelianin [Butterweck 2002b].

Hypericin at 1 µM had no significant activity (<10%) at the rat adenosine A_1 and A_2-receptors, the rat angiotensin AT_1 receptor, the cloned human bradykinin B_2 receptor, the unspecific mouse glucocorticoid receptor and the rat vasopressin V_1 receptor. Weak affinity was found at the rat CCK_A-receptor and the cloned human endothelin ET_A-receptor [Raffa 1998].

No inhibition of binding to nicotinic, prostanoid, vasopressin receptors V_1-V_3 and oxytocin receptors was found for hypericin, pseudohypericin, amentoflavone, quercitrin, isoquercitrin, hyperoside and miquelianin [Butterweck 2000].

Effects on receptor regulation
Exposure of confluent rat astrocytoma C6 cell cultures to an ethanolic (50%) extract over 8 days resulted in a dose-dependent β-adrenoreceptor (AR) down-regulation equal to desipramine [Kientsch 2001].

A dry extract (DER: 4-7:1; ethanol 57.9% V/V) demonstrated a reduction in β-AR numbers in cultured cells comparable with desipramine (DMI) and fluoxetine (FLU). Seven-day incubation of cell cultures with DMI resulted in a reduction of β-AR numbers of 65 ± 6% ($p<0.01$), an effect fully reversed by co-incubation with 100 µM α-tocopherol. The extract led to a down-regulation of 56 ± 9% compared to untreated controls ($p<0.001$). On co-incubation with α-tocopherol, receptor down-regulation was significantly inhibited (88.6±5.3% of control, $p<0.001$) following exposure to the extract [De Marchis 2006].

Pre-incubation of rat C6 glioblastoma cells with hyperforin and hyperoside for 3 days led to a significant reduction in β_2-AR sensitivity, comparable to desipramine, and a loss of cAMP generation after β_2-stimulation with terbutaline ($p<0.05$) [Prenner 2007].

Similar results were obtained with a methanolic (80%) extract, which caused a dose-dependent reduction in the expression of serotonin (5-HT) receptors in a rat neuroblastoma cell line (PC-12) after short-term exposure over 2 to 6 hours [Müller 1993].

In a further study, the same extract affected cellular mRNA levels of the glucocorticoid receptors GR_α and its inhibitory counterpart GR_β at clinically relevant concentrations of hyperforin (125 ng/mL) via a primary and transient up-regulation of GR_α mRNA levels (at a concentration of 2.5 µg/mL) and a down-regulation of GR_β mRNA levels (at a concentration of 10 µg/mL) after 16 h of treatment [Enning 2011].

Neurotransmitter re-uptake inhibition
Inhibition of neurotransmitter re-uptake from the synaptic cleft has long been assumed to be the principal biochemical mechanism underlying the therapeutic effects of antidepressants such as tricyclic antidepressants, selective 5-HT re-uptake inhibitors (SSRIs), norepinephrine (NE) re-uptake inhibitors (NRIs) and 5-HT–NE reuptake inhibitors (SNRIs).

A methanolic (80%) extract inhibited serotonin re-uptake by rat synaptosomes with an IC_{50} of 6.2 µg/mL [Perovic 1995].

In mice synaptosomal preparations a CO_2 extract (38.8% hyperforin) demonstrated pronounced reuptake inhibition of 5-HT (IC_{50} 0.26 µg/mL), NE (IC_{50} 0.25 µg/mL), DA (IC_{50} 0.056 µg/mL), GABA (IC_{50} 0.12 µg/mL) and glutamate (IC_{50} 2.83 µg/mL) and for hyperforin (IC_{50} 0.11 µg/mL). IC_{50} values of a methanolic extract (1.5% hyperforin) were about 10 times higher than those of the CO_2 extract [Chatterjee 1998a].

A hydromethanolic extract (hyperforin content < 5%) inhibited the accumulation of [^{3}H]-5-HT in rat brain cortical synaptosomes with an IC_{50} of 7.9 µg/mL, compared to 1.8 µg/mL for hyperforin. The extract at 3-10 µg/mL and hyperforin at 0.3-1 µg/mL induced marked tritium release from synaptosomes preloaded with [^{3}H]-5-HT [Gobbi 1999].

A hydromethanolic extract (0.3% hypericin, 4.9% hyperforin) dose-dependently inhibited 5-HT and NE uptake in astrocytes with IC_{50} values of 25 µg/mL and 10 µg/mL respectively [Neary 1999]. Another hydromethanolic extract inhibited synaptosomal uptake of 5-HT, DA and NE with IC_{50} values of 2.43, 0.85 and 4.47 µg/mL respectively [Müller 1997].

Hyperforin was tested on voltage- and ligand-gated ionic conductances to measure neuronal responses to stimulation of NMDA or AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors. At concentrations between 3 and 100 µM, antagonistic effects on the NMDA receptor and on responses mediated by AMPA or GABA were seen [Chatterjee 1999]. Hyperforin non-competitively inhibited the synaptosomal uptake of [^{3}H]-glutamate and [^{3}H]-GABA [Wonnemann 2000].

An ethanolic (50% m/m) extract led to a dose-dependent

inhibition of NE- and 5-HT-uptake into rat brain slices. The extract was more selective for the uptake of NE than for that of 5-HT. The maximal extent of uptake inhibition by the extract was comparable to that of the positive controls imipramine, desimipramine and fluvoxamine, but lower for NE transport than the controls [Kientsch 2001].

A study assessed the effects on neurotransmitter reuptake of two different extracts (extracts 1 and 2: 3.34% and 0.56% hyperforin, 0.59% and 0.05% adhyperforin, respectively), methanolic and *n*-heptane fractions, as well as purified hyperforin and adhyperforin. Adhyperforin was shown to have a slightly more potent inhibitory effect than hyperforin on the uptake of DA (IC_{50} 1.8 and 6 ng/mL), 5-HT (IC_{50} 15 and 65 ng/mL) and NE (IC_{50} 7.9 and 18 ng/mL) [Jensen 2001].

A methanolic (80%) extract inhibited uptake of 5-HT and NE in cultures of rat cortical astrocytes. The extract also inhibited 5-HT uptake in neuronal cultures from serotonergic-rich raphe nuclei. The extract was 25 times more potent in inhibiting 5 HT uptake in neurons than in astrocytes. The extract also inhibited NE re-uptake in SK-N-SH cells, a human neuroblastoma cell line enriched in NE transporters. Hyperforin was about 10 to 20 times more potent than the extract in inhibiting neurotransmitter uptake in astrocytes and neuronal cells; this is consistent with the concentration of 5% hyperforin in the extract. The extract stimulated a persistent activation of extracellular signal regulated protein kinase (ERK) [Neary 2001].

The effects of a methanolic (80%) extract (10 µg/mL) and pure hyperforin (300 nM) on human blood platelet 5-HT uptake and efflux were compared to those of amitriptyline (AMI), venlafaxine (VEN), and reserpine. In contrast to AMI and VEN, the extract and hyperforin did not inhibit 5-HT uptake in platelet-rich plasma. In contrast to controls and AMI, a loss of accumulated [^{14}C]-5-HT after a prolonged incubation period was observed, suggesting that both hyperforin and the extract might affect the storage of 5 HT in platelet vesicles. In the presence of a comparably high concentration of reserpine (2.5 µM), the degree of 5-HT depletion in storage vesicles was lower than in the presence of 1 µM hyperforin [Uebelhack 2001].

The effect of hyperforin on 5 HT re-uptake was examined in Sprague-Dawley rat cortex synaptosomal preparations. Total re uptake of radiolabelled choline was significantly inhibited by 10 µM hyperforin (-32%, $p<0.05$). The effect was exclusively based on the high affinity system, but not the non-sodium-dependent low affinity re-uptake (total inhibition 68%, IC_{50} of hyperforin 8.5 µM) [Buchholzer 2002].

A hyperforin-rich heptane extract inhibited the sodium-dependent uptake of 5-HT, DA and NE into rat brain synaptosomes. Hyperforin inhibited the uptake of all three monoamines non-competitively, which was in contrast to the competitive inhibition exhibited by FLU, GBR12909 and DMI. Hyperforin had no inhibitory effect on the binding of [^{3}H]paroxetine, [^{3}H]-GBR12935 and [^{3}H]-nisoxetine to membrane presynaptic transporters for 5-HT, DA and NE respectively. Hyperforin also non-competitively inhibited the uptake of these monoamines to rat brain synaptic vesicles but did not affect the direct binding of [^{3}H]-dihydrotetrabenazine, a selective vesicular monoamine transporter ligand, to rat forebrain membranes [Roz 2002].

The mechanism of inhibition of synaptic vesicle transport of monoamines by hyperforin was investigated. Since the pH gradient across the synaptic vesicle membrane, induced by vacuolar type H^+-ATPase, is the major driving force for vesicular monoamines uptake and storage, proton transport was measured. Adding Mg-ATP to the medium containing and synaptic vesicles caused an immediate decrease in fluorescence and the addition of 1 µM carbonyl-cyanide *p*-trifluromethoxyphenylhydrazone (FCCP) abolished this effect. H^+-ATPase dependent proton pumping was inhibited by hyperforin in a dose dependent manner (IC_{50} = 1.9×10^{-7} M). Hyperforin acted similarly to the protonophore FCCP, abolishing the ATP induced fluorescence quenching (IC_{50} = 4.3×10^{-7} M). Hyperforin and FCCP had similar potencies for inhibiting rat brain synaptosomal uptake of [^{3}H]-monoamines as well as vesicular monoamine uptake. The efflux of [^{3}H]-5-HT from synaptic vesicles was sensitive to both drugs, thus 50% of preloaded [^{3}H]-5-HT was released in the presence of 2.1×10^{-7} M FCCP and 4×10^{-7} M hyperforin [Roz 2003].

The efficacy of different commercial extracts was investigated by measuring their activity to inhibit 5-HT uptake. The products with different concentrations of pharmaceutically relevant ingredients also differed in their IC_{50} values between 3.07 and 17.9 µg/mL. The activity of the products was correlated to their amount of hyperforin ($p<0.05$) [Schulte-Löbbert 2004].

The immediate effects of an ethanolic extract (50% m/m) with a low content of hyperforin on the specific DA uptake were studied in rat striatal brain slices and compared with the effects on NE and 5-HT uptake in rat cortical brain slices. Specific DA uptake was inhibited in a dose-dependent manner. The NE uptake inhibition was around 30-times higher than that for 5-HT, and seven times higher than that of the DA uptake [Ruedeberg 2010].

Adhyperforin inhibited uptake of [^{3}H]-5-HT, [^{3}H]-NE, and[^{3}H]-DA (IC_{50} 4.1, 2.64, and 0.89 µg/mL, respectively) in synaptosomes of the frontal cortex, and displayed robust binding affinities to the 5-HT, NE but not DA transporters (K_i values 18.75 and 4.03 µg/mL respectively) [Tian 2014].

Membrane interaction

The effects of hyperforin on the fluidity of crude brain membranes from young guinea pigs was investigated using three different fluorescent probes. Diphenylhexatriene (DPH) and trimethylammonium-diphenylhexatriene (TMA-DPH) anisotropy measurements were inversely correlated with membrane flexibility (annular and bulk fluidity). Incubation of brain membranes with 10 µM hyperforin sodium salt resulted in increased DPH ($p<0.05$) and decreased TMA-DPH ($p<0.01$) anisotropy, respectively, indicating that hyperforin modifies specific membrane structures in different ways. It decreases the flexibility of fatty acids in the membrane hydrocarbon core, but fluidises the hydrophilic region of membrane phospholipids. Lower concentrations (0.3 µM) significantly ($p<0.05$) decreased the annular fluidity of lipids close to membrane proteins. However, bulk fluidity was unchanged by this low hyperforin concentration [Eckert 2001].

An ethanolic (60%) extract (containing 5% hyperforin) and hyperforin sodium salt were given at single oral doses of 300 mg/kg or 15 mg/kg, respectively, to female NMRI mice. Three hours later in the isolated brains the properties of murine brain membrane fluidity were determined. Oral administrations resulted in hyperforin brain levels of 29.5 ± 18.7 pmol/g and 53.7 ± 18.7 pmol/g. Treatment of mice with hyperforin lead to decreased annular- ($p<0.05$) and bulk fluidity ($p<0.001$) and increased ($p<0.01$) acyl-chain flexibility of brain membranes [Eckert 2004].

Effects on ion channels

In rat cerebellar Purkinje neurons, hyperforin inhibited ion currents at the NMDA and AMPA receptors (IC_{50} 3.2 µM and 4.6 µM respectively) in a dose- and time-dependent manner [Chatterjee 1999].

Spasmolytic properties of hyperforin were studied in the hamster *vas deferens* smooth muscle cell line DDT1-MF2. Hyperforin (0.3-10 µg/mL) caused a concentration-dependent increase of intracellular calcium concentration ($[Ca^{2+}]_i$) and extracellular acidification rate (ECAR). Both effects were independent of extracellular Ca^{2+}. Since the ECAR stimulation was inhibited by the intracellular Ca^{2+} chelator BAPTA, as well as by inhibitors of plasmalemmal and mitochondrial Na^+/Ca^{2+} exchangers, but not by inhibitors of the Na^+/H^+ antiporter, the intracellular Ca^{2+} increase seems to be essential for this hyperforin effect [Koch 2001].

Isolated patch-clamped rat central and peripheral neurons exposed to rapid changes in the compartment of external medium were used to investigate the modulation of various voltage- and ligand-gated channels by hyperforin and other constituents of *Hypericum perforatum*. At nanomolar concentrations, hyperforin induced inhibition of various ion channels. On P-type Ca^{2+} channels hyperforin acted via interaction with calmodulin or through calmodulin-activated pathways involving at least one second messenger [Krishtal 2001].

In rat PC12 cells, hyperforin (10 µM) increased intracellular concentrations of Na^+ and Ca^{2+}. This increase was dependent on the activation of a non-selective cation channel, TRPC6, a transient receptor potential channel. Blockade of TRPC6 channels inhibited the hyperforin effect in PC12 cells [Leuner 2007]. This was confirmed by experiments in cortical neurons from embryonic murine brain [Gibon 2010].

Hyperforin inhibited various ion channels in the patch-clamp model in peripheral and central neuronal cells isolated from Wistar rats. In P-type Ca^{2+} channels it was confirmed that hyperforin acts via an interaction with calmodulin or through calmodulin-activated pathways involving at least one second messenger. An extract (5% hyperforin) inhibited almost all tested ligand-gated ion channels. Quercitrin was detected as a potent inhibitor of ATP-induced conductance; biapigenin and hyperoside inhibited ATP- and AMPA-induced conductances, whereas hypericin was inactive in all cases [Krishtal 2001; Leuner 2007; Treiber 2005; Wang 2010].

The effect of an unspecified extract and hypericin on cell viability, human chorionic gonadotrophin (hCG) production, Ca^{2+} uptake, and Ca^{2+} transport proteins expression in JEG-3 cells was analysed. Toxicity was seen at high concentrations (≥150 µg/mL), but with no effect on hCG production with the extract (25 µg/mL) or hypericin (7.5 and 75 ng/mL). Cells treated with the extract or hypericin exhibited increased intracellular Ca^{2+} concentrations after 24 h but not 10 min of incubation. A significant ($p<0.01$) decrease in translationally controlled tumour protein Ca^{2+} handling protein was only caused by the extract. Hypericin increased the protein expression of the transient receptor potential vanilloid 6 Ca^{2+} channel and 28-kDa calcium-binding protein and decreased that of plasma membrane Ca^{2+}-ATPase 1/4 [da Conceicao 2010].

Hyperforin induced TRPC6-independent H^+ currents in HEK-293 cells, cortical microglia, chromaffin cells and lipid bilayers. The latter demonstrated that hyperforin acts as a protonophore. This activity caused cytosolic acidification, which strongly depended on the holding potential, and fueled the plasma membrane sodium-proton exchanger. Thereby the free intracellular sodium concentration increased and the neurotransmitter uptake by Na^+ co-transport was inhibited. Additionally, hyperforin depleted and reduced loading of large dense core vesicles in chromaffin cells, which requires a pH gradient in order to accumulate monoamines [Sell 2014].

Neutrophils from patients with Behçet's disease (BD) were divided into three subgroups and incubated with either an unspecified extract (containing 3.6% hyperforin), voltage-gated calcium channel (VGCC) blockers (verapamil+diltiazem, V+D) or a non-specific TRPM2 channel blocker (2-aminoethyl diphenylborinate, 2-APB). They were subsequently stimulated with fMLP as a Ca^{2+} agonist and oxidative stress generator. The high cytosolic-free Ca^{2+} $[Ca^{2+}]_i$, caspase-3, and caspase-9 values found in the patient groups decreased markedly after treatment with the extract and low GSH and GSH-Px values increased. The $[Ca^{2+}]_i$ also decreased in the groups with V+D and 2-APB incubations. The extract induced protective effects on oxidative stress by modulating Ca^{2+} influx in BD patients. [Naziroglu 2014a; Naziroglu 2014b].

Cholinergic effects

The effect of hyperforin on choline (Ch) uptake was studied in brain synaptosomes from Sprague-Dawley rat cortices. Hyperforin inhibited high-affinity choline uptake with an IC_{50} of 8.5 µM, whereas low-affinity uptake was not affected [Buchholzer 2002].

The spontaneous release of Ach in the neuromuscular junction of Charles River male mice was unaffected by a hydroalcoholic extract at doses from 0.2 to 1.0 mg/mL indicating that neither presynaptic nor postsynaptic functions were modified by the extract. Both the frequency and amplitude of the miniature end-plate currents (mepcs) were unmodified by the extract. The mepcs decay time, i.e. the apparent cholinergic channel life time, was significantly increased after treatment. The amplitude of the evoked end-plate currents was constantly and in a dose-dependent manner increased by the extract [Re 2003].

Smooth muscle function

Contractions of rat bladder strips were induced by electrical field stimulation (EFS) or by exogenous α,β-methylene ATP. An unspecified hydromethanolic extract was significantly ($p<0.001$) more active in inhibiting the EFS-induced contractions than the α,β-methylene ATP-induced contractions. The inhibitory effect of the extract on EFS-induced contractions was unaffected by methysergide, haloperidol, phentolamine and propranolol (antagonists blocking the action of 5-HT, DA, and NE on their own receptors), the L-type calcium channel antagonist verapamil, capsazepine (blocking the vanilloid receptor), or cannabinoid CB_1 receptor antagonist SR141716A. However, the opioid receptor antagonist naloxone significantly ($p<0.001$) reduced the inhibitory effect of the extract on EFS-induced contractions [Capasso 2004].

An unspecified hydromethanolic extract (1-300 µM) exhibited a concentration-dependent decrease in the amplitude of contractions in isolated rat and human *vas deferens* induced by both EFS and α,β-methylene ATP [Capasso 2005].

Immunological effects

A hydromethanolic extract caused pronounced suppression of interleukin-6 (IL-6) release in human blood samples after stimulation with phytohaemagglutinin [Thiele 1993]. Hypericin at the micromolar range inhibited phorbol myristate acetate- and TNF-α-induced activation of NF-κB, whereas hyperforin and an ethanolic extract (60%, w/w; 0.15% hypericin and 5% hyperforin) were inactive [Bork 1999]. The myeloperoxidase-catalysed dimerisation of enkephalins was inhibited by a methanolic extract [Denke 1999].

Mononuclear cells from healthy donors were incubated with a hydromethanolic extract or with SSRIs and tested for natural killer cell activity. The extract was tested at very low concentrations (0.5-20 ng/mL), paroxetine (PAR) at 120 ng/mL

and norfluoxetine at 100 ng/mL. In contrast to the SSRIs, the extract failed to enhance natural killer cell activity at these concentrations [Helgason 2000].

Seven unspecified commercial extracts were studied for their effect on substance P (SP)-induced synthesis of IL-6 in human astrocytoma cells. A potent and dose-dependent inhibition of SP-induced IL-6 synthesis by various extracts was demonstrated [Fiebich 2001].

Autoimmune inflammatory demyelination
Hyperforin inhibited IFN-γ production, with down-regulation of a Th1 gene expression marker and upregulation of a Th2 marker, on IL-2/PHA-activated T cells. In parallel, a strong down-regulation of the chemokine receptor CXCR3 expression on activated T cells was shown. *In vivo*, hyperforin (150 mg/kg in food pellets) significantly ($p<0.001$) attenuated the severity of symptoms of experimental allergic encephalomyelitis, a Th1-mediated autoimmune disease of the CNS [Cabrelle 2008].

Neuroprotection
The effect of hyperforin was studied on the processing of the amyloid precursor protein (APP) in PC12 cells, stably transfected with human wild type APP. A transiently increased release of secretory APP fragments was observed upon hyperforin treatment (50 nM - 10 μM). These results suggest that hyperforin is an activator of secretory processing of APP [Froestl 2003].

The neuroprotective role of an ethanolic (80%) extract and hexane, ethyl acetate, and butanol fractions in amyloid-β peptide (Aβ)-induced cell death in rat cultured hippocampal neurons was investigated. Induced lipid peroxidation was significantly ($p<0.05$) inhibited by fractions containing flavonol glycosides, flavonol and biflavone aglycones, and by a fraction containing mainly chlorogenic acid-type phenolics (21%, 77% and 98% respectively). Lipid peroxidation evaluated after incubation with 25 μM Aβ, was significantly ($p<0.05$) inhibited by the extract. The extract, and fractions containing flavonol glycosides, flavonol and biflavone aglycones, reduced Aβ-induced cell death by 65%, 58% and 59% respectively. Aβ induced a decrease in cell volume, chromatin condensation and nuclear fragmentation, effects not evident in the presence of the extract and fractions containing hypericins (hypericin = 11.0 μM), or fractions containing flavonoids (quercetin = 21.1 μM). Dendritic lesions were observed following insult with Aβ, but prevented after exposure to the peptide plus the fractions [Silva 2004].

An unspecified extract protected PC12 cells from H_2O_2-induced damage in a dose-dependent manner within 24-hour treatment. Intra- and extra-cellular ROS levels decreased significantly to 71.9% and 50.0% of the control at 20 μg/mL, respectively. The extract blocked DNA fragmentation and prevented H_2O_2-induced apoptosis of the cells at concentrations of 10 to 100 μg/mL [Lu 2004].

An ethanolic extract (50% V/V; 0.37% hypericins, 10.1% total flavonoids and 0.7% hyperforin) was evaluated for its antioxidative properties in glutamate-induced cell death of hippocampal HT22 cells. The maximum protective effect was observed at 0.1%. The effects were related to an attenuation of calcium fluxes and cellular energy statuses [Breyer 2007].

In a microglial cell line pre-treated with an ethanolic (50%) extract, cell death evoked by treatment with $Aβ_{25-35}$ and $Aβ_{1-40}$ was attenuated significantly ($p<0.01$ to $p<0.001$) in a dose-dependent manner. Investigation of the single compounds revealed that (+) catechin and (-)-epicatechin increased cell viability slightly, whereas the quercetin, rutin, hyperoside and quercitrin showed no effect on cell viability. At the same concentration, the flavonoids reduced the formation of amyloid-induced ROS in microglia. No influence of the extract on the capacity of microglia to phagocytose sub-toxic concentrations of fibrillar $Aβ_{1-40}$ was observed [Kraus 2007].

The neuroprotective effect of a methanolic extract was investigated in astrocytes from male Swiss albino mice. Treatment with the extract for 7 days (300 mg/kg/day p.o.) following a challenge on day 1 with 4 i.p. injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (20 mg/kg in 2 h intervals) resulted in reduced MPTP-induced striatal area astrocyte activation and a signfiicant inhibition of MAO-B activity ($p<0.05$) compared to MPTP alone [Mohanasundari 2007].

Quercetin, kaempferol and biapigenin significantly ($p<0.05$) reduced chemically induced neuronal death in hippocampal neurons from Wistar rat embryos. Neuroprotection was correlated with prevention of delayed calcium deregulation and with the maintenance of mitochondrial transmembrane electric potential in brain mitochondria. The compounds reduced mitochondrial lipid peroxidation ($p<0.05$ to $p<0.01$) and loss of mitochondrial transmembrane electric potential caused by oxidative stress. Biapigenin significantly ($p<0.05$) affected mitochondrial function and decreased the capacity of mitochondria to accumulate calcium [Silva 2008a].

Hypericin significantly interacted with peptides in β-sheet conformation by hindering the formation of fibrils and thereby interfering with early stages of the polymerisation process responsible for neurodegenerative disorders [Bramanti 2010].

An unspecified extract (31.25 ng/mL-5 μg/mL) significantly ($p<0.05$) decreased the survival of human SH-SY5Y neuroblastoma cells at the highest dosage, whereas lower concentrations exerted no significant effects. The treatment of cells with 5 μg/mL led to a significant ($p<0.05$) increase of ATP levels, whereas treatment with a concentration of 500 ng/mL had no significant effect. The extract did not show any cytotoxic activity [Schmidt 2010].

Quercetin and kaempferol stimulated depression-related signalling pathways involving brain-derived neurotrophic factor (BDNF), phosphorylation of cAMP response element binding protein (CREB), postsynaptic density proteins PSD95, and reduced amyloid-β peptide (Aβ) in neurons isolated from double transgenic AD mice (TgAPPswe/PS1e9). In addition, enhanced BDNF expression and reduction of Aβ oligomers were confirmed in the hippocampus [Hou 2010].

Hyperoside (2.5, 5, 10 or 20 μM) significantly inhibited cytotoxicity and apoptosis in $A\text{-}β_{25-35}$-induced primary cultured cortical neurons from embryonic Sprague-Dawley rat foetuses by reversing $A\text{-}β_{25-35}$-induced mitochondrial dysfunction, including mitochondrial membrane potential decrease, ROS production ($p<0.01$), and mitochondrial release of cytochrome c ($p<0.01$). The compound activated the PI3K/Akt signalling pathway, resulting in inhibition of the interaction between Bad and Bcl_{XL} and inhibited the mitochondria-dependent downstream caspase-mediated apoptotic pathway ($p<0.05$ to $p<0.01$). It protected Aβ-induced primary cultured cortical neurons via PI3K/ Akt/ Bad/ Bcl_{XL}-regulated mitochondrial apoptotic pathway [Zeng 2011].

An ethanolic extract (60% V/V; 0.3% hypericin and 0.61% hyperforin) administered to male Wistar rats for 21 days at a dose of 300 mg/kg per day demonstrated increased mRNA levels for mdr1 by 275% ($p<0.001$), for oatp1c1 by 41% ($p<0.05$), and a reduced mRNA level (by 68.5%, $p<0.05$)) for mrp1 in hippocampal tissue [Mrozikiewicz 2014].

Anti-inflammatory effects
Hypericin (1, 5, 10 µg/mL) significantly ($p<0.05$) and dose-dependently inhibited IL-12 production in LPS-activated macrophages from female DBA/2 mice (IC_{50} = 1.45 µg/mL) and potently inhibited the activation of an IL-12 gene promoter [Kang 2001].

A methanolic (80%) extract and hyperforin (0.03, 0.1, 0.3, 1, 3 and 10 µM) were investigated for inhibition of 5-lipoxygenase (5-LO) and cyclooxygenases (COX). Hyperforin directly inhibited 5-LO (IC_{50} ~ 90 nM) in freshly isolated human polymorphonuclear leukocytes (PMNL). In thrombin- or ionophore-stimulated human platelets, hyperforin suppressed COX-1 activity with IC_{50} values of 0.3 and 3 µM respectively, being about 3- to 18-fold more potent than aspirin. Hyperforin did not interfere with COX-2 product formation and did not significantly inhibit 12-LO in platelets or 15-LO in leukocytes. Inhibition of 5-LO was slightly more prominent for equimolar amounts of the extract (calculated for hyperforin; IC_{50} 22 µg/mL) [Albert 2002].

A methanolic (80%) extract exhibited antiinflammatory activity on PMNL myeloperoxidase (MPO) activity. The extract reduced the peroxidative and chlorinating activity of MPO in a concentration-dependent manner by 18.5% (at 20 µg/mL) to 44.1% (at 40 µg/mL) [Pabuccuoglu 2003].

Hyperoside exerted an anti-inflammatory action through suppressed production of TNF-α, IL-6, and NO in LPS-stimulated mouse peritoneal macrophages. The maximal inhibition rates of TNF-α, IL-6, and NO production by 5 µM hyperoside were 32.31 ± 2.8%, 41.31 ± 3.1%, and 30.31 ± 4.1% respectively. In addition, hyperoside inhibited NF-κB activation and IκB-α degradation [Kim 2011].

Various extracts (acetonic, 96% ethanolic and 50% ethanolic) and isolated constituents (flavonoids and dianthrones) showed a comparable and dose-dependent inhibition (>80% inhibition at 0.15%) of 5-LO. The strongest effect was seen for hyperoside (IC_{50} 3.89×10^{-6} M), whereas quercetin, quercitrin, isoquercitrin and rutin were less active (IC_{50} of $3.7 – 9.0 \times 10^{-5}$ M). The weakest effects were seen for hypericin and pseudohypericin (IC_{50} of 1.5 and 6.3×10^{-4} M) [Oblozinsky 2006].

In isolated PMNL hyperforin inhibited the generation of reactive oxygen species (ROS) as well as the release of leukocyte elastase with an IC_{50} of about 3 µM and blocked receptor-mediated Ca^{2+} mobilisation (IC_{50} 0.4 and 4 µM) in PMNL and monocytic cells [Feisst 2004].

The inhibition of prostaglandin E_2 (PGE_2) production by various extracts and some constituents was investigated in RAW 264.7 mouse macrophage cells. The anti-inflammatory activity of all extracts was light-independent. The extracts significantly reduced LPS-induced PGE_2 production by 73 to 82% (each $p<0.0001$) [Hammer 2007]. An extract fraction used in the previous study, and a combination of four synergistic constituents at levels identified from that fraction called the 4 component-system (0.07 µM amentoflavone, 0.08 µM quercetin, 0.2 µM chlorogenic acid and 0.03 µM pseudohypericin), were studied to identify key gene targets involved in the anti-inflammatory activity. The reduction in PGE_2 in LPS-stimulated macrophages observed with both treatments may be accounted for by genes involved in Janus kinase, as well as a signal transducer and activator of transcription (JAK–STAT) and eicosanoid pathways. COX-2 appeared to be a major target of both the 4-component-system and the fraction, and upstream pathways leading to COX-2 expression were also affected by both treatments [Hammer 2010].

The inhibitory effect of the 4 component system on LPS-induced prostaglandin E_2 and NO production in RAW 264.7 mouse macrophages was compared to ethanolic extracts and extract fractions. Pseudohypericin was found to be the most important for anti-inflammatory activity. Most of the extracts reduced LPS-induced PEG_2 and NO production [Huang 2012].

Hyperoside exerted an anti-inflammatory effect through inhibition of iNOS expression and suppressed production of TNF-α, IL-6 and NO in LPS-stimulated mouse peritoneal macrophages. Furthermore, it inhibited NFκB activation by inhibiting LPS-induced degradation of the inhibitory subunit IκB-α, a key step for NFκB-induced transcription of certain proinflammatory genes including iNOS [Kim 2011].

An unspecified extract was studied at doses of 10, 25 and 50 µg/mL on adipocyte differentiation and for anti-inflammatory effects in 3T3-L1 pre-adipocytes. The extract promoted adipocyte differentiation, while immunoblots indicated that the extract increased the expression of peroxisome proliferator activated receptor gamma (PPARγ), a nuclear receptor regulating adipocyte differentiation, and adiponectin, an anti-inflammatory adipokine. The anti-inflammatory activity of the extract was demonstrated by its inhibition of the activation of NF-κB. Stimulation of mature 3T3 L1 adipocytes by TNF-α decreased the expression of the NF-κB inhibitor IκBα, and increased its phosphorylation. Treatment with the extract decreased the TNF-α induced perturbation in IκBα expression and phosphorylation, indicating that the extract mediated the inhibition of NF-κB activation. In addition, the extract decreased the TNF-α induced increase in the mRNA levels of pro-inflammatory adipokines, IL-6, and monocyte chemoattractant protein-1 [Hatano 2014].

Tissue repairing effects
The tissue repairing effects of an alcoholic (50%) extract were evaluated in comparison with dexpanthenol on cultured chicken embryonic fibroblasts. The extract exhibited a tissue repairing activity mainly due to an increase in the stimulation of fibroblast collagen production and the activation of fibroblast cells in polygonal shape [Öztürk 2007].

Metabolic effects
In the INS-1E β-cell line an unspecified hydroalcoholic extract and hyperforin (at 1-3 µM) prevented cytokine (IFN-γ, 150 U/mL + IL-1β 10 U/mL + TNF-α 50 U/mL) induced impairment of glucose-stimulated insulin secretion, and protected cells against apoptosis in a dose-dependent manner. iNOS expression ($p<0.05$) as well as cytokine-induced activations of STAT-1 and NF-κB were down-regulated by the extract and hyperforin (range 0.5-5 µM) [Menegazzi 2008].

Ethanolic extracts, one from leaves and one from flowers, inhibited differentiation of 3T3-L1 adipocytes. Both extracts substantially inhibited insulin sensitive glucose uptake in mature 3T3-L1 adipocytes. Treatment with the flower extract resulted in a time- and dose-dependent inhibition of insulin stimulated glucose uptake in mature adipocytes [Amini 2009].

An unspecified flower extract at a dose of 50 µg/mL significantly ($p<0.02$) reduced insulin-sensitive glucose uptake in human adipocytes compared to control. The extract inhibited insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation in both murine and human fat cells. Hypericin (0.02–0.3 µM) and hyperforin (0.17–2.8 µM) did not modulate adipocyte development and insulin activity in mature adipocytes [Richard 2012].

A hydroalcoholic extract (4.1% hyperforin) at doses of 100 and 200 µg/mL and hyperforin at 1 and 2 µM were applied to

isolated rat and human islets exposed to cytokines (rat or human IFN-γ 1000 µg/mL + IL-1β 100 U/mL + TNF-α 1000 U/mL) over 20 h. In both rat and human islets, the extract and hyperforin counteracted cytokine-induced functional impairment and down-regulated mRNA expression of pro-inflammatory target genes, such as iNOS, CXCL9, CXCL10 and COX-2. Cytokine-induced NO production from cultured islets was significantly ($p<0.01$ to $p<0.001$) reduced in the presence of the extract and hyperforin. The increase in apoptosis and necrosis following 48-h exposure to cytokines was fully prevented by the extract, but only partially by hyperforin. Ultrastructural morphometric analysis in human islets exposed to cytokines for 20 h showed that the extract or hyperforin avoided early beta-cell damage [Novelli 2013].

Antiviral effects
Hypericin showed antiretroviral activity against equine infectious anaemia virus (EIAV). Treatment with 10 µg/mL hypericin reduced the virus production completely [Kraus 1990].

In primary human lymphocytes hypericin exhibited anti-HIV-1 activity via an inhibition of HIV-1 reverse transcriptase [Schinazi 1990].

Hypericin and protohypericin inhibited virus replication of human cytomegalovirus strain AD-169 in human foreskin (Hs-68) and lung fibroblast cells (MRC-5) with IC_{50} values of 1.8 and 5.7 µM respectively [Barnard 1992].

Hypericin (500 ng/mL) exhibited strong antiviral activity against vesicular stomatitis virus, *Herpes simplex* virus types 1 and 2, parainfluenza virus and vaccinia virus (up to 3.5 $\log_{10}$ reduction of viral production) [Andersen 1991].

Hypericin (0.12 - 0.25 µg/mL) inactivated murine cytomegalovirus, sindbis virus, and human immunodeficiency virus type 1 (HIV-I), especially on exposure to fluorescent light [Hudson 1991].

Hypericin was active against duck hepatitis B virus (DHBV). After 1 h incubation with hypericin at 15 µg/mL, cells stably-transfected with DHBV stopped virus replication for several days, though virus-like particles continued to be released into the culture medium. Hypericin was only slightly virucidal against DHBV, and culture medium from treated cultures did not block initiation of infection when added to DHBV susceptible cultures prior to a challenge with infectious DHBV [Moraleda 1993].

An ethanolic extract at doses of 0.32 to 40 µg/mL was tested for anti-hepatitis B virus (HBV) activity in HepG2 2.2.15 cells. The extract effectively suppressed the secretion of HBsAg and HBeAg in a dose-dependent manner, as well as the extracellular HBV DNA. In addition, the extract selectively inhibited the activity of HBV promoter Fp [Pang 2010].

Antimicrobial effects
Various extracts were evaluated for their antibacterial activity against *Bacillus subtilis*, *P. aeruginosa*, *Sarcina lutea* and *E. coli* at concentrations of 1, 10, 25 and 50 µg/µL. The *n*-hexane and dichloromethane extracts were inactive against all tested organisms. The *n*-butanol and ethyl acetate extracts showed activity against all the tested bacterial strains at concentrations of 25 and 50 µg/µL while the methanolic extract was only active against *B. subtilis* at concentrations 25 and 50 µg/µL [Naeem 2010].

Twenty-one samples of traditionally-prepared and commercial St. John's Wort olive oil macerates were screened for their antimicrobial and antiprotozoal activity. In the antiprotozoal assays, ten of the oils inhibited *Trypanosoma brucei rhodesiense* (IC_{50} 15.9-64.5 µg/mL), while only one oil exerted antimicrobial activity towards *Staphylococcus aureus* (IC_{50} 88.7 µg/mL) [Orhan 2013].

Hypericin (0-2.5 µM) resulted in a dose- and light-dose dependent (1 – 8 J/cm^2) cytotoxicity (cellular destruction and induction of early apoptosis as a prominent mode of cell death) in CNE-2 nasopharyngeal carcinoma cells [Xu 2010].

Various extracts (MeOH; petroleum ether; chloroform, ethyl acetate) showed growth inhibition of Gram-positive bacteria. The ethyl acetate extract was the most active (MIC 12.5 - 50 µg/mL). Incubation of selected microorganisms with flavonoids, hypericins or hyperforins resulted in a growth inhibition by hypericin (MIC 12.5 - >1000 µg/mL), hyperforin (MIC 25 – 100 µg/mL) and its dicyclohexilammonium salt (MIC 25 – 100 µg/mL). Flavonoids appeared to be inactive [Avato 2004].

Photosensitising and photodynamic effects
The methanolic fraction (PMF) of an unspecified extract was examined for activity against urinary bladder carcinoma using the T24 (high grade metastatic cancer) and RT4 (primary low grade papillary transitional cell carcinoma) human bladder cancer cells. PMF was excited using 630 nm laser light. PMF exhibited significant photocytotoxicity in both cell lines at a concentration of 60 µg/mL, with 4-8 J/cm^2 light dose, resulting in apoptosis from 80% to 86%. At 20 µg/mL PMF was not active in either cell line. Photofrin®, a clinically approved photosensitiser, exhibited less photocytotocixity at 4 µg/mL than PMF in both tested cell lines [Stavropoulos 2006].

In human epidermoid carcinoma cells (A431) the time- and dose-dependence of hypericin-photodynamic treatment (PDT)-based induction of cytotoxicity and apoptotic cell death was studied. Induction of apoptosis by hypericin-PDT was dose-dependent with necrotic cell death at higher doses. Time-course analysis of an almost homogenous apoptotic population of cells (at 1.44 J/cm^2) showed a rapid increase in nuclear fragmentation and activation of caspases reaching a maximum 5 hours after irradiation. Using specific caspase substrates, significant activation of caspase-2, -3, -6 and -9 was found. Mitochondrial involvement during hypericin-PDT-induced apoptosis was proven by a rapid reduction of the mitochondrial membrane potential; the level of intracellular adenosine-5'-triphosphate (ATP) remained at control level for up to 6 hr post irradiation suggesting upregulation of glycolysis as a compensating mechanism for energy supply [Berlanda 2006].

The modulation of hypericin-PDT by pre-treatment with *n*-3 and *n*-6 fatty acids was studied in HT-29 and HeLa tumour cells. A stimulation of cytotoxic effects by docosahexaenoic acid (*n*-3) and arachidonic acid (*n*-6) was observed in both cell lines. Treatment with either fatty acids or photodynamic therapy alone induced apoptosis in a dose- and time-dependent manner. The effect was even more striking with the combination applied as pre-treatment with fatty acids prior to PDT. The combination induced changes in membrane lipid composition leading to alteration in cell membrane fluidity. Increased toxicity from the combined treatment was also confirmed by the presence of oxidative stress as demonstrated by ROS production, RNS accumulation and increased presence of lipoperoxides [Kello 2010].

A hydroalcoholic extract was assessed for its anti-proliferative activity in A375 human melanoma cells after irradiation with UVA (1.8 J/cm^2) and demonstrated phototoxicity at IC_{50} 78 µg/mL. The extract caused a dose-related inhibition of NO production in murine monocytic macrophage cell line RAW 264.7 with an IC_{50} of 342 µg/mL [Menichini 2013].

Antiproliferative effects
Hypericin and pseudohypericin showed a strong inhibitory effect on protein kinase C (IC_{50} of 1.7 and 15 µg/mL respectively) which resulted in an anti-proliferative effect in BALB 3T3/H-ras cells with IC_{50} values of 6.3 and 28 µg/mL respectively; associated with an antiretroviral effect [Takahashi 1989].

Rather than stabilising topoisomerase II (topo II) in covalent complexes with DNA (cleavage complexes), hypericin inhibited the enzyme prior to DNA cleavage. The assays indicated that hypericin is a potent antagonist of cleavage complex stabilisation by etoposide and amsacrine. This antagonism appears to be due to the ability of hypericin to intercalate or distort DNA structure, thereby precluding topo II binding and/or DNA cleavage. Supporting its non-DNA damaging, catalytic inhibition of topoisomerase II, hypericin was shown to be equitoxic to both wild-type and amsacrine-resistant HL-60 leukaemia cell lines. Hypericin was incapable of stimulating DNA damage-responsive gene promoters that are activated by etoposide. As with the topo II assay, antagonism of DNA damage stimulated by 30 µM etoposide was evident in leukaemia cells pre-treated with 5 µM hypericin [Peebles 2001].

Photoactivated hypericin and paclitaxel (PXL) similarly increased the activity of caspase-8 and caspase-3. Drug-induced apoptosis was completely blocked by inhibitors of caspase-8 and caspase-3. The involvement of death receptors was analysed using neutralising monoclonal antibodies against Fas (SM1/23), FasL (NOK-2) and TNF-R1 (MAB225), and a polyclonal rabbit anti-human TNF-related apoptosis-inducing ligand (TRAIL) antiserum. TRAIL antibody blocked TRAIL-induced and photo-activated hypericin-induced, but not PXL-induced apoptosis of Jurkat cells. In contrast, PXL-induced, but not hypericin-induced apoptosis was blocked by the SM1/23 and NOK-2 antibodies. Anti-TNF-R1 antibody had no effect. These findings suggest that hypericin photo-induced apoptosis of Jurkat cells is mediated in part by the TRAIL/TRAIL-receptor system and subsequent activation of upstream caspases [Schempp 2001].

Hyperforin inhibited growth of various human and rat tumour cell lines with IC_{50} values between 3 – 15 µM. Treatment with hyperforin resulted in a dose-dependent generation of apoptotic oligonucleosomes, typical DNA-laddering and apoptosis-specific morphological changes. In MT-450 mammary carcinoma cells hyperforin increased the activity of caspase-9 and caspase-3, and hyperforin-mediated apoptosis was blocked by the caspase inhibitor zVAD.fmk. Hyperforin, but not paclitaxel, induced a rapid loss of the mitochondrial trans-membranal potential $\Delta \psi m$, and subsequent morphological changes such as homogenisation and vacuolisation of mitochondria. Hyperforin-mediated mitochondrial permeability transition was not prevented by zVAD.fmk. The compound was capable of releasing cytochrome *c* from isolated mitochondria. These findings suggest that hyperforin activates a mitochondria-mediated apoptosis pathway [Schempp 2002a].

Photoactivated hypericin did not cause Bcl-2 degradation but induced Bcl-2 phosphorylation in a dose- and time-dependent manner in HeLa cells. Bcl-2 phosphorylation was induced by sublethal PDT doses; increasing the photodynamic stress immediately led to apoptosis, during which Bcl-2 was neither phosphorylated nor degraded. The co-localisation of hypericin with α-tubulin and the aberrant mitotic spindles observed following sublethal PDT doses suggested that photodamage to the microtubule network provokes the G_2/M phase arrest. Bcl-2 phosphorylation is selectively suppressed by the cyclin-dependent protein kinase (CDK)-inhibitor roscovitine, completely blocked by the protein synthesis inhibitor cycloheximide and enhanced by the over-expression of CDK1, suggesting a role for this pathway [Vantieghem 2002].

The effect of an unspecified extract on antiproliferative activity of anticancer drugs and the expression of MDR1 mRNA were examined using HeLa and its MDR1-overexpressing subline. The effects on MDR1-mediated transport were evaluated using [^{3}H]-digoxin and MDR1 overexpressing LLC-GA5-COL150 cells. The effects of hypericin were compared with those of the extract. The extract (60 µg/mL containing 0.1 µM hypericin), but not hypericin, reversed the cytotoxicity of PXL and of daunorubicin slightly, down-regulated MDR1 mRNA, and inhibited MDR1-mediated transport [Wada 2002].

Hyperforin inhibited the growth of leukaemia K562 and U937 cells, brain glioblastoma cells LN229 and normal human astrocytes with GI_{50} values between 14.9 and 19.9 µM. Hyperforin and hypericin acted synergistically on leukaemic (K562, U937) cell growth. Cell death occurred by apoptosis after 24 h treatment with hyperforin. A dose-dependent loss of membrane phospholipid asymmetry associated with apoptosis was induced in all cell lines [Hostanska 2003b].

Different extracts (A: ethanol-water macerate of fresh flowering tops, B: macerate of flowering tops dried in the dark, C: aqueous-ethanolic percolation of the light-dried whole aerial parts), containing various amounts of hyperforin (A: 3.25%; B: 2.21%; C: 0.21% w/w) and flavonoids (A: and B: 5.3%; C: 10% w/w), but similar amounts of naphthodianthrones (0.3%), inhibited growth of K562 and U937 cell lines in the WST-1 assay. The GI_{50} for the extracts was 248.3-621.3 µg/mL in K562 and 378.2 911.7 µg/mL in U937 cells. The values of the three main constituents were 1.6 3.9 µM for naphthodianthrones, 1.0-40.7 µM for phloroglucinols and 30.5 68.5 µM for flavonoids [Hostanska 2003a].

The influence of a methanolic extract and hypericin on the human erythroleukaemic cell line K562 was tested. After 1 h exposure to increasing concentrations (as hypericin content: 0.5 to 5 µg/mL) of both agents in the dark, leukaemic cells were grown for 24 h and 48 h. Hypericin showed only a weak inhibitory effect on cell growth and no effect in inducing apoptosis. In contrast, the extract showed a significant ($p<0.05$) concentration-dependent inhibition of cell growth, and induced apoptosis [Roscetti 2004].

A lipophilic petroleum ether extract induced apoptosis with LC_{50} values of 5 and 7 µg/mL in T24 and NBT-II bladder cancer cells respectively. The most active fraction from this extract displayed 60% cell growth inhibition at a concentration of 0.95 µg/mL. Hyperforin concentrations in the extract and its most active fraction were 0.94 µg/mL, and 0.17 µg/mL respectively; considerably lower than the active cytotoxic concentration of pure hyperforin which appeared to be in the range of 1.8 µg/mL - 5.0 µg/mL [Skalkos 2005].

Hypericin inhibited protein kinase C in H-ras transfected mouse BALB 3T3 cells and induced apoptosis. This mechanism seemed to be linked to the photoactivated state, whereas in the dark no relevant cytotoxicity was observed [Kubin 2005; Lavie 1995; Takahashi 1989].

Hyperforin promoted apoptosis in B-CLL cells, as shown by time- and dose-dependent stimulation of phosphatidylserine externalisation and DNA fragmentation, by disruption of the mitochondrial transmembrane potential, caspase-3 activation and cleavage of the caspase substrate PARP-1 and induced down-regulation of the two antiapoptotic proteins Bcl-2 and Mcl-1. Hyperforin also down-regulated two proteins overexpressed by patients' B-CLL cells, the cell cycle inhibitor p27kip1 through

caspase-dependent cleavage into a p23 form, and the inducible NO synthase resulting in a reduction of NO production, known to be antiapoptotic in B-CLL cells. Preventing effects of the caspase inhibitor z-VAD-fmk indicated that hyperforin-promoted apoptosis of B-CLL cells was mostly caspase dependent. B lymphocytes from healthy donors appeared less sensitive to hyperforin-induced apoptosis than B-CLL cells [Quiney 2006].

Hyperforin-mediated induction of the mitochondrial pathway of caspase-dependent apoptosis was associated with up-regulation of Noxa, a BH3-only protein of the Bcl-2 family. Hyperforin was shown to induce an interaction between Noxa and a pro-survial protein Mcl-1, with the proapoptotic protein Bak becoming activated after being displaced from its complex with Mcl-1 [Zaher 2011]. These steps are known to cause mitochondrial membrane permeabilisation, accompanied by release of apoptogenic factors, and subsequent caspase cascade leading to apoptosis [Merhi 2011].

Hypericin induced an enhanced degradation of hypoxia-inducible factor 1α (HIF-1α) in two human tumour cell lines (U87-MG glioblastoma cells and RCC-C2VHL-/- renal cell carcinoma) and promoted ubiquitin ligation of HIF-1α mediated by lysosomal cathepsin-B enzymes through a hypericin-mediated reduction in intracellular pH [Barliya 2011].

The effect of hyperforin in the up-regulation of Noxa, a mitochondrial protein with pro-apoptotic activity, was investigated in cells from patients with chronic lymphocytic leukaemia CLL and the chronic B-cell leukaemia cell line MEC-1. Hyperforin caused an increase in Noxa expression in a time- and concentration-dependent manner. Noxa silencing by siRNA partially reduced hyperforin-elicited apoptosis [Zaher 2011].

The effects of hyperforin on acute myeloid leukaemia (AML) cell dysfunction were investigated in cell lines defining distinct AML subfamilies and primary AML cells. Hyperforin inhibited the growth of AML cell lines (U937, OCI-AML3, NB4, HL-60) by inducing apoptosis in a time- and concentration-dependent manner. It also induced apoptosis in primary AML blasts, whereas normal blood cells were not affected. The apoptotic process in U937 cells was accompanied by down-regulation of anti-apoptotic Bcl-2, upregulation of pro-apoptotic Noxa, mitochondrial membrane depolarisation, activation of procaspases and cleavage of the caspase substrate PARP 1. The caspase inhibitor Z-VAD-fmk and the caspase-9- and -3-specific inhibitors, but not caspase-8 inhibitor, significantly ($p<0.05$) attenuated apoptosis. Hyperforin-mediated apoptosis was associated with dephosphorylation of active Akt1 (at Ser(473)) and Akt1 substrate Bad (at Ser(136)) which activates Bad pro-apoptotic function. Hyperforin suppressed the kinase activity of Akt1, and combined treatment with the allosteric Akt1 inhibitor Akt-I-VIII significantly enhanced apoptosis of U937 cells [Merhi 2011].

A dried aqueous extract showed protective effects against oxidative DNA damage in HT29 colon cells (1 and 5 µg/mL). The extract and its main phenolic compounds quercetin and rutin (10 µM) resulted in a DNA protection of 40% ($p<0.05$). The alkylating damage repair was increased significantly ($p<0.05$) by 1 µg/mL extract (50%) and chlorogenic acid (80%) by base excision repair [Ramos 2013].

Anti-leukaemic effects of hyperforin were shown by induction of apoptosis in chronic lymphoid leukaemia (CLL) and acute myeloid leukaemia (AML) cells known for their resistance to chemotherapy. In AML cells, hyperforin inhibited the kinase activity of the serine/ threonine protein kinase B/AKT1, leading to activation of the pro-apoptotic Bcl-2 family protein Bad via non-phosphorylation by AKT1. In primary CLL cells, hyperforin acted by stimulating the expression of the pro-apoptotic Bcl-2 family member Noxa. Other hyperforin targets included matrix metalloproteinase-2 in AML cells and VEGF and matrix metalloproteinase-9 in CLL cells. Hyperforin also lowered the activity of Pgp which is involved in the resistance of leukaemia cells to chemotherapeutic agents [Billard 2013].

In this study hypericin was absorbed by melanoma cells and partially co-localised in the endoplasmic reticulum, mitochondria, lysosomes and melanosomes, but not the nucleus. Light activation of hypericin induced a rapid, extensive modification of the tubular mitochondrial network into a beaded appearance, loss of structural details of the endoplasmic reticulum and concomitant loss of hypericin co-localisation. The opposite was found for lysosomal-related organelles, suggesting that melanoma cells might use these intracellular organelles for hypericin-PDT resistance. However, hypericin-PDT was effective in killing both unpigmented (A375 and 501mel) and pigmented (UCT Mel-1) melanoma cells by specific mechanisms involving the externalisation of phosphatidylserines, cell shrinkage and loss of cell membrane integrity. The treatment resulted in extrinsic (A375) and intrinsic (UCT Mel-1) caspase-dependent apoptotic cell death, as well as a caspase-independent apoptotic mode that did not involve apoptosis-inducing factor (501 mel) [Kleemann 2014].

Antiangiogenic effects

Hyperforin blocked angiogenesis of human dermal microvascular endothelial cells (HDMEC) and reduced proliferation of HDMEC in a dose-dependent manner, without displaying toxic effects or inducing apoptosis [Schempp 2005].

In bovine aortic endothelial cells hyperforin treatment resulted in strong inhibitory effects on angiogenesis (IC_{50} 7 ± 3 µM). Capillary tube formation on Matrigel was abrogated completely by 2.5 µM hyperforin. Hyperforin also exhibited a clear inhibitory effect on the invasive capabilities of endothelial cells at 10 µM. Zymographic assays showed that hyperforin treatment produced a complete inhibition of urokinase and a remarkable inhibition of matrix metalloproteinase 2 [Martinez-Poveda 2005].

Hyperforin inhibited neutrophil and monocyte chemotaxis and angiogenesis induced by angiogenic chemokines (CXCL8 or CCL2) [Lorusso 2009].

Apoptosis

Pre-treatment with an unspecified extract significantly ($p<0.05$) decreased apoptosis in human SK-N-MC neuroblastoma cells as shown in different assays. In addition, pre-treatment with the extract inhibited H_2O_2-induced increase in caspase-3 activity [Jang 2002].

Antioxidant effects

Two extracts, standardised to either 0.3 – 0.5% hypericin or 3% hyperforin (not further specified), diluted between 1:1 to 1:20, were evaluated in a xanthine/xanthine oxidase assay in both cell-free and human vascular tissue. A pro-oxidant effect was seen at the highest concentration. Lower concentrations revealed dose-dependent antioxidant properties of the extracts with the strongest suppression at the most diluted concentration [Hunt 2001].

Strong antioxidant effects were observed for hyperforin in the oxidative burst of polymorphonuclear cells after stimulation with *N*-formyl-methionyl-leucyl-phenylalanine (IC_{50} 1.8 µM). No antioxidant activity was found after stimulation with opsonised zymosan or in the H_2O_2/ horseradish peroxidase system [Heilmann 2003].

Pre-incubation with an ethanolic extract for 24 h at 1-100 µg/mL significantly ($p<0.05$) prevented the marked reduction in cell survival observed following 8 h exposure of PC12 cells to H_2O_2 (300 µM). Different concentrations of the standardised extract (0.1-100 µg/mL) also attenuated the increase in caspase-3 activity and suppressed the H_2O_2–induced reactive oxygen species generation [Benedi 2004].

The free radical-scavenging properties of an ethanolic extract were studied using DPPH (EC_{50} = 49 µg dwb/mL), 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) (EC_{50} = 50 µg dry weight biomass (dwb)/mL) and lipid peroxidation induced by ascorbate/iron (EC_{50} = 28 µg dwb/mL). The extract was able to scavenge NO and HOCl [Silva 2008b].

The antioxidant activity of an unspecified flavonoid-rich extract was investigated to demonstrate protection against hydrogen peroxide-induced apoptosis in PC12 cells. After exposure of PC12 cells to H_2O_2, a significant ($p<0.001$) decrease in cell viability and increased levels of lactate dehydrogenase (LDH) release were observed. Pre-treatment of PC12 cells with the extract elevated cell viability, decreased levels of LDH release, decreased occurrence of apoptotic cells and inhibited DNA laddering in PC12 cells [Zou 2010].

Pronounced antioxidant activity was observed for fractions of an extract in the DPPH assay (lowest IC_{50} 0.52 µg/mL), for NO scavenging (6.11 µg/mL), superoxide scavenging (1.86 µg/mL), lipid peroxidation (0.0079 µg/mL) and ferric reducing ability of plasma (highest reduction capacity 104 mg Fe^{2+} equivalents/g). The activity of the fractions was attributed to flavonoids and phenolic acids, while phloroglucinols and naphthodianthrones showed no significant activity [Orcic 2011].

The antioxidant activities of ethanolic, acetonic and petroleum ether extracts were determined in the DPPH assay. The IC_{50} values were <7.8, 105.9, 5.99 and 12.77 µg/mL for the ethanolic extract, the acetonic extract, and the two reference antioxidants, ascorbic acid and butylated hydroxytoluene (BHT), respectively. The petroleum ether extract did not demonstrate antioxidant activity [Mašković 2011].

An H_2DCFDA-assay was performed in porcine HaCaT keratinocytes irradiated with solar simulated radiation. Hyperforin (IC_{50} 0.7 µM) was much more effective than Trolox (IC_{50} 12 µg/mL) and *N* acetylcysteine (IC_{50} 847 µg/mL), without showing phototoxicity. The radical protection factor of a cream (containing 1.5% (m/m) hypericin-free CO_2 extract with 44.3% hyperforin) was 200×10^{14} radicals/mg, indicating a high radical scavenging activity. The cream *ex vivo* on porcine ear skin significantly ($p<0.05$) reduced radical formation after infrared irradiation [Meinke 2012].

An ethanolic (70%) extract was investigated for phototoxicity in A375 human melanoma cells. Linoleic acid peroxidation and DPPH tests were used to assess antioxidant activity, while the MTT assay allowed evaluation of anti-proliferative activity of A375 cells after irradiation with a UVA dose of 1.8 J/cm^2. Inhibition of NO production of macrophages was also investigated. The extract showed greater antioxidant activity in the beta-carotene bleaching test than in the DPPH assay (IC_{50} = 0.89 µg/mL); significant phototoxicity in A375 cells at 78 µg/mL concentration resulted in cell destruction of 50%. The extract caused significant ($p<0.05$) dose-related inhibition of NO production in murine monocytic macrophage cell line RAW 264.7 with an IC_{50} value of 342 µg/mL [Menichini 2013].

An unspecified ethanolic extract was assessed for its protective role on Ca^{2+} entry and oxidative stress through modulation of TRPM2 channels in dorsal root ganglion (DRG) neurons from male Wistar rats using whole-cell patch-clamp, Fura-2 and antioxidant experiments. TRPM2 current densities and $[Ca^{2+}]_i$ in the neurons were reduced by the extract at doses of 10, 20, 30 and 40 µg/mL (2 and 24 h after administration). Cytosolic Ca^{2+} mobilization was reduced by the extract and VGCC blockers (verapamil+diltiazem, V+D). Glutathione peroxidase activity and GSH values in the DRG were high for the extract, and lipid peroxidation was low [Naziroglu 2014a].

Penetration of blood-brain barrier

The penetration of [^{3}H]-amentoflavone through the blood-brain barrier (BBB) was studied in primary cell cultures of porcine brain capillary endothelial cells (PBCEC). The concentration-dependent uptake (37 2000 nM) was neither saturable nor temperature-sensitive, indicating passive diffusion as the major uptake mechanism as confirmed by transport experiments through PBCEC monolayers (> 2% of applied dose was transported after 30 min). Co-administration of an extract increased amentoflavone transport significantly (amentoflavone only: permeability coefficient P_{app} 4.59×10^{-6} cm/s; amentoflavone + extract: P_{app} 6.74×10^{-6} cm/s) indicating that the extract enhanced amentoflavone transport. In experiments with the Pgp over-expressing cell line P388-MDR, amentoflavone uptake was significantly enhanced by addition of the Pgp inhibitor verapamil [Gutmann 2002].

PBCEC were used as a model of the BBB and epithelial cells of the plexus chorioidei as a model of the blood-CSF barrier (BCB). Results indicated no active transport of miquelianin in one direction. Although moderate, the permeability coefficients (BBB: $Pc = 1.34 \pm 0.05 \times 10^{-6}$ cm/s; BCB: $Pc = 2.0 \pm 0.33 \times 10^{-6}$ cm/s) indicated the ability of miquelianin to cross both barriers to reach the CNS [Juergenliemk 2003].

The effects of a methanolic (80%) extract and selected constituents on the BBB transporter Pgp and on protein kinase C (PKC) were assessed in cultured PBCEC and freshly isolated pig brain capillaries from. Pgp function was studied in PBCEC using a calcein-AM uptake assay and in isolated pig brain capillaries using the fluorescent ciclosporin (CyA) derivative NBD-CSA and confocal microscopy. The extract (0.1 to 5 µg/mL) and the constituents hyperforin, hypericin and quercetin (0.1 to 10 µM) decreased Pgp transport activity in a dose- and time-dependent manner. The extract and hyperforin directly inhibited Pgp activity, whereas hypericin and quercetin modulated transporter function through a mechanism involving protein kinase C. Quercetin decreased Pgp transport activity at high concentrations, but increased transporter function at low concentrations [Ott 2010].

Effects on nitric oxide synthase

Quercetin and hyperoside showed concentration-dependent inhibitory activity on nitric oxide synthase (NOS) in blood and cerebral homogenate of rat. The IC_{50} values of quercetin in rat cerebral homogenate and blood were 63.06 and 57.54 µM, and those of hyperoside 56.23 and 158.49 µM, respectively [Luo 2004].

In vivo experiments

Biochemical findings

After acute and repeated oral treatment of mice with 10 mg/kg of an extract, the levels of serotonin and the serotonin metabolite 5-hydroxyindolacetic acid (5-HIAA) were increased in the hypothalamus and hippocampus indicating increased turnover; 5-HIAA levels were also increased in other regions of the brain [Yu 2000].

The effects of acute oral administration (24 h and 1 h before

testing) of a methanolic extract (62.5-500 mg/kg; 0.3% hypericin, 6% flavonoids) or an extract enriched in flavonoids (62.5-500 mg/kg; 0.3% hypericin, 50% flavonoids) on the levels of tryptophan, 5-HT, 5-HIAA, NE and DA in the cortex, diencephalon and brainstem of rats were evaluated. Results were compared with the effects of fluoxetine (10-80 mg/kg). The two extracts and fluoxetine increased 5-HT levels in the cortex. The flavonoid-enriched extract increased 5-HT and 5-HIAA levels in the diencephalon, and 5-HT and NE levels in the brainstem. Both extracts increased NE and DA levels in the diencephalon [Calapai 1999]. Comparable results for the flavonoid-enriched extract were obtained in a subsequent study using similar techniques; it did not modify tryptophan content, but significantly enhanced 5-HT and 5-HIAA levels in the cortex, diencephalon and brainstem at 125-500 mg/kg, and increased NE and DA in the diencephalon and NE in the brainstem at 250-500 mg/kg [Calapai 2001].

Rats were treated orally with 300 mg/kg of a hydromethanolic extract three times (23.5 h, 5 h and 1 h before forced swimming test). The extract significantly reduced immobility time ($p<0.01$). No significant changes in monoamine levels were detected in cortical or hippocampal brain regions 1 hour and 24 hours after the last dose [Calapai 2001].

The effects of imipramine (IMI; 15 mg/kg), an 80% methanolic extract (500 mg/kg) and hypericin (0.2 mg/kg) on the levels of 5-HT, NE, DA and their metabolites in the hypothalamus and hippocampus were investigated after short-term (2 weeks) and long-term (8 weeks) daily oral administration to rats. All three treatments significantly increased 5-HT levels in the hypothalamus ($p<0.05$) after 8 weeks, but not after 2 weeks; levels in the hippocampus were unchanged. 5-HT turnover (the ratio of 5-HIAA to 5-HT) was significantly reduced in both brain regions (both $p<0.05$) after 8 weeks of treatment with the extract but not with hypericin; IMI reduced 5-HT turnover only in the hippocampus. Consistent changes in catecholamine levels were only detected in hypothalamic tissues after long-term treatment. Comparable to IMI, the extract and hypericin significantly decreased 3,4-dihydroxyphenylacetic acid and homovanillic acid levels in the hypothalamus ($p<0.01$). The data showed that long-term, but not short-term, administration of St. John's wort and its active constituent hypericin modified levels of neurotransmitters in brain regions involved in the pathophysiology of depression [Butterweck 2002a].

The effects of acute and repeated administration of an undefined extract on extracellular levels of 5-HT, NE, DA, and their major acidic metabolites, in the brain of anaesthetised male Sprague Dawley rats were studied using *in vivo* microdialysis in the prefrontal cortex (PFC), ventral hippocampus and striatum. The extract inhibited the reuptake of DA, 5-HT and NE after i.p. administration of 60 mg/kg, whereas a single oral dose of 300 mg/kg only showed an effect on DA in the PFC. In rats treated for 14 days with a daily dose of 300 mg/kg p.o., the last dose of the extract increased the extracellular concentrations of all monoamines in the order DA >> 5-HT > NE, whereas the levels of metabolites were reduced in the order 3,4-dihydroxyphenylacetic acid << homovanillic acid < 5-hydroxyindoleacetic acid. The most pronounced effect of hypericum in both the acute and subchronic regimens was seen on the re-uptake and metabolism of DA [Kehr 2002].

The effects of a hydroalcoholic extract on extracellular 5-HT, NE and DA levels and the acidic metabolites (3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-HIAA) were examined by *in vivo* microdialysis in the prefrontal cortex of awake rats. Single doses of the extract (60 mg/kg i.p. or 300 mg/kg p.o.) increased DA concentrations to 165 and 140% of control values respectively, and increased locomotor activity in non-habituated rats. DOPAC and HVA levels were markedly reduced. 5-HT concentrations were elevated moderately, while the NE levels were not affected by any treatment. Whole-tissue analysis revealed that the extract increased, whereas the MAO A/B inhibitor phenelzine decreased, DA and 5-HT turnover [Yoshitake 2004].

Neurotransmitter receptor density in the brain
Sprague-Dawley rats were treated daily for 26 weeks with an 80% methanolic extract (2700 mg/kg p.o.). In preparations of the brain without cerebellum and brain stem, the number of both 5-HT_{1A} and 5-HT_{2A} receptors were significantly ($p<0.01$) increased as compared to controls, whereas the affinity of both serotonergic receptors remained unaltered [Teufel-Mayer 1997].

Following sub-acute oral treatment of rats with 240 mg/kg of an 80% methanolic extract for 14 days, β-adrenoreceptor down-regulation was observed in the frontal cortex, a common characteristic finding for many antidepressant drugs. The simultaneous up-regulation of 5-HT_2 receptors observed was in contrast to the 5-HT_2 down-regulation observed with many classic antidepressants [Müller 1997].

Using the push-pull superfusion technique, neurotransmitter concentrations in the *locus coeruleus* of rats were studied after i.p. injection of hyperforin at 10 mg/kg. Extracellular concentrations of DA, NE, 5-HT and glutamate increased, whereas 5-HIAA, GABA, taurine, aspartate, serine and arginine remained unchanged [Kaehler 1999].

The short- (2 weeks) and long-term (8 weeks) effects of an 80% methanolic extract (500 mg/kg p.o.), a CO_2 extract (15 mg/kg p.o.), hypericin (0.2 mg/kg p.o.), hyperoside (0.6 mg/kg p.o.) and hyperforin-trimethoxybenzoate (8 mg/kg p.o.) on β-adrenoceptor (β-AR) binding were investigated by quantitative radioligand receptor-binding-studies in the frontal cortex of CD rats, and compared to IMI (15 mg/kg p.o.) and FLU (10 mg/kg p.o.). [^{125}I]-cyanopindolol binding to β-AR was decreased after short as well as long-term treatment with IMI (36%, $p<0.01$; 40%; $p<0.01$). Short-term treatment with FLU decreased the number of β-AR (17%; $p<0.05$) while it elicited an increase (14%; $p<0.01$) in β-AR-binding in long-term treatment. This effect was comparable to that of the CO_2 extract which decreased β-AR-binding (13%; $p<0.05$) after two weeks and slightly increased the number of β-AR's after 8 weeks (9%). Short-term treatment with the methanolic extract decreased β-AR-binding (14%); no effects were observed after 8 weeks. Treatment with hypericin led to a significant down-regulation (13%; $p<0.05$) of β-AR's in the frontal cortex after 8-weeks, but not after 2 weeks; hyperforin-trimethoxybenzoate and hyperoside were ineffective in both cases [Simbrey 2004].

Animal models of depression
Various test models of depression allow the detection of antidepressant activity. Behavioural experiments have indicated that St. John's wort extracts are centrally active, with a variety of extracts showing antidepressant-like properties in different animal models of depression. Antidepressant-like effects comparable in magnitude with those induced by classical antidepressants have been obtained in the rat forced swimming test (FST) and in related models, such as the mouse FST, the mouse tail suspension test (TST) and the rat learned helplessness test. The extracts also induced an antidepressant-like profile with respect to in vivo monitored field potentials in freely moving rats.

Forced swimming test
An 80% methanolic extract (0.24-0.32% total hypericin), at an oral dose of 125-1000 mg/kg b.w., significantly reduced

($p<0.05$ to $p<0.001$) the duration of immobility in the FST in male Sprague Dawley rats. The effects on immobility time were eliminated by dopamine D_2 antagonists [Winterhoff 1995].

A 50% ethanolic extract (250 mg/kg i.p.) was tested in mice in the FST, with DMI (30 mg/kg i.p.) and trimipramine (TRI) (20 mg/kg i.p.) serving as controls. All treatments significantly decreased swimming time of male albino mice ($p<0.05$) [Öztürk 1997; Öztürk 1996].

Fractionation of an 80% methanolic extract yielded two fractions with high activity in the FST with male CD rats, one contained mainly flavonoids and the other naphthodianthrones. Hypericin was inactive in the FST at a dose of 0.8 mg/kg. However, procyanidins (mainly procyanidin B_2) present in the naphthodianthrones fraction markedly increased the solubility and bioavailability of hypericin; in combination with procyanidin B_2 even 0.009 mg/kg p.o. hypericin was significantly active. Comparable results were obtained with pseudohypericin. In addition to hypericins, a flavonoid fraction from a methanolic extract and the flavonoids hyperoside, isoquercitrin and miquelianin showed significant activity in the FST after acute and sub-acute administration [Butterweck 2000; Butterweck 1998; Butterweck 1997].

The effect of oral administration of a CO_2 extract (38.8% hyperforin) was compared to an ethanolic extract (4.5% hyperforin) in the FST in rats [Chatterjee 1998a] and mice [Bhattacharya 1998]. Both extracts were active, with the effect of 5, 15 and 30 mg/kg of the CO_2 extract being comparable to that of 50, 150 or 300 mg/kg of the ethanolic extract.

Immobility time in the rat FST was significantly reduced by acute i.p. administration of 80% methanolic ($p<0.05$) and 50% ethanolic $p<0.01$) extracts. Results for these extracts (5-40 mg/kg) were comparable to i.p. administration of IMI (3-30 mg/kg) and FLU (3-30 mg/kg) [De Vry 1999].

Daily administration for 3 days of a 50% ethanolic extract, at 100 and 200 mg/kg p.o. to CF rats and Wistar mice, demonstrated comparable effects to acute administration of IMI at 15 mg/kg i.p. in various animal models of depression [Kumar 1999].

An extract (0.3% hypericin, 3.8% hyperforin) was administered intragastrically to male msP rats at 3 doses of 250 mg/kg. The anti-immobility effect of the extract in the FST was completely suppressed by i.p. pre-treatment with the σ-receptor antagonist rimcazole. Intracerebroventricular pre-treatment with 5,7-dihydroxytryptamine, which produced a marked depletion of brain 5-HT, also reduced the anti-immobility effect [Panocka 2000].

After acute oral administration to rats at 250-500 mg/kg, an unspecified extract enriched in flavonoids (0.3% hypericin, 4.5% hyperforin and 50% flavonoids) significantly and dose-dependently decreased immobility time ($p<0.001$ compared to saline). Concomitant treatment with the extract (500 mg/kg) and sulpiride (a DA receptor antagonist), metergoline (a serotonin receptor antagonist) or 6-hydroxydopamine (which destroys NE-containing neurons) significantly increased the period of immobility by 22-57%, the largest effect was observed with metergoline (57%; $p<0.001$) [Calapai 2001].

Several ethanolic (60%) and methanolic (80%) extracts demonstrated a significant ($p<0.01$) increase in activity in male Sprague-Dawley rats in the FST, with the exception of one methanolic extract containing a reduced level of rutin. Addition of rutin to this extract produced a pharmacological effect comparable to the other extracts [Nöldner 2002].

In the 24h preceding a FST, male CD-COBS rats were administered three i.p. injections of either one of two methanolic extracts (containing hyperforin at 4.5% (A) or 0.5% (B)), the dicyclohexylammonium (DCHA) salt of hyperforin or a vehicle. Immobilisation time was significantly decreased by extract A at doses of 3.12 and 6.25 mg/kg ($p<0.05$) and hyperforin DCHA at doses of 0.19 and 0.38 mg/kg ($p<0.01$), but not by extract B [Cervo 2002].

Three extracts were tested in the FST in male CD rats. Extract A (3.2% hyperforin, 0.15% hypericin) was prepared with 60% ethanol. Extract B (0.15% hypericin) was prepared from extract A by removal of hyperforin. Extract C (approximately 12% flavonoids) was prepared from extract B by removing naphthodianthrones. Immobility time was significantly reduced by oral administration of all three extracts (500 mg/kg) and IMI (30 mg/kg). Only extract C significantly reduced immobility time when the dose was reduced to 250 mg/kg. However, after 14 days of pre-treatment, similar effects were observed for all three extracts [Butterweck 2003a].

The effects of hyperoside at single i.p. doses of 7, 35 and 70 mg/kg was investigated in the FST in male BIC_{57} mice. With the increasing doses, immobility time increased by 10.1%, 25.8% and 38.6% respectively, as compared to controls [Bach-Rojecky 2004].

An ethanolic (60%; V/V) extract, rutin and isorhamnetin were compared to IMI in the FST after oral administration for 9 days. The extract and the two flavonoids were found to significantly decrease the duration of immobility, with isorhamnetin (3 and 10 mg/kg) exhibiting the strongest effect, more pronounced than after IMI administration. The administration of 200 mg/kg or more of the extract was comparable to 30 mg/kg IMI [Paulke 2008].

The effects of an ethanolic (A: 50%; (m/m), 0.08% hyperforin) and a methanolic (B: 80%; V/V, 4.5% hyperforin) extract were evaluated and compared to FLU (1 mg/kg p.o.) in male Wistar rats in the FST and an open field test over 28 days. The rats were given an oral daily dose of either one of four different doses of extracts A (1.78, 3.57, 7.14, and 14.28 mg/kg) or B (3.21, 6.43, 12.86, and 27.71 mg/kg), FLU or vehicle. The tests were carried out before treatment commenced and on days 7, 14, 21 and 28 of treatment. After 21 and 28 days of treatment, both extracts significantly ($p<0.05$) increased the latency to the first immobility and decreased the total immobility time in a dose-dependent manner. FLU significantly ($p<0.05$) increased the latency to first immobility and total immobility time. FLU exhibited antidepressant activity after 14 days, whereas with both extracts 21 days were required [Lozano-Hernandez 2010].

Adult male CF1 mice were treated with hyperoside (10, 20 or 40 mg/kg i.p.), IMI (20 mg/kg i.p.), or vehicle 30 min before the FST. Compared to vehicle, a significant ($p<0.001$) antidepressant-like effect was observed for hyperoside at doses of 10 and 20 mg/kg, comparable to IMI. In male Wistar rats, hyperoside (1.8 mg/kg/day p.o.) and IMI both showed a significantly (both $p<0.01$) decreased immobility time. The antidepressant-like effect in rats was prevented by the D_2 antagonist sulpiride (50 mg/kg i.p.) [Haas 2011; Schulte Haas 2011].

An unspecified extract at an oral dose of 25 mg/kg was compared to FLU (10 mg/kg i.p.) in male albino rats. Both treatments showed a significant ($p<0.05$) antidepressant effect in all behavioural studies and also significantly increased brain neurotransmitter levels, especially that of 5-HT. The effects of the extract were comparable to FLU [Kulkarni 2012].

The effects of a standardised extract (not further specified) at doses of 30-90 mg/kg i.p. were compared with standard

antidepressants in the FST when given 1 h before the test in male rats. The extract caused a dose-dependent reduction in immobility time with the maximal effect of 53% at 90 mg/kg ($p<0.001$). This effect was reversed at higher doses (100 mg/kg) showing a U-shaped dose response curve. FLU and IMI (30 -70 mg/kg i.p.) produced a similar reduction in the immobility time in rats, whereas VEN exhibited only a 34% reduction [Bukhari 2013].

Adhyperforin at an oral dose of 16 mg/kg significantly ($p<0.05$) decreased the immobility time of male Swiss mice [Tian 2014].

Escape deficit test (learned helplessness)
Male Charles Foster rats received either a CO_2 extract (no hypericin, no polyphenols, 38.8% hyperforin) or an ethanolic extract (0.3% hypericin, 4.5% hyperforin) before the inescapable electrical shock test. Control animals, trained to the inescapable shock situation, exhibited marked increases in escape failures under escapable conditions (72.5%), as compared to animals not previously trained to helplessness. Dose-dependent decreases of escape failures were observed for both extracts [Bhattacharya 1998; Chatterjee 1999].

Sprague-Dawley rats received 250, 500, 1000 or 1500 mg/kg of a hydroethanolic extract (0.3% hypericin) p.o., 60 minutes before an inescapable stress test. A significant dose-dependent reduction in escape deficit 24 h later was found for the 1000 mg ($p<0.05$) and 1500 mg ($p<0.01$) doses as compared to control. A tail-flick test and hot plate test confirmed that the result was not caused by a non-specific analgesic effect. Co-administration of the DA D_1 receptor antagonist SCH 23390 or the 5-HT_{1A}- and β-AR antagonist pindolol significantly reduced the efficacy of the extract. After pre-treatment of rats with daily doses of 500, 1000 or 2000 mg/kg p.o. for 15 days or IMI (5 mg/kg, i.p., twice daily), the extract at 1000 mg showed significantly ($p<0.05$) greater activity (increased escapes) than control, and the 2000 mg dose had a significant effect comparable with IMI ($p<0.01$) [Gambarana 1999].

Tail suspension test (TST)
A methanolic (80%, V/V) extract at an oral dose of 500 mg/kg caused a significant decrease in immobilisation time of NMRI mice. The effect was completely abolished by D,L butyrolactone (an inhibitor of dopaminergic neuron function), and by α-methyltyrosine (an inhibitor of DA and adrenaline synthesis) [Butterweck 1997].

Three different extracts were studied at an oral dose of 500 mg/kg in female NMRI mice: (A) an ethanolic (60%) extract (3.2% hyperforin, 0.15% of hypericin); (B) a CO_2 extract (no hyperforin, 0.14% hypericin) and (C) a sub-fraction of extract (B) (no hyperforin or hypericin, approximately 12% flavonoids). IMI (30 mg/kg p.o.) was used as control. Extracts B and C reduced immobility time significantly ($p<0.05$) and were comparable to IMI, whereas extract A was less active. Hyperforin produced a U-shaped dose-response curve: below 4 and above 8 mg/kg hyperforin remained inactive [Butterweck 2003a].

The effects of hyperoside at single oral doses of 7, 35 and 70 mg/kg were investigated in the TST in adult male CF1 mice. With the increasing doses, the antidepressant effect increased by 12.7%, 16.5% and 24.5% compared to controls [Bach-Rojecky 2004].

The administration of rutin (0.3 - 3 mg/kg, p.o.) reduced the immobility time of male Swiss mice in the TST without a change in locomotor activity. Pre-treatment of mice with p-chlorophenylalanine methyl ester (100 mg/kg, i.p., for 4 consecutive days) or alpha-methyl-p-tyrosine (100 mg/kg, i.p.), representing inhibitors of serotonin or NE synthesis respectively, prevented the effect of rutin in the TST [Machado 2008].

Hot plate
No activity of hyperoside (10, 20 and 40 mg/kg i.p.) was found on the hot-plate in male Swiss mice [Schulte Haas 2011].

Open field test (Locomotor activity)
Oral application for 3 days of a methanolic extract (80%, V/V; 500 mg/kg/day) or of hypericin (1.5 mg/kg/day) had no impact on the spontaneous motility of male and female CD rats [Winterhoff 1995].

The effect of an ethanolic (50%) extract on locomotor activity in the rota-rod test was assessed. Male albino mice received i.p. either the extract (250 mg/kg), DMI (30 mg/kg) or TRI (20 mg/kg). The extract demonstrated a significant decrease in the walking time of mice on the rotating rod ($p<0.05$). While DMI and TRI caused a more significant decrease ($p<0.005$) [Öztürk 1997; Öztürk 1996].

Oral doses of pseudohypericin (166 µg/kg) and hypericin (9 -28 µg/kg) were administered to male CD rats concomitantly with a fraction of flavonoids. The number of line crossings in comparison to the control group were not increased, suggesting that reported positive results in the FST (see above) were not related to stimulation of locomotor activity [Butterweck 1998].

Hyperoside, miquelianin and isoquercitrin at an oral dose of 0.6 mg/kg did not change locomotor activity in male CD rats [Butterweck 2000].

In contrast, a lyophilised aqueous infusion administered i.p. to male Charles River mice at doses of 25, 50, and 100 mg/kg significantly diminished the number of crossings and rearings ($p<0.01$), whereas immobility times were raised ($p<0.05$) [Coleta 2001].

A methanolic extract (80%, V/V; 500 mg/kg) or hypericin (0.1 mg/kg p.o.), administered alone or in combination with procyanidin B_2, did not induce changes in locomotor activity of male CD rats [Butterweck 2001a].

An unspecified methanolic extract was tested at doses from 2.5 to 200 mg i.p. in adult male CD1 mice. The extract increased motor activity at 10 mg/kg i.p. and decreased it at the highest dose [Diana 2007].

The effects of an ethanolic (50%) extract were tested on caffeine-induced locomotor activity in male Swiss-Webster mice. Caffeine (4 - 16 mg/kg i.p.) produced significant ($p<0.0001$) increases in locomotor activity. The extract (6 - 24 mg/kg i.p.) significantly ($p=0.016$) blocked the caffeine-induced locomotor hyperactivity. Pre-treatment with L arginine (1 g/kg) reversed the inhibitory effect of the extract (6 mg/kg i.p.) without any significant effect when administered alone [Uzbay 2007].

The effects of an ethanolic (50%) extract on behavioural changes were assessed in streptozotocin (STZ)-diabetic Wistar rats. In horizontal and vertical spontaneous locomotor activities, a diabetes mellitus-induced decrease was observed which was not restored by insulin (2 IU/kg/day). Treatment with the extract (125 and 250 mg/kg) for seven days resulted in a significant ($p<0.05$ and $p<0.01$) improvement [Can 2011b].

Adult male CF1 mice treated with single doses of hyperoside at 20 and 40 mg/kg i.p. showed reduced ($p<0.001$) exploratory behaviour in the open field test compared to controls [Schulte Haas 2011].

The effects of a standardised extract (details unknown) were compared with antidepressants in the open field test at doses of 1-30 mg/kg i.p. in rats. The extract caused a dose-dependent reduction in locomotor counts with a maximal effect of 80% at 30 mg/kg ($p<0.001$) compared to vehicle. FLU (30-70 mg/kg i.p.), dothiepin (30 mg/kg i.p.) and VEN (50 mg/kg i.p.) produced similar reductions in the number of locomoter counts (89%, $p<0.001$; 70%, $p<0.001$ and 62%, $p<0.001$, respectively) [Bukhari 2013].

Elevated plus maze
A CO_2 extract (no hypericin, 38.8% hyperforin) and an ethanolic extract (0.3% hypericin, 4.5% hyperforin) were tested in Wistar mice in the elevated plus maze (EPM). Both extracts at the highest dose (300 mg/kg) significantly ($p<0.05$) reduced the time spent in the open arm [Bhattacharya 1998].

Lyophilised aqueous extracts at i.p. doses of 25, 50 and 100 mg/kg were tested in the elevated plus maze in male Charles River mice. The extract significantly reduced ($p<0.01$) the total number of entries and the number of closed entries [Coleta 2001].

A diabetes mellitus-induced decrease in exploratory behaviour, indicating increased anxiety in STZ-diabetic Wistar rats, was not restored by daily insulin (2 IU/kg/day i.p.), but was significantly ($p<0.001$) improved by treatment with an ethanolic (50%) extract (125 and 250 mg/kg, i.p.) for seven days [Can 2011a].

Other pharmacological effects

Stress response
A 14-day mild, unpredictable and inescapable foot shock stress (FSS) test induced depression, suppressed male sexual behaviour, cognitive dysfunction, marked gastric ulceration and a significant increase in adrenal gland weight with concomitant decrease in spleen weight in albino rats. All these FSS-induced perturbations were attenuated dose-dependently by the extract at 100 and 200 mg/kg p.o. [Kumar 2001b].

Administration of a dried St. John´s wort preparation (350 mg/kg/day p.o.) for 21 days significantly enhanced recall of passive avoidance behaviour, but had no effect on the acquisition of conditioned avoidance responses in chronically (2 h daily for 21 days) stressed rats. Impaired recall of passive avoidance behaviour following chronic administration of corticosterone (5 mg/kg/day for 21 days) was also reversed [Trofimiuk 2006].

Male laca mice were sleep deprived for 72 hours using the grid suspended over water method. An ethanolic extract (60%; 0.3% hypericin, 4.1% hyperforin, 3.3% rutin, 1.8% hyperoside, 1.0% isoquercitrin, 1% quercetin, 0.3% bioapigenin) or IMI were administered for five days, starting two days before sleep deprivation. Sleep deprivation significantly altered body weight and locomotor activity, and produced anxiety-like behaviour and oxidative damage (depleted reduced GSH and CAT activity; increased lipid peroxidation and nitrite activity) as compared to naive animals placed on sawdust ($p<0.05$). Treatment with the extract (200 and 400 mg/kg p.o.) or with IMI (10 mg/kg i.p.) significantly improved body weight, locomotor activity, antianxiety and antioxidant effects as compared to the sleep deprived control ($p<0.05$). Co-administration of the extract (200 mg/kg p.o.) with IMI (10 mg/kg i.p.) further improved body weight, locomotor activity and the antianxiety effect, as well as reducing oxidative damage in sleep-deprived animals as compared to their effect *per se* ($p<0.05$) [Kumar 2007].

The effect of a methanolic (80%) extract on gastric mucosal damage induced by hypothermic restraint stress (HRS) was investigated in Wistar rats: group 1 was exposed to HRS, group 2 was the untreated control, groups 3-5 received the extract (25, 50 and 100 mg/kg/day p.o.), and group 6 received ranitidine (50 mg/kg s.c.) as the positive control. Macroscopic analysis of the stomach lesions showed that treatment with the extract at each dose significantly reduced lesions compared to stressed controls by 65, 95 and 75% respectively ($p=0.001$). Treatment with ranitidine also improved ulcers significantly. Histopathologic analyses indicated that 50 mg/kg/day of the extract produced the most significant effect [Cayci 2009].

In a restraint stress model, male Albino mice were immobilised for a period of 6 h resulting in severe anxiety like behaviour, antinociception and impaired locomotor activity as compared to unstressed animals ($p<0.05$). An unspecified extract was administered at doses of 50 and 100 mg/kg i.p. for five days 30 min before acute immobilised stress. Various behavioural parameters for anxiety, locomotor activity and nociceptive threshold were assessed, followed by biochemical assessments (malondialdehyde (MDA) level, GSH, catalase activity (CAT) and nitrite). Biochemical analyses revealed an increase in MDA and nitrites concentration, and depletion of reduced GSH and CAT, as compared to unstressed animal brain. Treatment with the extract significantly (all $p<0.05$) attenuated restraint stress-induced behavioural changes (improved locomotor activity, reduced tail flick latency and anxiolytic effect) and oxidative damage as compared to control [Kumar 2010].

An ethanolic (80%) extract was studied at doses of 125 to 750 mg/kg p.o. in a chronic restraint stress model. Stressed rats showed decreased time in open field activity compared to unstressed rats; an effect reversable by the extract and FLU (10 mg/kg p.o.). Chronic restraint stress significantly decreased thymus and spleen indices ($p<0.05$). The extract produced a significant and dose-dependent increase in both thymus and spleen indices compared to the stressed controls ($p<0.05$), as did FLU. Both treatments significantly reduced stress-induced increases in plasma ACTH and corticosterone levels ($p<0.05$ to $p<0.01$) as well as TNF-α levels ($p<0.05$) [Grundmann 2010].

Post-stress grooming
Post swim grooming behaviour of Wistar mice was significantly ($p<0.01$) increased by IMI (10 mg/kg i.p.), and dose-dependently and significantly ($p<0.05$ to <0.001) increased by oral administration of an ethanolic extract (0.3% hypericin, 4.5% hyperforin; 50-300 mg/kg/day), whereas a CO_2 extract (no hypericin, 38.8% hyperforin; 5-30 mg/kg/day) had a significant effect only at the highest dose ($p<0.05$) [Bhattacharya 1998].

Influence on body temperature
The decrease in body temperature after i.p. application of reserpine to female NMRI mice was counteracted by oral administration of a hydroalcoholic extract (0.015% hypericin) [Winterhoff 1993].

Oral treatment of male Charles Foster rats with either a CO_2 (no hypericin, 38.8% hyperforin) or an ethanolic (0.3% hypericin, 4.5% hyperforin) extract did not induce changes in body temperature, except for a slight increase at the highest dose of 300 mg/kg/day. Reserpine-induced hypothermia was completely antagonised by 15 mg/kg/day of the CO_2 extract and 300 mg/kg/day of the ethanolic extract. The activity of both extracts in reserpine-treated animals was similar to IMI (10 mg/kg i.p.) [Bhattacharya 1998].

A methanolic (80%; V/V) extract was administered orally to female NMRI mice (500 mg/kg). One hour after administration basal body temperature was significantly elevated ($p<0.005$) [Winterhoff 1995].

Exposure of male BL6/C57J mice to open field stress significantly increased body temperature by 1.8 ± 0.13°C ($p<0.05$). Oral administration of an ethanolic extract (80%; V/V; 0.3% hypericin, 4% hyperforin and 9.4% flavonoids) significantly reduced the body temperature increase at doses of 250 and 500 mg/kg ($p<0.01$). Hypericin (0.1 mg/kg p.o.) administered 60 min prior to testing significantly reduced the increase ($p<0.001$), whereas hyperforin (1 10 mg/kg p.o.) had no effect. Hyperoside, isoquercitrin and quercitrin (all at 0.6 mg/kg p.o.) and rutin (1 mg/kg p.o.) only partially blocked open field-induced hyperthermia ($p<0.05$). Miquelianin (1.2 mg/kg p.o., $p<0.05$) and amentoflavone (0.1 mg/kg p.o., $p<0.001$) also significantly decreased the stress-induced hyperthermia. Diazepam (5 mg/kg p.o; $p<0.001$) and the 5-HT_{1A} receptor agonist buspirone (10 mg/kg p.o.; $p<0.01$) significantly reduced the temperature increase, whereas IMI (10 mg/kg p.o.) and fluoxetine (10 mg/kg p.o.) had no effect [Grundmann 2006].

Influence on sleeping time
Oral pre-treatment of female NMRI mice with 500 mg/kg of an ethanolic extract (containing 0.015% hypericin), 2 h prior to injection of ketamine, significantly ($p<0.05$) reduced sleeping time [Winterhoff 1993].

Oral administration of a methanolic extract (80%, V/V; 50 to 1500 mg/kg) demonstrated dose-dependent activity in the same model, with fully expressed activity at 500 mg/kg ($p<0.001$). Dose reduction to 200 mg/kg led to a less pronounced reduction in sleeping time ($p<0.05$), while the effect with 50 mg was not significant. IMI (20 mg/kg) led to a prolongation of sleeping time, whereas bupropion (20 mg/kg), a dopamine re-uptake inhibitor, had the same effect as the extract. Hypericin, at doses equivalent to that found in the tested extract, exhibited less reduction in sleep duration when compared to the extract [Winterhoff 1995].

Effects on EEG
Power changes in the frontal cortex and hippocampus of male Fisher-344 rats were detected after oral administration of an ethanolic (50%, m/m) extract at doses of 0.5 and 1 mg. Frequencies in these brain areas changed predominantly in the direction of effects known from antidepressant drugs [Dimpfel 1997].

Continuous EEG was recorded in male rats after oral application of a CO_2 extract (30.1% hyperforin, practically no naphthodianthrones and flavonoids) and a methanolic extract (80%, V/V; 4.7% hyperforin). The dosage was adjusted for the application of identical amounts of hyperforin. Both extracts produced nearly identical patterns of electrical power changes during the first two hours. The changes mainly consisted of reproducible power increases within the α_1 band of the striatum, similar to the reaction following the application of SSRIs. The methanolic extract developed a late action (increased δ-activity) not seen with the CO_2 extract, which matched data obtained with NMDA-antagonists like MK 801 or memantine [Dimpfel 1998].

Effects on neurotransmitters and hormones
Three weeks of oral treatment with 125 mg/kg/day of a methanolic (80%, V/V) extract significantly reduced basal prolactin ($p<0.05$) and corticosterone levels ($p<0.01$) in male CD rats [Winterhoff 1995].

The effects of a methanolic extract (80%, V/V; 200 mg/kg i.p.), hypericin (0.4 mg/kg i.p.) and hyperforin (9.3 mg/kg i.p.) on 5-HT and DA-mediated neuroendocrine responses were measured in male Sprague-Dawley rats. Plasma corticosterone was significantly ($p<0.05$ to $p<0.001$) and equivalently increased in all three treatment groups. The effects were blocked by the 5-HT_2 receptor antagonist ketanserin, but not by the 5-HT_{1A} receptor antagonist WAY-100635. The effects were less pronounced after sub-chronic treatment with the extract (20 and 100 mg/kg/day for two weeks). Acute treatment with the extract and hyperforin significantly decreased plasma prolactin (PRL), whereas sub-chronic treatment with the extract at 100 mg/kg/day significantly ($p<0.01$) increased PRL [Franklin 2000; Franklin 2001].

Oral treatment of male Sprague-Dawley rats for 2 weeks with a methanolic (80%, V/V) extract (in a diet containing 3 g/kg) resulted in significantly reduced corticosterone ($p<0.002$) and PRL ($p<0.04$) responses to the post-synaptic 5-HT_{2A} receptor agonist 2,5-dimethoxy-4-iodophenyl-2-aminopropane [Franklin 2003].

Brain concentrations of corticosterone and cortisol (COR) were significantly reduced in rats receiving an oral dose of 3 g/kg of a methanolic (80%, V/V) extract for 2 weeks, whereas serum concentrations remained unaffected [Franklin 2004].

Using *in situ* hybridisation histochemistry, the effects of short-term (2 weeks) and long-term (8 weeks) oral administration of a CO_2 extract (containing about 23.7% hyperforin and 6.2% adhyperforin) and hyperforin-trimethoxybenzoate were studied in male CD rats. FLU and haloperidol were used as positive and negative controls respectively, to assess the expression of genes involved in the regulation of the hypothalamic-pituitary-adrenal (HPA). FLU (10 mg/kg p.o.) daily for 8 weeks, but not 2 weeks, significantly ($p<0.05$) decreased levels of CRH mRNA by 22% in the paraventricular nucleus of the hypothalamus and tyrosine hydroxylase (TH) mRNA by 23% in the locus coeruleus. FLU increased ($p<0.05$) levels of mineralocorticoid (MR) (17%), glucocorticoid (GR) (18%) and 5-HT_{1A} receptor (21%) mRNAs in the hippocampus at 8, but not 2, weeks. Comparable to haloperidol (1 mg/kg p.o.), neither the hyperforin-rich CO_2 extract (27 mg/kg p.o.) nor hyperforin-trimethoxybenzoate (8 mg/kg p.o.) altered mRNA levels in brain structures relevant for HPA axis control at either time point [Butterweck 2003c].

An ethanolic extract (DER 3.6:1; containing 0.13% hypericin and pseudohypericin, 3.03% hyperforin) was given orally at 20 to 240 mg/kg to male Sprague-Dawley rats over 8 weeks. Chronic IMI treatment (5 mg/kg/day i.p. for 8 weeks) significantly ($p<0.05$ both) down-regulated circulating plasma levels of adrenocorticotropic hormone (ACTH) and corticosterone compared to controls. The extract had no effect on plasma ACTH or corticosterone, even at the highest doses tested [Frost 2003].

Hypericin (0.2 mg/kg), hyperoside (0.6 mg/kg), isoquercitrin (0.6 mg/kg), miquelianin (0.6 mg/kg) and IMI (15 mg/kg), were administered daily to male CD rats by gavage for two weeks. The treatments significantly down-regulated circulating plasma levels of ACTH ($p<0.05$) and corticosterone ($p<0.05$ to $p<0.01$) by 40 - 70%. However, none of the compounds had an effect on plasma ACTH and corticosterone levels after chronic treatment over 8 weeks [Butterweck 2004].

In a study on the expression of genes involved in the regulation of the hypothalamic-pituitary-adrenal axis, short (2 weeks) and long term (8 weeks) oral administration to male CD rats of a methanolic extract (80%, V/V; 500 mg/kg), hypericin 0.2 mg/kg) and IMI (15 mg/kg) resulted in decreased levels of mRNA for CRH ($p<0.01$) by 16 - 22% in the paraventricular nucleus, and mRNA for 5-HT_{1A}-receptor ($p<0.05$ to $p<0.01$) by 11 - 17% in the hippocampus. Only IMI decreased tyrosine hydroxylase mRNA levels in the *locus coeruleus* by 23% ($p<0.01$) after 8 weeks [Butterweck 2001b].

An ethanolic extract (0.3% hypericin, 4.5% hyperforin), but not a CO_2 extract (no hypericin, 38.8% hyperforin), dose-

dependently and significantly ($p<0.001$) potentiated L-DOPA-induced changes in behaviour of Wistar mice. Treatment with apomorphine (0.5 mg/kg i.p.) led to stereotypical behavioural changes which were increased by IMI (10 mg/kg i.p.) ($p<0.01$). The ethanolic extract, and to a lesser extent the highest dose of the CO_2 extract, significantly ($p<0.001$ and $p<0.05$ respectively) increased the apomorphine stereotypy score [Bhattacharya 1998].

The effects of a methanolic extract (80%, V/V; 4.67% hyperforin; 31.25, 62.5 and 125 mg/kg/day) and a CO_2 extract (30.14% hyperforin; 2.42, 4.84 and 9.68 mg/kg/day) were investigated in male CD rats as single oral doses acutely and over 14 days. IMI (20 mg/kg/day p.o.) and vehicle served as controls. Both extracts increased DA and 5-HT levels, as measured by *in vivo* microdialysis. The dose-response curve followed an inverse U-shape. Repeated administration caused a rapid tolerance of DA but not of 5-HT neurons. Similar changes were observed after acute and repeated administrations of IMI. The potency of hyperforin surpassed that of imipramine in the acute release of both DA and 5-HT by the nucleus accumbens. The effect of hyperforin correlated with its inhibiting potency on high-affinity DA and 5-HT uptake. The inhibition of neuronal GABA transport occurred at higher concentrations of hyperforin [Rommelspacher 2001].

The effect of hyperforin on striatal ACh release and associated choline levels was studied using microdialysis in adult Sprague-Dawley rats. Local infusion of hyperforin (10 µM and 100 µM) via the dialysis probe caused changes in brain ACh and choline levels. Systemic administration of hyperforin (1-10 mg/kg i.p.) led to therapeutic plasma levels of hyperforin and caused a significant elevation of striatal ACh release. The higher hyperforin dose (10 mg/kg) revealed a reduction of locomotor activity in CD1 mice [Buchholzer 2002].

Male rats were treated orally with two different doses (30 and 100 mg/kg) of an unspecified extract for 3 or 15 days. A 3-day treatment was not able to modify PRL serum levels, whereas a 15-day treatment with the extract at the higher dose significantly ($p<0.01$) inhibited PRL production [Di Carlo 2005].

The effects of chronic treatment with intragastric injections of a methanolic (80%V/V) extract on neurochemical markers of 5-HT, DA and opioid systems in mesolimbic regions of fawn-hooded male FH rats were investigated by quantitative autoradiography. After 10 days' treatment, the extract increased [^{3}H]-citalopram binding to 5-HT transporters in multiple mesolimbic regions. In contrast, the extract resulted in a region-specific alteration of [^{3}H]-mazindol binding to DA transporters, such as increased binding of [^{3}H]-mazindol in the olfactory tubercle and decreased binding in the ventral tegmental area. In addition, the extract resulted in differential modulation of the binding properties of 5-HT_{1A}-, 5-HT_{2A}- and µ-opioid receptors in a region-specific manner [Chen 2003].

Cognitive effects

The effect of a 50% ethanolic extract was investigated in various experimental models of learning and memory, *viz*. transfer latency (TL) on elevated plus-maze, passive avoidance (PA), active avoidance (AA), and scopolamine- and sodium nitrite-induced amnesia (SIA & NIA) in albino rats. The extract was administered orally (100 and 200 mg/kg) for three days, while piracetam (500 mg/kg i.p.), a nootropic agent, was administered acutely. The extract and piracetam significantly ($p<0.01$) shortened TL on days 2 and 9, and antagonised the amnesic effects of scopolamine and sodium nitrite on TL. The extract had no significant effect on the retention of PA. The higher dose produced a significant ($p<0.05$) reversal of scopolamine-induced PA retention deficit but no significant reversal was observed with sodium nitrite. The extract at both doses and piracetam facilitated the acquisition and retention of AA and attenuated the scopolamine and sodium nitrite-induced impaired retention of AA [Kumar 2000].

The effect of an ethanolic (80%, V/V) extract was tested on memory retrieval 24 h after training on a one-trial passive avoidance task in male Swiss albino mice. Acute i.p. administration at doses of 4.0, 8.0, 12.0, and 25.0 mg/kg before retrieval testing increased ($p<0.01$) the step-down latency during the test session. The same doses of the extract failed to reverse scopolamine-induced amnesia of a two-trial passive avoidance task. Pre-treatment of the animals with serotonergic 5-HT_{1A} receptor antagonist (-)-pindolol (0.3, 1.0, and 3.0 mg/kg s.c.), serotonergic 5-HT_{2A} receptor blocker spiperone (0.01, 0.03, and 0.1 mg/kg i.p.), α-AR antagonist phentolamine (1, 5, and 10 mg/kg i.p.), β-AR antagonist propranolol (5, 7.5, and 10 mg/kg i.p.), dopaminergic D_1 receptor antagonist SCH 23390 (0.01, 0.05, and 0.1 mg/kg i.p.), and dopaminergic D_2 receptor antagonist sulpiride (5, 7.5, and 10 mg/kg i.p.) revealed the involvement of adrenergic and serotonergic 5-HT_{1A} receptors in the facilitatory effect of the extract on retrieval memory [Khalifa 2001].

The effects of a methanolic (80%, V/V) extract and hyperforin sodium salt were evaluated in rat and mouse avoidance tests. In a conditioned rat avoidance response, 7 days oral administration of the extract (50 mg/kg/day) and hyperforin (1.25 mg/kg/day) improved learning ability from the second day until day 7. The memory of the learned responses acquired during the 7 consecutive days of administration and training was largely retained after 9 days without further treatment or training. A single dose of the extract at 25 mg/kg did not reveal any significant effects in the passive avoidance response test in mice, whereas a single oral dose (1.25 mg/kg) of hyperforin almost completely reversed scopolamine-induced amnesia [Klusa 2001].

The Morris water maze (MWM) was used to test the effects of St. John's wort tablets containing dried crude herb standardised to 0.3% hypericin, administered orally for 9 weeks at doses equivalent to 4.3 and 13 µg/kg hypericin to male Wistar rats. In the acquisition phase (days 1 to 4) latencies were significantly ($p<0.001$) reduced due to training. The learning effect was greater ($p<0.05$) in the high dose group than in the low dose group or control. In the probe trial on day 5, the high dose group crossed the correct annulus in the target quadrant significantly ($p<0.05$) more often than the other groups. After completion of the experiment, the concentrations of monoamines and metabolites were determined in selected brain regions (prefrontal cortex, hippocampus and hypothalamus) demonstrating significant ($p<0.05$ to $p<0.001$) differences in their contents between the treatment groups and control [Widy-Tyszkiewicz 2002].

Hippocampal acetylcholine levels were increased ($p<0.01$ to $p<0.001$) by 50-100% in adult Sprague-Dawley rats after i.p. administration of hyperforin at doses of 1 and 10 mg/kg. The effect was almost completely suppressed by local perfusion with calcium-free buffer or with tetrodotoxin (1 µM) [Kiewert 2004].

The potential for prevention of working memory impairments was tested in male Wistar rats orally administered 350 mg/kg of an extract (solvent not specified; 0.05-0.3% hypericin, 2-4.5% hyperforin, 2-4% flavonoids and 8-12% procyanidins) for 21 days. Spatial working memory impairments caused by chronic restraint stress or medication with exogenous corticosterone in the Barnes maze and MWM were prevented ($p<0.001$). The treatment significantly improved hippocampus-dependent spatial working memory in comparison with control ($p<0.01$) and alleviated other negative effects of stress on cognitive functions [Trofimiuk 2008; Trofimiuk 2005].

Middle-aged male Wistar rats (18 months) displayed a decline in the acquisition of spatial working memory in the MWM. Chronic oral administration of the same extract (350 mg/kg for 21 days) significantly ($p<0.001$) improved processing of spatial information and increased the levels of phosphorylated cAMP response element binding protein in the hippocampus ($p<0.01$). Locomotor impairments caused by ageing as tested in the OFT remained unaffected [Trofimiuk 2010].

An ethanolic (50%) extract significantly improved glucose metabolism in STZ-induced diabetic Wistar rats when given at i.p. doses of 125 and 250 mg/kg over one week and significantly improved learning parameters of animals in a shuttle-box test ($p<0.01$ to $p<0.001$) compared to controls [Can 2011a].

The effect of an aqueous dry extract (3% (m/m) hyperforin and > 20% (m/m) flavonoids) on diabetes-induced learning and memory impairment was assessed in streptozotocin-induced diabetic rats. After oral administration of 6, 12 and 25 mg/kg for 30 days, passive avoidance learning and memory were significantly ($p<0.05$ to $p<0.001$) improved by the two highest doses [Hasanein 2011].

The potential for alleviation of stress- and corticosterone-related memory impairments by restoring levels of the synaptic plasticity proteins, neuromoduline (GAP-43) and synaptophysin (SYP), in hippocampus and prefrontal cortex was tested in male Wistar rats which displayed a decline in the acquisition of spatial working memory in the Barnes maze. Chronic oral administration of a dried St. John's wort preparation (350 mg/kg for 21 days) potently improved processing of spatial information in stressed and corticosterone-treated rats ($p<0.001$). Treatment significantly increased levels of GAP-43 and SYP in hippocampus and prefrontal cortex ($p<0.05$) and prevented the deleterious effects of both chronic restraint stress and prolonged corticosterone administration on working memory. It significantly ($p<0.01$) improved hippocampus-dependent spatial working memory in comparison with control and alleviated other negative effects of stress on cognitive functions [Trofimiuk 2011].

The effect of an ethanolic extract (80%; 250 and 500 mg/kg p.o.) and FLU (10 mg/kg p.o.) on genes involved in the pathogenesis of depression were studied using a chronic restraint stress model in rats. Hypothalamic and hippocampal tissues were analysed using the Affymetrix gene chip Rat Genome 230 2.0 Array. Genes involved in the pathways of inflammatory processes (Mapk8), oxidative stress (Gpx3, Gstm3, Sod3) and Alzheimer's disease (Sncb, Apbb1ip) were altered by both FLU and the extract [Jungke 2011].

Anxiety
A methanolic (80%, V/V) extract was given to male Wistar rats at doses of 62.5-500 mg/kg p.o., either acutely or as chronic treatment for 14 days. The animals were tested in the elevated T-maze (ETM), the light/dark transition, and the cat odour test. The results showed that acute treatment (125 mg/kg) impaired ETM inhibitory avoidance without altering escape performance. Chronic treatment (62.5-250 mg/kg) enhanced avoidance latencies only in animals pre-exposed to the open arms of the maze, with pre-exposure shortening escape latency. In contrast to IMI (15 mg/kg p.o.), chronic treatment with the extract did not impair escape from the open arms of the maze. Similarly to IMI, the extract increased the number of transitions between the two compartments in the light/dark transition model. Treatment regimens with the extract and IMI did not alter behavioural responses of rats to a cloth impregnated with cat odour [Flausino 2002].

A methanolic extract (80%, V/V; 0.3% hypericin, 3.3% hyperforin) was administered orally at doses of 150 and 300 mg/kg acutely, subchronically (7 days) and chronically (21 days) to adult male Swiss albino mice. PAR at 5 mg/kg was used as a positive control. Anxiolytic-like and antipanic-like effects were assessed with the mouse defence test battery. On day 21 both treatments showed reduced flight reactions to the presence of the predator. After 21 days PAR increased the number of approaches/withdrawals and reduced the number of upright postures, while the extract only reduced the number of upright postures [Beijamini 2003].

A hydroalcoholic extract (not further specified) was tested for the treatment of co-morbid mood disturbances and anxiety in diabetic rats. Type 2 diabetes mellitus was induced by a single i.p. injection of STZ (65 mg/kg), 15 min after an i.p. injection of nicotinamide (120 mg/kg). The extract was administered orally (100 and 200 mg/kg) to diabetic animals for 14 days. Anxiolytic activity was evaluated using the open-field exploration test (OFT) and elevated plus maze (EPM) test. Diabetic rats showed a significant increase in anxiety in OFT ($p<0.05$) and EPM ($p<0.05$) compared to non-diabetic control rats. Treatment with the extract significantly ($p<0.001$) improved anxiety symptoms in the OFT and ($p<0.001$) reduced elevated blood glucose levels in diabetic rats [Husain 2011a].

Neuroprotection
In male Sprague-Dawley rats hyperforin (6 μM) injected in the hippocampus bilaterally (i) decreased Aβ deposit formation after injection of amyloid fibrils in the hippocampus, (ii) decreased the neuropathological changes and behavioural impairments in a model of amyloidosis, and (iii) prevented Aβ-induced neurotoxicity in hippocampal neurons both from amyloid fibrils and Aβ oligomers, avoiding the increase in ROS associated with amyloid toxicity. The effects were explained by the capacity of hyperforin to disaggregate amyloid deposits in a dose and time-dependent manner and to decrease Aβ aggregation and amyloid formation [Dinamarca 2006].

The effect of an unspecified extract (0.34% hypericin, 4.1% hyperforin, 5% flavonoids, 10% tannins) given at an oral dose of 30 mg/kg was evaluated in an experimental model of spinal cord injury in adult CD1 mice. The degree of (a) spinal cord inflammation and tissue injury (histological score), (b) nitrotyrosine, (c) poly(adenosine diphosphate-ribose), (d) neutrophils infiltration, and (e) the activation of signal transducer and activator transcription 3 were markedly reduced in spinal cord tissue [Genovese 2006b].

Wistar rats (18 months old) displayed a decline in the acquisition of spatial working memory ($p<0.001$) in the MWM. Administration of a dried St. John´s wort preparation (0.3 % hypericins, 6% hyperforin, 2-4% flavonoids and 8-12% procyanidins), at an oral dose of 350 mg/kg for 21 days, significantly improved the processing of spatial information ($p<0.001$). The preparation also increased pCREB in the hippocampus ($p<0.01$). Aging caused significant locomotor impairments in the OFT ($p<0.001$) but no changes in the MWM test; factors unaffected by the treatment [Trofimiuk 2010].

Neurogenesis
A methanolic extract was administered at an i.p. dose of 30 mg/kg/day for 3 weeks to adult CD1 mice with corticosterone-induced stress. Treatment with the extract reversed the decrease in hippocampal progenitor cell proliferation induced by corticosterone. Stressed mice also exhibited a reduced dendritic spine density that was significantly ($p<0.05$) ameliorated by the extract [Crupi 2011].

Alzheimer's disease
Quercetin and kaempferol stimulated depression-related

signalling pathways involving brain-derived neurotrophic factor (BDNF), phosphorylation of cAMP response element binding protein CREB and postsynaptic density proteins PSD95, and reduced Aβ peptide in neurons isolated from double transgenic AD mice (TgAPPswe/PS1e9). Administration of the flavonols enhanced BDNF expression and reduced Aβ oligomers in hippocampus of the mice; this correlated with cognitive improvement [Hou 2010].

A methanolic extract (80%, V/V; 5% hyperforin) was given orally (1250 mg/kg/day) to 30-day-old male C57BL/6J-APP/PS1(+/-) mice for 60 or 120 days. When compared to the untreated controls, mice receiving the extract showed significant ($p<0.05$ to $p<0.01$) reductions in plaque load for both $A\beta_{1-40}$ and $A\beta_{1-42}$, and significant ($p<0.001$ to 0.0001) increases in cerebrovascular Pgp expression [Brenn 2014].

Parkinson's disease
A study evaluated the protective effect of chronic administration of two standardised extracts, (A) rich in hyperforin (6%) and (B) poor in hyperforin (0.2%), and quercetin, against neurodegeneration induced by chronic administration of rotenone in rats.The animals received pre-treatment with extract A (4 mg/kg i.p.), extract B (4 mg/kg i.p.) or quercetin liposomes (25 and 100 mg/kg i.p.) 60 min before rotenone injection (2.5 mg/kg) for 45 days. Pre-treatment with both extracts efficiently blocked deleterious toxic effects of rotenone, revealing normalisation of catalepsy and amelioration of neurochemical parameters. Extract A also reduced neuronal damage by diminishing the substantia nigra dopaminergic cell death caused by rotenone and inhibited the apoptotic cascade by decreasing Bax levels. Extract A was more active than extract B [Gomez del Rio 2013].

Movement disorders
The effects in male Wistar rats of an unspecified aqueous extract (300 mg/kg/day p.o.) were evaluated in models of abnormal movements induced by fluphenazine (25 mg/kg/day i.m.) or reserpine (0.5 mg/kg, s.c. every 2 days for 6 days). The number of vacuous chewing movements (VCMs) and locomotor activity were measured. In experiment 1, rats received a single dose of fluphenazine enanthate and/or the extract for 7 days. In experiment 2, rats received reserpine and/or the extract for 16 days beginning 10 days before the first administration of reserpine. The extract failed to protect against orofacial movements induced by fluphenazine or reserpine [Reis 2013].

An ethanolic (70%) extract was tested in a rat model of Parkinson's Disease. Unilateral intrastriatal lesions were induced by 6-hydroxydopamine (6-OHDA) in adult male Wistar rats. Treatment with the extract (200 mg/kg/day i.p.) was started 1 week pre-surgery and continued for 1 week post-surgery. The extract attenuated apomorphine-induced rotational behaviour, decreased the latency to initiate and the total time in the narrow beam task, lowered striatal level of malondialdehyde, enhanced striatal catalase activity and reduced GSH content, normalised striatal expression of glial fibrillary acidic protein and tumour necrosis factor alpha with no significant effect on mitogen-activated protein kinase, lowered nigral DNA fragmentation, and prevented damage of nigral dopaminergic neurons with a higher striatal tyrosine hydroxylase immunoreactivity [Kiasalari 2015].

Effects on seizures
The effects of different fractions of an ethanolic (80%) extract (A: water, B: ether, C: *n*-butanol; at 100 mg/mL i.m.) on the kindling epileptic discharges in Chinchilla rabbits were analysed using electrodes implanted in cortical structures and hippocampus. The effect correlated with the polarity of the fractions. The most polar constituents found in the water fraction exerted the highest antiepileptic activity in 100% of animals. The butanol fraction repressed the epileptic manifestations in 40% of animals, whereas lipid-soluble constituents in the ether fraction potentiated the epileptic activity [Ivetic 2002].

The anticonvulsant activity of undefined aqueous and ethanolic extracts was studied in mice in the pentylenetetrazole (PTZ) and the maximal electroshock seizures (MES) tests. In the PTZ test, the extracts (0.1-1 g/kg i.p.) delayed the onset of tonic convulsions and protected mice against mortality. In the MES test, neither extract demonstrated anti-seizure activity. L-NAME (1-10 mg/kg i.p.) reduced the anticonvulsant activity of the extracts [Hosseinzadeh 2005].

Water, *n*-butanol and ether fractions of an ethanolic (70%) extract were tested on epileptogenesis in Chinchilla rabbits of both sexes in the kindling model of epilepsy. Epileptic focus was induced by stimulation of the hippocampus. Animals treated with the ether fraction required significantly weaker minimum current strengths for development of epileptogenic focus, and displayed longer after-discharge (AD) times, while the number of electro-stimulations necessary for full kindling was less. In contrast, animals treated with water and n-butanol fractions required increased electro-stimulations for the development of epileptic discharge, and displayed shortened AD durations versus controls [Ivetic 2011].

A methanolic (80%) extract was given at doses of 25 to 200 mg/kg i.p. to male adult mice. After 20 min, seizures were induced by 10 mg/kg i.p. picrotoxin. Latency to seizure increased with the extract at 50 mg/kg ($p<0.01$). The highest dose (200 mg/kg) significantly decreased duration of seizure ($p<0.01$) and death latency ($p<0.01$) [Etemad 2011].

The effect of a standardised extract (not futher specified) on PTZ-induced convulsions was compared with standard antidepressants (FLU 30 and 50 mg/kg i.p.; dothiepin 30 and 50 mg/kg i.p.; VEN 30 mg/kg i.p.). Male mice were injected with either saline, the extract (50-500 mg i.p.) or the antidepressants. Sixty minutes later, animals received 60 mg/kg i.p. pentylenetetrazole. In a dose-dependent manner, the extract significantly ($p<0.05$ to $p<0.001$) reduced latency to convulsion, with the maximum reduction of about 43% at 100 and 200 mg/kg. The extract also dose-dependently increased mouse mortality, with 100% mortality occurring at 400 mg/kg. FLU, dothiepin and VEN at 30 mg/kg produced maximum reductions in latency of 33, 44 and 37.6% respectively. VEN caused 80% mortality at 30 mg/kg, while at the same dose FLU and dothiepin caused 20 and 40% mortality respectively [Bukhari 2013].

Migraine
The pain-relieving property of an unspecified extract was measured in male Swiss albino mice. The NO donors nitroglycerin (GTN; 10 mg/kg i.p.) and sodium nitroprusside (SNP; 0.5, 1 and 2 mg/kg i.p.) induced allodynia (cold plate test) and hyperalgesia (hot plate test). A single oral dose of the extract (5 mg/kg p.o.) produced a prolonged relief from pain hypersensitivity. Pre-treatment with the extract delayed the onset of hyperalgesia and reduced the duration of the painful symptomatology. No behavioural side effects or altered locomotor activity were observed [Galeotti 2013a].

An unspecified extract (5 mg/kg p.o.) reduced meningeal nociception in mice induced by GTN and SNP, which produced a delayed meningeal inflammation, as shown by the upregulation of IL-1β and iNOS, and a prolonged cold allodynia and heat hyperalgesia with a time-course consistent with NO-induced migraine attacks. A single oral administration of the extract counteracted the nociceptive behaviour and the

over-expression of IL-1β and iNOS. The increased expression and phosphorylation of pro-inflammatory factors was prevented by the extract and hypericin (10 µg/kg p.o.) [Galeotti 2013b].

Antinociceptive effects
The i.p. administration of 30-100 mg/kg of an unspecified extract produced significant analgesic effects in acetic acid-induced writhing (up to 88% inhibition, $p<0.001$) and formalin licking tests (up to 83% inhibition, $p<0.01$) in NMRI mice. The effect was twice as potent as ibuprofen (100 mg/kg i.p.) in the acetic acid-induced writhing test [Bukhari 2004].

Oral administration of an unspecified extract (25 mg/kg) displayed antinociceptive activity in the tail electric stimulation ($p<0.05$) and hot plate ($p<0.01$) tests. Acetic acid-induced writhing was significantly ($p<0.05$) reduced by oral FLU (50 mg/kg) or etodolac (72 mg/kg), but not by the extract. The nociceptive response caused by i.p. injection of capsaicin (1.6 µg/paw) was unaffected by the extract, but reduced by FLU ($p<0.05$). Injection of the extract (50, 125 or 250 mg/kg s.c.) to pylorus-ligated rats decreased gastric acid secretion, but increased indomethacin-induced gastric mucosal lesions dose-dependently [Abdel-Salam 2005].

An unspecified hydroethanolic extract was studied in acetic acid-induced abdominal constriction in Balb-C mice. The extract (10-20 mg/kg i.p.), opium (10-30 mg/kg i.p.), morphine (0.75-3.0 mg/kg i.p.) and acetylsalicylic acid (50-100 mg/kg i.p.) showed dose-dependent antinociceptive effects. In animals treated with naloxone (0.5 mg/kg s.c.), the antinociceptive effect of the extract was significantly ($p<0.05$ to $p<0.01$) reduced similar to that of opium, while the effect of acetylsalicylic acid remained unchanged [Subhan 2007].

A study investigated the antinociceptive properties of an unspecified extract in male ICR mice. Oral pre-treatment with the extract (100 1000 mg/kg) significantly ($p<0.05$) attenuated licking/biting times, in both the first and second phases following formalin injection in a dose-dependent manner. Naloxone (5 mg/kg s.c.) did not attenuate the effect. Formalin injection resulted in an increase in nitrites/nitrates (NOx) in mouse spinal cord which was significantly ($p<0.05$) attenuated by the extract. Pre-treatment with the extract significantly ($p<0.01$) potentiated the antinociceptive effect of morphine (0.3 mg/kg s.c.), although concentrations of morphine in plasma and brain were not significantly changed [Uchida 2008].

An unspecified dried extract induced a prolonged antinociceptive effect for 120 minutes after intracerebroventricular (i.c.v.) injection to male Swiss albino mice in acute thermal and chemical pain. Thermal antinociception was prevented by naloxone and by the protein kinase C (PKC) activator PMA, whereas the chemical antinociception was prevented only by PMA. A chloroform (CHL) and a methanol (MET) fraction increased pain threshold with a time course comparable to the extract. The CHL antinociception was antagonised by naloxone, the MET antinociception by PMA. Hyperforin and hypericin showed an antinociceptive efficacy comparable to CHL and MET respectively. The flavonoids remained without effect. The presence of hypericin was fundamental to inducing thermal and chemical antinociception through the inhibition of the PKC activity, whereas hyperforin selectively produced a thermal opioid antinociception [Galeotti 2010a].

Neuropathic pain
An unspecified dried chloroform extract was investigated for its effect on neuropathic pain due to chronic constriction injury or the repeated administration of oxaliplatin (3.4 mg/kg i.p.) in male Sprague Dawley albino rats. Acute administration of the extract at low doses (30 60 mg/kg p.o.) reversed mechanical hyperalgesia up to 180 min after administration. Hyperforin and hypericin were responsible for the antihyperalgesic properties whereas flavonoids were ineffective. Treatment with the extract, hyperforin and hypericin did not result in any behavioural side effects or signs of altered locomotor activity [Galeotti 2010b].

The antinociceptive effect of an unspecified extract on hypersensitivity induced by GTN and SNP was assessed by cold and hot plate tests in male Swiss albino rats. A single oral administration of low doses of the extract or hypericin reversed the induced prolonged allodynia and hyperalgesia, whereas hyperforin and flavonoids were ineffective. The increased CREB and STAT1 phosphorylation, the activation of NF-κB in periaqueductal grey matter and thalamus, and the increased expression and phosphorylation of PKCγ/ε isoforms after GTN and SNP administration were prevented by the extract and hypericin [Galeotti 2012].

Alcohol withdrawal
In cAA rats, a genetic model of alcoholism, acute i.p. administration of IMI (3-30 mg/kg), FLU (1-10 mg/kg) or an ethanolic extract (50% m/m; 10-40 mg/kg) dose-dependently reduced alcohol intake in a 12-h limited access two-bottle [ethanol 10% (V/V) *versus* water] choice procedure; with minimal effective doses of 30, 5 and 20 mg/kg respectively. The anti-alcohol effects of FLU and the extract appeared to be specific, as reductions in alcohol intake co-incided with reductions in alcohol preference [De Vry 1999].

Upon administration of single oral doses of 100, 200, 400, 600 and 800 mg/kg of a methanolic extract (80%, V/V; 0.22% hypericin, 4.05% hyperforin), alcohol intake was dose-dependently reduced in alcohol-preferring rats. Repeated application of 400 mg/kg for 15 days did not induce tolerance of this effect [Rezvani 1999].

Acute intragastric administration of 125 - 250 mg/kg of an ethanolic extract (50%; 0.3% hypericin) reduced alcohol intake of alcohol-preferring male msP rats by 30 - 40% [Perfumi 1999].

The application of an ethanolic extract (50%; 0.3% hypericin and 3.8% hyperforin) by 3 intragastric administrations of 250 mg/kg to male msP rats produced positive results in the model of the alcohol-preferring rat. The inhibition observed was neither modified by the σ receptor antagonist rimcazole nor by the neurotoxin 5,7-dihydroxytryptamine [Panocka 2000].

Administration of a methanolic extract (0.3% hypericin, 3.8% hyperforin; E1) or a CO_2 extract (24.33% hyperforin, very low hypericin content; E2) to alcohol preferring male msP rats dose-dependently reduced ethanol intake, with E2 being approximately 8-times more potent than E1. Food and water intakes were not affected [Perfumi 2001].

In the same model the effect on ethanol intake of msP rats was investigated 1h after intragastric administration of a CO_2 extract. Naloxone (NAL:1, 3, or 5 mg/kg) or naltrexone (NTX; 0.5, 1, or 3 mg/kg) were given by i.p. injection 10 min before the extract. The extract alone reduced ethanol intake at 31 or 125 mg/kg, but only at 7 mg/kg when combined with NAL. These doses neither modified food or water intake, nor reduced 0.2% saccharin intake. NAL alone significantly reduced ethanol and food intake at 3 or 5 mg/kg, but not at 1 mg/kg. When NAL was combined with each dose of the CO_2 extract, the attenuation of ethanol intake was more pronounced than after the administration of the extract alone. NTX alone reduced ethanol intake at 1 and 3 mg/kg, but not at 0.5 mg/kg. When

NTX 0.5 mg/kg was combined with the CO_2 extract at 7 mg/kg, ethanol intake was markedly reduced [Perfumi 2003].

In C57BL/6J alcohol-preferring mice oral administration of a crude methanolic extract was compared with i.p. administration of a hyperforin-rich CO_2 extract (45% hyperforin). The dose of the hyperforin-rich extract required to significantly ($p<0.05$) reduce 10% ethanol intake was 5 mg/kg, and was 125-fold less than that required for the methanolic extract (625 mg/kg), and comparable to the dose of FLU (10 mg/kg i.p.). None of the treatments significantly affected water intake [Wright 2003].

Intragastric administration of a CO_2 extract (24.33% hyperforin, 0.08% hypericin) significantly reduced ethanol self-administration in alcohol preferring male msP rats at doses of 31 or 125 mg/kg but not 7 mg/kg, when given 1h prior to ethanol access. It did not modify saccharin self-administration. The extract at 31 and 125 mg/kg abolished the increased ethanol intake following ethanol deprivation ($p<0.05$ and $p<0.01$ respectively) [Perfumi 2005b].

In a similar study, repeated intragastric administration of a CO_2 extract, alone or combined with NTX (0.5 or 3 mg/kg i.p.) daily for 12 days, markedly reduced ethanol intake at 125 mg/kg ($p<0.01$), but not at 7 mg/kg. The same dose of the CO_2 extract slightly reduced the simultaneous intake of food, but only on days 3 and 11 of treatment. Rats promptly recovered baseline ethanol intake when treatment did not precede access to ethanol (on day 8) or after the end of treatment (days 13 and 14). Repeated i.p. treatment with NTX reduced ethanol intake at 3 mg/kg ($p<0.001$), but not at 0.5 mg/kg. The extract (7 mg/kg) combined with NTX (0.5 mg/kg) synergistically evoked a significant ($p<0.001$) reduction of ethanol intake. The effect on ethanol intake of the combined treatment remained stable over the 12 days of treatment [Perfumi 2005a].

In ethanol-dependent adult male Wistar rats an ethanolic extract (50%; 25-200 mg/kg i.p.) given just before ethanol withdrawal produced a dose-dependent and significant ($p<0.05$) inhibition of locomotor hyperactivity 2 and 6 hours after withdrawal, and significantly ($p<0.05$) reduced the number of stereotyped behaviours. The extract produced significant inhibitory effects on tremor (50 and 100 mg/kg; $p<0.05$) and audiogenic seizures (100 mg/kg, $p<0.05$) during the withdrawal period [Coskun 2006].

Nicotine withdrawal
Male Swiss mice received nicotine (2 mg/kg, s.c. four injections daily) for 14 days. An extract was administered at various doses (125-500 mg/kg p.o.) immediately after the last nicotine injection. In a further experiment a dose of 500 mg/kg of extract was administered with nicotine at various points during the study period. The reduction of locomotor activity induced by nicotine withdrawal was abolished by the extract, which also significantly ($p<0.05$ to $p<0.01$) and dose-dependently reduced the total nicotine abstinence score when injected after nicotine withdrawal [Catania 2003].

Male Swiss mice were divided into several treatment groups (n=6 each): Group 1: saline i.p. injection, 4 times daily for 14 days; Group 2: nicotine (2 mg/kg i.p.) 4 times daily for 14 days to induce nicotine dependence; Groups 3-5: nicotine protocol plus administration (125 to 500 mg/kg p.o.) of a flavonoid-enriched ethanolic extract (60%; 0.3% hypericin, 4.5% hyperforin, 50% flavonoids) immediately after the last nicotine injection for 30 days after nicotine withdrawal; Group 6: saline protocol plus the extract at 500 mg/kg after the last saline injection for 30 days (no nicotine). In a further group, animals treated with nicotine (14 days), alone or with the extract, were co-administered with a single s.c. dose of saline or the selective 5-HT receptor antagonist WAY 100635 (1 mg/kg i.p.). Brain 5-HT metabolism was evaluated in the cortex 30 days after nicotine withdrawal through evaluation of 5-HT, 5-HIAA and the 5-HIAA/5-HT ratio. The total abstinence score was significantly ($p<0.01$) reduced in mice given the extract (500 mg/kg) after nicotine withdrawal compared to the nicotine control group. WAY 100635 significantly ($p<0.01$) inhibited the reduction of total abstinence score induced by the extract. A reduction of 5-HT content was observed after nicotine withdrawal. A significant increase of cortical 5-HT content was found in animals treated with the extract, with a concomitant significant increase of 5-HT_{1A} receptors [Mannucci 2007].

Opioid withdrawal
In morphine-dependent adult male Wistar rats signs of withdrawal were induced by naloxone (3 mg/kg i.p.) given 4 h after the last injection of morphine on day 7 of morphine treatment. The manifestations of withdrawal were significantly ($p<0.001$) inhibited by co-administration of the morphine (dose increased daily from 2.5 to 50 mg/kg) with an unspecified aqueous extract (p.o. 0.4 mL/200 g, 0.8 mL/200 g and 1.2 mL/200 g per gavage) or clonidine (0.2 mg/kg i.p.). Clonidine was more effective than the extract at 0.4 mL/200 g. There was no significant difference in the mean frequency of withdrawal signs between clonidine and the extract at 0.8 mL/200 g. The extract at a dose of 1.2 mL/200 g exhibited significantly ($p<0.05$) greater attenuation of morphine withdrawal signs than clonidine [Feily 2009].

Sprague-Dawley rats were treated orally with an opium extract (increasing from 80–650 mg/kg) along with an ethanolic St. John's wort extract (70%; 20 mg/kg), either twice daily for 8 days (chronic use), or acutely as a single dose 1 h before induction of opium withdrawal with naloxone. Chronic use of the extract significantly ($p<0.05$) reduced withdrawal signs including stereotype jumps and wet dog shake number when compared to saline control, and decreased rectal temperature at 30 min ($p<0.05$), 60 min ($p<0.01$) and 120 min ($p<0.05$). Acute use of the extract reduced withdrawal-induced diarrhoea ($p<0.05$ vs. saline) [Khan 2014].

Antiaggressive effects
After oral administration of hydroethanolic preparations with defined amounts of hypericin (equivalent to 2-12 mg/kg), aggressive behaviour was significantly reduced in mice after 3 weeks of daily treatment with extract equivalent to 6 or 12 mg/kg of hypericin [Okpanyi 1987].

The antiaggressive activity of hyperforin was investigated in several rodent models using adult male Charles Foster rats and male Wistar mice. Animals were screened for aggressive behaviour, divided into three groups and treated once daily, for seven consecutive days, with A: lorazepam (2.5 mg/kg i.p.), B: hyperforin (10 mg/kg i.p.) or C: vehicle. Hyperforin treatment significantly ($p<0.001$) reduced various aggressive parameters in isolation induced aggression, resident-intruder aggression and foot shock-induced aggression. In the water competition test, hyperforin treatment significantly ($p<0.001$) reduced the duration of water consumption and frequency of water spout possession [Kumar 2009].

Sexual dysfunction
A hyperforin-rich CO_2 extract was examined in an experimental model for the expulsion phase of ejaculation in anesthetised Sprague Dawley rats. The ejaculation model involved inducing rhythmic bulbospongiosus (BS) muscle contractions in male rats under urethane anaesthesia (1.2 g/kg s.c.) by transiently raising the internal urethral pressure with saline infusion. Injection of the 5-HT_{1A} agonist 8-hydroxy-2-(di-*N*-propylamino)

tetralin (8-OH-DPAT) (0.4 mg/kg s.c.) intensified the BS muscle contractions induced by the increases in urethral pressure. The extract (5 to 80 mg/kg i.p.) reduced the effects of 8-OH-DPAT on ejaculation with maximum effect at a dose of 10 mg/kg i.p. The reduction in the amplitude of bursts with the extract remained unchanged after a midthoracic spinal transsection, suggesting that the action of hyperforin is either at the spinal ejaculation generator or directly on the neurons innervating the BS muscles [Thomas 2007].

Anti-inflammatory effects
Anti-inflammatory effects of St. John's wort extracts were studied in carrageenan-induced paw oedema test [Savikin 2007; Zdunic 2009], the indomethacin-induced gastric mucosa damage test [Zdunic 2009] in rats and the croton-oil induced ear oedema test in mice [Sosa 2010] after topical [Sosa 2010], oral [Savikin 2007; Zdunic 2009] and i.p. [Feisst 2009] administration.

A standardised ethanolic (50%) extract was examined for its putative anti-inflammatory and analgesic activity at the daily doses of 100 and 200 mg/kg p.o. The experimental paradigms used were carrageenan-induced paw oedema and cotton pellet-induced granuloma for anti-inflammatory activity; the tail flick, hot plate, and acetic acid-induced writhing methods were used to assess analgesic activity. Indomethacin (20 mg/kg i.p.) was used as an anti-inflammatory active control, while pentazocine (10 mg/kg i.p.) and aspirin (25 mg/kg i.p.) were used as analgesic active controls. The extract showed anti-inflammatory and analgesic activity at both dose levels, in all the paradigms used. In addition, the extract potentiated the anti-inflammatory activity of indomethacin and analgesic activities of pentazocine and aspirin [Kumar 2001a].

A hydroethanolic extract reduced croton oil-induced ear oedema in rodents by 50% ($p<0.05$). From fractionation it was concluded that the anti-inflammatory principle is concentrated in the lipophilic fractions [Brondz 1982].

Administration of an unspecified extract to Sprague-Dawley rats at 50, 150 or 300 mg/kg s.c. dose-dependently produced a significant ($p<0.001$) inhibition of carrageenan-induced oedema, by 53.7, 61.3 and 75.3% respectively, compared to 90% with FLU at 50 mg/kg s.c. and 60.7% with etodolac (ETO) at 72 mg/kg s.c. [Abdel-Salam 2005].

A methanolic extract (0.34% hypericin, 4.1% hyperforin, 5% flavonoids, 10% tannins) ameliorated acute pancreatitis induced by caerulein in male CD mice. The degree of (a) pancreatic inflammation and tissue injury (histological score), ($p<0.05$), (b) expression of ICAM-1, (c) the concentration of nitrotyrosine and poly(ADP-ribose), and (d) MPO activity was markedly reduced after administration of the extract (30 mg/kg p.o.). Moreover, the treatment significantly ($p<0.01$) reduced the mortality rate 5 days after caerulein administration [Genovese 2006a].

The same extract was given at 30 mg/kg p.o. prior to the carrageenan in male CD mice with carrageenan-induced pleurisy. All parameters of inflammation were attenuated by the extract. The carrageenan-induced upregulation of the expression of ICAM-1, and the increases in nitrotyrosine and poly (ADP-ribose), were all significantly ($p<0.01$) reduced by the extract. The extract also inhibited inflammation-induced activation of NF-κB and STAT-3 in the lung [Menegazzi 2006].

The effect of a methanolic (80%) extract on various pro-inflammatory cytokines (IL-1β, IL-2, IL-6, IFN-γ, TNF-α and on intestinal epithelium apoptosis, was investigated in rats treated with irinotecan (60 mg/kg i.v.) for 4 days in combination with the extract or vehicle (400 mg/kg p.o.) for 8 days. The extract markedly reduced irinotecan-induced diarrhoea ($p=0.002$) and intestinal lesions ($p<0.05$) and significantly suppressed the intestinal epithelial apoptosis induced by irinotecan over days 5-11 ($p<0.05$ to $p<0.001$). The extract further significantly inhibited the expression of TNF-α mRNA in the intestine over days 5-11 ($p<0.01$ and $p<0.001$) [Hu 2006].

In the carrageenan-induced rat paw oedema test, ethanolic extracts from various *Hypericum* species were each administered to adult male Wistar rats at doses of 25, 50, 100 and 200 mg/kg p.o. and compared to indomethacin at 1, 2, 4 and 8 mg/kg p.o. or vehicle. All tested doses of the *Hypericum perforatum* extracts demonstrated significantly ($p<0.001$) greater anti-inflammatory activity than the vehicle-treated controls with mean ED_{50} between 47.6 mg/kg and 50.9 mg/kg. The ED_{50} of indomethacin was 2.48 mg/kg. Anti-inflammatory activity was not found to be correlated with the hypericin content of the extracts [Savikin 2007].

An unspecified extract improved the inflammatory and immune response of colonic mucosa in male Wistar albino rats with inflammatory bowel disease (IBD) induced by intracolonic administration of 25 mg 2,4,6-trinitrobenzene sulfonic acid. Treatment with the extract (50, 150 and 300 mg/kg/day i.p.) resulted in significant ($p<0.05$ to $p<0.001$) improvement in the macroscopic scoring of colonic damage, blood levels of catalase, GSH, tissue levels of GSH reductase (GR), MPO activity and GSH-Px [Dost 2008].

Hyperforin (4 mg/kg i.p.) significantly reduced the exudate volume (63%, $p<0.001$), the number of migrating cells (50%, $p<0.001$) and the leukotriene B_4 (LTB_4) formation in pleural exudates (50%, $p<0.001$) of carrageenan-treated rats [Feisst 2009].

Of the three different oil extracts tested, an extract prepared by maceration with 96% ethanol followed by extraction with sunflower oil, given at an oral dose of 1.25 mL/kg, exhibited the highest anti-inflammatory (95.24 ± 11.66%) and gastroprotective activity (gastric damage score of 0.21 ± 0.12) in carrageenan-induced rat paw oedema and indomethacin-induced rat gastric mucosa damage. Its major components quercetin and I3,II8-biapigenin (at 8 mg/kg p.o.) showed a significantly ($p<0.05$ and $p<0.01$ respectively) greater anti-inflammatory activity than the sunflower oil control and comparable with indomethacin (8 mg/kg p.o.; $p<0.001$), as well as significant gastroprotective activity ($p<0.05$), compared to indomethacin [Zdunic 2009].

Topical anti-inflammatory effects of a hydroalcoholic extract, a lipophilic extract and an ethylacetate fraction, as well as isolated constituents, were investigated in croton oil-induced ear oedema in mice. The hydroalcoholic extract (100 - 1000 μg/cm^2), the lipophilic extract (100 - 1000 μg/cm^2), and the ethylacetate fraction (30 - 1000 μg/cm^2) were applied topically to mouse ears. The hydroalcoholic extract was less potent than the lipophilic extract and ethylacetate fraction (ID_{50} > 1000 μg/cm^2, 220 μg/cm^2 and 267 μg/cm^2 respectively). Amentoflavone, hypericin, hyperforin dicyclohexylammonium salt and adhyperforin showed ID_{50} values of 0.16 μmol/cm^2, 0.25 μmol/cm^2, 0.25 μmol/cm^2 and 0.30 μmol/cm^2 respectively. Indomethacin resulted in an ID_{50} of 0.26 μmol/cm^2 [Sosa 2010].

In carrageenan-induced rat paw oedema in male Wistar Han rats administration of hyperforin (4 mg/kg i.p.) significantly reduced the exudate volume (64%; $p<0.001$) and suppressed LTB_4 formation (50%; $p<0.01$) in pleural exudates. The effects were similar to i.p. administration of 10 mg/kg of the 5-LO inhibitor zileuton (77%, $p<0.001$ and 65%, $p<0.001$, respectively) [Feisst 2009].

Various extracts inhibited several members of the immunoglobulin superfamily of cell adhesion molecules, specifically intercellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) [De Paola 2005; Genovese 2006a; Menegazzi 2006] as well as P-selectin in mice and rats [De Paola 2005].

A methanolic extract (0.34% hypericin, 4.1% hyperforin, 5% flavonoids, 10% tannins) was given orally for eight days at a dose of 2 mg/kg/day to male Sprague Dawley rats following ligature-induced periodontitis. The extract exerted potent anti-inflammatory effects, significantly ($p<0.05$ to $p<0.01$) reducing all measured parameters of inflammation [Paterniti 2010].

An olive oil extract (A), an ethanolic (96%) extract (B) and fractions of the ethanolic extract (*n*-hexane, chloroform and ethyl acetate) were assessed for their anti-inflammatory activity in acetic acid-induced increased capillary permeability. A dose-dependent anti-inflammatory activity was found for the ethanol extract, the ethyl acetate subfraction and fractions containing hyperoside, isoquercitrin, rutin, (-)-epicatechin and hypericin [Süntar 2010].

An unspecified extract (150, 300 and 450 mg/kg p.o.) given for 26 days improved irritable bowel syndrome (IBS) induced by 5-day restraint stress in male Wistar rats. FLU and loperamide (LOP) were used as positive controls. Gastric emptying, small bowel and colon transit, as well as the levels of TNF-α, MPO, lipid peroxidation, and antioxidant power in colon homogenates were determined. A significant reduction in small bowel ($p<0.05$) and colonic ($p<0.001$) transit (450 mg/kg), TNF-α ($p<0.001$), MPO ($p<0.001$), and lipid peroxidation ($p<0.05$ to $p<0.001$) and an increase ($p<0.001$ each) in antioxidant power in all extract-treated groups were seen as compared with the control group [Mozaffari 2011].

Administration of hyperforin (4 mg/kg i.p.) to rats reduced exudate volume and leukocyte numbers in carrageenan-induced pleurisy associated with reduced PGE_2 levels, and inhibited carrageenan-induced mouse paw oedema formation with an ED_{50} (1 mg/kg) superior to that of indomethacin (ED_{50} = 5 mg/kg) [Koeberle 2011].

An unspecified ethanolic extract (30-300 mg/kg i.p.) dose-dependently reduced paracetamol-induced lethality in male Swiss mice ($p<0.05$). The paracetamol-induced increase in plasma AST and ALT concentrations, in hepatic MPO activity and IL-1β, TNF-α and IFN-γ concentrations, as well as reduced GSH concentrations and decreased capacity to reduce 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate radical cation) were inhibited ($p<0.01$) by the extract at 300 mg/kg [Hohmann 2015].

Wound healing

An olive oil extract (A), an ethanolic (96%) extract (B), and fractions of the ethanolic extract (*n*-hexane, chloroform and ethyl acetate), showed wound healing activity in excision and incision wound models in male Sprague-Dawley rats. Extract A had a significant wound healing effect on excision (5.1-82.6% inhibition, $p<0.001$) and circular incision (20.2-100.0% inhibition, $p<0.001$) wounds. The ethyl acetate fraction was the most active, inhibiting excision wounds by 17.9% to 100.0% and incision wounds by 9.4% to 100.0% [Süntar 2010].

A study investigated the therapeutic efficacy of the topical and systemic administration of an unspecified extract on chemo-therapy induced-mucositis. Oral mucositis was induced in 72 male golden hamsters by administration of 5-fluorouracil (5-FU; 60 mg/kg i.p.) on days 0, 5 and 10 of the study. The cheek pouch was scratched with a sterile needle on days 1 and 2. The extract was administered orally (300 mg/kg) and topically (10% extract gel) on days 12-17. Both of the extract treatment groups demonstrated a significant (both $p<0.01$) relief in oral mucositis compared to the control and base gel group [Tanideh 2014].

The efficacy of an unspecified extract for prevention of post-operative left uterine horn adhesion formation was studied in female Wistar rats. Group 1 (control): closure of abdominal incision. Group 2: closure after i.p. administration of Ringer's lactate (RL) solution. Group 3: closure after i.p. administration of olive oil (vehicle). Group 4: closure after i.p. administration of the extract. Fourteen days later, re laparatomy was performed and surgical adhesion scores, inflammation and fibrosis scores were determined. Groups did not differ in inflammation and fibrosis scores [Hizli 2014].

Bone formation

The effect of an aqueous extract on bone formation in the expanded premaxillary suture model was studied in male Wistar rats. Four groups of rats were investigated: control (A); only expansion (B); the extract given (21 mg/day p.o.) only during the expansion and retention period (a total of 17 days) (C); and the extract given during the nursery phase before expansion (40 days), and during the expansion and retention periods (a total of 57 days) (D). After the 5-day expansion period was completed, the rats in the B, C, and D groups underwent 12 days of mechanical retention, following which they were killed, and their premaxilla dissected and fixed. Histological examination was performed to determine the number of osteoclasts and capillaries, as well as the number of osteoblasts, inflammatory cell infiltration, and the amount of new bone formation. The number of osteoclasts and capillaries, and the inflammatory cell infiltration, as well as new bone formation, were higher in the extract groups C and D than in the other groups. Among these two groups, all parameters were higher ($p<0.01$) in group D than C [Halicioglu 2015].

Gastrointestinal motility

The effect on gastric motility of a standardised extract (details unknown) at doses of 100, 300 and 600 mg/kg/day p.o. was investigated in male Wistar rats. The extract delayed gastric emptying ($p<0.05$). The effect of the extract on electrical field stimulation (EFS)-induced contractions was unaffected by drugs inhibiting intrinsic inhibitory nerves or by tachykinin antagonists, but reduced by the 5-HT antagonist methysergide. The inhibitory effect of the extract on ACh-induced contractions was reduced by the sarcoplasmic reticulum Ca^{2+}-ATPase inhibitor cyclopiazonic acid ($p<0.001$), but not by the L-type Ca^{2+} channel blocker nifedipine or by methysergide. Among the chemical constituents of the extract, hyperforin ($p<0.05$ to $p<0.001$) and, to a lesser extent, the flavonoids kaempferol ($p<0.01$) and quercitrin ($p<0.05$), inhibited ACh-induced contractions [Capasso 2008].

Gastroprotective effects

Injection of an unspecified extract (50, 125 or 250 mg/kg s.c.) to pylorus-ligated rats decreased gastric acid secretion ($p<0.05$), but dose-dependently increased ($p<0.05$) indomethacin-induced gastric mucosal lesions [Abdel-Salam 2005].

Three oil extracts, prepared according to traditional prescriptions, were studied in the indomethacin-induced rat gastric mucosa damage test. All extracts possessed gastroprotective activity. The extract prepared by maceration with 96% ethanol, followed by extraction with sunflower oil, had the highest content of quercetin and I3,II8-biapigenin (129 ± 9 µg/mL and 52 ± 4 µg/mL respectively). Quercetin and I3,II8-biapigenin given separately at a dose of 8 mg/kg p.o. exhibited significant (both $p<0.05$) gastroprotective activity [Zdunic 2009].

Gastric mucosal damage induced by hypothermic restraint stress in Wistar rats was improved by an unspecified extract (25, 50 or 100 mg/kg/day p.o.) given over 3 days. The extract significantly reduced lesions compared to placebo by 65, 95 and 75% respectively (p=0.001). Treatment with ranitidine (5 mg/kg s.c.) also reduced ulcers significantly (p=0.001) compared with the placebo groups [Cayci 2009].

Metabolic effects
The hypocholesterolaemic effect of a flavonoid-rich extract (FEHP) was studied in male Wistar rats receiving either a cholesterol-rich (2%) or a standard diet for 16 weeks combined with a daily oral dose of FEHP (0, 25, 75 and 150 mg/kg). The cholesterol-rich diet induced hypercholesterolemia with elevated serum levels of total cholesterol (TC) and triglycerides (TG), and lowered LDL. Daily medium (75 mg/kg) and high doses (150 mg/kg) of FEHP decreased (all $p<0.05$) the serum levels of TC, TG and LDL, and increased ($p<0.05$) serum levels of HDL. Serum and liver contents of MDA were decreased ($p<0.05$) after FEHP compared with cholesterol-rich diet alone. FEHP increased ($p<0.05$) the activity of SOD in serum and liver, whereas the activity of CAT was elevated only in the liver [Zhou 2005].

A study evaluated the effect of an unspecified extract (0.1% hypericin, 3.8% hyperforin) in an experimental model of binge eating (BE). BE for highly palatable food (HPF) was evoked in female Sprague-Dawley rats by three 8-day cycles of food restriction/re-feeding and acute stress on the test day (day 25). Stress was induced by preventing access to HPF for 15 min, while rats were able to see and smell it. Only rats exposed to both food restrictions and stress exhibited BE. The oral doses of 250 and 500 mg/kg but not 125 mg/kg of the extract significantly ($p<0.05$ and $p<0.01$ respectively) reduced the BE episode. The same doses did not affect HPF intake in the absence of BE [Micioni Di Bonaventura 2012].

Ovariectomised (OVX) rats were treated orally for 6 weeks with a 70% ethanolic extract of whole plants (A) or a 70% ethanolic extract of flowers and leaves (B), β-oestradiol-3-benzoate (50 µg/kg/day) or vehicle. OVX increased body weight gain and adiposity, showed higher food efficacy ratio and increased serum cholesterol as well as insulin resistance while reducing uterus weight and uterine epithelial proliferation rate. Extracts A and B showed an oestrogen-like effect on body weight gain, adipose tissue weight and food efficacy ratio in OVX rats. The extracts prevented hypercholesterolaemia induced by OVX more effectively ($p<0.05$) than β-oestradiol-3-benzoate [You 2014].

An ethanolic (50%) extract significantly improved glucose metabolism in STZ-induced diabetic Wistar rats ($p<0.01$, $p<0.001$; 125 and 250 mg/kg i.p. for seven days) [Can 2011a].

In STZ-diabetic Charles Foster rats, an ethanolic (50%) extract given orally at 100 and 200 mg/kg significantly ($p<0.001$) reduced diabetes-induced elevated blood glucose levels [Husain 2011a].

Male STZ-induced diabetic albino rats received an ethyl acetate extract at 50, 100 and 200 mg/kg p.o. for 15 days. At the end of the treatment the extract showed a dose-dependent decrease in fasting blood glucose (FBG). Thirty minutes after administration, FBG was reduced significantly ($p<0.01$) when compared with controls. The extract caused a significant reduction in ($p<0.05$ to $p<0.01$) plasma glucose level, serum total cholesterol, triglycerides and glucose-6-phosphatase levels. Tissue glycogen content, HDL and glucose-6-phosphate dehydrogenase were significantly ($p<0.01$) increased compared with diabetic controls [Arokiyaraj 2011].

A hydroalcoholic extract (50%; 3% hyperforin, 0.3% hypericin) given at doses of 100 and 200 mg/kg orally over 15 days led to lowered total cholesterol and low-density cholesterol in normal Charles Foster rats. In high-fat-fed rats, the extract significantly ($p<0.001$) inhibited weight gain. In fructose-fed rats, the extract normalised the fructose-induced dyslipidaemia ($p<0.01$) and improved insulin sensitivity ($p<0.001$) [Husain 2011b].

A 95% ethanolic extract was given orally to diet-induced obese (DIO) C57BL/6J mice at doses of 50 and 200 mg/kg. The extract significantly improved glucose and lipid metabolism, lowered glucose AUC ($p<0.01$ and $p<0.001$) and fasting insulin levels (both $p<0.01$), improved insulin receptor sensitivity (HOMA-IR) ($p<0.01$ and $p<0.001$) and decreased serum triglyceride levels ($p<0.05$ and $p<0.01$) as compared to control [Tian 2015].

Reperfusion injury
The effect of an unspecified extract on splanchnic artery occlusion (SAO) shock-mediated injury was studied in male Sprague-Dawley rats. SAO shock was induced by clamping the superior mesenteric artery and the celiac trunk for 45 min. After 1 h of reperfusion, SAO-shocked rats developed a significant ($p<0.01$) fall in mean arterial blood pressure. Treatment of rats with an unspecified extract (applied at 25 mg/kg i.v. over 15 min before reperfusion) significantly ($p<0.01$) reduced the fall in mean arterial blood pressure and the migration of polymorphonuclear cells caused by SAO-shock. The extract also attenuated the ileum injury (histology) as well as the increase in the tissue levels of MPO ($p<0.01$) and MDA caused by SAO shock in the ileum. The extract markedly reduced the intensity and degree of P-selectin ($p<0.01$) and ICAM 1 ($p<0.01$) in tissue section from SAO-shocked rats. The extract also significantly ($p<0.01$) improved survival [De Paola 2005].

An ethanolic (70%) extract was studied for its effects on hepatic ischaemia-reperfusion (I/R) injury in rats which were subjected to 45 min of hepatic ischaemia followed by 60 min of reperfusion. The extract (50 mg/kg i.p. 15 min prior to hepatic ischemia) significantly (all $p<0.05$) decreased the ALT, AST, LDH and MDA levels, and markedly increased ($p<0.05$) activities of CAT and GPx in tissue homogenates compared to I/R-induced rats without treatment [Bayramoglu 2013].

The effects of montelukast and an unspecified extract against I/R-induced intestinal damage were investigated. Hamsters were divided into 4 groups after midline abdominal laparatomy: control, I/R group, montelukast and I/R (MIR) group, and extract and I/R (HPIR) group. After 60 min of ischaemia through obstruction of the superior mesenteric artery, 24 h of reperfusion was maintained. Ten minutes prior to the reperfusion period, the MIR group received montelukast (7 mg/kg i.p.) and the HPIR group received the extract (7 mg/kg i.p.). Montelukast and the extract significantly reduced MDA levels and increased GSH levels compared to the I/R group ($p<0.008$). A significant difference was also found between the I/R group and MIR and HPIR groups in terms of MPO levels ($p<0.008$). The MIR and HPIR groups showed increased cardiotrophin-1 levels compared to the control and I/R groups ($p<0.008$ for all) and significantly lower histological scores compared to the I/R group ($p=0.03$ and $p=0.007$ respectively) [Ocak 2014].

Albino rats were subjected to 45 min of hepatic ischaemia followed by a 60 min reperfusion period. An ethanolic extract (70%; 50 mg/kg) was injected as a single i.p. dose, 15 min prior to hepatic ischaemia. Biochemical investigations of serum and liver tissue resulted in significantly increased ALT, AST, LDH and MDA levels whereas CAT and GPx were significantly decreased in I/R-induced control compared to normal control ($p<0.05$). Treatment with the extract significantly decreased

the ALT, AST, LDH and MDA levels, and markedly increased activities of CAT and GPx compared to I/R-induced rats (all $p<0.05$) [Bayramoglu 2014].

The effect of an ethanolic (96%) extract on I/R injury of the liver was investigated in male Wistar-albino rats divided into five groups: Sham group, I/R group (who underwent partial liver I/R protocol), carboxymethyl cellulose (CMC) group administered 0.5 % carboxymethyl cellulose for a week before I/R, and the extract group (HP) receiving 0.5 % carboxymethyl cellulose with the extract (400 mg/kg) for a week before I/R. Blood levels of ALT, TNF-α, IL-6 and MDA were significantly ($p<0.05$) lower in the extract group compared with the I/R and CMC groups ($p<0.05$). There was no difference between the liver injury scores of I/R and CMC groups [Aydin 2014].

Nephroprotection
Male Wistar rats were treated with an unspecified extract (400 mg/kg/day p.o.) for 10 consecutive days, and were injected with cisplatin (5 mg/kg i.v.) one day after the final extract administration. Cisplatin treatment increased ($p<0.01$) the serum creatinine level to 1.5 ± 0.2 mg/dL (mean±SE) from 0.3 ± 0.1 mg/dL (control) on day 5 after injection. This increase was improved significantly ($p<0.01$) to 0.9 ± 0.1 mg/dL by pre-treatment with the extract. Cisplatin-induced histological abnormality of the kidney was blocked by the pre-treatment. Extract administration resulted in increases in renal metallothionein (MT) and hepatic multidrug resistance protein 2 (Mrp2) to 164.8 ± 13.0% and 220.8 ± 39.3% of control levels respectively. GSH levels in the kidney and liver were not changed. Total and free cisplatin concentration in serum was not influenced by the extract [Shibayama 2007].

Antiviral effects
A dose-dependent anti-viral effect of hypericin and pseudohypericin against retrovirus infection with Friend leukaemia virus was confirmed in BALB/cJ, BALB/cBy and C57BL/6J mice after a single i.v. dose of up to 150 µg. Both compounds inactivated infectivity and reverse transcriptase activity of intact viruses. Hypericin was more effective than pseudohypericin [Lavie 1989]. In a further experiment the efficacy of both compounds against radiation leukaemia virus was shown [Meruelo 1988].

The efficacy of an unspecified extract (50 to 200 mg/kg b.w. p.o. twice daily for 5 days) against influenza A virus (IAV) was investigated in BALB/c mice. Protection rate, mean survival days, lung index and viral titre, as well as the secretion of IL-6, IL-10, TNF-α and IFN-γ in lung tissue and serum were measured on days 3 and 5 post-infection. The extract dose-dependently reduced the lung index and viral titre ($p<0.05$ to $p<0.01$) of infected mice, decreased mortality, and prolonged the mean survival time ($p<0.01$). It decreased the concentration of IL-6 at the highest dose ($p<0.05$) and TNF-α ($p<0.01$) in lung tissue and serum on day 5 post-infection. The lung and serum levels of IL-l0 ($p<0.01$) and IFN-γ ($p<0.01$) on days 3 and 5 post-infection were enhanced in a dose-dependent manner [Xiuying 2012].

The immune-regulatory effect of an ethanolic (95%) extract was evaluated in A549 lung epithelial cells and BALB/c mice exposed to influenza A/PR/8/34 H1N1 virus. In A549 cells, the extract (30 µg/mL) significantly ($p<0.05$) inhibited influenza virus induced monocyte chemotactic protein (MCP)-1 and IFN-γ induced protein 10 kD (IP-10) ($p<0.05$), but significantly ($p<0.05$) increased IL-6. In mice inoculated intranasally with a high dose of of influenza A/PR/8/34 H1N1 ($10^{7.9}$ EID_{50}), daily oral treatment with the extract at 110 mg/kg increased lung viral titre, bronchoalveolar lavage (BAL), pro-inflammatory cytokine and chemokine levels, and the infiltration of pro-inflammatory cells in the lung 5 days post-inoculation, as compared to ethanol vehicle treated mice. Suppressor of cytokine signalling 3 (SOCS3) transcription was increased by the extract both in A549 cells and BALB/c mice, which could have interrupted the anti-viral immune response and thus led to the inefficient viral clearance and increased lung inflammation. The extract resulted in a minor reduction of viral titre without affecting body weight when mice were inoculated with a lower dose (~$10^{5.0}$ EID_{50}) and the extract was applied in the later phase of infection. Mice challenged intranasally with the high dose of influenza virus suffered from a higher mortality rate when dosed with the extract [Huang 2013].

Immunomodulatory effects
The immumodulatory potential of an ethanolic (95%) extract was investigated in male BALB/c-mice randomly allocated to two groups and immunised with sheep red blood cells (SRBCs) and complete Freund's adjuvant. Mice treated with the extract (110 mg/kg/day i.p.) for the duration of the 2 week study showed a significant ($p<0.001$) increase in the level of anti-SRBC antibody and a significant decrease ($p<0.01$) in the level of cellular immunity (a lower percentage of foot pad thickness) compared to control [Froushani 2015].

Antiproliferative effects
Treatment with hypericin (10 mg/kg i.p.) reduced the growth rate of metastases in breast adenocarcinoma (DA3) in female BALB/c mice and squamous cell carcinoma (SQ2) in male BALB/c mice after resection of primary tumours at stages with presence of lung micro-metastases. Long-term survival in DA3 tumour-excised groups increased ($p<0.001$) from 15.6% in controls to 34.5% after treatment with hypericin (5 mg/kg on day 1, 2.5 mg/kg on day 2 and 1.25 mg/kg on days 3-5 i.p.) prior to surgery. In mice bearing SQ2 tumour metastases, therapy with hypericin increased animal survival from 17.7% in tumour resected controls to 46.1%. Median survival time was prolonged ($p<0.001$) from 52.5 days to 97 days (resected group) and to 190 days in the combination group (surgery + hypericin). The hypericin content in lungs bearing metastases was approximately 2-fold higher than in the lungs of healthy animals, demonstrating the tendency of hypericin to accumulate in primary and metastatic tumours [Blank 2004].

Hyperforin (daily injections of 0.2 µM over 14 days) inhibited the growth of autologous MT-450 breast carcinoma in immunocompetent female Wistar Furth rats to a similar extent as paclitaxel, without any signs of acute toxicity [Schempp 2002a].

Antiangiogenic effects
After s.c. injection with MT-450 mammary carcinoma cells, Wistar rats were treated with peritumoural injections of hyperforin or solvent. Hyperforin (1 – 5 µg/mL) significantly ($p<0.05$) inhibited tumour growth, induced apoptosis ($p<0.05$) of tumour cells and reduced ($p<0.05$) tumour vascularisation [Schempp 2005].

In Wistar albino rats, extensive angiogenesis induced in the cornea and iris by intraocular administration of basic fibroblast growth factor (FGF-2) was effectively inhibited by treatment with hypericin (2 mg/kg i.p.) [Lavie 2005].

An unspecified extract (15 mg/kg/day) or hypericin (15, 45 or 135 µg/kg/day) were given orally to C57BL/6 neonatal mice to investigate retinal neo-vascularisation. Treatment with the extract or hypericin significantly inhibited the degree of retinal neovascularisation ($p<0.05$ to $p<0.001$), but did not affect the area of retinal vasoobliteration. Treatment with the extract or hypericin reduced (both $p<0.05$) phosphorylation of extracellular signal-regulated MAP kinases (ERK) in the retina [Higuchi 2008].

Antioxidant effects
A dry ethanolic extract administered orally to rats at 250 mg/kg/day significantly reduced immobilisation stress-induced lipid peroxidation (measured ex vivo in liver homogenate; 42% protection after 30 days, $p<0.001$) [Tripathi 1999].

The effects of an unspecified extract (0.34% of hypericin, 4.1% of hyperforin, 5% flavonoids, 10% tannins) on the development of zymosan-induced multiple organ dysfunction syndrome were investigated in male CD1 mice. Treatment with the extract (30 mg/kg i.p.), 1 and 6 h after zymosan (500 mg/kg i.p.), attenuated the zymosan-induced peritoneal exudation and migration of polymorphonuclear cells, pulmonary, intestinal and pancreatic injury, and renal dysfunction as well as the increase in MPO in the lung and intestine. Immunohistochemical analysis showed that the increase of iNOS, nitrotyrosine, and poly(ADP-ribose) in lung and intestine tissues from zymosan-injected mice was markedly reduced in mice treated with the extract [Di Paola 2007].

Male albino Wistar rats received pre-treatment with an unspecified extract (4 mg/kg) or quercetin liposomes (25 and 100 mg/kg) 60 min before injection with rotenone (2 mg/kg). All treatments were given i.p. for 45 days. Rotenone increased activities of SOD, GSH GPx and levels of MDA, and decreased the content of reduced GSH ($p<0.05$). Rotenone significantly ($p<0.05$) induced the gene expression of CuZnSOD, MnSOD, CAT and GPx in brain. In contrast, the extract exerted an antioxidant effect which was related to a decrease of MnSOD activity and mRNA levels of some antioxidant enzymes. Treatment with quercetin liposomes resulted in a preservation of the activities of antioxidant enzymes and a decrease ($p<0.05$) in the mRNA levels of these enzymes [Sanchez-Reus 2007].

In a chronic restraint stress model, inflammatory markers, hormonal levels and enzymatic markers of antioxidant capacity such as SOD, GPx and CAT were determined in the hippocampus and hypothalamus of male Sprague-Dawley rats. Oral treatment with an ethanolic (80%, V/V) extract at doses of 125-750 mg/kg resulted in normalisation of the activities of antioxidant enzymes [Grundmann 2010].

The protective properties of an unspecified extract on oxidative stress, apoptosis, and Ca^{2+} entry through transient receptor potential melastatin 2 (TRPM2) and transient receptor potential vanilloid 1 (TRPV1) channels in spinal cord injury (SCI)-induced dorsal root ganglion (DRG) neurons of rats were investigated. Rats received 30 mg/kg p.o. of the extract for three successive days after SCI induction. The SCI-induced TRPM2 and TRPV1 currents and cytosolic free Ca^{2+} concentration were reduced by the extract. The SCI-induced decrease in GPx and cell viability were ameliorated by the extract, as well as the SCI-induced increase in apoptosis, caspase 3, caspase 9, cytosolic ROS production, and mitochondrial membrane depolarisation values in DRG of SCI [Ozdemir 2015].

Photosensitising and photodynamic effects
The time course and skin histopathology of the phototoxic response after a single topical application of hypericin (0.1-1%) and hypericin acetate (0.015-1.5%), with subsequent irradiation (10 J/cm^2, 20 mW/cm^2) were investigated. Methyl-aminolaevulinic acid (Me-ALA) was used as positive control. Application of hypericin on mouse ears resulted in limited phototoxicity, probably due to confined penetration into the epidermal layers. Extended penetration achieved by administration of hypericin acetate evoked a more severe and prolonged response after irradiation, characterised by intense erythema and ear swelling. Skin damage induced by 0.15% hypericin acetate completely recovered without scar formation over 14 days. Hypericin acetate dose-dependently induced equal or more severe phototoxic responses compared with Me-ALA [Boiy 2008].

Pharmacological studies in humans
The effect of a methanolic extract (80%, V/V; 3 × 300 mg/day p.o. for 14 days) on plasma concentrations of testosterone, dihydrotestosterone (DHT), dehydroepiandrosterone sulfate, sex hormone-binding globulin and the combined concentrations of androsterone sulfate (AoS) and epiandrosterone sulfate (epiAoS) was determined in 12 healthy subjects (6 males, 6 females). The extract significantly decreased the combined concentrations of AoS and epiAoS in all subjects ($p=0.02$), and in males ($p=0.04$). The testosterone to DHT ratio was increased without significance in both men and women [Donovan 2005].

A double-blind, randomised, crossover study examined whether a methanolic (80%) extract could reverse low-dose ketamine-induced changes in auditory evoked potentials (AEP) in healthy subjects. Sixteen healthy subjects were given either 2 × 750 mg of the extract or placebo for 1 week. On day 7, a test-battery including AEP, the oculodynamic test (ODT) and a cognitive test were performed before and after an infusion with 4 mg of S-ketamine over a period of 1 hour. S-ketamine led to a significant ($p=0.004$) decrease in the N100-P200 peak to peak (ptp) amplitude after placebo treatment, whereas ptp was significantly increased by S-ketamine infusion in subjects treated with the extract. Cognitive testing and the ODT revealed no significant effect of ketamine-infusion and, therefore, no interaction between the treatments [Murck 2006].

Receptor binding
Patients with major depressive disorder (n=13) were treated twice daily for 6 weeks with either an unspecified extract (600 mg) or the MAO-A inhibitor moclobemide (300 mg). Assessment with [^{11}C]-harmine positron emission tomography found MAO-occupancy of less than 5% for the extract, but 74% for moclobemide [Sacher 2011].

Smoking cessation
In an open, uncontrolled pilot study, 28 smokers of 10 or more cigarettes per day for at least one year were randomised to receive a methanolic (80%) extract at 300 or 600 mg daily for one week before and continuously for 3 months after a target quit date. In addition, all participants received motivational/behavioural support from a trained pharmacist. The point prevalence and continuous abstinence rates were both 18% at 3 months and 0% at 12 months [Barnes 2006].

A methanolic (80%) extract was assessed for its feasibility of use and efficacy in reducing withdrawal symptoms after smoking cessation. A one-arm, uncontrolled study utilised a two-stage group sequential design with a 1-week run-in period between the start of the extract treatment and the designated quit date in smokers (smoking ≥ 10 cigarettes/day). The extract (450 mg) was taken orally twice daily along with cessation counselling messages. Smoking status was determined through self-report and bioverification using carbon monoxide (CO) testing. Among evaluable subjects, the 12-week quit rate was 37.5% (9/24). The extract was generally well tolerated [Lawvere 2006].

In a double-blind study, smokers stopping smoking were randomised to oral treatment with 900 mg methanolic (80%) extract or placebo daily. All participants received weekly behavioural support. The primary endpoints were biochemically confirmed prolonged abstinence and mean weight gain in abstinent smokers 4 weeks after quitting. Six of 71 (8.5%) participants treated with the extract and 9/72 (12.5%) on placebo achieved prolonged abstinence at 4 weeks. At 6 months, 4.2%

of the extract group and 8.3% placebo participants were still abstinent [Parsons 2009].

A standardised hydroalcoholic extract (0.3% hypericin) was given orally to 118 participants at doses of 900 or 1800 mg per day in a randomised, blinded, placebo-controlled, three-arm, dose-ranging clinical trial together with behavioural interventions over 12 weeks. The dropout rate was high (43%). By intention-to-treat analysis, no significant differences were observed in abstinence rates at 12 and 24 weeks between the extract and placebo. The extract neither attenuated withdrawal symptoms in abstinent subjects nor reduced abstinence rates [Sood 2010].

The neurocognitive effects of an ethanolic (50% m/m) extract were compared with nicotine replacement therapy (NRT) and the combination of NRT plus extract during smoking abstinence in 20 smokers aged between 18 and 60 years over a period of 10 weeks during smoking cessation. A spatial working memory (SWM) task was completed at baseline, 4 weeks prior to quitting, as well as at the completion of the study, following the 10 weeks of treatment. Brain activity was recorded during the completion of the SWM task using steady-state probe topography. Reaction time and accuracy on the SWM task were not significantly different between treatment groups at retest. Differences in the steady state visually evoked potentials (SSVEP) treatment profiles were noted: a stronger SSVEP amplitude increase in posterior-parietal regions for the extract and NRT groups, and greater fronto-central SSVEP phase advance in the extract group [Camfield 2013].

Cognitive function
Thirteen healthy volunteers received a methanolic extract (80%, V/V; 900 or 1800 mg/day), AMI (25 mg/day, positive control) or placebo in a double-blind, placebo-controlled study. Cognitive and psychomotor tests, and subjective measures of sedation, were evaluated before and 1, 2 and 4 hours after drug administration. AMI impaired performance on a battery of psychological tests, which included critical flicker fusion, choice reaction time, digit symbol substitution test, profile of mood states and the line analogue rating scale, while the extract did not influence performance in these tests [Timoshanko 2001].

In a randomised, double-blind, crossover study healthy male volunteers received oral doses of either 255-285 mg of an un-specified extract (900 µg hypericin), 25 mg AMI or placebo, three times daily for periods of 14 days each with at least 14 days wash out. Psychometric tests and quantitative EEG (qEEG) were performed before start of medication and on the last treatment day. Neither the extract nor AMI had an influence on cognitive performance such as choice reaction, psychomotor co-ordination, short-term memory and responsiveness to distractive stimuli. AMI, but not the extract, decreased self rated activity ($p<0.05$). Both drugs caused significant qEEG changes. The extract increased theta power density ($p<0.05$). AMI increased theta as well as fast α power density ($p<0.05$) [Siepmann 2002].

Effect on stress hormones
The effects of daily oral administration of a methanolic extract (80%; 300 or 600 mg) or placebo on COR, GH and PRL secretion were examined in twelve healthy subjects. No PRL stimulation was observed. A small but significant ($p<0.05$) elevation in GH AUC values occurred after 300 mg of the extract. After 600 mg of the extract, a distinct COR stimulation was observed ($p<0.05$ to $p<0.01$), occurring from 30 up to 90 min after administration [Schüle 2001].

In a similar study, the effects of oral administration of a methanolic (80%) extract, at doses of 600, 900 and 1200 mg on 4 different days, on the COR, ACTH, GH and PRL secretions were examined in 12 healthy male volunteers. A significant ($p=0.044$) stimulatory effect of the extract on ACTH secretion was observed, whereas COR, GH and PRL secretions were not significantly influenced [Schüle 2004].

In a double-blind, randomised, placebo-controlled, crossover study, 16 healthy subjects (11 men and 5 women) received either an ethanolic extract (60%; 300 mg three times daily) or placebo for 7 days. IMI treatment (50 mg three times daily) in 7 subjects served as a positive control. The extract had no effect on plasma concentrations of NE and its main metabolite, dihydroxyphenylglycol, whereas plasma DOPAC (the main metabolite of DA) concentrations were significantly ($p=0.04$) increased (1661 ± 924 pg/mL) compared to placebo (1110 ± 322 pg/mL) [Schroeder 2004].

The effects of two doses of a methanolic (80%) extract on evening salivary cortisol and NE-mediated melatonin were studied. Twenty healthy male volunteers received either a low (2 x 300 mg/day) or high (2 × 900 mg/day) dose of the extract for 7 days. The extract significantly ($p<0.01$) increased salivary COR in the low dose group but had no discernible effect in the high dose group. Salivary melatonin was not increased in either group [Franklin 2006].

Autonomic nervous function
In a randomised, double-blind, placebo-controlled study in 12 healthy male subjects, the effects of an unspecified extract (standardised to 900 µg hypericin) on the autonomic responses of blood vessels and sweat glands were compared with those of AMI and placebo. Subjects received oral doses of 255 to 285 mg of the extract, 25 mg of AMI and placebo 3 times daily for 14 days each, with at least 14 days washout. The extract affected neither the vasoconstrictory response (VR) of cutaneous blood flow nor the skin conductance response (SR). Whereas AMI led to a reduction of SR and a prolonged dilation phase of VR ($p<0.05$) [Siepmann 2004].

The effects on heart rate variability of the same extract and AMI (at the same dosages) were compared to placebo in 12 healthy male volunteers in a randomised, double-blind, crossover study. The extract did not affect heart rate variability, whereas AMI significantly ($p<0.001$) decreased it [Siepmann 2002].

Skin protection
A cream containing 1.5% w/w of a hyperforin-rich (44.3%) CO_2 extract was tested on 20 volunteers in a randomised, double-blind, vehicle-controlled study. It significantly ($p<0.01$) reduced UVB-induced erythema as compared to vehicle. Occlusive application of cream on non-irradiated test sites did not cause any skin irritation [Meinke 2012].

Topical treatment with a 1.5% hyperforin-rich cream increased the radical protection of the skin during VIS/NIR irradiation in 11 volunteers. After a single application, VIS/NIR-induced radical formation was completely inhibited by both verum and placebo ($p<0.05$). After application for 4 weeks, radical formation was significantly reduced by 45% ($p<0.05$) by placebo and 78% ($p<0.01$) by verum. Skin lipids in both verum and placebo groups increased directly after a single application but only significantly ($p<0.05$ and $p<0.01$) for ceramide [AP], ceramide [NP1] and squalene. After long-term application, concentration of cholesterol and ceramides increased without significance [Arndt 2013; Haag 2013; Haag 2014].

Clinical studies: depressive disorders
The efficacy and safety of standardised hydroalcoholic St. John's Wort extracts have been assessed in 54 controlled, double-blind

studies involving 8,327 patients [Anghelescu 2006; Behnke 2002; Bjerkenstedt 2005; Brenner 2000; Engesser 1996; Fava 2005; Gastpar 2005; Gastpar 2006; Halama 1991; Hänsgen 1996; Häring 1996; Harrer 1994; Harrer 1991; Harrer 1999; 2002; Hoffmann 1979; Hübner 1994; Kalb 2001; Kasper 2006; Kasper 2007a; Kasper 2008; Kugler 1990; Laakmann 1998; Lecrubier 2002; Lehrl 1993; Lenoir 1999; Mannel 2010; Martinez 1994; Montgomery 2000; Moreno 2006; Philipp 1999; Quandt 1993; Randløv 2006; Rapaport 2011; Sarris 2012; Schlich 1987; Schmidt 1989; Schrader 2000; Schrader 1998; Shelton 2001; Singer 2011; Sommer 1994; Szegedi 2005; Uebelhack 2004; van Gurp 2002; Vorbach 1997; Vorbach 1994; Warnecke 1986; Werth 1989; Wheatley 1997; Witte 1995; Woelk 2000a] and 15 open studies, drug monitoring studies and numerous case reports [Albrecht 1994; Brattström 2009; Demling 2004; Findling 2003; Grube 1999; Holsboer-Trachsler 1999; Hübner 2000; Meier 1997; Melzer 2010; Mueller 1998; Rychlik 2001; Schakau 1996; SDSGrube 1996; Sepehrmanesh 1999; Simeon 2005] have involved a further 15,359 patients.

The major indication in most of the studies was mild or mild to moderate depressive disorders. Three studies were designed for treatment of severe depressive disorders [Davidson 2002; Shelton 2001; Vorbach 1997]. A significant improvement in main symptoms (mood, loss of interest and activity) and other symptoms of the depressive syndrome (sleep, concentration, somatic complaints) has been demonstrated in many of these trials. The activity has been studied in comparison to placebo and various antidepressants (AMI, IMI, maprotiline, FLU, sertraline (SERT), citalopram (CIT)). Nine meta-analyses [Kim 1999; Linde 2005a; Linde 2009; Linde 1996; Liu 2013; Liu 2014; Raak 2012; Rahimi 2009; Whiskey 2001] and 18 systematic reviews [Clement 2006; Gaster 2000; Hammerness 2003; Hazell 2009; Henderson 2002; Izzo 2001; Izzo 2009; Knüppel 2004; Linde 1998; Linde 2005b; Linde 2005c; Linde 2009; Linde 2011; Posadzki 2013; Raak 2012; Whiskey 2001; Whitten 2006] of clinical trials with different selection criteria have confirmed the efficacy of various St. John's wort extracts in mild to moderate depression, but not in severe depression.

The studies summarised below are grouped according to the preparations tested. Comparability is assumed for hydroalcoholic dry extracts within the range 60% ethanol to 80% methanol on the basis of their similar active constituent profiles [Gaedcke 2000]. An overview of the controlled studies is given in Table 1.

*Clinical studies and post-marketing surveillance studies in **mild to moderate depression** performed with hydromethanolic extracts (80% methanol)*

The DER of the extract used in the following studies was 3-6:1

In a randomised, placebo-controlled study involving 50 patients suffering from psychovegetative depressive symptoms (ICD-09: neurotic depression or depressive mood of short duration), 3 × 300 mg daily, in some cases reduced to 2 × 300 mg after week 2, of an extract led to a substantial improvement after 4 weeks. A reduction of at least half from their initial HAMD total score or a score below 10 after 4 weeks was achieved by 50% of patients under verum ($p<0.01$), but by none in the placebo group. Other parameters, such as the von Zerssen Complaint Score and CGI score, showed similar differences between verum and placebo [Halama 1991].

In a multicentre, placebo-controlled study, 50 patients (mean age 49 years) with depressive symptoms (ICD-09: 300.4 neurotic depression; or 309.0 brief depressive reaction) were treated daily for 4 weeks with either 3 × 300 mg of an extract or placebo. HAMD scores improved from 23.7 to 17.4 in the verum group and from 21.6 to 16.8 in the placebo group, the difference was not significant. In an evaluation of cognitive performance using the short test for general basic capacities of information processing, patients on verum showed slightly better improvement than those on placebo ($p<0.1$) [Lehrl 1993].

In a multicentre, uncontrolled drug surveillance study, 1060 patients (mean age 51 years) with mild to moderate depressive symptoms were assessed over a 4 week period of treatment with 3 × 300 mg daily of an extract. By the end of the study, the average HAMD score had decreased from 18.4 to 5.4 and the D-S score from 21.1 to 7.3. According to CGI evaluation, 66% of patients had improved and 27% were symptom-free [Albrecht 1994].

In a multicentre, randomised, double-blind study, 105 patients (mean age 45 years) with depressive symptoms (ICD-09: 300.4 or 309.0) were allocated to 3 × 300 mg of an extract, or placebo daily for 4 weeks. By the end of the study initial HAMD scores (15.8 points in both groups) had dropped to 7.2 in the verum group and 11.3 in the placebo group ($p<0.01$). Responder rates, defined by a total endpoint score of less than 10 or a

ABBREVIATIONS used in the summaries of clinical studies

BDI	Beck Depression Inventory [Beck 1972]
D-S	Depression scale (von Zerssen) [von Zerssen 1973]
CGI	Clinical Global Impressions scale [Guy 1967]
DSM-III-R	Diagnostic and Statistical Manual of Mental Disorders, 3rd ed., revised [Ocak 2014]
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th ed. [Martinez 1994]
GAF	Global Assessment of Functioning [Hall 1995]
HAMA	Hamilton Anxiety Scale [Hamilton 1959]
HAMD	Hamilton Depression Scale [Williams 1988]
ICD-09	International Classification of Diseases, Ninth Revision: Mental Disorders [WHO 1975]
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision [Sell 2014; WHO 2010].
ITT	Intention-to-treat
MADRS	Montgomery-Asberg Scale for Depression [Williams 2008]
MDD	Major depression disorder
MRS	Menopause Rating Scale [Greene 2002]
MOS	Medical Outcome Study (older form of SF-36) Functioning scores
PHQ-9	Patient Health Questionnaire [Kroenke 2001]
SF-36	The Short Form (36) Health Survey [Jenkinson 1996]

TABLE 1: OVERVIEW OF CONTROLLED CLINICAL STUDIES

First author Year	Number of patients	Type of preparation Extraction solvent	Daily dosage	Reference therapy	Duration (days)	Mean initial HAMD [2)]	Severity of depression	Superiority ?
Halama 1991	50	Dry extract, 80% methanol	3 × 300 mg [1)]	Placebo	28	18.3	Mild to moderate	Yes
Lehrl 1993	50	Dry extract, 80% methanol	3 × 300 mg [1)]	Placebo	28	22.7	Mild to moderate	No
Sommer 1994	105	Dry extract, 80% methanol	3 × 300 mg[1)]	Placebo	28	15.8	Mild	Yes
Hübner 1994	39	Dry extract, 80% methanol	3 × 300 mg	Placebo	28	12.5	Mild	Yes
Hänsgen 1996	102	Dry extract, 80% methanol	3 × 300 mg	Placebo	42	20.7	Mild to moderate	Yes
Häring 1996	28	Dry extract, 80% methanol	3 × 300 mg	Placebo	Not stated	Not stated	Mild	
Montgomery 2000	248	Dry extract, 80% methanol	3 × 300 mg	Placebo	84			No
Shelton 2001	200	Dry extract, 80% methanol	3-4 × 300 mg	Placebo	56	> 20.0	Severe	No
Kasper 2006	332	Dry extract, 80% methanol	1 × 600 mg 2 × 600 mg	Placebo	42	22.8 22.6	Mild to moderate	Yes Yes
Kasper 2007a	161	Dry extract, 80% methanol	1 × 600 mg 2 × 600 mg	Placebo	120 Relapse	22.5-23.3	Mild to moderate	No
Kasper 2008	425	Dry extract, 80% methanol	3 × 300 mg	Placebo	546	23.8	Mild to moderate	Yes
Kasper 2007b		Dry extract, 80% methanol	600 – 1200 mg	Placebo	217	≤20	Mild	Yes
Mannel 2010	206	Dry extract, 80% methanol	2 × 300 mg	Placebo	56	12.4	Mild	Yes
Harrer 1994	102	Dry extract, 80% methanol	3 × 300 mg	Maprotiline	28	20.5	Mild to moderate	No
Vorbach 1994	135	Dry extract, 80% methanol	3 × 300 mg	Imipramine	42	20.2	Mild to moderate	No
Wheatley 1997	165	Dry extract, 80% methanol	3 × 300 mg	Amitriptyline	42	20.6	Mild to moderate	No
Vorbach 1997	209	Dry extract, 80% methanol	3 × 600 mg	Imipramine	42	25.3	Severe	No
Brenner 2000	30	Dry extract, 80% methanol	2-3 × 300 mg	Sertraline	49	21.3	Mild to moderate	No
HDTSG 2002 Sarris 2012 [Continuation HDTSG 2002]	340 124	Dry extract, 80% methanol	3-5 × 300 mg	Sertraline Placebo	8 26	23.1	Moderate to severe	No No No No
Lecrubier 2002	375	Dry extract, 80% methanol	3 × 300 mg	Placebo	42	24.2	Mild to moderate	Yes
Bjerkenstedt 2005	163	Dry extract, 80% methanol	3 × 300 mg	Fluoxetine Placebo	28	24.9	Mild to moderate	No No
Fava 2005	135	Dry extract, 80% methanol	3 × 300 mg	Fluoxetine; Placebo	84	19.6	Mild to moderate	Yes Yes
Szegedi 2005	251	Dry extract, 80% methanol	3 × 300 mg 3 × 600 mg	Paroxetine	42	25.5	Moderate to severe	Yes
Anghelescu 2006	133	Dry extract, 80% methanol	3 × 300 mg 3 × 600 mg	Paroxetine	145	25.3	Moderate to severe	No
Gastpar 2006 Singer 2011 [extra analysis of Gastpar 2006]	388 154	Dry extract, 80% ethanol	900 mg 900 mg	Citalopram Placebo Citalopram Placebo	42 180	21.9	Mild to moderate	No Yes No No
Uebelhack 2004	140	Dry extract, 80% ethanol	900 mg	Placebo	42	22.8	Mild to moderate	Yes
Laakmann 1998	147	Dry extract, 60% ethanol [WS 5572; 5% hyperforin] [WS 5573; 0.5% hyperforin]	3 × 300 mg	Placebo	42	20.8	Mild to moderate	 Yes No
Philipp 1999	263	Dry extract, 60% ethanol	3 × 350 mg	Imipramine Placebo	56	22.7	Moderate	No Yes
Harrer 1999	149	Dry extract, 60% ethanol	2 × 400 mg	Fluoxetine	42	16.9	Mild	
Lenoir 1999	348	Fresh plant extract 60% ethanol	30, 60, 180 mg	-	42	16.4-16.9	Mild	Yes
Kalb 2001	72	Dry extract, 60% ethanol	3 × 300 mg	Placebo	42	19.9	Mild to moderate	Yes

First author Year	Number of patients	Type of preparation Extraction solvent	Daily dosage	Reference therapy	Duration (days)	Mean initial HAMD [2)]	Severity of depression	Superiority ?
Witte 1995	97	Dry extract, 50% ethanol	2 × 100-120 mg	Placebo	42	24.6	Moderate	Yes
Schrader 1998	162	Dry extract, 50% ethanol	2 × 250 mg	Placebo	42	19.5	Mild to moderate	Yes
Schrader 2000	240	Dry extract, 50% ethanol	2 × 250 mg	Fluoxetine	42	19.6	Mild to moderate	No
Woelk 2000a	324	Dry extract, 50% ethanol	2 × 250 mg	Imipramine	42	22.4	Mild to moderate	No
Gastpar 2005	161	Dry extract, 50% ethanol	615 mg	Sertraline	168	22.0	Mild to moderate	Yes
Behnke 2002	70	Unspecified	2 × 150 mg	Fluoxetine	42	20.0	Mild to moderate	No
Moreno 2006	72	unspecified	900 mg	Fluoxetine Placebo	8	-	Mild to moderate	No No
Randløv 2006	150	unspecified	270 mg	Placebo	6	13.9/14.1	Mild to moderate	No
Rapaport 2011		unspecified	810 mg	Citalopram	84	13.6	Mild (minor depression)	No
van Gurp 2002	87	unspecified	900 to 1800 mg	Sertraline	84	16	Mild to moderate	No
Warnecke 1986	60	Tincture, 50% ethanol	3 × 1.5 ml	Diazepam	94	---	Mild	
Hoffmann 1979	60	Tincture, 50% ethanol	3 × 1.5 ml	Placebo	42	---	Mild	
Schlich 1987	49	Tincture, 49% ethanol	3 × 1 ml	Placebo	28	23.5	Mild	
Schmidt 1989	40	Tincture, 49% ethanol	3 × 1.5 ml	Placebo	28	29.4	Mild to moderate	
Harrer 1991	120	Tincture, 49% ethanol	3 × 1.5 ml	Placebo	42	21.3	Mild to moderate	
Quandt 1993	88	Tincture, 49% ethanol	3 × 1.5 ml	Placebo	28	17.6	Mild	
Werth 1989	30	Tincture, 49% ethanol	3 × 1.5 ml	Imipramine	23	---	Mild	
Kugler 1990	80	Tincture, 49% ethanol	3 × 1.5 ml	Bromazepam	28	---	Mild	
Lenoir 1999	348	Dry ethanolic extract from fresh shoot tips	3 × 60 mg	Different dose levels of same extract	42	16.5	Mild	
Engesser 1996	19	Tea preparation	Not stated	Milfoil tea	14	---	Mild	

[1)] Daily dosage was up to 50% lower due to up to 50% of excipients in the amount stated [Schulz 1998].
[2)] In the St. John's wort group

drop of at least 50% from baseline values, were 67% and 28% respectively [Sommer 1994].

In a placebo-controlled, randomised, double-blind study, 40 patients (mean age 51 years) suffering from mild depression with somatic symptoms (ICD-09: 300.4; 309.0) were treated with 3 × 300 mg of an extract or placebo daily for 4 weeks. Initial mean HAMD scores of 12.5 dropped to 5.5 in the verum group compared to 10.8 in the placebo group ($p<0.05$). Patients in the placebo group had an improved score of 9 after 2 weeks, but this subsequently increased. Responder rates, defined by an endpoint score of < 10 or a drop of at least 50% from baseline value, were 70% and 47% respectively [Hübner 1994].

In a 4-week randomised, double-blind, comparative study, the efficacy and tolerability of 3 × 300 mg daily of an extract was compared with 3 × 75 mg daily of maprotiline in 102 patients (mean age 46 years) with moderate depression (ICD-10: F 32.1). Initial 17-item HAMD scores decreased from 20.5 to 12.2 in the St. John's wort group and from 21.5 to 10.5 in the maprotiline group. Responder rates, defined by a total endpoint score of less than 10 or a drop of at least 50% from baseline values, were 61% and 67% respectively, with no significant differences between groups [Harrer 1994].

In a 6-week multicentre, randomised, double-blind, comparative study, 3 × 300 mg daily of an extract was compared to 3 × 25 mg daily of IMI. The patients (n = 135; mean age 53 years) had diagnoses in accordance with DSM-III-R criteria of depression with a single episode (296.2), several episodes (296.3), depressive neurosis (300.4) or adjustment disorder with depressed mood (309.0). Initial HAMD scores declined from 20.2 to 8.8 in the extract group and from 19.4 to 10.7 points in the IMI group. Group comparisons showed no significant differences. Analysis of von Zerssen depression scale (D-S) and CGI patterns in the two groups also revealed comparable results. Subgroups with initial HAMD scores of > 21 performed significantly better ($p<0.05$) with the extract (n = 26) than IMI (n = 25) with regard to HAMD score and CGI [Vorbach 1994].

In a multicentre, uncontrolled drug surveillance study, 3250 patients (mean age 51 years), of whom 49% had mild, 46% moderate and 3% severe depressive symptoms, were treated for 4 weeks with 3 × 300 mg daily of an extract. D-S scores dropped from 23.2 to 11.8 over the 4 week period. At the end of treatment, 82% of patients were assessed as improved or symptom-free by the physicians and 79% by self assessment. The therapeutic effect was slightly better in patients less than 50 years old. Cases of mild or moderate depression responded to treatment to the same extent, while severe cases improved less [Woelk 1994].

In an open, randomised, single-blind study involving patients (mean age 46 years) suffering from seasonal affective disorder

(major depression with a seasonal pattern in accordance with DSM-III-R), daily treatment for 4 weeks with 3 × 300 mg of an extract was combined with phototherapy: either bright light (3000 lux; n = 10) or dim light (< 300 lux, n = 10) for 2 hours per day. Average HAMD scores at baseline decreased significantly in both groups ($p<0.001$) [Martinez 1994].

In 102 outpatients (mean age 52 years) with depression of mild or moderate severity (DSM-III-R: 296.2, 296.3), daily treat ment with 3 × 300 mg of an extract for 4 weeks was compared to placebo. Average HAMD scores at baseline were 21 in the verum group and 20.4 in the placebo group. After 4 weeks the average score was significantly lower ($p<0.001$) in the verum group (8.9) than in the placebo group (14.4). Similar results were obtained on the von Zerssen depression scale (D-S) ($p<0.001$). Responder rates were 70% under verum and 24% under placebo ($p<0.001$). The randomised, double-blind period was followed by a further 2-week period when all patients were openly treated with the extract; HAMD scores decreased further, to 6 in the former verum group and more substantially to 8.7 in the former placebo group [Hänsgen 1996].

In a prospective, placebo-controlled, double-blind pilot study, 28 outpatients undergoing chemotherapy for solid tumours were randomly assigned to 3 × 300 mg of an extract or placebo daily for 2-3 chemotherapy treatment cycles. Although the average HAMD score in the verum group showed a slight trend towards improvement, results from the two treatment groups were comparable with respect to a quality of life analysis, the D-S scale and the CGI scale [Häring 1996].

In a 5-week drug monitoring study, the efficacy of an extract (3 × 135-225 mg providing 900 µg of total hypericin daily) was assessed in 114 patients (mean age 48 years) with mild depressive symptoms. The treatment was evaluated by the D-S scale, the distribution of characteristic psychic and somatic symptoms and a global rating by the physician. From an initial score of 21 the D-S score dropped to 15 after 2 weeks and to 2 after 5 weeks. The physicians rated 39% of patients as improved and 35% as symptom-free after the 5 weeks [Grube 1996].

In a 12-week uncontrolled drug-monitoring study, the efficacy of an extract (3 × 135-225 mg, providing 900 µg of total hypericin daily) was assessed in 111 women (mean age 52 years) with menopausal symptoms. Treatment was evaluated by the menopause rating scale (MRS), a questionnaire for assessing sexuality and the CGI scale. Menopausal complaints diminished or disappeared completely in 79% of the women as rated by the physicians (CGI rating better or symptom-free). Sexual well-being was favourably affected in approximately 60%. The average MRS total score decreased from an initial 63.4 (corresponding to a marked intensity of symptoms) to 23.5 (slight intensity) after 12 weeks [Grube 1999].

In an uncontrolled drug-surveillance study, 647 patients with mild to moderate depression were treated daily for 6 weeks with 3 × 300 mg of an extract. Improvement was shown in 75% of the patients. The D-S score dropped from 19.8-21.2 at baseline to 8.1-9.3 after 6 weeks. Patients older than 65 years improved at a slightly slower rate but the severity of depression did not appear to affect the outcome [Holsboer-Trachsler 1999].

In a 6-week multicentre, randomised, double-blind, comparative study, 165 mildly to moderately depressed patients (mean age 40 years) diagnosed in accordance with DSM-IV were treated with 3 × 300 mg daily of an extract or 3 × 25 mg daily of amitriptyline. Initial 17-item HAMD scores were between 17 and 24 (mean 20.7). No significant difference between groups was observed with respect to the response rate (defined as an endpoint HAMD score of less than 10 or a drop of at least 50% from baseline). HAMD scores dropped to 10 in the extract group and to 6 in the amitriptyline group after 6 weeks, a difference significantly in favour of amitriptyline ($p<0.05$). Analysis of the Montgomery-Asberg rating scale for depression (MADRS) also favoured amitriptyline ($p<0.05$) [Wheatley 1997].

In a 6-week multicentre, randomised, double-blind comparative trial, 209 patients (mean age 49 years) were allocated to 3 × 600 mg daily of an extract or 3 × 50 mg daily of IMI. The primary target parameter, mean 17-item HAMD scores, dropped from 25.3 to 14.4 points in the extract group and from 26.1 to 13.4 points in the IMI group, significantly in favour of IMI ($p=0.021$). Equivalence of the two treatments was shown with respect to response rates, defined as the percentage of patients showing a reduction of at least 50% in HAMD total score over the study period: 35.3% in the extract group and 41.2% in the IMI group [Vorbach 1997].

In a hospital-based, double-blind, randomised study, 30 outpatients (18 to 65 years) with an initial 17-item HAMD score ≥17 and a DSM-IV diagnosis of major depressive disorder (single or recurrent episodes), dysthymic disorder, adjustment disorder with depressed mood, or depressive disorder not otherwise specified, were given daily doses of either 2 × 300 mg of an extract or 50 mg SERT. After one week, the extract dose was increased to 3 × 300 mg and SERT to 75 mg daily for a further 6 weeks. Mean HAMD and CGI scores significantly improved during both treatments ($p<0.01$), and no significant differences were observed between the treatments. Comparable clinical response (≥ 50% reduction in HAMD scores) was obtained in 47% of patients receiving the extract and 40% receiving SERT [Brenner 2000].

Adult outpatients (n = 200; mean age 42 years) suffering from recurrent depression (64%) or a single episode of depression (35%) or melancholia (40%) with a DSM-IV diagnosis of major depression and an initial HAMD total score (17-item scale) of at least 20 participated in a randomised, double-blind, placebo-controlled study. The duration of the current major depressive disorder episode was more than 2 years on average and onset of the initial major depressive disorder was more than 10 years ago. After a 1-week single-blind run-in with placebo, patients were treated for 8 weeks with either an extract, 3 × 300 mg daily for 4 weeks, increased thereafter to 4 × 300 mg daily in the absence of adequate response, or placebo. The primary outcome measure (rate of change in HAMD score over the treatment period) was significantly decreased by both treatments ($p<0.001$), with no differences between treatments ($p=0.16$). Similar results were obtained for the secondary outcome measures, HAMA and CGI. 26.5% of patients in the verum group and 18.6% in the placebo group were responders. A significantly higher ($p=0.02$) remission rate (defined as an endpoint HAMD score of 7 or less and CGI-I score of 1 or 2) was determined for the extract (14.3%) compared to placebo (4.9%) [Shelton 2001].

In a multicentre, randomised, placebo-controlled, double-blind, three-armed study, 340 patients (mean age 42 years) with major depression of moderate severity (according to Clinical Interviews for Axis I DSM-IV disorders, initial 17-item HAMD score ≥ 20 and a Global Assessment of Functioning (GAF) score ≤60) were assigned to one of three treatments daily for 8 weeks: 3 × 300 mg of an extract (standardised to 0.12-0.28% hypericin and 3.1% of hyperforin), 50 mg of SERT (divided into 3 doses) or placebo. Responders at week 8 could continue blinded treatment for another 18 weeks. Daily dosage was increased after week 3 to 1500 mg of the extract or to 100 mg of SERT if warranted; the mean highest daily doses prescribed during the 8-week period were 1299 mg of the extract, 75 mg of SERT or

placebo equivalent. With respect to the change from baseline HAMD score and full response rates, defined as an endpoint HAMD score of 8 or less and a CGI-I score of 1 or 2, neither the extract nor SERT were significantly different to placebo. Mean reductions in HAMD scores were 8.7 for the extract, 10.5 for SERT and 9.2 for placebo. Full response rates were 23.9% for the extract, 24.8% for SERT and 31.9% for placebo [HDTSG 2002].

Continuation data from the above study was used to analyse the 124 participants (responders) that continued treatment with an extract (900 – 1500 mg), SERT (50 – 100 mg) or placebo from week 8 until week 26. At week 26, the primary outcome HAMD scores were: extract (6.6 ± 4.5), SERT (7.1 ± 5.4) and placebo (5.7 ± 5.4), with a significant effect for time (p=0.036). Differences between all treatments were not significant (p=0.61). The effects on BDI, CGI-severity, CGI-improvement, and on intention-to-treat analyses were also non-significant [Sarris 2012].

In a double-blind, randomised, placebo-controlled study, 163 out-patients (mean age 50.3 years) with major depressive disorder (MDD) received, after a 3 to 7 day placebo run-in period, one of the following treatments for 4 weeks: 3 × 300 mg daily of an extract, 20 mg daily fluoxetine or placebo. Patients had a current major depressive episode with mild to moderate intensity and a minimal total score of 21 on the 21-item HAMD score, as well as a history of at least two episodes of non-psychotic MDD. During the four weeks treatment, a reduction in HAMD total score was observed from 24.9 to 15.0 in the extract group, from 23.8 to 14.9 in the fluoxetine group and from 25.2 to 9.7 in the placebo group. There was no significant difference for HAMD and treatment response between the treatments. However, with regard to remission, the extract (24%; p=0.02) and fluoxetine (28%; p=0.005) were significantly superior to placebo (7%). Almost identical results for the three groups were attained with the MADRS and CGI [Bjerkenstedt 2005].

In a double-blind, randomised, placebo-controlled study, 135 out-patients (mean age 37.3 years) with MDD received one of the following treatments for 12 weeks after a 1 week placebo run-in period: 3 × 300 mg daily of an extract, 20 mg daily fluoxetine (FLU) or placebo. Patients had a current MDD episode according to DSM-IV of at least 2 weeks duration and a total 17-item HAMD score ≥ 16. In comparison to baseline, treatment with the extract was associated with a significantly ($p<0.05$) greater decrease in HAMD scores compared with fluoxetine at all post-baseline visits, except visit 5 (week 8). There was no significantly greater reduction in HAMD score in the extract and FLU groups when compared with placebo and non-significantly higher rates of remission (HAMD < 8) in the extract group (38%) compared to FLU (30%) or placebo (21%). No significant differences were observed for CGI-S, CGI-I and BDI score between the treatments [Fava 2005].

In an 8-week double-blind, placebo-controlled, randomised trial, 600 mg daily of an extract or placebo were given to patients with vegetative features of atypical depression, e.g. hyperphagia or hypersomnia. One-hundred patients with a mild- and another 100 patients with a moderate severity major depressive episode according to ICD 10, who met a score of 2 in at least one of the items 22–26 of the 28-item HAMD scale, and had an episode duration of at least 3 months, were randomized for treatment. The absolute reduction of the 17-item HAMD was significantly ($p<0.05$) greater for the extract than placebo. No significant benefit was observed for the sum score of the atypical vegetative items of the 28-item HAMD; however, the sum score of the hypersomnia items showed a significant superiority for the extract. The HAMA, PHQ-9, and CGI-I scales demonstrated superiority of the extract ($p<0.01$) [Mannel 2010].

The DER of the extract used in the following studies was 3-7:1

In a double-blind, randomised, placebo-controlled study, 375 out-patients (aged 18 to 25 years) after a one-week placebo run-in period received 3 × 300 mg daily of an extract (standardised to 3-6% hyperforin and 0.12-0.28% hypericin) or placebo for 6 weeks. Patients had a current major depressive episode of at least 2 weeks duration according to DSM-IV (mild or moderate depression, single or recurrent episode), and a total score on the 17-item HAMD score between 18 and 25 and a score on item 1 ("depressed mood") of 2 or higher at screening and baseline. The HAMD score changed from 21.9 to 12.0 in the verum group and from 21.9 to 13.8 in the placebo group (p=0.03). Significantly (p=0.03) more patients had a treatment response or remission in the verum group (52.7%) compared to placebo (42.3%). There was a trend towards greater reduction in the MADRS score for the verum group (p=0.06). The depression sub-score of SCL-58 was not significantly different between treatments [Lecrubier 2002].

In a double-blind, randomised, active-controlled, double-dummy, non-inferiority study, 251 out-patients (mean age 47.3 years) with MDD received one of the following treatments over 6 weeks: 3 × 300 mg daily of an extract (standardised to 3-6% hyperforin and 0.12-0.28% hypericin) or 20 mg daily PAR. Patients had single or recurrent moderate or severe episodes of MDD according to DSM-IV persisting between two weeks and one year. At screening and baseline all participants had to have a total score ≥ 22 points on the 17-item HAMD scale and ≥ 2 points for the item "depressive mood". HAMD decreased from 25.5 to 11.1 in the extract group and from 25.5 to 14.1 in the PAR group, demonstrating that the extract was as effective as PAR. At the end of the treatment, 71% of patients in the extract group and 60% in the PAR group had responded to treatment (p=0.08), and 50% and 35% showed remission (p=0.02) respectively. Patients in the St. John's wort group showed a significantly greater decrease of the MADRS (p=0.01) and BDI scores (p=0.01). Item 1 of the CGI score was signficiantly (p=0.02) improved in ≥ 2 categories in the extract group [Szegedi 2005].

In a multicentre, randomised, double-blind, active controlled study, 133 patients received after a 3-7 days run-in phase, 2 × 300 mg daily of an extract or 20 mg daily PAR for 6 weeks. Patients suffered from single or recurrent episodes of moderate or severe intensity MDD, without psychotic features, according to DSM-IV with a duration of at least two weeks and not more than 1 year. A total score of at least 22 points on the 17-item HAMD scale and an item score of at least 2 for 'depressive mood' were required for inclusion. Patients with a HAMD total score decrease of ≥ 50% during the 6 weeks of acute treatment were asked to continue the treatment for another 16 weeks to prevent relapse. Patients who received the extract in the acute phase were randomised to maintenance doses of either 900 (n = 33) or 1800 mg of the extract daily (n = 38). Patients who received PAR in the acute phase were randomised to maintenance doses of either 20 (n = 28) or 40 mg daily (n = 34) of PAR. Between baseline of the acute phase and end of continuation treatment the total HAMD score decreased from 25.3 ± 2.5 (mean ± SD) to 4.3 ± 6.2 points for the extract and from 25.3 ± 2.6 to 5.2 ± 5.5 points for PAR (p=0.49). Remission (total HAMD score < 8) occurred in 81.6% of the patients in the extract group and in 71.4% of the PAR group (p=0.29). Three patients relapsed (HAM-D increase > 5 points during continuation treatment) in the extract group and two patients in the PAR group. None of the secondary parameters showed any significant difference between the treatments [Anghelescu 2006].

In a double-blind, randomised, placebo-controlled, multicentre study an extract at two doses (600 mg/day; 1200 mg/day) or

placebo were given for 6 weeks to 332 patients with mild to moderate, single or recurrent, major depressive episodes as defined by the DSM-IV. Average total HAMD scores decreased in all three groups between baseline and day 42 ($p<0.001$ each). Both verum groups showed a significantly ($p<0.001$) greater decrease than placebo, from 22.8 to 11.2 (600 mg/day) and from 22.6 to 11.8 (1200 mg/day) versus 23.6 to 17.6 (placebo). CGI, MADRS, SF-36 and global self-rating scores improved significantly ($p<0.001$) for both verum groups compared to placebo [Kasper 2006].

In a double-blind, randomised, placebo-controlled study, relapse of depressive symptoms following 6-weeks single blind treatment with an extract was studied in 426 patients (18–65 years old) suffering from a recurrent episode of major depression (ICD-10 F33.0 or F33.1, and DSM-IV 296.3), and with a history of 2 or 3 previous episodes according to ICD-10 and DSM-IV criteria. After a 1 week single-blind placebo run-in period, the 6 weeks single blind acute treatmen took place. Patients classified as responders to acute treatment were randomised (2:1) to continue in a double-blinded manner with either 3 × 300 mg extract or placebo for 26 weeks. Patients who did not relapse during continuation treatment were entered into a 52-week double-blind maintenance treatment phase. Patients under placebo continued on the same regimen, those treated with the extract were re-randomised at a ratio of 1:1 to maintenance treatment with either 3 × 300 mg/day extract or placebo. Relapse was defined as a 17-item HAMD score ≥ 16, or clinical diagnosis of a depressive episode according to ICD-10 criteria, or premature treatment termination related to lack of efficacy. In the intention-to-treat (ITT) analysis, 51 of 282 (18.1%) patients on verum and 37 of 144 (25.7%) on placebo relapsed ($p=0.035$). The mean time until relapse was 177 days (verum) and 163 days (placebo), $p=0.034$. Compared to baseline, HAMD scores were decreased after the 6 week acute treatment phase from 23.8 to 8.6 in the group randomised to receive verum continuation treatment and from 23.6 to 8.6 in the group randomised to receive placebo. In the continuation treatment period, verum signficantly ($p=0.04$) reduced the HAMD score by a further 1.3, compared to 0.1 with placebo. CGI improved significantly ($p=0.01$) during verum continuation treatment, whereas BDI was comparable [Kasper 2008].

The DER of the extract used in the following studies was 4-7:1

In an uncontrolled, multicentre, drug monitoring study involving 1606 patients (mean age 52 years) suffering from typical depressive symptoms such as low mood, disturbed sleep, loss of interest and energy, more than 90% were treated 2-3 times daily with 300 mg of an extract (standardised to 900 µg of total hypericin). After a mean treatment period of 5 weeks, the intensity of dominating symptoms was markedly reduced. The efficacy was rated as very good or good by 81% of the physicians and 76% of patients. No age-dependent correlation of efficacy was reported [Sepehrmanesh 1999].

In an uncontrolled, multicentre, drug monitoring study, 313 patients received an extract at a dose of 3 × 300 mg daily for 12 months. Patients were suffering from mild to moderate MDD (DSM-IV 296.2 and 296.3), dysthymic disturbances (300.4) or adaptation disturbances (309.0) and had a baseline 17-item HAMD score between 12 and 22, as well as a CGI score of 4 to 6. At the end of treatment, the HAMD score decreased from 17.4 to 7.1 ($p<0.001$). Responder rate (HAMD score < 10 and HAMD reduction of at least 50%) was 68.7% [Hübner 2000].

In an uncontrolled study an extract was given for 4 to 6 weeks to paediatric patients (n = 101; 1 to 12 years) with symptoms of depression and psychovegetative disturbances. Doses were determined individually by the responsible physician. Relief of symptoms, tolerability and satisfaction with the therapy was assessed by physicians and parents by questionnaires. Physicians rating of effectiveness as 'good' or 'excellent' was 72% after 2 weeks, 97% after 4 weeks and 100% after 6 weeks. The ratings by parents were very similar [Hübner 2001].

*Clinical studies and post-marketing surveillance studies in **mild to moderate depression** performed with hydroethanolic extracts (ethanol 80%)*

The DER of the extract used in the following studies was 3-6:1

In a post-marketing surveillance study in 4188 patients suffering from psychovegetative disorders, depressive states, anxiety and/or nervous unrest, the effect of an extract (900 mg per day p.o.) was studied over 12 weeks. The HAMD score decreased from an average of 15.8 to 9.5 after 4 weeks and finally to 4.6. The percentage of responders (decrease in HAMD-score ≥ 50%) was about 78%. In the subgroup of patients with a HAMD-baseline score of ≥ 17 the percentage of responders was about 77%; of this group, two-thirds of the patients older than 65 years and two-thirds of those with an illness duration > 1 year experienced an improvement. The efficacy of the extract was rated "very good" or "good" by 81.1% of the doctors and 76.6% of the patients. The tolerability was rated "very good" or "good" by 95.6% of the doctors and 92.4% of the patients [Demling 2004].

In a double-blind, randomised, placebo-controlled, prospective study, an extract at a dose of 900 mg daily was compared to placebo. Out-patients (n=140) suffering from moderate MDD according to ICD-10 (F32.1 or F33.1) and DSM-IV with a total 17-item HAMD score of 20 to 24 were included. Following a single-blind placebo run-in period of 7 days, the patients were randomised to the extract or placebo for 6 weeks. While the total HAMD score decreased in both groups compared to baseline, from 22.8±1.1 to 11.8±4.4 in the verum group and from 22.6±1.2 to 19.2±3.8 in the placebo group, there was a significantly greater reduction in the verum group ($p<0.001$). Comparable differences in favour of the extract were revealed by the D-S and CGI scales and a global efficacy assessment [Uebelhack 2004].

Patients (n=388) suffering from a first episode or recurrent MDD defined by ICD-10 (F32.1 or F33.1) and DSM-IV (major depressive (296.2 x) and recurrent major depression (296.3 x) with a total 17-item HAMD score of 20-24 received 900 mg extract or 20 mg CIT or placebo daily for 6 weeks. From baseline values of 21.9 ± 1.2 points (extract), 21.8 ± 1.2 points (CIT) and 22.0 ± 1.2 points (placebo), the total HAMD scores were reduced to 10.3 ± 6.4 (extract), 10.3 ± 6.4 (CIT) and 13.0 ± 6.9 (placebo). A therapeutic equivalence of the extract to CIT and superiority over placebo ($p<0.0001$) were demonstrated. At the end of treatment, 54.2% (extract, $p<0.003$ vs. placebo), 55.9% (CIT, $p<0.001$ vs. placebo) and 39.2% (placebo) of the patients were assessed as therapy responders (HAMD ≤10). No significant difference in the responder rate was observed between both active treatments. Regarding changes in D-S and CGI scales, the extract was not significantly different from CIT, but was significantly ($p<0.001$) superior to placebo [Gastpar 2006].

In the above study, 188 patients were classified as responders to treatment, as defined by a final HAMD score of ≤10 at the end of the trial. Duration of response and occurrence of relapse/recurrence was measured in 154 patients by re-analysing data from the clinical trial. In total, 30 (19.5%) of the responders were diagnosed with a relapse. The number of patients exhibiting relapse was the highest in the CIT group (14 of 54), whereas

patients who were treated with the extract showed the lowest relapse rate (8/54); patients from the placebo group showed a relapse rate of 8/46. No difference in the severity of relapse was observed. The duration of response was longest for the extract group (1817 days), intermediate for the CIT group (1755 days) and shortest for the placebo group (802 days) [Singer 2011].

A controlled observational study with a follow-up duration of six months involved 514 patients with moderate depression (intention-to-treat group). Of the included patients, 362 (70.4%) received an extract and 152 (29.6%) were treated with an SSRI, in most cases CIT. Outcome measures were efficacy and tolerability of treatment under day-to-day conditions. Efficacy was comparable between the groups, assessed by physicians as "good" to "very good" in 86.1% of extract group patients and 87.9% of SSRI group patients. The extract was significantly ($p<0.001$) better tolerated than the SSRIs, with tolerability judged as "very good" to "good" in 97.0% of extract patients compared to 82.1% of SSRI patients [Kresimon 2012].

Clinical studies and post-marketing surveillance studies in ***mild to moderate depression*** *performed with hydroethanolic extracts (ethanol 60%)*

The DER of the extract used in the following studies was 5-7:1

In an uncontrolled drug surveillance study, 2404 patients (mean age 50 years) with depressive symptoms of varying severity were treated with an extract at an average dose of 120-180 mg twice daily for an average treatment period of 5 weeks. Typical symptoms of depression decreased in both frequency and intensity, the response rates were 73% for depressed mood, 81% for loss of interest, 66% for reduced self-esteem and self-confidence, 69% for lack of concentration and 68% for anxiety. Overall, 90% of patients responded to treatment and more than 50% noticed an improvement in symptoms after 2 weeks of therapy. Using the CGI scale, efficacy was rated as good or very good in 77% of patients (about 80% for patients under 54 years, decreasing to 73% with older patients). A correlation was evident between severity of condition and response rates (82% for mild, 79% for moderate, 64% for marked severity) [Schakau 1996].

In a multicentre, randomised, double-blind, three-armed study, 263 patients (mean age 47 years) with moderate depression (ICD-10: F32.1 and F33.1), and a mean initial total 17-item HAMD score of 22.6 points, were allocated daily treatment for 8 weeks with either 3 × 350 mg of an extract (0.2-0.3% total hypericin, 2-3% hyperforin), 100 mg of IMI (50 mg + 25 mg + 25 mg) or placebo. The extract reduced HAMD scores more effectively than placebo (mean decrease after 6 weeks of 13.4 versus 10.3). Mean decreases from baseline at 8 weeks were similar for the extract and IMI (15.4 versus 14.2). More patients receiving the extract had ≥ 50% improvement in HAMD scores than patients receiving placebo ($p=0.027$); whereas the proportions did not differ between the extract and IMI groups ($p=0.14$). Comparable results were found for HAMA and CGI scores, and were most pronounced for the Zung self-rating depression score. Quality of life, as measured by the SF-36 standardised mental component scale, was improved to a greater extent by both active treatments than by placebo (40.5% by the extract, 30% by IMI). Using the SF-36 physical component scale, compared with placebo quality of life was markedly improved only by the extract (74.5% as compared to 23% by IMI) [Philipp 1999].

The effects of 2 × 400 mg of an extract or 2 × 10 mg of FLU daily were compared in a 6 week randomised, double-blind, comparative trial in 149 elderly outpatients (mean age 69 years) suffering from a first episode of mild or moderate depression (ICD-10: F32.0 or F32.1). During treatment, average HAMD scores decreased from 16.6 to 7.9 in the extract group and from 17.2 to 8.1 in the FLU group. The efficacy of the two medications was found to be equivalent in both mild and moderate depressive episodes. Responder rates (total HAMD score < 10 or a reduction of at least 50% from initial score) were 71% in the extract group and 72% in the fluoxetine group [Harrer 1999].

In a further uncontrolled drug monitoring study, 607 patients (mean age 50.5 years) with depressive symptoms were treated once or twice daily for 6 weeks with 425 mg of an extract. The initial mean 17-item HAMD score of 19.2 decreased to 7.4 points by the end of the treatment. Similar results were obtained for the D-S scale score decreasing from 22.2 to 8.9 points. Core symptoms such as low mood, lack of energy and concentration improved by 50% [Mueller 1998].

The DER of the extract used in the following studies was 2.5-5:1.

In a multicentre, randomised, double-blind, placebo-controlled study, 147 patients (mean age 49 years) suffering from mild or moderate depression in accordance with DSM-IV criteria, and with initial total 17-item HAMD scores ≥ 17, were treated daily for 6 weeks with placebo or 3 × 300 mg of one of two extracts (5% hyperforin or 0.5% hyperforin). The average HAMD score at baseline was 20.8. At the end of the study, the group receiving the extract with 5% hyperforin had the largest reduction in HAMD score from baseline (10.3 ± 4.6), followed by the group with the lower content of hyperforin (8.5 ± 6.1) and the placebo group (7.9 ± 5.2). The proportions of responders were 49% in the 5% hyperforin group, 39% in the 0.5% hyperforin group and 33% in the placebo group. HAMD score reduction was significantly superior to placebo only in the 5% hyperforin group ($p=0.004$). Results were similar for other outcome measures (CGI and D-S depression scales) [Laakmann 1998].

In a multicentre, randomised, double-blind, placebo-controlled study, 72 patients with mild to moderate MDD (in accordance with DSM-IV) were given either a daily dose of 3 × 300 mg of an extract (5% hyperforin) or placebo for 6 weeks. Group differences in favour of the verum were significant after 4 weeks ($p=0.011$) and 6 weeks ($p<0.001$). Average 17-item HAMD scores decreased from 19.7 to 8.9 after verum and from 20.1 to 14.4 after placebo. Analysis of responders showed a reduction of at least 50% from HAMD baseline scores in 62.2% of the verum group and 42.9% of the placebo group ($p=0.10$). The difference was larger when '60%-responders' were considered (51.4% vs. 17.1%) [Kalb 2001].

In a multicentre, observational study, the effectiveness of an extract was examined in 2166 patients (average age 50 years) suffering from mild to moderate depression. Most of the patients were suffering from a first episode, with one third suffering from recurrent depression. Initially, 1385 patients took 600 mg and 781 patients 1200 mg of the extract daily. Moderate depression at baseline (based on CGI) was reduced to less than "mild" after an average observation time of seven weeks in 83.7% of patients at 600 mg and 88.6% at 1200 mg. The improvement in symptoms as measured by 17-item HAMD scores was of clinical relevance [Rychlik 2001].

The DER of the extract used in the following studies was 3.5-6:1

In an observational study, 1778 patients with depressive symptoms received an extract (425 – 1700 mg) for 12 weeks. At inclusion, the mean daily dose was 822.5 mg and at the last visit 754.4 mg. At the last visit, the ICD-10 sum score had

decreased by 63.1% and the proportion of patients described as 'normal to mildly ill' (GCI-S) had increased from 21.6% to 72.4%. Regarding the GCI-I, 77% of the patients had improved 'very much' or 'much', consistent with their self-assessment (76%). Lower age and shorter duration of the disorder were associated with significantly better outcomes [Melzer 2010].

Clinical studies and post-marketing surveillance studies in ***mild to moderate depression*** *performed with hydroethanolic extracts (50% ethanol)*

The DER of the extract used in the following studies was 5-8:1

In a randomised, double-blind, multicentre trial, 97 patients (mean age 43 years) with a moderate depressive episode (ICD-10, F32.1) received 2 × 100-120 mg of an extract or placebo daily for 6 weeks. Initial HAMD scores dropped from 24.6 to 7.9 points in the verum group and from 22.6 to 10.3 in the placebo group (p=0.019). Responder rates (total score at the endpoint < 10 or decrease of at least 50% from initial score) were 79% and 56% respectively [Witte 1995].

The DER of the extract used in the following studies was 4-7:1

In an open, uncontrolled, multicentre drug surveillance study, 170 patients (mean age 49 years) with masked, mild, moderately severe or severe depression received 2 × 250 mg of an extract (0.2% total hypericin) daily for an average period of 66 days. HAMD scores were evaluated in 84 patients. Treatment response was satisfactory in patients with masked, mild and moderate depression, but not in those with severe depression. Typical depressive symptoms decreased by 40% in the first group but only by 12.5% in the subgroup with severe depression. In the group of patients whose HAMD scores were evaluated, the mean initial score of 36.3 dropped to 27.2 by the end of treatment. In a further subgroup analysis, severe cases did not significantly benefit from the treatment (p=0.46). Global efficacy, assessed in 94 of the patients, was rated as good or very good in 78%, slight in 3% and insufficient in 19% [Meier 1997].

In a randomised, double-blind, multicentre trial with 162 patients (mean age 43 years) suffering from mild to moderate depression (ICD-10: F32.0, F32.1) the treatment was either 2 × 250 mg of an extract (0.2% total hypericin) or placebo daily for 6 weeks. Over the course of the study 21-item HAMD scores dropped from 20.1 to 10.5 in the verum group and from 18.8 to 17.9 in the placebo group (p<0.001). Responder rates (defined as reduction in HAMD ≥ 50% and/or actual HAMD score of ≤10) were 56% and 15% respectively [Schrader 1998].

In another randomised, double-blind, multicentre study, involving 240 patients (mean age 47 years) with mild to moderate depression (ICD-10: F32.0, F32.1) and 21-item HAMD scores of 16 to 24 (mean initial score 19.6), the treatment was either 2 × 250 mg of an extract (0.2% total hypericin) or 1 × 20 mg of FLU daily for 6 weeks. After 6 weeks, HAMD scores dropped significantly from 19.65 to 11.5 in the extract group compared to 19.5 to 12.2 in the FLU group (p<0.09). The extract was superior to fluoxetine with respect to responder rates (defined as reduction in HAMD ≥ 50% and/or actual HAMD score of ≤ 10) (p=0.005; 60% for the extract and 40% for fluoxetine) and CGI item 1 score (p<0.03) [Schrader 2000].

In a randomised, double-blind, multicentre study, 324 outpatients (mean age 46 years) with mild to moderate depression (ICD-10: F32.0, F32.1, F33.0, F33.1) were treated daily for 6 weeks with either 2 × 250 mg of an extract (0.2% total hypericins) or 2 × 75 mg of IMI. The 17-item HAMD scores decreased from 22.4 to 12.0 in the extract group and from 22.1 to 12.75 in the IMI group. None of the differences between treatment groups reached significance, except for a difference in HAMD anxiety-somatisation subscale scores in favour of the extract (p=0.03). Responder rates, defined by a drop of at least 50% of the baseline HAMD values, were 43% in the extract group and 40% in the IMI group [Woelk 2000b].

An observational study in 11,296 patients, diagnosed in most cases with mild to moderate depression, was performed using an extract. After eight weeks of treatment symptom scores (modified HAMD) were improved by 60%. Efficacy was rated as "good" to "very good"by the physicians in 80.7% of cases. Mild adverse effects were reported in only 0.2% of cases, and did not show a systematic pattern [Volz 2000].

A 12-week observational study assessed the effects on quality of life and safety of a once daily dosing scheme (dose not specified) of an extract in 4337 patients with mild to moderate depression. Quality of life was assessed using the SF-12 symptom score, and showed a significant improvement, reaching average values of the normal population. Only four patients (0.15%) reported mild adverse events [Rudolf 2004].

A controlled, double-blind, multicentre study aimed to demonstrate the non-inferiority of an extract (612 mg once daily; n = 123) compared to SERT (50 mg; n = 118) in patients with a diagnosis of moderate depressive disorder (according to ICD-10 criteria). The initial study duration was 12 weeks, followed by an additional 12 weeks of treatment for 81 participants in the extract group and 80 participants in the SERT group. As measured by the primary efficacy variable, the 17-item HAMD total score at the end of the first 12 weeks of treatment, the extract was found not to be inferior to SERT (p<0.0001) [Gastpar 2005].

The long-term safety and effects of an extract were evaluated in 440 out-patients suffering from mild to moderate depression according to ICD-10 in an open multicentre study. Patients were treated for up to 1 year with 500 mg of the extract per day. Mean HAMD scores decreased from 20.58 at baseline to 12.07 at week 26 and to 11.18 at week 52. Mean CGI scores decreased from 3.99 to 2.20 at week 26 and 2.19 at week 52 [Brattström 2009].

Clinical studies performed with tinctures (49-50% ethanol)

In a randomised, double-blind, placebo-controlled study, 60 depressive patients (mean age 49 years) were treated daily for 6 weeks with 3 × 1.5 mL of a tincture (50% ethanol, daily dose equivalent to 0.9 mg of total hypericins) or placebo. Using a self-developed, graded scale with 52 symptoms, a considerable reduction of 61.4% in the total score for the verum group compared to only 15.8% for the placebo group. Responder rates, defined by the investigators' assessment of good or very good improvement, were 63% in the verum group and 10% in the placebo group [Hoffmann 1979].

In another randomised, double-blind, placebo-controlled study, 49 patients (mean age 42.3 years) with mild to moderate depressive symptoms were treated with 3 × 1 mL of a tincture (49% ethanol, 0.4:1) or placebo daily for 4 weeks. In the placebo group the mean number of symptoms increased from 24 to 29.6 after 4 weeks, whereas patients treated with the tincture experienced an average reduction from 22.9 to 16.4 symptoms (p<0.05) [Schlich 1987].

In a two-centre, double-blind, placebo-controlled study, 40 patients (mean age 47 years) with depressive symptoms received daily either 3 × 1.5 mL of a tincture (49% ethanol, 0.4:1) or placebo. After 4 weeks the initial HAMD total scores had

decreased from 29.25 to 9.75 in the verum group (n = 16) and from 29.5 to 19.5 in the placebo group (n = 12). Responder rates (a reduction of at least 50% from baseline HAMD scores or an endpoint score of < 10) were 62.5% in the verum group and 33.3% in the placebo group [Schmidt 1989].

In a multicentre, double-blind, placebo-controlled study, 120 patients (mean age 48.5 years) suffering from mild depressive symptoms (ICD-09: 304.4 and 309.9; initial HAMD scores of 16-20) were treated with 3 × 1.5 mL of a tincture (49% ethanol; 0.25 mg of total hypericins per mL) or placebo daily for 6 weeks. Results from 116 patients showed a marked reduction of 57.9% in HAMD total scores in the verum group compared to only 18.1% in the placebo group. Comparable results were obtained using the HAMA and D-S scale. Responder rates (total HAMD score < 10 at endpoint or a drop of at least 50% from baseline scores) were 65.9% after verum and 25% after placebo [Harrer 1991].

In another randomised, double-blind, placebo-controlled, multicentre study, 88 patients (mean age 43.3 years) suffering from mild to moderate depressive symptoms (ICD-09: 300.4; HAMD score at least 16) were treated with either 3 × 1.5 mL of a tincture (49% ethanol; 0.25 mg of total hypericins per mL) or placebo daily for 4 weeks. HAMD scores decreased from 17.8 to 5.2 in the verum group ($p<0.001$) and from 17.3 to 15.5 in the placebo group. Responder rates (defined as reduction in HAMD ≥ 50% and/or actual HAMD score of ≤10) were 70.7% after verum and only 7.1% after placebo [Quandt 1993].

In other studies, the same preparation at the same daily dosage was compared to 50 mg of IMI daily in 30 patients with depressive states after surgery [Werth 1989], and to 6 mg of bromazepam daily in 80 patients suffering from psychogenic depressive symptoms [Kugler 1990]. Comparable efficacy results for the tincture and the reference medication were reported in both studies.

In an open, comparative study, patients (mean age 52.9 years) with climacteric complaints were treated daily with either 3 × 1.5 mL of a tincture (50% ethanol; daily dose equivalent to 0.9 mg of total hypericins; n = 40) or 3 × 2 mg of diazepam (n = 20). In the investigator's judgement, 77.5% of patients treated with the tincture and 50% of patients treated with diazepam were fully remitted after 3 months. The score for the depression component of the HAMA scale dropped from 2.80 to 1.73 after 1 month and to 0.79 after 3 months in the tincture group, and from 2.95 to 1.86 after 3 months in the diazepam group. Similar results were obtained from D-S score evaluation [Warnecke 1986].

Clinical studies performed with other preparations

In a randomised, double-blind, multicentre, three-armed study involving 348 outpatients suffering from mild to moderate depression, diagnosed in accordance with ICD-10, three preparations containing a dry extract (DER 4-5:1; standardised to 0.17 mg, 0.33 mg or 1 mg of total hypericins per day) were assessed for 6 weeks. The highest daily dose corresponded to 3 × 60 mg of the extract. Initial average HAMD scores of 16-17 dropped to 8-9 after 6 weeks in all three groups. Response rates (defined as reduction in HAMD ≥ 50% and/ or actual HAMD score of ≤10) were 62%, 65% and 68% respectively. No significant differences between the three groups were detected [Lenoir 1999].

In a randomised, double-blind, crossover study, the anti-depressant effect of a tea taken twice daily (at least 0.28 mg of total hypericins per day) was assessed in 19 patients (at least 60 years old) and compared to milfoil tea (*Achillea millefolium*) as a control. Each treatment period lasted 14 days, separated by a wash-out period of 3 days. Thirteen patients reported better results with St. John's wort tea, while five patients had better results with milfoil tea and one patient showed no difference. The pilot study indicated a trend towards a better mood in patients treated with St. John's wort tea ($p=0.06$) [Engesser 1996].

In a randomised, controlled, double-blind study, 70 patients (mean age 49.7 years) with mild to moderate depression received twice daily for 6 weeks either an unspecified extract (150 mg) or FLU (20 mg). Both treatments significantly ($p<0.001$) decreased the total HAMD score (extract: 50%; FLU: 58%) and the D-S scale (42% and 52% respectively). The extract achieved 83% (HAMD) and 78% (D-S) of the effects of fluoxetine, with the differences not significant ($p=0.23$). Assessments by physicians and patients indicated considerable improvement with no differences between the treatments [Behnke 2002].

In a double-blind, randomised trial, treatment for 12 weeks with an unspecified extract (900 to 1800 mg/day) was compared with SERT (50 to 100 mg/day) in 87 men and women with major depression and an initial HAMD score of ≥ 16. No differences in the change to mean HAMD and BDI scores (using intention-to-treat analysis), with and without adjustment for baseline demographic characteristics, were observed between the two groups at 12 weeks. Significantly ($p<0.05$) more side effects were reported in the SERT group than in the extract group at 2 and 4 weeks' follow up [van Gurp 2002].

A pilot study examined the efficacy, safety, tolerability, and pharmacodynamics of an unspecified extract in the treatment of 33 outpatient youths (6 to 16 years; mean (SD) age 10.5 (2.9)) diagnosed with major depressive disorder (DSM-IV). In this 8-week, prospective, open-label study, patients were initially prescribed 150 mg of the extract three times daily. At the end of week 4, in the 22 patients who did not meet the *a priori* response criteria, the dose was increased to 300 mg three times daily. Twenty-five of the patients met the response criteria (Children's Depression Rating Scale – Revised (CDRS-R) score ≤ 28 and a CGI-S score ≤ 2) after 8 weeks of treatment. Overall, the extract was well tolerated [Findling 2003].

An uncontrolled study evaluated the efficacy of treatment for 8 weeks with an unspecified extract (0.3% hypericin, 3% hyperforin; 3 × 300 mg daily) in 26 adolescents with MDD (12–17 years, mean age 14.8 years). Of the 11 patients completing the study, 9 (82%) showed significant clinical improvement based on CGI change scores. Of the 15 patients (58%) who did not complete the study, 8 were non-compliant and 7 were discontinued because of persisting or worsening depression [Simeon 2005].

An unspecified extract was compared with FLU in a randomised, double-blind trial in 72 patients with mild to moderate depression. Patients were treated for 8 weeks with either a standardised extract (not futher specified; 900 mg/day), FLU (20 mg/day) or placebo. Intention-to-treat analysis showed no differences in changes to the mean scores of HAMD-21, Montgomery-Asberg Rating Scale and CGI between the three groups. Patients receiving the extract had the lowest remission rates (12%, $p=0.016$) compared to FLU (34.6%) and placebo (45%) [Moreno 2005].

In a three-arm, randomised clinical trial, the effects of treatment for 12 weeks with an unspecified extract (810 mg/day) were compared with CIT (20 mg/day) and placebo in 81 patients with minor depression or milder forms of MDD (with at least

of two to four symptoms of major depression according to DSM-IV). Additional criteria included HAMD scores between 10 and 17, a GAF score of less than 70, and either an MOS Social Functioning score of 75% or less or an MOS Emotional Role Functioning score of 67% or less. Due to a high placebo response on all outcome measures, neither the extract nor CIT differed from placebo [Rapaport 2011].

Clinical studies - other conditions

Attention-deficit hyperactivity disorder (ADHD)
An unspecified standardised extract (0.3% hypericin) was administered in a randomised, double-blind, placebo-controlled trial to children aged 6 to 17 years who met DSM IV criteria for ADHD. After a placebo run-in phase of 1 week, participants were randomly assigned to receive either placebo or 300 mg of the extract 3 times daily for 8 weeks. No significant difference was found in the change in ADHD Rating Scale-IV scores from baseline to week 8 between the treatment and placebo groups: inattentiveness improved by 2.6 points (95% confidence interval [CI], -4.6 to -0.6 points) with the extract vs. 3.2 points (95% CI, -5.7 to -0.8 points) with placebo (p=0.68) and hyperactivity improved by 1.8 points (95% CI, -3.7 to 0.1 points) with the extract vs. 2.0 points (95% CI, -4.1 to 0.1 points) with placebo (p=0.89). There was also no significant difference in the percentage of participants who met criteria for improvement (score ≤2) on the CGI Improvement Scale. No difference between groups was found for adverse effects [Weber 2008].

An unspecified extract was given at a daily oral dose of 30 mg for 4 weeks in an open trial to three 14-16-year-old male psychiatric outpatients with ADHD. After this treatment, patients received a placebo for a further 4 weeks. Assessed by the Conner Scale and by the Continuous Performance Test, at baseline and at the end of each treatment, patients' mean scores improved for Conners' hyperactivity, inattention and immaturity factors [Niederhofer 2010].

Somatoform disorder
The efficacy of a methanolic (80%) extract was studied in a multicentre, double-blind, placebo-controlled trial in 151 out-patients suffering from somatisation disorder (ICD-10: F45.0), undifferentiated somatoform disorder (F45.1), or somatoform autonomic dysfunctions (F45.3). Patients received the extract (600 mg/day p.o.) or placebo for 6 weeks. The extract demonstrated significantly (p=0.001) greater efficacy in the primary outcome criterion Hamilton Anxiety Scale, subfactor somatic anxiety (HAMA-SOM) [decrease from 15.39 to 6.64 in the extract group and from 15.55 to 11.97 in the placebo group]. The extract was also significantly more effective as measured by the CGI (p=0.006), HAMA-total score (p=0.0001), subscore psychic anxiety (p=0.001), HAMD (p=0.001), Self-Report Symptom Inventory 90 items – revised (SCL-90-R; p=0.001), and SCL-90-R, subscore somatic anxiety (SCL-90-R-Som; p=0.001). Tolerability of the extract was excellent [Volz 2002].

In a prospective, randomised, double-blind, placebo-controlled, parallel group study, outpatients with somatisation disorder (ICD-10 F45.0), undifferentiated somatoform disorder (F45.1) and somatoform autonomic dysfunction (F45.3), received either 300 mg of a methanolic (80%) extract twice daily or placebo for 6 weeks. Somatoform Disorders Screening Instrument-7 days (SOMS-7), somatic subscore of the HAMA, SCL-90-R, subscores "improvement" and "efficacy" of the CGI, and the global judgment of efficacy by the patient were measured. In the intention-to-treat population, for each of these measures as well as for the combined test, significant superiority of treatment over placebo was demonstrated (p<0.0001). Of the treated patients, 45.4% were classified as responders compared with 20.9% for placebo (p=0.0006). Tolerability of the extract treatment was equivalent to placebo [Müller 2004].

Social phobia
Forty patients with generalised social anxiety disorder were randomised to 12 weeks of treatment with a flexible dose (600-1800 mg) of a methanolic (80%) extract or placebo. Subjects with co-morbid depression (HAMD>16) were excluded. No significant difference (p=0.27) in mean change on the Liebowitz Social Anxiety Scale was observed between the extract (11.4) and placebo (13.2) [Kobak 2005b].

Obsessive-compulsive disorder
In a randomised, double-blind, placebo-controlled, parallel group study sixty subjects with obsessive-compulsive disorder (OCD) were randomised to 12 weeks of treatment with a methanolic (80%) extract in a flexible-dose schedule (600-1800 mg/day p.o.) or placebo. The mean change in the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) with the extract (3.43) as well as Y-BOCS subscales were not significantly different from the mean change with placebo (3.60). The percentage of patients rated as 'much' or 'very much' improved at endpoint was not significantly different between the extract treatment (17.9%) and placebo (16.7%) (p=0.905) [Kobak 2005a].

Autism
An unspecified extract was assessed in an open trial in three male patients with autistic disorder. Parent and mentor ratings on the Aberrant Behaviour Checklist, irritability, stereotypy and inappropriate speech factors improved slightly during treatment. Ratings such as the Psychiatric Rating Scale Autism, Anger and Speech Deviance factors, Global Assessment Scale and CGI efficacy did not improve significantly [Niederhofer 2009].

Menopause
Women aged from 40 to 65 years (n=47) experiencing hot flushes (≥ 3 per day, Heart and Estrogen/Progestin Replacement Study scale) were randomly assigned to receive an ethanolic extract (300 mg 3 times daily) or placebo. After 12 weeks of treatment, a non-significant difference favouring the extract group was observed in the daily hot flush frequency (extract -2.3 ± 3.6; placebo -1.0 ± 2.2; p=0.11) and the hot flush score (3.8 ± 8.3 and -1.8 ± 6.5 respectively; p=0.10). After 3 months of treatment, the women in the extract group reported significantly better quality of life (p=0.01) and significantly less sleep problems (p=0.05) compared to placebo [Al-Akoum 2009].

Women (n=100, mean age 50.4 years) with climacteric complaints according to the Blatt-Kupperman Index were treated with 20 drops 3 times per day of an extract (containing 0.2 mg/ml hypericin; not further specified) or placebo for 8 weeks. Although both groups responded to the interventions, the differences between the groups in frequency, duration, and severity of hot flushes were significant (p<0.05). The duration of hot flushes was significantly decreased in the extract group compared with placebo in week 8 (p<0.001), but not in week 4 (p=0.085). The decrease in frequency of hot flushes was significantly greater in the extract group compared to placebo in weeks 4 and 8 (p=0.05 and p<0.001 respectively). The decrease in the severity of flushes was more evident in the extract group in weeks 4 and 8 (p=0.004 and p<0.001 respectively) [Abdali 2010].

St. John's wort was found to be significantly superior to placebo (standard mean difference = -1.08; 95% confidence interval: 1.38 to -0.77) in a meta-analysis comparing the efficacy and adverse events for extracts of St. John's wort, combination products and placebo in menopausal women [Liu 2013].

Premenstrual symptoms (PMS)
Women with premenstrual syndrome (n=19) took a methanolic (80%) extract for two complete menstrual cycles (300 mg extract per day standardised to 900 µg hypericin). The Hospital Anxiety and Depression (HAD) scale and a modified Social Adjustment Scale were measured at baseline and after one and two menstrual cycles. There were significant ($p<0.05$) reductions in all outcome measures. The degree of improvement in overall premenstrual syndrome scores between baseline and the end of the trial was 51%, with over two-thirds of participants demonstrating at least a 50% decrease in symptom severity [Stevinson 2000].

In a randomised, double-blind, placebo-controlled, crossover study 36 women (18-45 years) with regular menstrual cycles (25-35 days), who were prospectively diagnosed with mild PMS, underwent a two-cycle placebo run-in phase. They then received either 900 mg/day of a methanolic extract (80%; standardised to 0.18% hypericin; 3.38% hyperforin) or placebo for two menstrual cycles. After a placebo wash-out month the treatments were crossed over for two further cycles. The extract was superior to placebo in improving physical and behavioural symptoms of PMS ($p<0.05$) but not superior for mood- and pain-related symptoms. Plasma hormone (FSH, LH, E_2, progesterone, PRL and testosterone) and cytokine (IL-1β, IL-6, IL-8, IFN-γ and TNF-α) levels, as well as weekly reports of anxiety, depression, aggression and impulsivity, did not differ significantly between treatments ($p>0.05$) [Canning 2010].

Patients with PMS (n=170) received an unspecified extract (two 680 µg "hypericin tablets" per day) or placebo for 8 weeks. Treatment with the extract resulted in significantly lower PMS scores compared with baseline ($p<0.001$) and control ($p<0.001$). The biggest improvements in score occurred for crying (71%) and depression (52%). The drop-out rate due to adverse events was higher in the extract group ($p=0.02$), although there were no significant differences in adverse events. Symptom ratings were reduced by 40% after extract treatment [Ghazanfarpour 2011].

Irritable bowel syndrome (IBS)
An unspecified extract was given for 8 weeks at an oral dose of 900 mg/day to 30 female patients with IBS. Patients and 20 healthy controls underwent serial autonomic nervous system function tests before and after 8 weeks of treatment. Five-minute-short-time heart rate variability spectral analysis was assessed at rest and during three different types of stress test (somatic, psychological and visceral stimuli). HAMD and HAMA scores were used to measure depression and anxiety; and patients completed a gastrointestinal (GI) symptom diary once daily during the 2 weeks before therapy and for 2 weeks up to the last scheduled therapy session. Compared to the untreated control group, the IBS-group had higher initial scores in the HAMA and HAMD (both $p<0.01$); with these scores significantly decreasing post-treatment ($p<0.05$). Compared to the resting state, the IBS group exhibited significant increases in the low frequency band/high frequency band ratio (L/H) during each period of stress testing ($p<0.01$). Treatment with the extract for 8 weeks led to a significantly decreased ($p<0.05$) L/H response to the various stressors when compared to baseline. GI symptoms of IBS were also significantly ($p<0.05$) relieved by the treatment [Wan 2010].

In a randomised, double-blind, placebo-controlled trial, 70 patients with IBS received either an unspecified extract at an oral dose of 900 mg or placebo for 12 weeks. Of the patients, 29% had constipation dominated IBS (C-IBS), 37% diarrhoea dominated IBS (D-IBS), and 31% had mixed IBS. Both groups reported decreases in overall bowel symptom score from baseline, though the placebo arm had significantly lower scores at 12 weeks ($p=0.03$) compared with the extract. The percentage of patients with adequate relief was also significantly higher in the placebo group ($p=0.02$) [Saito 2010].

Burning mouth syndrome (BMS)
A double-blind, randomised, placebo-controlled, single-centre study evaluated patients with BMS (n=39) receiving oral doses (300 mg, 3 times daily) of an unspecified extract (hypericin 0.31%, hyperforin 3.0%) or placebo for 12 weeks. Burning pain, measured using a visual analogue scale (VAS), was only slightly more improved in the extract treated group [Sardella 2008].

Antiviral effects
In a phase I dose escalation study, the antiviral activity of hypericin was assessed in 19 patients with chronic HCV infection. For 8 weeks, 12 patients received 0.05 mg/kg and 7 patients 0.1 mg/kg hypericin orally once a day. At the end of the 8 week period, plasma HCV RNA level did not change more than 1.0 $\log_{10}$ units. No anti-HCV activity was noted [Jacobson 2001].

Antibacterial effects
In a randomised, placebo-controlled, double-blind study, 21 patients with mild-to-moderate atopic dermatitis (mean SCORAD score 44.5) were treated twice daily for 4 weeks with either a cream containing an unspecified extract (1.5% hyperforin) or placebo (vehicle). The intensity of the eczematous lesions was significantly ($p<0.05$) improved with the verum compared to the vehicle at all clinical visits (days 7, 14, and 28). Skin colonisation with *Staphylococcus aureus* was reduced by verum and placebo treatments, with a trend towards better antibacterial activity for the verum ($p=0.064$) [Schempp 2003a].

Wound healing
In a randomised, double-blind clinical trial (n=144), ointments containing either an oily extract or placebo were applied topically to surgical wounds 3 times daily for 16 days, starting from 24 h after caesarean section. An additional control group remained without any post-operative intervention. Compared to both placebo and control groups, there were significant improvements observed in the extract group for wound healing on the 10th day ($p<0.005$) and scar formation on the 40th day postpartum ($p<0.0001$). Significantly lower pain ($p<0.001$) and pruritus ($p<0.001$) were reported by the treatment group compared with the placebo and control groups on day 40 [Samadi 2010].

Pharmacokinetic properties

Pharmacokinetics in vitro
Variations in the matrices of different extracts affected their absorption and efflux as shown for three extracts submitted to a human intestinal Caco-2 cell transport experiment. Transepithelial transport across the Caco-2 cell monolayer was different for rutin, hyperoside and isoquercitrin founds in extracts when compared to the respective pure compounds. The results clearly revealed that the product matrices affected permeability of the key components [Gao 2010].

The metabolism of hyperforin was characterised using human liver microsomes and CYPs, CYP-selective chemical inhibitors and correlation with CYP-recombinant heterologously expressed CYP enzymes. Among 57 detected metabolites six mono¬hydroxylated compounds (M1-M6) were identified, while others were higher hydroxylated. A combined approach of cDNA-expressed recombinant specific marker activities indicated a central role of the CYP2C and CYP3A families in the metabolism of hyperforin [Hokkanen 2011].

Interactions
After administration of St. John's wort extracts and some of its

components, two types of qualitatively different pharmacokinetic interactions have been observed:

- After acute administration: inhibition of metabolic enzymes and drug transporters [Budzinski 2000; Chaudhary 2006; Dostalek 2011; Dostalek 2005; Foster 2004; Hokkanen 2011; Komoroski 2005; Komoroski 2004; Lee 2006; Obach 2000; Ott 2010; Patel 2004; Schwarz 2011; Tuna 2010; Volak 2010; Wada 2002; Wang 2004a; Weber 2004].

- After subchronic or chronic administration: induction of several enzymes of the cytochrome P450 (CYP) enzyme family (CYP1A1, CYP1A2, CYP3A, CYP2B6, CYP2C8 and CYP2C9, C2C19 and CY2E1) [Chen 2004; Gutmann 2006; Hellum 2009; Komoroski 2005; Komoroski 2004; Krusekopf 2005; Sahi 2009], UDP-glucuronyltransferases (UGT1A1, UGT1A6) [Volak 2010], and several drug efflux transporters from the ATP-binding cassette transporters superfamily (ABC transporters, such as P-glycoprotein (MDR1/ABCB1) or MRP2 (ABCC2), MRP3 (ABCC3)) [Gutmann 2006; Ott 2010; Weber 2004], shown *in vitro* and in animal trials. One mechanism of the interactions is the activation of the pregnane X receptor (PXR) by hyperforin [Moore 2000; Watkins 2003; Wentworth 2000]. The extent of PXR mediated gene induction was proportional to the extent of hyperforin in the extracts [Gödtel-Armbrust 2007; Mueller 2009; Mueller 2006].

Acute administration
Unspecified extracts in methanolic (1%) solution were examined (using microsomal enzyme preparations) for their inhibition towards CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. CYP2D6 was the most sensitive. In the assay of single components, CYP1A2 was inhibited by I3,II8-biapigenin (IC_{50} 3.7 µM) and quercetin (IC_{50} 7.5 µM), CYP2C9 by hyperforin (IC_{50} 4.4 µM), I3,II8-biapigenin (IC_{50} 4.0 µM) and hypericin (IC_{50} 3.4 µM), CYP2C19 by hyperforin (IC_{50} 31 µM), I3,II8-biapigenin (IC_{50} 28 µM) and hypericin (IC_{50} 37 µM), CYP2D6 by hyperforin (IC_{50} 1.6 µM), I3,II8-biapigenin (IC_{50} 5.7 µM), quercetin (IC_{50} 24 µM) and hypericin (IC_{50} 8.5 µM) and CYP3A4 by hyperforin (IC_{50} 2.3 µM), I3,II8-biapigenin (IC_{50} 0.082 µM), quercetin (IC_{50} 22 µM) and hypericin (IC_{50} 8.7 µM) [Obach 2000].

Two ethanolic extracts (A: 50% m/m; B: 60% V/V) and a methanolic extract (C: 80% m/m), as well as hypericin, pseudohypericin, hyperforin, rutin and quercetin, were investigated for their inhibitory effects on carcinogen activation by CYP1A1 using purified enzyme preparations. The extracts potently inhibited CYP1A1 activity (IC_{50} values: 24.0 ± 3.9 µg/mL (A), 8.0 ± 1.0 µg/mL (B), 1.3 ± 0.2 µg/mL (C)). All constituents, except rutin, possessed strong inhibitory potencies with IC_{50} values of 0.5 ± 0.1 µM (hypericin), 8.0 ± 0.1 µM (pseudohypericin), 1.2 ± 0.2 µM (hyperforin) and 1.5 ± 0.2 µM (quercetin) [Schwarz 2003].

The inhibition of human cytochrome P450 enzymes by 18 St. John's wort products containing a wide variety of hyperforin, hypericin and pseudohypericin levels was assessed. The aqueous extracts markedly inhibited cytochrome P450-mediated (CYP 2C9*1, 2C9*2, 2C19, 2D6, 3A4, 3A5 and 3A7) metabolism and inhibited P-glycoprotein (Pgp) activity [Foster 2004].

In MDR1 transfected MDCK cells, hypericin and quercetin at concentrations of 5 to 200 µM showed a significant ($p<0.05$) and dose-dependent inhibition of Pgp-mediated efflux of [^{3}H]-ritonavir and inhibited CYP3A4 activity (hypericin IC_{50} 0.87 µM, quercetin IC_{50} 0.53 µM, kaempferol IC_{50} 0.26 µM) [Patel 2004].

In primary cultures of human hepatocytes, hyperforin at high concentrations of 5 and 10 µM, 1 hour before and along with probe substrate, inhibited CYP3A4 activity. Hypericin had no effect on any of the enzymes tested [Komoroski 2005; Komoroski 2004].

Human hepatocytes were exposed to hyperforin (0.1, 0.5 or 1.5 µM) or rifampin (10 µM) for 48 hours. The medium was then replaced by one containing docetaxel (100 µmol/L). Docetaxel metabolism was characterised by HPLC/MS after 1 hour: metabolism induced by rifampin was 6.8- to 32-fold higher than that in control cultures. Hyperforin induction was dose-dependent and, at maximum, was 2.6- to 7-fold greater than that in controls. CYP3A4 and CYP2C8 were involved in metabolite formation [Komoroski 2005].

Seven flavonoids in St. John's wort and apigenin were screened for their inhibition of recombinant human CYP1B1 and CYP1A1. While myricetin, apigenin, kaempferol, quercetin, amentoflavone, quercitrin and rutin were slightly more selective for CYP1B1 ethoxyresorufin-*O*-deethylase (EROD) inhibition (K_is 0.06 - 5.96 µM) compared to CYP1A1 (K_is 0.20 - 1.6 µM), the differences in K_is for the P450s were not significantly different. Rutin did not inhibit CYP1A1 at concentrations up to 10 µM. In kinetic analyses apigenin and amentoflavone were competitive inhibitors of CYP1B1, while quercetin showed mixed type inhibition. Five flavonoids were studied further for their ability to inhibit TCDD-induced EROD activity in 22Rv1 human prostate cancer cells. 22Rv1 cells expressed constitutive and TCDD-inducible CYP1A1 and CYP1B1 mRNA. The IC_{50} values were similar to those measured for the recombinant CYP1A1 except for amentoflavone. Quercetin (IC_{50}: 4.1 µM), kaempferol (3.8 µM), myricetin (3.0 µM) and apigenin (3.1 µM) caused significant ($p<0.05$) inhibition of EROD activity whereas amentoflavone did not cause inhibition [Chaudhary 2006].

Further investigations confirmed this activity of hyperforin, demonstrating the inhibition of recombinant CYP3A4 (IC50 0.63 µM) [Lee 2006] and of CYP3A4 (IC_{50} 4.4 - 9.6 µM) and CYP2D6 (IC_{50} 7.3 µM) [Hokkanen 2011].

A study of the inhibition of CYP1A1-mediated 2-hydroxylation of oestradiol by a methanolic (80%, V/V) extract and single components found IC_{50} values of 19.5 µg/mL for the extract, 9.8 µM (hypericin), 6.8 µM (pseudohypericin), 3.2 µM (quercetin), 2.2 µM (kaempferol) and 2.2 µM (myricetin). No effects were seen for hyperforin and rutin [Schwarz 2011].

The effects of hyperforin and hypericin on Pgp activity were compared to CyA in the MDR1 over-expressing cell line NIH-3T3-G 185. Both compounds inhibited Pgp activity with an IC_{50} of about 30 µM, which was about 15-fold higher than for ciclosporin (2 µM) [Wang 2004a].

A methanolic (80%, m/m) extract and some of its components were investigated for their effect on Pgp activity in the calcein-AM assay using porcine brain capillary endothelial cells (PBCEC) as a model of the blood-brain barrier as well as in a human MDR1 over-expressing lymphocytic leukaemia-derived cell line VLB 100. The extract inhibited Pgp activity above a concentration of 100 µg/mL ($p<0.05$) in both cell lines. Hyperforin (1 - 10 µM), hypericin (0.03 - 30 µM), quercetin (0.01 - 3 µM), hyperoside (0.3 and 3 µM), rutin (10 µM) and biapigenin (10 and 30 µM) showed significant ($p<0.05$ to $p<0.001$) inhibitory effects on Pgp activity in PBCEC. Amentoflavone activated P-glycoprotein at 3 and 30 µM ($p<0.05$), quercetin and biapigenin activated Pgp at the lowest concentration and inhibited it at the higher concentrations ($p<0.05$). In VLB 100 cells, only 10 µM hyperforin inhibited Pgp ($p<0.01$) [Weber 2004].

A methanolic extract (80%, V/V; 0.1 – 5 µg/mL), hyperforin

(0.1 – 10 µM), hypericin (1 – 50 µM) and quercetin (1 – 50 µM) decreased Pgp transport activity in a dose- and time-dependent manner in PBCEC cells and freshly isolated porcine brain capillaries. The extract and hyperforin directly inhibited Pgp activity, whereas hypericin and quercetin modulated transporter function through a mechanism involving protein kinase C. Quercetin decreased Pgp transport activity at high concentrations (> 10 µM), but increased transporter function at low concentrations (≤10 µM) [Ott 2010].

Hypericin bound and inhibited the major glutathione-S-transferase isoforms GST-α (K_i 0.16 ± 0.02 µM to 1.91 ± 0.21 µM) and GST π (0.55 ± 0.07 µM to 2.46 ± 0.43 µM) [Tuna 2010].

Many UDP-glucuronosyltransferases (UGTs) require phosphorylation by protein kinase C (PKC) for glucuronidation activity. Hypericin, a non-selective PKC inhibitor, inhibited paracetamol glucuronidation by human LS180 cells (IC_{50} = 7.1 ± 0.6 µM). In UGT1A6-infected Sf9 insect cells, hypericin showed a three times more potent inhibition of serotonin glucuronidation in treated whole cells versus cell lysates (IC_{50} = 0.59 ± 0.05 µM) [Volak 2010].

Sub-chronic or chronic administration
LS180 cells were exposed for 72 hours to a methanolic (80%) extract at concentrations of 3 to 300 µg/mL or hyperforin (0.03 to 3 µM) for 3 days. Pgp expression was strongly and dose-dependently induced by the extract (400% increase at 300 µg/mL) and by hyperforin (700% at 3 µM). In Caco-2 cell monolayers, the extract and hyperforin caused a moderate inhibition of Pgp-mediated transport at the highest concentration [Perloff 2001].

LS180 cells were cultured for 48 hours in the presence and absence of three doses of an extract (not further specified) containing 5.61% hyperforin. The extract increased the expression of CYP1A2 in a dose-dependent manner [Karyekar 2002].

In primary cultures of human hepatocytes, after 48 hours incubation with 10 µM hyperforin or rifampin, CYP2B6 expression was induced approximately 4-fold and 15-fold respectively [Goodwin 2001]. Treatment for 96 h resulted in significant ($p<0.05$) increases in mRNA and protein expression, as well as activity of CYP3A4 and CYP2C9, but had no effect on CYP1A2 or CYP2D6 [Komoroski 2005; Komoroski 2004].

HepG2 cells were treated with 0.1% (V/V) of an ethanolic (50% m/m) extract or 0.1 µM hyperforin for 24 hours and RNA was subjected to gene array analysis. The extract and hyperforin affected the expression of genes that mediate metabolism (e.g. CYP3A4, CYP1A1, CYP1A2) and transport exogenous and endogenous compounds (e.g. MRP2), but also of genes involved in energy metabolism, intracellular calcium regulation, cell proliferation and apoptosis [Krusekopf 2005].

An extract and hyperforin but not hypericin increased the expression of Pgp in LS180 cells. Incubation with the extract (20 to 75 µg/mL) for 24 hours led to a dose-dependent increase of MDR1 mRNA expression that exceeded the positive control rifampin (10 µM). Removal of the extract resulted in restoration of basal Pgp levels within 48 hours [Tian 2005].

An ethanolic extract (50% m/m, low hyperforin content) did not affect MDR1 or CYP3A4 expression in LS180 cells. MDR1 expression was significantly ($p<0.01$) up-regulated by hypericin and hyperforin (each 10 µM), and CYP3A4 expression by hypericin, quercitrin and hyperforin [Gutmann 2006].

An ethanolic (60%) extract incubated for 72 hours increased the metabolic activity of CYP2C19 in primary human hepatocytes at 8 and 80 µg/mL ($p<0.05$), while a strong inhibition was observed at 800 µg/mL ($p<0.05$). CYP2E1 activity was increased by 65% ($p<0.05$) at 8 µg/mL and completely inhibited at 800 µg/mL [Hellum 2009].

Pharmacokinetics in animals
After intravenous injection of 350 µg hypericin (about 17.5 mg/kg), peak plasma concentrations in BALB/c mice were 27.8 µg/mL at 10 minutes after administration, which decreased to 0.010 µg/mL after 240 h. The AUC was 285 µg×h/mL, the volume of distribution 12.6 mL and the elimination half-life 38.5 h [Liebes 1991].

After oral administration of [^{14}C]-labelled hypericin and pseudohypericin to 8 female mice, radioactivity was measured in the GI tract and in liver, kidney, muscle, blood and brain. At 1.5 and 6 h after administration, 42% and 19% of the hypericin dose and 69% and 44% of the pseudohypericin dose were detected in the gastrointestinal tract. A proportion of 31% and 11% radioactivity from hypericin was found in muscle after 1.5 and 6 hours. In blood and brain minor quantities were found for both compounds, in the brain 0.7% and 0.6% of the hypericin dose and 0.4% and 0.9% of the pseudohypericin dose at 1.5 and 6 hours respectively [Stock 1991].

After an i.v. administered dose of [^{14}C]-hypericin and [^{14}C]-pseudohypericin in mice, 80% of hypericin and 60% of pseudohypericin was absorbed. Tissue distribution was followed for 2, 4, 6, 8, 24, 72 and 168 hours. The highest tissue concentrations were observed in the lung with over 3.5 µg/g at 4 h, gradually declining to less than 2% of the maximum after 7 days. The concentrations in the tissues were: lung > spleen > liver > blood > kidney > heart > gut > tumour > stomach > skin > muscle > brain [Chung 1994].

The tissue distribution of hypericin (2, 5 or 20 mg/kg i.p.) in DBA/2 mice bearing subcutaneously implanted P388 lymphoma cells resulted in a very high uptake in liver and spleen. Clearance from plasma followed a two-phase exponential decay: a first phase of rapid clearance (half-life 6.9 hours) was followed by a slower phase (half-life 37.9 hours) [Chen 2000].

After oral administration of an ethanolic extract containing 5% hyperforin to rats at 300 mg/kg, maximum plasma levels of 370 ng/mL hyperforin were reached after 3 hours. Estimated half-life and clearance values were 6 hours and 70 mL/min/kg [Biber 1998].

In Sprague-Dawley rats, i.p. administration of 10 mg/kg hyperforin led to plasma levels in the low micromolar range (3 - 5 µM). No hyperforin was detected in the cerebrospinal fluid. At 1 mg/kg the compound was detected in the plasma [Buchholzer 2002].

In a study of the metabolic profile of hyperforin in liver microsomes from male and female Sprague-Dawley rats, with or without induction by phenobarbital or dexamethasone, four major phase I metabolites (19-hydroxyhyperforin, 24 hydroxyhyperforin, 29-hydroxyhyperforin and 34-hydroxyhyperforin) were identified. Results suggested hydroxylation as a major biotransformation of hyperforin in rat liver, mainly by inducible CYP3A and/or CYP2B [Cui 2004].

In male CD rats, procyanidin B_2 increased the oral bioavailability of hypericin by approximately 58% and hyperoside by 34%. The compounds had a different influence on the plasma kinetics of hypericin; median maximal plasma levels of hypericin were detected after 360 min (C_{max} 8.6 ng/mL) for B_2, and after 150 min (C_{max} 8.8 ng/mL) for hyperoside [Butterweck 2003b].

Unspecified extracts (containing either 4.5 or 0.5% hyperforin) and hyperforin dicyclohexylammonium (DCHA) were given to male CD-COBS rats. The 4.5% extract, administered as three i.p. injections in 24 h (3.12-6.25 mg/kg), resulted in dose-related plasma concentrations of hyperforin which were of similar magnitude to those found following the administration of hyperforin DCHA that contained hyperforin at a level equivalent to the 4.5% extract (0.14 and 0.28 mg/kg). Hyperforin was undetectable in rat brain [Cervo 2002].

The determination of plasma levels of hypericin in rats in the presence and absence of procyanidin B_2 or hyperoside showed increased oral bioavailability of hypericin by ca. 58 % procyanidin B_2 and 34% (hyperoside). Median maximal plasma levels of hypericin were detected after 360 min (C_{max}: 8.6 ng/mL) for procyanidin B_2, and after 150 min (C_{max}: 8.8 ng/mL) for hyperoside [Butterweck 2003b].

In mice treated with 15 mg/kg hyperforin, either as the sodium salt or as a 5% extract, average brain tissue concentrations of hyperforin were found to be higher for the sodium salt group (28.8±10.1 ng/g) than the extract group (15.8±10.9 ng/g) [Keller 2003].

After administration of 300 mg/kg p.o. of an ethanolic extract (4.5% hyperforin) to male CD1 mice, hyperforin rapidly reached the systemic circulation, with a C_{max} of approximately 1 µM within 60 min. Blood levels peaked faster (30 minutes) after the application of hyperforin DCHA salt at 18.1 mg/kg. Hyperforin was quantifiable up to 4 - 6 hours post administration. Interestingly, the C_{max} of hyperforin was reduced by approximately 50% after repeated dosing (4 days), correlating with an induction of CYP3A enzymes by hyperforin [Cantoni 2003].

Wistar rats treated with an unspecified extract (1000 mg/kg/day) for 1, 3 or 7 days were administered a single oral dose of midazolam at 10 mg/kg after their final extract dose. Oral clearance of midazolam in the extract treated rats increased time dependently, and was significant ($p<0.01$) after 7 days of treatment with the extract. The midazolam-1-hydroxylation activity in liver microsomes obtained from the extract treated rats was significantly ($p<0.01$) higher than in controls. Linear correlation was observed between oral clearance and midazolam-1-hydroxylation activity in the liver microsomes, suggesting that CYP3A induction in the liver mainly decreased the midazolam concentration in plasma. Immunoblotting revealed that CYP3A was induced within 3 days of extract treatment [Qi 2005].

After single oral doses of an ethanolic extract (60%; 1600 mg/kg) and isoquercitrin (100 mg/kg) to male Sprague Dawley rats, maximal CNS levels for quercetin (340 ng/g) and isorhamnetin/tamarixetin (50 ng/g) were found at 4 h. With repeated daily doses over 8 days the maximal cumulation for quercetin (367 ng/g) occurred after 5 days, whereas isorhamnetin/tamarixetin (640 ng/g) did not reach its maximal cumulation level within the 8-day test period [Paulke 2008].

After administration of an unspecified extract, brain concentrations of amentoflavone were below the limit of quantification. Levels were consistently detected only after i.p. amentoflavone (10 mg/kg), with a mean brain-to-plasma ratio of about 0.02. These concentrations were possibly related to the compound's contribution from residual blood, but in any case are too low to support any interaction with central mechanisms so far tested [Colovic 2008].

In mice treated for a month with an unspecified extract (1500 mg/kg p.o.), hypericin plasma levels were significantly ($p<0.05$) more elevated in females (93 ± 7 ng/mL) than in males (32 ± 3 ng/mL) [Radu 2009].

An experiment investigating fractions of extracts for their antidepressant activity showed that the amount of hypericin needed for significant antidepressant activities in the FST was very high (0.23 mg/kg p.o.), whereas a hypericin-rich fraction was active at doses of 0.028 - 0.16 mg/kg. The fraction contained procyanidin B_2 which increased solubility of hypericin in water, which for hypericin alone was 0.5 µg/mL compared to more than 60 µg/mL with increasing amounts of procyanidin B_2. Higher solubility of hypericin led to increased bioavailability and to increased *in vivo* activity as shown by co-administration of hypericin with procyanidin B_2, thereby increasing the AUC to 160% [Nahrstedt 2010].

Hypericin was administered as an i.v. bolus of 2 mg/kg (n = 3) or 5 mg/kg (n = 1) to male Rhesus monkeys. Plasma and cerebrospinal fluid (CSF; ventricular or lumbar) were sampled prior to administration and at frequent intervals for up to 50 h after administration of the drug. Mean peak plasma concentration of hypericin following the 2 mg/kg dose was 142 ± 45 µM. Elimination of hypericin from plasma was biexponential, with an average distribution half-life of $t_{½,\alpha}$ of 2.8 ± 0.3 h and an average terminal half-life of $t_{½,\beta}$ of 26 ± 14 h. The 2 mg/kg dose was sufficient to maintain plasma concentrations above 10 µM for up to 12 h. No hypericin was detected in the CSF of any animal (LOD 0.1 µM) [Fox 2001].

Interactions

The effects of a co-administered methanolic (80%) extract on the pharmacokinetics of CPT-11 were evaluated in rats pre-treated with the extract (400 mg/kg p.o. for 8 days, 6 mL/kg) or vehicle. CPT-11 was given at a dose of 60 mg/kg per day by i.v. injection via tail vein for 4 consecutive days. Co-administration with the extract significantly ($p<0.05$) altered the pharmacokinetics of both CPT-11 and its metabolite SN-38, and reduced the AUC of the SN-38-glucuronide by 31.2% [Hu 2005].

Swiss Webster mice received either A) an unspecified ethanolic extract for 2 weeks (oral dose not clearly defined); B) the same dose of the extract for 1 week; C) a single dose of the extract; D) no extract; E) hypericin (10 µg); or F) control. All groups received a single dose of procainamide (100 mg/kg p.o.), 24 h after the last extract dose in groups A and B, and 1 h later in group C. The mean procainamide concentration 1 h after administration was highest in group C (11.59 µg/mL) followed by group A (9.92 µg/mL), whereas group B (7.44 µg/mL) and the control group D (7.36 µg/mL) showed comparable values. The concentration in group C was significantly higher than in the control group D ($p=0.03$). *N*-Acetyl procainamide concentrations and estimated half-life of procainamide were comparable between the groups. With hypericin, a significant ($p<0.05$) increase in bioavailability (53%) of procainamide was observed compared to control group F [Dasgupta 2007].

Oral administration of either 150 or 300 mg/day of an unspecified extract for 15 days significantly reduced plasma levels of indinavir. A perfusion study demonstrated that both the small intestine and the liver contributed significantly (both $p<0.05$) to the reduction of indinavir bioavailability. The effect was flow rate-dependent. The small intestine was the major site for the pre-systemic metabolism of indinavir, with or without extract pre-treatment [Ho 2009].

An unspecified extract was given at an oral dose of 400 mg/kg/day to male Wistar rats. Daily administration for 10 days increased respectively the amounts of MRP2, glutathione S-transferase-P (GST-P) and CYP1A2 in the liver to 304%, 252% and 357%

of control levels, the amounts of P-gp and MRP1 were not changed. MRP2 did not appear to increase in the kidney. The increase in the levels of each protein was maximal at 10 days after extract treatment and lasted for at least 30 consecutive days [Shibayama 2004].

Male Sprague-Dawley rats were given a methanolic extract (80%; 1000 mg/kg/day p.o.) for 14 days. On day 15, livers were isolated and perfused in a recirculating system with fexofenadine (FEXO; 2 µg/mL), either alone or following addition of CyA (0.5 µg/mL) 5 min before the application of FEXO. Administration of the extract significantly ($p<0.05$) increased biliary clearance with respect to perfusate, and biliary clearance with respect to the concentration in the liver, by 74% and 71% respectively. This was reversed by the Pgp inhibitor CyA [Turkanovic 2009].

In male Wistar rats i.p. administration of 100 mg/kg/day of a methanolic (80%) extract for 10 days resulted in significant induction of CYP2D2 ($p<0.001$) and CYP3A2 ($p<0.001$) as well as inhibition of CYP2C6 ($p<0.001$) and CYP1A2 ($p<0.001$) activity in an isolated perfused rat model [Dostalek 2011; Dostalek 2005].

A methanolic (80%) extract was given to male Wistar rats for 10 days at an i.p. dose of 100 mg/kg prior to liver preparation. Paracetamol formation from phenacetin (12.5 mg/L) was used as marker for CYP1A2 activity in this isolated perfused liver model. The formation rate of acetaminophen was significantly inhibited by pre-treatment with the extract ($p<0.001$) [Dostalek 2011].

A methanolic (80%) extract at a dose of 300 mg was given orally to four dogs every 24 h for 14 days. A single dose of CyA (5 mg/kg) was given orally 7 days before and 7 and 14 days after start of the extract administration. Control dogs received a single oral dose of CyA (5 mg/kg). The maximum whole-blood concentration and AUC(0-infinity) of the extract group were significantly lower and the CL_{tot}/F and V_d/F were significantly higher (all $p<0.05$) than those in the control group 7 and 14 days after the start of extract administration [Fukunaga 2011].

Pharmacokinetic parameters of etoricoxib (EXB) were significantly altered by oral co-administration to rats of an unspecified extract (25 mg/kg/day) alongside EXB (15 mg/kg/day) for 3 weeks. The steady state peak plasma concentration and terminal half-life were reduced by 32% and 91% respectively, due to a more than 3-fold increase in its apparent clearance which was a concentration and time dependent effect [Radwan 2012].

Rats were given single oral doses of methotrexate (MTX; 5 mg/kg), alone or co-administered with either an unspecified extract (300 or 150 mg/kg) or diclofenac (25 mg/kg). The extract at a dose of 300 mg/kg significantly increased (both $p<0.05$) the AUC(0-t) and C_{max} of MTX, by 163% and 60% respectively, and at 150 mg/kg significantly ($p<0.05$) increased the AUC(0-t) of MTX by 55%. Diclofenac enhanced the C_{max} of MTX by 110% [Yang 2013].

Pharmacokinetics in humans

A study of the bioavailability of hypericin in 2 healthy volunteers after oral administration of an extract (300, 600 and 1200 mg at intervals of 7 days) demonstrated that plasma levels of hypericin were dose-dependent. After ingestion of a single dose of 600 mg of the extract by 12 volunteers, the following parameters were determined for hypericin: t_{max} 2.5 hours, C_{max} 4.3 ng/mL and a plasma half-life of about 6 hours [Weiser 1991].

A methanolic extract was administered orally as single doses of 900, 1800 and 3600 mg, containing 2.81, 5.62 and 11.25 mg of total hypericin. Maximum plasma concentrations of total hypericin, observed about 4 hours after administration, were 0.028, 0.061 and 0.159 mg/L respectively [Brockmöller 1997].

Oral administration to 13 healthy volunteers (at intervals of at least 10 days) of single doses of a methanolic extract containing 250, 750 and 1500 µg of hypericin and 526, 1578 and 3135 µg of pseudohypericin, respectively gave peak plasma levels of 1.3, 7.2 and 16.6 µg/L for hypericin and 3.3, 12.2 and 29.7 µg/L for pseudohypericin. C_{max} and AUC values for the lowest dose were disproportionately lower than those for the higher doses. Lag-times were determined as 1.9 hours for hypericin and 0.4 hours for pseudohypericin. Mean half-lives for absorption, distribution and elimination were 0.6, 6.0 and 43.1 hours after 750 µg of hypericin, and 1.3, 1.4 and 24.8 hours after 1,578 µg of pseudohypericin. After 14 days of oral treatment with 250 µg of hypericin and 526 µg of pseudohypericin, steady state levels of 7.9 µg/L for hypericin and 4.8 µg/L for pseudohypericin were achieved. Kinetic parameters in two subjects after intravenous administration resembled those after oral administration. Hypericin and pseudohypericin were initially distributed into volumes of 4.2 and 5.0 L respectively; at steady state the mean distribution volumes were 19.7 L for hypericin and 39.3 L for pseudohypericin; systemic bioavailability from the methanolic extract was about 14 and 21% respectively [Kerb 1996].

Hypericin levels in serum and skin blister fluid were determined in volunteers after oral administration of a hydromethanolic extract as a single dose of 6 tablets or 3 x 1 tablet daily for 7 days. Each tablet contained 300 mg extract, standardised to 900 µg of total hypericin. Six hours after the single high dose, mean levels of total hypericin were 43 ng/mL in serum and 5.3 ng/mL in skin blister fluid. After 3 tablets daily for one week, mean levels were 12.5 ng/mL in serum and 2.8 ng/mL in skin blister fluid [Schempp 1999].

Plasma levels of hyperforin were measured over a 24-hour period in volunteers treated with 300 mg of an ethanolic extract containing 14.8 mg of hyperforin. Maximum plasma levels of about 150 ng/mL were reached 3.5 hours after oral administration. Half-life and mean residence time of hyperforin were 9 and 12 hours respectively. Up to 600 mg of the extract, hyperforin kinetics were linear [Biber 1998].

In a phase I dose escalation study, patients received oral doses of hypericin once daily for 8 weeks, at either 0.05 mg/kg (n=12) or 0.1 mg/kg (n=7). At the end of the 8 week period, pharmacokinetic data revealed a long elimination half-life (mean values of 36.1 and 33.8 h respectively) and mean AUC determinations of 1.5 and 3.1 mg×h/mL respectively [Jacobson 2001].

In a placebo-controlled, randomised, double-blind study, 33 healthy volunteers received a methanolic (80%) extract alongside various co-medications (placebo, cimetidine and carbamazepine) for 7 days, after a run-in period of 11 days with extract alone. No significant differences in AUC(0-24), C_{max}, and t_{max} values for hypericin and pseudohypericin were observed between the treatment groups [Johne 2004].

The bioavailability of five constituents from an ethanolic (80%) extract was determined in two open clinical trials in 18 healthy male volunteers. Subjects received the extract (612 mg/day p.o.) either as a single oral dose or as a daily dose over a period of 14 days. Concentration/time curves were determined for hypericin, pseudohypericin, hyperforin, quercetin and isorhamnetin for 48 h after single dosing and for 24 h on day 14 at the end of 2 weeks of continuous daily dosing. After single dose administration, the key pharmacokinetic parameters were determined as follows: hypericin: AUC(0-infinity) = 75.96 ng×h/mL, C_{max} = 3.14 ng/mL,

t_{max} = 8.1 h, $t_{1/2}$ = 23.76 h; pseudohypericin: AUC(0-infinity) = 93.03 ng$_x$h/mL, C_{max} = 8.50 ng/mL, t_{max} = 3.0 h, $t_{1/2}$ = 25.39 h; hyperforin: AUC(0-max) = 1009.0 ng$_x$h/mL, C_{max} = 83.5 ng/mL, t_{max} = 4.4 h, $t_{1/2}$ = 19.64 h. Quercetin and isorhamnetin showed two peaks of maximum plasma concentration separated by about 4 h. Quercetin: AUC(0-infinity) = 318.7 ng$_x$h/mL, C_{max}, 1 = 47.7 ng/mL, t_{max}, 1 = 1.17 h, C_{max}, 2 = 43.8 ng/mL, t_{max}, 2 = 5.47 h, $t_{1/2}$ = 4.16 h; isorhamnetin: AUC(0-infinity) = 98.0 ng$_x$h/mL, C_{max}, 1 = 7.6 ng/mL, t_{max}, 1 = 1.53 h, C_{max}, 2 = 9.0 ng/mL, t_{max}, 2 = 6.42 h, $t_{1/2}$ = 4.45 h. Similar results were obtained under the steady-state conditions reached during multiple dose administration [Schulz 2005].

Interactions

The acute effects of an unspecified extract on CYP2D6 and CYP3A4 activity were studied in 7 healthy volunteers given a dose of 3×300 mg/day p.o. for 3 days. Enzyme activity was assessed by phenotyping, using as probes 30 mg dextromethorphan (DM) for CYP2D6 and alprazolam (ALPZ) for CYP3A4. Each probe was given before and during co-administration with the extract. Pharmacokinetics of ALPZ were measured in plasma, while DM and its metabolite dextrorphan (DX) were measured in urine after collection for 8 hours, and the metabolic ratio DM-DX was determined. Compared to baseline, no significant differences were observed for ALPZ or DM-DX ratios when probes were given during treatment with the extract [Markowitz 2000].

The effects of a methanolic extract (80%, V/V; 3 × 300 mg/day for 14 days) on the activity of CYP3A4 and CYP2D6 were assessed in 13 healthy volunteers by determination of the urinary 6-β-hydrocortisol/cortisol and dextromethorphan–dextrorphan (DM/DX) ratios at baseline and at the end of treatment [Roby 2001]. The CYP2D6 participants received an additional 30 mg of dextromethorphan hydrobromide on day 15. On the last day of both studies urine was collected over 24 hours and concentrations of 6-β-hydroxycortisol and cortisol and urinary DM–DX ratios were determined. The mean urinary 6-β-hydroxycortisol/cortisol ratio significantly increased (p=0.003) from a baseline value of 7.1 ± 4.5 to 13 ± 4.9. No significant change was seen for DM-DX ratios [Roby 2000].

The effects of a methanolic (80%) extract on the urinary excretion of D-glucaric acid, 6β-hydroxycortisol and free cortisol were investigated in order to assess its impact on the activity of hepatic xenobiotic metabolising enzymes. Forty-eight healthy volunteers (25 males and 23 females) received a daily dose of 1800 mg of extract for 14 days. Urinary excretion of D glucaric acid was unaffected after the 14-day treatment, whereas excretion of 6β-hydroxycortisol significantly increased from a mean baseline value of 254 µg/day to 369 µg/day (p<0.0001). The excretion of free cortisol was unaltered, but the ratio of 6β-hydroxycortisol to free cortisol changed significantly (p<0.001) from 9.9 at baseline to 14.3. Thus, high-dose treatment with the extract induced CYP3A activity in healthy volunteers as evidenced by increased 6β-hydroxycortisol excretion [Bauer 2002].

In a patient under long-term treatment with CyA (125 – 150 mg/day) and prednisolone (5 mg/day), stable blood levels of CyA between 100 and 130 pg/L suddenly decreased to around 70 pg/L, despite the daily dose being raised to 250 mg. The patient had started to drink a herbal tea containing St. John's wort to control seasonal depressive symptoms. Five days after cessation of the tea, CyA blood levels increased from 70 to 170 µg/L (250 mg/day), and a subsequent dose reduction to 175 mg/day resulted in blood levels of around 130 pg/L [Alscher 2003].

Treatment of four patients on methadone medication with an unspecified extract (900 mg/d) for between 14 and 47 days resulted in a major reduction of (R,S)-methadone concentration-to-dose ratios, with a median decrease to 47% of the original concentration (range: 19% - 60% of the original concentration). Two patients reported symptoms that suggested a withdrawal syndrome [Eich-Hochli 2003].

Twelve healthy men, 6 extensive metabolisers of CYP2C19 (2C19*1 / 2C19*1) and 6 poor metabolisers (4 with 2C19*2 / 2C19*2 and 2 with 2C19*2 / 2C19*3), were enrolled in a two-phase, randomised, crossover study. All subjects took oral doses of either an unspecified extract (0.3% hypericin and a minimum 4% hyperforin; 300 mg) or placebo three times daily for 14 days, and the activities of CYP2C19 and CYP1A2 were measured using mephenytoin and caffeine. The extract significantly increased CYP2C19 activity in CYP2C19 wild-genotype subjects, with urinary 4'-hydroxymephenytoin excretion raised by 151.5% ± 91.9% (p=0.0156), whereas no significant alteration was observed for CYP2C19 poor metabolisers. Repeated extract administration did not affect the CYP1A2 phenotypic ratio for either CYP2C19 genotype subjects [Wang 2004b].

In a trial involving 340 outpatients with major depression, treated with either a methanolic extract (80%; 900-1500 mg/day), SERT (50-100 mg/day) or placebo for 8 weeks, treatment adherence was assessed by measuring plasma hyperforin. Samples from the placebo and extract groups were assayed for hyperforin, and samples from the SERT group for SERT/N-desmethyl-SERT. Of the 104 patients randomised to placebo, 18 (17%) had detectable plasma hyperforin. Of the 97 patients randomised to the extract, 17 (17%) had no detectable plasma hyperforin. All the assayed SERT patients (n = 91) had detectable plasma SERT/N-desmethyl-SERT plasma concentrations. The clinical trial conclusions remained unchanged when only patients with plasma assay consistent with random assignment were included in the analyses [Vitiello 2005].

Healthy volunteers (n = 18) received a methanolic (80%, V/V) extract at a dose of 3 × 300 mg daily for 14 days. The CYP2D6 substrate debrisoquine (5 mg) was administered before and at the end of treatment. CYP2D6 activity was assessed using 8-hour debrisoquine urinary recovery ratios (DURR): [4-hydroxydebrisoquine/(debrisoquine + 4-hydroxydebrisoquine)]. No significant effect on CYP2D6 activity was found [Gurley 2008a].

The effects of an unspecified extract (3 x 300 mg/day p.o.) on oral contraceptive (OC) therapy (1 mg norethindrone and 20 µg ethinyl oestradiol), ovarian activity and break-through bleeding were studied in sixteen healthy women treated for two consecutive 28-day cycles in a single-blind sequential trial. Treatment with the extract resulted in a significant 13-15% (p=0.021) reduction in the dose exposure to the contraceptive. Breakthrough bleeding increased in the treatment cycles, as did evidence of follicle growth and probable ovulation [Murphy 2005].

To investigate whether administration of a methanolic (80%) extract at an oral dose of 3×300 mg together with an OC (20 µg ethinyl oestradiol and 1 mg norethindrone acetate) modified elevated serum androgen levels in OC treatment of hirsutism and acne, 15 healthy women received OC alone for 2 menstrual cycles, followed by two cycles with addition of the extract. No significant differences in testosterone, free testosterone, androstendione, dehydroepiandrosterone, 3α-diol-glucuronide, dehydrotestosterone and or sex hormone binding globulin were observed during the co-administration [Fogle 2006].

The time for recovery to baseline activity of CYP3A after induction by an unspecified extract was studied in 12 healthy males. The volunteers received an oral dose of 5 mg midazolam and from

the next day 3 × 300 mg/day of the extract p.o. for 14 days. On the last day of treatment, and 3 and 7 days later, they received the same dose of midazolam. Compared to baseline, oral clearance of midazolam was significantly (p=0.001) increased after the extract, from 65.3 ± 8.4 L/h to 86.8 ± 17.3 L/h. It returned to the control level 7 days after the last dose of extract (day 7; 59.7 ± 3.8 L/h) [Imai 2008].

Five cancer patients received irinotecan (IT) at a dose of 350 mg/m^2 i.v. once every 3 weeks. An unspecified extract was given at a daily dose of 3 x 300 mg for 14 days, from before the first IT administration until 4 days after IT administration. Myelosuppression was clearly more pronounced with IT alone (decrease in leukocyte and neutrophil counts of 56% and 63% respectively) than after co-administration with the extract (8.6% and 4.3% respectively). The AUC of the active metabolite decreased by 42% (p=0.033) [Mathijssen 2002].

In a study involving 16 healthy volunteers, the effects of a methanolic extract (80%, V/V; 3 × 300 mg/day p.o. for 14 days) on the activities of CYP1A2, CYP2D6, CYP3A4, *N*-acetyltransferase 2 (NAT2) and xanthine oxidase (XO) were assessed by phenotyping before and at the end of treatment. Probes for enzyme activities were ratios of metabolites 17MX/137MX from caffeine (CYP1A2), DM/DX (CYP2D6), endogenous 6-hydroxycortisol/cortisol (CYP3A4), AFMU/1MX from caffeine (NAT2) and 1MU/1MX from caffeine (XO), found in saliva or urine. From these metabolic ratios an induction of CYP3A4 by the extract was confirmed. CYP1A2 was induced only in females. The activities of CYP2D6, NAT2 and XO were not affected [Wenk 2004].

In a controlled, open-label study, acute and long-term effects of a methanolic extract (80%, V/V; 3 × 300 mg/day p.o. for 15 days) on the pharmacokinetics of voriconazole (VCZ) were investigated in 16 healthy male subjects. Since the extensive metabolism of VCZ is primarily mediated by CYP2C19 and CYP3A4, and to a minor extent by CYP2C9, subjects were genotyped for the CYP2C19 alleles *2 and *3. A single dose of VCZ (400 mg) was given on days -3 (baseline), 1 and 15. On days 1 and 15, the extract was administered 60 min before VCZ adminis¬tration. On day 1 of extract administration the AUC(0-10h) of VCZ was increased by 22% compared with control (15.5 ± 6.84 h×µg/mL versus 12.7 ± 4.16 h×µg/mL; p=0.02). After 15 days intake, the AUC(0-10h) was reduced by 59% compared with control (9.63 ± 6.03 h×µg/mL versus 23.5 ± 15.6 h×µg/mL; p=0.0004), with a corresponding increase in oral VCZ clearance (CL/F) from 390 ± 192 to 952 ± 524 mL/min (p=0.0004). The baseline CL/F of VCZ and the absolute increase in CL/F were smaller in carriers of 1 or 2 deficient CYP2C19*2 alleles compared with wild-type individuals (p<0.03) [Rengelshausen 2005].

The net effect of simultaneous administration of an inducer of CYP3A4 (St. John's wort) and an inhibitor (ritonavir) was investigated in 12 healthy volunteers in an open, fixed sequence study design. The CYP3A4 probe midazolam (MDZ) was given orally (4 mg) as a single dose in 4 different study phases: (i) at baseline, (ii) after a single oral dose of either a methanolic extract (80%, V/V; 300 mg) or ritonavir (3 × 100 mg) (n = 6 each), (iii) after 14 days of co-administration of ritonavir (2 × 300 mg daily) and the extract (3 × 300 mg daily), and (iv) at 2 days after cessation of both the extract and ritonavir. Each oral MDZ administration was followed by an intravenous MDZ injection of 2 mg 6 h later ('*semi-simultaneous administration*'). Combined administration of inducer and inhibitor resulted in a predominance of enzyme inhibition: co-administration of the extract and ritonavir with i.v. administration of MDZ showed an increase in the MDZ AUC(0–8h) to 180% of baseline value (p<0.05), whereas with orally administered MDZ, the AUC0–6h increased to 412% of baseline value (p<0.05). After cessation of the co-administered drugs, the AUC(0–6h) of orally administered MDZ decreased to 6% of the level observed during combined administration, and the AUC(0–8h) of i.v. administered midazolam decreased to 33% of the values observed during combined administration (p<0.001 for each) [Hafner 2010].

A male patient with ADHD who had been successfully treated with methylphenidate (20 mg/day), as measured by a reduction in Conner's score (CS) from 75.3 to 68.4, began additional self-medication with an unspecified extract (600 mg/day) six months after starting on the methylphenidate. Treatment with the extract for 4 months diminished the efficacy of methylphenidate, demonstrated by an increase in the CS from 68.4 to 74.9. Three weeks after discontinuation of the extract, the CS dropped back down again to 70.3 [Niederhofer 2007].

The interaction of atorvastatin (10 – 40 mg daily) and an unspecified extract (300 mg twice daily) was studied in a controlled, randomised, open, crossover study involving 16 patients with hypercholesterolaemia. After a 4-week run-in period, patients on a stable atorvastatin dose were randomised to additional treatment with either the extract or control for 4 weeks. The extract significantly increased the serum level of LDL cholesterol compared with control (2.66 mmol/L vs. 2.34 mmol/L; p=0.004) and of total cholesterol (5.10 mmol/L vs. 4.78 mmol/L; p=0.02). No significant changes were observed in HDL cholesterol or in triglycerides [Andren 2007].

The interaction of simvastatin (10 – 40 mg daily) and an unspecified extract (300 mg twice daily) was investigated in a similar study involving 24 patients with hypercholesterolaemia. After a 4-week run-in period, patients on a stable simvastatin dose received either the extract or control for 4 weeks. Compared to control, total cholesterol, LDL-cholesterol and triglycerides increased significantly in the extract group after 4 weeks: 4.56 vs. 5.08 mmol/L (p<0.001), 2.30 vs. 2.72 mmol/L (p<0.0001) and 1.30 vs. 1.54 mmol/L (p=0.0155) respectively. No significant differences were observed for HDL-cholesterol [Eggertsen 2007].

Table 2 below summarises the interactions of St. John's wort extracts with other drugs from clinical studies:

Preclinical safety data
Systematic studies on single dose toxicity, reproductive toxicity and carcinogenicity of St. John's wort extracts have been carried out by major manufacturers, but not published.

Acute toxicity
No data available

Repeated dose and chronic toxicity studies
No significant tissue lesions or adverse events were observed in male Long-Evans rats fed St. John's wort (SJW; locally collected in the fully blooming stage) at 5% of their diet for 119 days. Feeding SJW in conjunction with *Senecio jacobaea* did not influence the chronic toxicity of *Senecio* as assessed by rat survival time. SJW did not affect hepatic copper levels [Garrett 1982].

No signs of toxicity were observed following administration of dried herb to rats at up to 2 g/kg/day for one year, at 3 g/kg/day for 28 days, and to dogs at 2 g/kg/day for 1 year. Tolerability levels of < 10 g/kg wet weight, eaten at the flowering stage, and < 2.65 mg/kg for hypericin were demonstrated in sheep. Non-specific toxic symptoms, including slight load damage to liver and kidneys, were observed after administration of 0.9 and 2.7 g/kg of a methanolic extract (80%, V/V) for 26 weeks in pivotal toxicological models in rats and dogs respectively [Morgan 2005].

TABLE 2: CHANGES IN THE PHARMACOKINETICS OF THE FOLLOWING DRUGS BY CO-ADMINISTRATION OF ST. JOHN'S WORT

Drug	Subjects	Type of preparation, posology (mg/day) and duration of administration	Effect on blood levels (AUC)	Presumed Mechanism	References
Alprazolam	Healthy subjects	900 mg of a methanolic (80%) extract for 12-14 days	53.6% decrease	CYP3A4	[Markowitz 2003]
Amitriptyline	Healthy subjects (n = 12)	900 mg of a methanolic (80%) extract for 12-14 days	22% decrease	CYP3A4	[Johne 2002]
Boceprevir	Healthy subjects (n = 17)	600 mg of an ethanolic (60%) extract for 14 days	No significant effect		[Jackson 2014]
Bupropion	Healthy subjects (n = 18)	975 mg of an unspecified extract for 14 days	14.3% decrease	CYP2B6	[Lei 2010]
Caffeine	Healthy subjects (n = 12)	900 mg of a methanolic (80%) extract for 2 weeks	No significant effect		[Wang 2001]
Carbamazepine	Healthy subjects (n = 8)	900 mg of a methanolic (80%) extract for 14 days	No significant effect		[Burstein 2000]
Ciclosporin	Transplant patients (n=7)	900 mg of a methanolic (80%) extract for 14 days	Increased clearance	CYP3A4, Pgp	[Ruschitzka 2000] [Mai 2000] [Karliova 2000] [Mandelbaum 2000] [Barone 2001]
Ciclosporin	1 patient	Unspecified extract	Significant decrease	CYP3A4, Pgp	[Barone 2000]
Ciclosporin	Kidney transplants (n = 11)	600 mg of a methanolic extract	42% decrease	CYP3A4, Pgp	[Bauer 2003]
Ciclosporin	Kidney transplant (n = 30)	Unknown	47% decrease	CYP3A4, Pgp	[Breidenbach 2000]
Ciclosporin	Healthy subjects (n = 21)	900 mg of a methanolic (80%) extract for 10 days	38.6% decrease	CYP, Pgp	[Dresser 2003]
Ciclosporin	Transplant patients (n = 10)	900 mg of a methanolic (80%) extract	51.9% decrease	CYP, Pgp	[Mai 2004]
Clozapine	1 Patient	900 mg unspecified extract for 3 weeks	65.2% decrease in trough levels	CYP1A2 CYP3A4	[Van Strater 2011]
Dextromethorphan	Healthy subjects (n = 12)	900 mg of methanolic (80%) extract	No significant effect	CYP2D6	[Wang 2001]
Desogestrel	Healthy women (n = 18)	600 and 900 mg of a methanolic (80%) extract for 4 weeks	43.9% decrease	CYP3A4	[Pfrunder 2003]
Digoxin	Healthy subjects (n = 13)	900 mg of a methanolic (80%) extract for 10 days	25% decrease	Pgp	[Johne 1999]
Digoxin	Healthy subjects (n = 96)	Different extracts for 10 days	mg/day hyperforin / % decrease 28.9 / 24.8 21.1/ 26.6 10.6/ 17.7 5.3 /1.8 3.56/ 7.3 2.6/ -1.5 0.83/ -5.6 0.13/ 5.2 0.3/ 9 0.04/0.3 Placebo/6.4	Pgp	[Mueller 2004]
Digoxin	Healthy subjects (n = 18)	900 mg of a methanolic (80%) extract for 14 days	23% decrease	Pgp	[Gurley 2008b]
Digoxin	Healthy subjects (n = 8)	900 mg of a methanolic extract (80%) for 14 days	18% decrease	Pgp	[Dürr 2000]
Ethinyloestradiol	Healthy women (n = 12)	900 mg of an unspecified extract (3% hyperforin) for 28 days	No significant effect		[Hall 2003]
Ethinyloestradiol	Healthy women (n = 18)	600 and 900 mg of a methanolic (80%) extract for 4 weeks	No significant effect		[Pfrunder 2003]

Drug	Subjects	Type of preparation, posology (mg/day) and duration of administration	Effect on blood levels (AUC)	Presumed Mechanism	References
Ethinyloestradiol	Healthy women (n = 16)	900 mg of an unspecified extract for two times 28 days	14.1% decrease	CYP, Pgp	[Murphy 2005]
Fexofenadine	Healthy subjects (n = 12)	900 mg of a methanolic (80%) extract for 14 days	14% decrease, not significant	Pgp	[Wang 2002]
Fexofenadine	Healthy subjects (n = 21)	900 mg of a methanolic (80%) extract for 10 days	48.5% decrease	Pgp	[Dresser 2003]
Finasteride	Healthy volunteers (n = 12)	600 mg of a methanolic (80%) extract for 2 weeks	58% decrease	CYP3A4	[Lundahl 2009]
Gliclazide	Healthy volunteers (n = 21)	900 mg/of a methanolic (80%) extract for 2 weeks	33% decrease	CYP2C9	[Xu 2008]
Ibuprofen	Healthy volunteers (n = 21)	900 mg of an methanolic (80%) extract	No significant decrease		[Bell 2007b]
Imatinib	Healthy volunteers (n = 12)	900 mg of a methanolic (80%) extract for 2 weeks	30% decrease	CYP3A4, Pgp, BCRP	[Frye 2004]
Imatinib	Healthy volunteers (n = 10)	900 mg of an unspecified extract for 2 weeks	32% decrease	CYP3A4, Pgp,	[Smith 2004a; Smith 2004b]
Indinavir	Healthy volunteers (n = 8)	900 mg of a methanolic extract for 2 weeks	57% decrease	CYP3A4, Pgp	[Piscitelli 2000]
Irinotecan	Cancer patients (n = 5)	900 mg of an unspecified extract for 18 days	42% decrease of SN-38	CYP3A4	[Mathijssen 2002]
Ivabradine	Healthy subjects (n = 12)	900 mg of a methanolic (80%) extract for 14 days	61.7% decrease	CYP3A4, Pgp	[Portoles 2006]
Methadone	2 Patients	900 mg of a methanolic extract (> 3% hyperforin) for 31 days	47% decrease	CYP3A4	[Eich-Hochli 2003]
Midazolam	Healthy subjects (n = 12)	900 mg of a methanolic (80%) extract for 2 weeks	52.3% decrease	CYP3A4	[Wang 2001]
Midazolam	Healthy subjects (n = 12)	900 mg of an unspecified extract for 28 days	Significant decrease	CYP3A4, Pgp	[Gurley 2002]
Midazolam	Healthy subjects (n = 21)	900 mg of a methanolic (80%) extract for 10 days	30.6% decrease	CYP3A4	[Dresser 2003]
Midazolam	Healthy women (n = 12)	900 mg of an unspecified extract (3% hyperforin) for 28 days	35% decrease	CYP3A4	[Hall 2003]
Midazolam	Healthy subjects (n = 42)	Different extracts for 14 days	mg/day hyperforin / % decrease 41.25 / 80.0 12.06 / 48.0 8.04 / 36.0 5.36 / 32 2.68 / 22 0.13 / 21	CYP3A4	[Mueller 2006]
Mycophenolate	Transplant patients (n = 10)	600 mg of a methanolic (80%) extract for 14 days	No significant effect		[Mai 2003]
Nevirapine	HIV patients (n = 5)	unknown	decrease	CYPs, Pgp	[De Maat 2001]
Nifedipine	Healthy subjects (n = 12)	900 mg of a methanolic extract for 14 days	45.2% decrease	CYP3A4, Pgp	[Wang 2007]
Norethindrone	Healthy women (n = 12)	900 mg of unspecified extract (3% hyperforin) for 28 days	About 14% decrease	CYP3A4	[Hall 2003]
Norethindrone	Healthy women (n = 16)	900 mg of unspecified extract for two times 28 days	12.6% decrease	CYP, Pgp	[Murphy 2005]
Omeprazole	Healthy subjects (n = 12)	900 mg of a methanolic (4% hyperforin) extract for 14 days	37.9% decrease	CYP3A4, CYP2C19	[Wang 2004c]
Oxycodon	Healthy subjects (n = 12)	900 mg of a methanolic (80%) extract for 15 days	50% decrease	CYP3A4	[Nieminen 2010]
Phenprocoumon	Healthy subjects (n = 10)	900 mg of a methanolic (4% hyperforin) extract for 14 days	17.4% decrease	CYP3A4	[Maurer 1999]

Drug	Subjects	Type of preparation, posology (mg/day) and duration of administration	Effect on blood levels (AUC)	Presumed Mechanism	References
Phenytoin	Healthy subjects (n = 12)	900 mg methanolic (4% hyperforin) extract for 14 days	Increased metabolism	CYP2C19	[Wang 2004b]
Pravastatin	Healthy subjects (n = 16)	900 mg of unspecified extract for 14 days	No significant effect		[Sugimoto 2001]
Prednison Prednisolone	Healthy subjects (n = 8)	900 mg of a methanolic extract (> 3% hyperforin) for 4 weeks	No significant effect		[Bell 2007a]
Quazepam	Healthy subjects (n = 13)	900 mg of an unspecified extract for 14 days	28.5% decrease	CYP2C9	[Kawaguchi 2004]
Repaglinide	Healthy subjects (n = 15)	975 mg of an unspecified extract for 14 days	No significant effect		[Fan 2011]
Simvastatin	Healthy subjects (n = 16)	900 mg of unspecified extract for 14 days	48% decrease	CYP3A4, Pgp	[Sugimoto 2001]
Tacrolimus	Transplant patients (n = 10)	600 mg of a methanolic (80%) extract for 14 days	57.8% decrease	CYP3A4, Pgp	[Mai 2003]
Tacrolimus	Healthy subjects (n = 10)	900 mg of a methanolic (80%) extract for 18 days	35.3% decrease	CYP3A4, Pgp	[Hebert 2004]
Talinolol	Healthy subjects (n = 9)	900 mg of a methanolic (80%) extract for 12 days	31% decrease	Pgp	[Schwarz 2007]
Theophylline	Healthy subjects (n = 12)	900 mg of an unspecified extract for 15 days	No significant effect		[Morimoto 2004]
Verapamil	Healthy subjects (n = 8)	900 mg of an unspecified extract for 14 days	78% (R-verapamil) and 80% (S-verapamil) decrease	CYP3A4, Pgp	[Tannergren 2004]
Voriconazole	Healthy subjects (n = 16)	900 mg of a methanolic (80%) extract for 15 days	59% decrease	CYP3A4, CYP2C9, CYP2C19	[Rengelshausen 2005]
Warfarin	Healthy subjects (n = 12)	3000 mg of an unspecified extract for 14 days	27% and 24.6% decrease for S- and R-warfarin	CYP3A4, CYP2C9, CYP1A2	[Jiang 2004]
Zolpidem	Healthy subjects (n = 14)	900 mg of a methanolic (80%) extract for 14 days	30.3% decrease	CYP3A4, CYP2C9 CYP1A2	[Hojo 2011]
Low-dose hyperforin					
Alprazolam	Healthy subjects (n = 28)	240 mg of a low hyperforin extract for 10 days	No significant effect		[Arold 2005]
Caffeine	Healthy subjects (n = 28)	240 mg of a low hyperforin extract for 10 days	No significant effect		[Arold 2005]
Ciclosporin	Transplant Patients (n = 10)	900 mg of an unspecified low hyperforin extract	No significant effect	CYP, Pgp	[Mai 2004]
Desogestrel	Healthy women (n = 16)	500 mg of a low hyperforin ethanolic extract (57.9%) for 14 days	No significant effect		[Will-Shahab 2008]
Digoxin	Healthy subjects (n = 28)	240 mg of a low hyperforin extract for 10 days	No significant effect		[Arold 2005]
Ethinylestradiol	Healthy women (n = 16)	500 mg of a low hyperforin ethanolic extract (57.9%) for 14 days	No significant effect		[Will-Shahab 2008]
Midazolam	Healthy subjects (n = 42)	900 mg of an unspecified low hyperforin extract for 14 days (Hyperforin 0.13 mg/day)	No significant effect		[Mueller 2006]
Midazolam	Healthy subjects (n = 20)	1000 mg of an unspecified low hyperforin extract or 14 days	No significant effect		[Mueller 2009]
Tolbutamide	Healthy subjects (n = 28)	240 mg of a low hyperforin extract for 10 days	No significant effect		[Arold 2005]

Mutagenicity and carcinogenicity

Genotoxicity
No increase in the extent of DNA fragmentation was observed on addition of a methanolic (80%, V/V) extract to rat cortical cells. The cells were incubated for 18 hours with concentrations up to 40 µg/mL [Perovic 1995].

Genotoxicity of a standardised aqueous ethanolic extract was investigated in various *in vivo* and *in vitro* test systems. No suggestion of mutagenicity was found. The testing involved: A) Hypoxanthine guanidine phosphoribosyl transferase-test in Chinese hamster V79 cells with extract concentrations up to 0.50 µg/mL (without S9-mix) or 4 µg/mL (with S9-mix); B) Unscheduled DNA synthesis assay in primary rat hepatocytes with extract concentrations up to 1.37 µg/Ml; C) Cell transformation test using S9-mix activated Syrian hamster embryo cells, the extract was added in concentrations up to 10 µg/Ml; D) Fur spot test in NMRI mice with oral doses of up to 10 mL/kg of test substance; and E) Chromosome aberration test with bone marrow cells of Chinese hamsters receiving oral doses of up to 10 mL/kg of the extract [Okpanyi 1990].

Carcinogenicity
No data are available

Reproductive toxicity

In vitro experiments
Hamster oocytes were incubated with an extract before sperm/oocyte interaction. Penetration was prevented by a very high dose of 0.6 mg/mL, but 0.06 mg/mL had no effect [Vandenbogaerde 1998].

In an explanted Sprague-Dawley rat embryo model, enabling the study of embryonic development during the critical period of organogenesis from 9.5 to 11.5 gestational days, exposures to high concentrations of hypericin (71.0 and 142.0 ng/mL) demonstrated a significantly lower total morphological score ($p=0.002$) and number of somites ($p<0.001$) compared to control. A significant negative linear trend in total morphological score ($p<0.001$), yolk sac diameter ($p=0.01$), and number of somites ($p<0.01$) was observed with increasing concentration of hypericin. No statistical difference was detected in crown-rump length [Chan 2001].

In vivo experiments
Exposure of Sprague-Dawley rats to diets containing 0, 180, 900, 1800 or 4500 ppm of an unspecified extract beginning on gestational day 3 and ending at off-spring weaning on postnatal day (PND) 21 did not reveal any effects on maternal weight gain, duration of gestation or extract-related behavioural alterations. Brain weights of offspring at adulthood indicated no significant effects of the extract [Cada 2001].

A methanolic (80%) extract was administered to female rats during the period of organogenesis (gestational days 9-15). Thirty inseminated Wistar rats received oral doses of saline or 36 mg/kg of the extract. No clinical signs of maternal toxicity were observed and none of the variables analysed in foetuses and placentas showed significant differences [Borges 2005].

The effect of treatment with high doses of an unspecified methanolic extract (100 and 1000 mg/kg/day), administered prenatally and during breastfeeding, on the level of transcripts of mdrla, mdr1b, mrp1, mrp2 and cyp3A2 genes was studied in Wistar rats. All transcripts were detected in the liver, and their level of expression increased from foetuses to adults. Extract administration, at both dosages, caused a significant ($p<0.01$) decrease of the levels of mdr1a, mdr1b, mrp1 and mrp2 in the livers of foetuses, and an increase ($p<0.01$) in the levels of mdrla, mdrlb, mrp2 and cyp3A2 in the mothers [Garrovo 2006].

High concentrations of hyperforin (> 1 µM) inhibited mouse embryonic stem (ES) cell population growth ($p<0.05$) and induced apoptosis ($p<0.05$) in fibroblasts (representing adult tissues). Hyperforin affected ES cell differentiation into cardiomyocytes in a dose-dependent manner ($p<0.05$). Analysis of tissue-specific marker expression also revealed that hyperforin at high concentrations partially inhibited ES cell differentiation into mesodermal and endodermal lineages [Nakamura 2013].

Effects on offspring
Treatment of male CD rats for 14 days with 500 mg/kg daily of a methanolic (80%, V/V) extract, hypericin 0.1 mg/kg, or hypericin combined with procyanidin B_2 1:25, did not lead to any changes in the reproductive organs [Butterweck 2001a].

The cognitive impact of prenatal exposure to an extract (0.3% hypericin, solvent not specified, 182 mg/kg/day) was studied in CD1 mice. The extract or a placebo was consumed in food bars for 2 weeks before mating and throughout gestation. One offspring per gender from each litter (verum 13, placebo 12) was tested using the juvenile runway with adult memory, adult Morris spatial water maze, adult passive avoidance and adult straight water runway, followed by a dry Cincinnati maze. Learning occurred in both genders in all tasks ($p<0.003$) with no significant differences between treatments at the final trial. Female offspring exposed to the extract, rather than to placebo, required more time to learn the MWM task ($p<0.05$). Post-learning sessions did not show any significant differences [Rayburn 2001a].

Female mice were treated orally with 180 mg/kg/day of the same extract or placebo for 2 weeks before conception and throughout gestation. Gestational age at delivery and litter size did not differ between the groups. Body weight, body length and head circumference from postnatal day 3 to adulthood did not differ from controls regardless of gender. The only difference between the groups was a temporary delay in the eruption of upper incisors in male offspring exposed to the extract. The extract did not affect reproductive capacity, perinatal outcomes, or growth and development of second-generation offspring [Rayburn 2001b].

A methanolic extract (0.3% total hypericin) given to Wistar rats at doses of 100 and 1000 mg/kg/day by gavage during pregnancy and lactation revealed severe damage in the livers and kidneys of new-born animals euthanised post-natally on days 0 and 21 at both doses. The lesions were more severe at the higher dose and in animals that were breastfed for 21 days [Gregoretti 2004].

A methanolic extract (80%, V/V) at a dose of 36 mg/kg was given to 30 pregnant Wistar rats during organogenesis (days 9 - 15 of pregnancy). No clinical signs of maternal toxicity were observed in the necropsy on day 21. The indices of implantation and resorption, and toxicological parameters (such as foetus and placenta weight), were not significantly different compared to control [Borges 2005].

In a systematic review on the safety of St. John's wort during pregnancy and lactation, no impact on maternal weight gain or duration of gestation were observed in Sprague-Dawley rats who were exposed to oral doses of St. John's wort 1 to 25 times the recommended human dose. Offspring body weights were similar to controls, although there was a tendency towards lower weight after treatment with St. John's wort. No alterations in behaviour were found [Dugoua 2006].

Organ mRNA expression and protein content of Abcb1 were determined following oral administration of an 80% methanolic extract (hypericin 0.3%) to pregnant female Wistar rats at 1 g/kg for 9 days during late pregnancy. Abcb1 expression was substantially lower in foetal than in maternal organs and significantly induced in the maternal jejunum and placenta [Saljé 2012].

An extract (0.3% hypericin; not further specified) was administered orally to pregnant Wistar rats at 36, 72 or 144 mg/kg per day.The highest dose demonstrated greater head-dipping activity in the hole-board test ($p<0.001$) and reduced immobility in the tail suspension test ($p<0.001$) and FST ($p<0.001$), at 10 and 60 days post-treatment [Vieira 2013b].

Maternal exposure to FLU (7.5 mg/kg) or an unspecified extract (100 mg/kg), given daily by gavage during pregnancy and lactation, showed that FL, but not the extract interfered with reproductive parameters in adult male rats [Vieira 2013a].

Hepatotoxicity

Hypericin and pseudohypericin at a dose of 50 µg (oral, i.v., i.p.) exhibited low toxicity in BALB/c mice and prevented viral-induced manifestations following retrovirus infection. Neither hypericin nor pseudohypericin had an impact on alkaline phosphatase, lactate dehydrogenase, AST, ALT or cholesterol. Haematology and blood biochemistry were unaffected [Meruelo 1988].

Intraperitoneal administration of 280 mg/kg/day of an extract (0.29% total hypericins; 2.29% hyperforin; solvent not specified), 1 mg/kg/d of hypericin or 10 mg/kg/d of hyperforin to male Swiss Webster mice for periods ranging from 4 days to 3 weeks did not induce changes in ALT, and no histological changes in the livers were observed [Bray 2002a; Bray 2002b].

Phototoxicity

Photosensitization caused by St. John's wort has mainly been reported in veterinary studies, especially in unpigmented skin of grazing animals [James 1992]. Dose-dependent phototoxic symptoms were observed in calves within 4 hours after single oral doses of dried St. John's wort at 3 – 5 g/kg [Araya 1981].

Merino ewes (an unpigmented breed, freshly shorn of wool), dosed orally with 5.7, 4.0 or 2.85 g/kg of dried St. John's wort (corresponding to 5.3, 3.7 or 2.65 mg/kg of hypericin) and then exposed to bright sunlight, had a tolerance level for hypericin of less than 2.65 mg/kg [James 1992].

Foetal calf serum or albumin strongly inhibited the photocytotoxic effects of pseudohypericin, but not those of hypericin, in A431 tumour cells [Bernd 1999]. Human keratinocytes were cultured in the presence of various concentrations of an extract and irradiated with UVA or UVB. A phototoxic effect was only seen with high hypericin concentrations (≥ 50 µg/mL) after irradiation with UVA [Rayburn 2001b].

The phototoxic potential of 3 unspecified extracts from various sources, as well as some of their main constituents, was investigated in an immortalised human keratinocyte cell line (HaCaT cells) under UVA irradiation (250 and 700 mJ/cm^2). Known phototoxic compounds like chloropromazine and psoralenes like 5-MOP served as controls. The extracts demonstrated cytotoxicity and photocytotoxicity in a dose- and UVA-dose dependent manner (IC_{50} without irradiation 35 to 74 µg/mL, with irradiation 24 to 84 µg/mL). Hypericin also evoked severe phototoxic effects and was the main phototoxic constituent. Among the tested flavonoids quercitrin and rutin were found to be cytotoxic. However, quercitrin co-administration with the extract diminished the phototoxicity of the latter [Wilhelm 2001].

The phototoxic and apoptosis-inducing capacity of pseudohypericin was compared to hypericin in human leukaemic lymphoma cells (Jurkat). Treatment with both photoactivated hypericin and pseudohypericin resulted in a dose-dependent inhibition of cell proliferation, whereas compounds without photoactivation had no effect at the concentrations tested. The IC_{50} of hypericin was lower (100 ng/mL) than pseudohypericin (200 ng/mL) ($p<0.05$). A dose-dependent increase of DNA fragmentation was observed following treatment with both photoactivated compounds in an apoptosis assay [Schempp 2002b].

Phototoxicity of hypericin to the eye was evaluated in human lens epithelial cells. Cells were exposed to 0.1 – 10 µM hypericin and irradiated with 4 J/cm^2 UVA or 0.9 J/cm^2 visible light. Neither hypericin alone nor light exposure alone reduced cell viability. In contrast, cells exposed to hypericin in combination with UVA or visible light underwent necrosis and apoptosis. The ocular antioxidants lutein and *N*-acetyl cysteine did not prevent damage [He 2004].

The cytotoxicity of various extracts (prepared with ethanol, water, chloroform, hexane) and purified compounds was examined in three cell lines. All extracts exhibited significant cytotoxicity; those prepared in ethanol (no hyperforin, 3.6 µM hypericin, 134.6 µM flavonoids) showed between 7.7 and 77.4% cell survival ($p<0.0001$ and 0.01), whereas the chloroform and hexane extracts (hyperforin, hypericin and flavonoids not detected) showed approximately 9.0% ($p<0.0001$) and 4.0% ($p<0.0001$) survival. Light-sensitive toxicity was observed primarily with the ethanol extracts sequentially extracted following removal of material extracted in either chloroform or hexane. The absence of light-sensitive toxicity with the extracts suggests that the hypericins were not playing a prominent role in the toxicity of the extracts [Schmitt 2006].

The phototoxic effects of 0.1 - 10 µM hypericin were investigated further in combination with visible light on the human retina. Fluorescence emission confirmed hypericin uptake by human retinal pigment epithelial cells (hRPE). When irradiated with 0.7 J/cm^2 of visible light ($\lambda > 400$ nm) loss of cell viability as measured by mitochondrial activity and cell membrane damage was observed. Hypericin in irradiated hRPE cells significantly changed the redox equilibrium of glutathione and decreased the activity of glutathione reductase. Increased lipid peroxidation was correlated to hypericin concentration in hRPE cells and visible light radiation [Wielgus 2007].

Mild phototoxicity was observed after topical administration of hypericin at a dose of 0.01 to 1% [Boiy 2008].

Neither hypericin exposure nor UVA irradiation alone reduced the cell viability of human pigmented and unpigmented melanoma cells, immortalised keratinocytes and melanocytes. An exposure to 1 µM UVA-activated hypericin did not change cell viability, while 3 µM activated hypericin induced necrosis in pigmented melanoma cells and melanocytes and apoptosis in cells without melanosomes (non-pigmented melanoma cells and keratinocytes). Hypericin localisation in the endoplasmic reticulum in these cells, shown by fluorescent microscopy, further supported a disruption in cellular processing and induction of cell death. It was demonstrated that intracellular accumulation of hypericin induced mitochondrial-associated caspase-dependent apoptosis [Davids 2008].

The photobiochemical properties of an extract and 19 known

constituents were characterised with a focus on generation of ROS, lipid peroxidation and DNA photocleavage as indicators of photosensitive, photoirritant and photogenotoxic potentials. The ROS assay revealed the photoreactivity of the extract and some constituents as evidenced by type I and/or II photochemical reactions under light exposure. Only hypericin, pseudohypericin and hyperforin exhibited *in vitro* photoirritant potential [Onoue 2011].

Fluorescence confocal microscopy was used to visualise binding between hypericin and α-crystallin, a major lens protein, in a human lens epithelial (HLE) cell line. UVA irradiation of hypericin-treated HLE cells resulted in a decrease in α-crystallin detection concurrent with an accumulation of the tryptophan oxidation product *N*-formylkynurenine (NFK). Examination of actin in HLE cells indicated that it accumulated NFK resulting from hypericin-mediated photosensitisation. Wavelengths < 400nm provided an incomplete protection against α-crystallin modification and NFK accumulation [Ehrenshaft 2013].

Clinical safety data

At therapeutic dose levels (up to the equivalent of 6 g of drug), occasional mild gastrointestinal disturbances, nausea, restlessness, fatigue, headache, insomnia or allergic reactions have been reported [Albrecht 1994; Bergmann 1993; Bernhardt 1996; De Maat 2001; Engesser 1996; Grube 1999; Halama 1991; Hänsgen 1996; Häring 1996; Harrer 1994; Harrer 1991; Harrer 1999; Hoffmann 1979; Holsboer-Trachsler 1999; Hübner 1994; Kaehler 1999; Kalb 2001; Kasper 2010; Knüppel 2004; Kugler 1990; Laakmann 1998; Lecrubier 2002; Lehrl 1993; Lenoir 1999; Martinez 1994; Meier 1997; Mueller 1998; Pieschl 1989; Quandt 1993; Rahimi 2009; Reh 1992; Röder 2004; Schakau 1996; Schlich 1987; Schmidt 1989; Schrader 2000; Schrader 1998; Schulz 2001; Sepehrmanesh 1999; Sommer 1994; Vorbach 1994; Warnecke 1986; Werth 1989; Wheatley 1997; Witte 1995; Woelk 2000a; Woelk 1994]. Onset of mania and hypomania were reported in several patients with latent bipolar disorders [Barbenel 2000; Fahmi 2002; Moses 2000; Nierenberg 1999; O'Breasail 1998; Saraga 2005; Schneck 1998; Stevinson 2004]. In one patient taking up to 3600 mg of a methanolic extract acute psychotic symptoms were observed that recovered completely after termination of treatment [Shimizu 2004].

A detailed overview of the pharmacological, toxicological and clinical literature on St. John's wort is provided in several reviews [Ernst 1995; Greeson 2001; Nathan 2001; Russo 2014].

In a meta-analysis of 35 double-blind randomised trials and observational studies of St. John's wort mono-preparations, drop-out and adverse event rates were extracted from 17 observational studies including 35,562 patients. Drop-out rates ranged between 0% and 5.9%. No serious adverse effects were reported. In 24 double-blind, randomised trials (n = 1,334 St. John's wort, n = 1,292 placebo) drop-out and adverse effect rates were similar to placebo (OR = 0.82 (CI = 0.64 to 1.06) and 0.79 (CI = 0.51 to 1.03) respectively). In 7 studies *versus* older antidepressants, drop-out and adverse effect rates were significantly lower in the St. John's wort group (OR = 0.25 (CI = 0.14 to 0.45) and 0.39 (CI = 0.31 to 0.50)). In 6 studies *versus* SSRIs, drop-out and adverse effect rates were slightly but not significantly lower in the St. John's wort group (OR = 0.60 (CI = 0.31 to 1.15) and 0.75 (CI = 0.52 to 1.08)) [Knüppel 2004].

The clinical efficacy of some standardised methanolic and ethanolic extracts in the treatment of mild, moderate and severe major depression has been demonstrated in 38 controlled clinical trials and two recent meta-analyses. Sixteen post-marketing surveillance studies with such preparations, based on a total of 34,804 patients, recorded an incidence of adverse events (AEs) among patients between 0% and 6%. Of these studies, the four large-scale surveillance studies with a total of 14,245 patients recorded a rate of AEs ranging from 0.1% to 2.4% and a drop-out rate due to AEs of 0.1-0.9%. AEs associated with St. John's wort treatment were mild and transient in nearly all cases [Schulz 2006b].

In an observational study 1778 patients with depressive symptoms received an extract (3.5-6:1; 425 – 1700 mg) for 12 weeks. The incidence of ADRs was 3.54% and decreased continuously from the first visit onwards; serious ADRs did not occur [Melzer 2010].

Spontaneous ADR reports (n = 84) reported to the Australian Therapeutic Goods Administration (TGA) between 2000 - 2013 were obtained for St. John's wort (SJW). The majority of reported ADRs for SJW were concerning females aged 26-50 years (28.6%). The central nervous system (45.2%) was most affected by ADRs to SJW [Hoban 2015].

Pregnancy and lactation

A prospective, observational, controlled cohort study followed three groups of pregnant women who contacted the Motherisk Program (a teratogen information service in Canada): 54 women using any St. John's wort product at any time during their pregnancy, a disease-match group of 54 women with depression under conventional antidepressant therapy and 54 healthy women not exposed to any known teratogen. Pregnancy outcomes were similar in the three groups. There were 3, 2, and 0 malformations in the St. John's wort, disease-matched and healthy groups respectively. The rate of spontaneous abortion was higher in the St. John's wort (n = 11) and disease-matched control (n = 7) groups compared to the healthy control group (n = 5). Neither finding reached statistical significance. The rate of malformation was as expected in the population [Moretti 2009].

In a cohort study, 33 breastfeeding women presenting to the teratogen/toxicant counselling service taking St. John's wort preparations (not further specified) at an average dose of 704.9 mg/day (range 225 - 2150 mg/day) (group 1) were compared to 101 disease-matched controls (group 2) and 33 age- and parity-matched healthy controls (Group 3). Whereas only 1 infant each in groups 2 and 3 was reported to be colicky, there were 2 cases of "colic", 2 of "drowsiness" and 1 of "lethargy" in group 1. No significant difference was observed in the frequency of maternal reports of decreased milk production among the groups, nor a difference in infant weight over the first year of life [Lee 2003].

In a case report, hypericin and hyperforin were measured in the breast milk of a woman receiving 300 mg of an 80% methanolic extract 3 times a day. Only hyperforin was excreted at minimal levels into breast milk, but not into infant´s plasma [Klier 2002].

An excretion of hyperforin into breast milk was confirmed in 5 other breastfeeding women. Low plasma concentrations of hyperforin were detected in breastfed infant plasma, with a milk-to-plasma ratio of 0.04 to 0.13 corresponding to relative doses of 0.9% to 2.5%. No adverse effects were observed in mothers or infants [Klier 2006].

Phototoxicity and photosensitization

A double-blind, placebo-controlled study with 40 volunteers showed that photosensitivity was not induced by therapeutically relevant dosages of total hypericin, i.e. up to 1 mg daily for 8 days [Wienert 1991].

In a study involving i.v. administration of synthetic hypericin to HIV-infected patients, reversible symptoms of phototoxicity

were observed at the highest dosage regime, which was 35-times higher than the highest oral dosage of total hypericin used in the therapy of depressive disorders [James 1992].

In a placebo-controlled, randomised clinical trial (n = 13), high daily oral doses of up to 3600 mg of a methanolic extract (80%, V/V; corresponding to 11.25 mg hypericin) were administered to volunteers. No dose-related trend in light sensitivity to solar stimulated irradiation (SSI) was observed. Sensitivity to UVA light was slightly but significantly (p=0.03) increased only after the highest dose (from 10.8 J/cm^2 (placebo) to 8.7 J/cm^2 (extract)). After multiple doses of 5.6 mg total hypericin for 15 days, the minimal erythema dose (MED) was assessed. A small but significantly increased SSI sensitivity was observed (decrease in MED from 0.17 to 0.16 J/cm^2, p=0.005) and an increased sensitivity to UVA light (decrease in MED from 9.9 to 7.8 J/cm^2, p<0.0001). In spite of the high doses used, frequency of side effects was equal to placebo medication and light sensitivity was only marginally increased [Brockmöller 1997].

After topical application of St. John's wort oil (110 µg/mL hypericin; n = 8) and St. John's wort ointment (30 µg/mL hypericin; n = 8) to the volar forearms of sixteen volunteers (with skin types II and III), solar simulated radiation was applied. No change of the MED was detected on visual assessment after either treatment (p>0.05). Using photometric measurement of skin erythema, an increase of the erythema-index was observed after treatment with the oil (p≤0.01) [Schempp 2000].

In a prospective randomised study the effect of a methanolic (80%) extract on skin sensitivity to ultraviolet B (UVB), ultraviolet A (UVA), visible light (VIS) and solar simulated radiation was determined. Volunteers of skin types II and III received either a single dose equivalent to 5.4 or 10.8 mg hypericin (n = 48) or an initial dose equivalent to 5.4 mg hypericin, and subsequently 3 x 2.7 mg hypericin per day for 7 days (n = 24). No significant influence on the erythema-index or melanin-index was detected after either dose regimen, with the exception of a marginal influence on UVB induced pigmentation (p=0.0471) in the single-dose study [Schempp 2003b].

Volunteers received 3 x 340 mg of an ethanolic (60%, V/V) extract daily corresponding to 3 mg hypericin over a period of 10 days. Even at this high hypericin concentration, and in spite of the observed decrease in visual and objective MED reduction from 20 to 14 J/cm^2 (p=0.047) and from 33.73 to 22.0 J/cm^2 (p=0.014) respectively, and an increase in erythema intensity (in 73% of subjects), the maximum slope of the dose-response curve (which is an indicator of an individual's susceptibility to burning) was not increased by the extract [Beattie 2005].

The effect of two different ethanolic extracts (50% and 80%) was assessed on photosensitivity with respect to MED after 14 days of treatment. Both open, multiple-dose, phase I studies were conducted in 20 healthy men receiving 612 and 900 mg of the extracts respectively. Mean MED at 24 h, in both studies, was not significantly different between baseline and after 14 days of treatment [Schulz 2006a].

The effect of extract use on cataractogenesis was investigated utilising data from a large, population-based sample. Self-reported data on St. John's wort use in the past 12 months and cataracts were obtained from the 2002 National Health Interview Survey, a nationally representative population-based sample. Crude and adjusted OR and 95% CI between SJW use and cataracts were estimated using logistic regression. After adjusting for potential confounding characteristics, participants that reported having cataracts were 59% more likely to report St. John's wort use (OR 1.59; 95% CI 1.02-2.46) [Booth 2009].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Supplement 2009
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Supplement 2009
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003

LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Second Edition, 2003
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
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ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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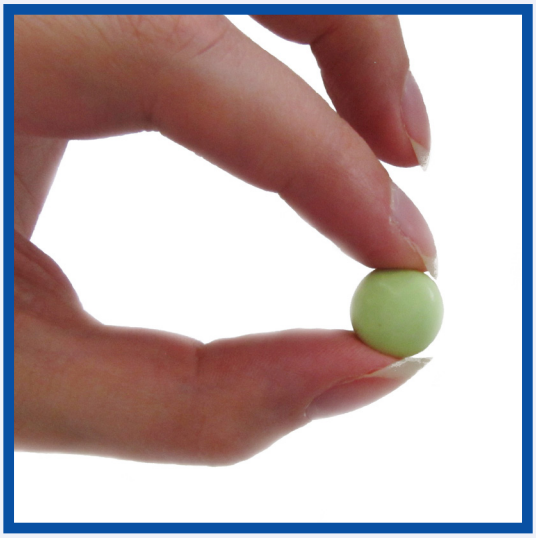

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FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books*, ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009,* but are not available online for copyright reasons.

After purchase of a single monograph*,* the specific items to be downloaded are:

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Information on the member organizations and people involved in the production of ESCOP monographs and other activities can be found on the website (www.escop.com):

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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-kB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-b	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

JUGLANDIS FOLIUM

2024

Walnut leaf

DEFINITION

Walnut leaf consists of the whole or cut dry leaves of *Juglans regia* L. (Juglandaceae). It contains not less than 2.0 per cent of tannins adsorbed by hide powder, expressed as pyrogallol ($C_6H_6O_3$, Mr 126.1) and calculated with reference to the dried drug. The material complies with the monograph of the Deutscher Arzneimittel-Codex [Wallnussblätter - Juglandis folium, 2023].

CONSTITUENTS

Characteristic constituents are ellagitannin-type tannins (approximately 10%) and flavonoids (approximately 3%, mainly quercetin, kaempferol, hyperoside and their derivatives) [Amaral 2004; Pereira 2007; Zhao 2014; Suschke 2014; Lichius 2016]. Phenolcarbonic acids e.g. caffeic, vanillic, ellagic and chlorogenic [Amaral 2004; Pereira 2007; Amaral 2008; Gutiérrez Ortiz 2018]. The contents of phenolic compounds in young leaves are higher than their amounts in the mature leaves [Salimi 2012; Cosmulescu 2012]. Further characteristic compounds include diarylheptanoids, juglone derivates, anthraquinones, megastigmanes and oligomeric proanthocyanidins [Forino 2016; Schwindl 2019].

Fresh leaves contain reduced naphthoquinones as glycosides and aglycones [Babula 2009], the major aglycone is juglone [Lichius 2016]. Glycosides are cleaved enzymatically to aglycones [Morant 2008]. Juglone is an unstable compound which is easily polymerized to pigments (from yellow to black), therefore in dry leaves, juglone is present only in traces and in some extracts prepared from the dry plant material it is undetectable [Amaral 2004; Lichius 2016].

Other constituents are traces of essential oil including monoterpenes α- and β-pinene [Fojtova 2008], sesquiterpenes including caryophyllene and germacrene D [Nahrstedt 1981; Buttery 1986; Farag 2008; Boukhari 2013]; organic acids including malic acid, shikimic acid, citric acid and ascorbic acid [Santos 2013].

CLINICAL PARTICULARS

Therapeutic indications
Minor inflammatory conditions of the skin, excessive perspiration of hands and feet [Jänicke 2003; Bäumler 2007]

In these indications, the efficacy is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Adult daily dose: 4-6 g of the comminuted herbal substance in 200 mL of boiling water as a decoction. To be applied topically as a compress or a partial bath [Suschke 2014, Schilcher 2016; Lichius 2016].

Method of administration
For cutaneous application.

Duration of use
No restriction.

Contraindications
Open wounds and extensive skin injuries. Not to be applied as an occlusive treatment [Bonamonte 2001; Suschke 2014; Corazza 2019].

Special warnings and special precautions for use
Topical application to large areas should be avoided. In rare cases, contact dermatitis may occur [Bonamonte 2001; Suschke 2014; Corazza 2019].

E/S/C/O/P MONOGRAPHS

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
None reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Hypoglycaemic activity
A dry methanolic extract (DER 4-6:1) was investigated for hypoglycaemic activity by assessing its effect on glucose uptake in C2C12 myocytes, protein tyrosine phosphatase 1B (PTP1B) inhibition and peroxisome proliferator-activated receptor gamma (PPARγ) activation. The extract enhanced the glucose uptake rate at 25 µg/mL compared to untreated cells. This activity may partly be explained by the inhibition of PTP1B in a concentration range of 10 to 100 µg/mL. PPARγ was not affected by the extract in the same range [Pitschmann 2014].

In an activity-guided isolation, two megastigmanes were shown to affect glucose uptake in HepG2 and Caco-2 cells. (3S,5R,6R,7E,9S)-3,5,6,9-tetrahydroxymegastigman-7-ene significantly ($p<0.001$) increased the uptake in HepG2 cells at 0.05 µg/µL, whereas 3,6,9-trihydroxymegastigman-7-ene was more effective in Caco-2 cells [Forino 2016].

Antioxidant and radical scavenging activity
The radical scavenging activities of acetone, ethyl acetate and methanolic extracts (not further specified) were assessed at a concentration of 62.5 µg/mL in the DPPH assay. The methanolic extract was the most effective DPPH radical scavenger with an inhibition of 67.8% [Miliauskas 2004].

A lyophilised 40% ethanolic extract (not further specified) demonstrated scavenging activity against all the reactive species tested. IC_{50} values for the ROS $O_2^{\bullet-}$ and H_2O_2 were 47.6 and 383 µg/mL respectively [Almeida 2008].

The antioxidant activities of methanolic and petroleum ether extracts (yield 27.7% and 1.1% respectively) were assessed by their ability to inhibit oxidative haemolysis of human erythrocytes induced by 2,2´-azobis(2-amidinopropane) dihydrochloride (AAPH). Under the oxidative action of AAPH, only the methanolic extract significantly ($p<0.05$) protected the erythrocyte membrane from haemolysis (IC_{50} 0.06 mg/mL) [Carvalho 2010].

A methanolic extract exhibited greater antioxidant activity than a decoction (not further specified). Respective EC_{50} values were 65.91 µg/mL vs. 78.97 µg/mL for DPPH scavenging activity, 75.87 µg/mL vs. 83.71 µg/mL for reducing power, 189.92 µg/mL vs. 269.27 µg/mL for µ-carotene bleaching inhibition and 20.36 µg/mL vs. 114.68 µg/mL for TBARS inhibition [Santos 2013].

A dry 95% ethanolic extract (yield 7.93%) had an ORAC value of 2543.5 trolox equivalents/g and reducing power with an IC_{50} value of 121.7 µg/mL [Wang 2015].

Enzyme inhibitory activity
The mushroom tyrosinase inhibitory activity of a dry 80% methanolic extract containing 16.25% ellagic acid was tested by monitoring the formation of dopachrome, an intermediate in melanogenesis. The IC_{50} of the extract was 505 µg/mL [Oezer 2007].

An aqueous dry extract (DER 8.65:1) inhibited acetylcholinesterase and butylcholinesterase by 40.09 and 56.23% respectively at a concentration of 0.1 mg/mL [Karakaya 2019].

Antibacterial activity
The antimicrobial activities of a purified extract (acetone–water 2:1) against acne-promoting microorganisms were determined using the disk diffusion method and compared to tea tree oil, doxycycline and clindamycin. The extract at concentrations of 10-20% exhibited significantly ($p<0.02$) larger inhibition zones than tea tree oil at concentrations of 10 to 20% when tested against *Propionibacterium acnes* and *Staphylococcus epidermidis*. Whereas only the 15% concentration extract had significantly ($p<0.03$) greater antimicrobial activity than tea tree oil against *S. aureus*. The extract had comparable activity with doxycycline (5 µg) or clindamycin (2 µg) against *Staphylococcus* species but less activity against *P. acnes*. There were considerably lower levels of microbial resistance for the extract than for tea tree oil [Qa'dan 2005].

A 75% ethanolic extract (not further specified) was active against *L. monocytogenes*, *L. ivanovii* and *L. murrayi* with IC_{50} values of 12.5, 25 and 12.5 µg/mL respectively [Altanlar 2006].

Lyophilized water extracts (not further specified) selectively inhibited the growth of Gram positive bacteria (*Bacillus cereus*, *B. subtilis*, *S. aureus*) with *B. cereus* being the most susceptible (MIC 0.1 mg/mL). The extracts were not active against Gram-negative strains [Pereira 2007].

A methanolic extract (not further specified) exhibited an antimycobacterial effect against *M. tuberculosis* strain H37Rv with an MIC of 125 µg/mL [Cruz-Vega 2008].

The hydrodistilled essential oil exhibited broad spectrum inhibition against all the bacteria tested, with Gram-positive strains (*S. epidermidis* MTCC-435, *Bacillus subtilis* MTCC-441, *S. aureus*; MIC 15.62 µg/mL) being more susceptible than Gram-negative (*Proteus vulgaris* MTCC-321, *Pseudomonas aeruginosa* MTCC-1688, *Salmonella typhi*, *Shigella dyssenteriae*, *Klebsiella pneumoniae* and *E. coli*; MIC 31.25-62.50 µg/mL) [Rather 2012].

A 70% ethanolic extract (not further specified) was active against *Staphylococcus epidermidis* with an MIC of 156.25 µg gallic acid equivalents/mL [Nicu 2018].

Antifungal activity
The antifungal activity of an 80% methanolic extract (not further specified) was evaluated against nineteen *Candida* strains, including *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis* species. The extract exerted an inhibitory effect against all the tested *Candida* strains (inhibition zones 9-13 mm), with an MIC_{50} value of 187.5 µg/mL for the most susceptible strain of *C. tropicalis* [Martins 2015].

Cardiovascular effect
An aqueous extract (not further specified) inhibited the maximal contractile response induced by noradrenaline on isolated rat

thoracic aorta in a concentration-dependent manner (IC_{50} 3.2 mg/mL) [Perusquía 1995].

Antiproliferative activity
A methanolic extract (yield 27.7%) was tested for antiproliferative activity using the human renal cancer cell lines A-498 and 769-P, and the colon cancer cell line Caco-2. The extract showed concentration-dependent growth inhibition towards all cell lines with IC_{50} values of 0.226, 0.352 and 0.229 mg/mL, respectively [Carvalho 2010].

A methanolic extract and a decoction (not further specified) exhibited similar antiproliferative activity on breast (MCF-7), colon (HCT-15) and hepatocellular (HepG2) carcinoma cell lines (50% growth inhibition (GI_{50}) 209-285 µg/mL) [Santos 2013].

A hexane extract (not further specified) exhibited dose-dependent antiproliferative activity against PC3 human prostate cancer cells in the concentration range of 5-100 µg/mL with an IC_{50} value of 48.4 µg/mL, and induced significant ($p<0.05$) apoptosis [Li 2015].

Bioassay-guided fractionation of a chloroform extract led to the isolation of 5-hydroxy-3,7,4′-trimethoxyflavone, lupeol, daucosterol, 4-hydroxy-α-tetralone, β-sitosterol, 5,7-dihydroxy-3,4′-dimethoxyflavone and regiolone. All compounds inhibited proliferation of MCF-7 and BHY cells in a concentration-dependent manner. 5,7-Dihydroxy-3,4′-dimethoxyflavone and regiolone showed cytotoxic effects on MCF-7 and BHY cells (IC_{50} 21–51 µM) yet were not toxic to normal NIH3T3 fibroblasts. MCF-7 growth inhibition was attributed to apoptosis. The caspase cascade was not involved in the apoptosis [Salimi 2014].

In vivo experiments

Antidiabetic effect
An 80% ethanolic extract (DER 1:16.9) and a water decoction (DER 1:20) were administered orally to mice and assessed in the oral glucose tolerance test. While the ethanolic extract increased glycaemia, the decoction exhibited hypoglycaemic activity [Neef 1995].

Sprague Dawley rats with alloxan-induced diabetes were fed a diet supplemented with 60 g/kg b.w./day dry walnut leaf for 15 days, while an untreated diabetic group and a non-diabetic group received only the standard diet. Walnut leaf reduced the blood glucose level significantly compared with the untreated diabetic group (9.03 vs. 14.36 mmol/l, $p<0.05$). The decreased volume density of islets in pancreatic tissue, percent of β cells and islets size in untreated diabetic control rats was improved by the supplementation [Jelodar 2007].

The effects of an aqueous extract on serum glucose and lipid levels were reported in two similar studies each involving male Wistar albino rats divided into three groups, a healthy control group and two groups with streptozotocin-induced diabetes. One of the diabetic groups also received the extract (400 mg/kg p.o.) for 4 weeks. The extract significantly ($p<0.05$) attenuated the diabetes-induced increases in glucose, triglycerides, cholesterol, LDL-C and the induced decrease in HDL-C. The LDL-C/HDL-C ratio and total cholesterol/HDL-C ratio were also significantly ($p<0.05$) decreased compared with the diabetic control group [Gholamreza 2008; Divband 2010].

An ethanolic extract (not further specified) was examined in male Wistar rats divided into four groups: non-diabetic rats, alloxan-induced diabetic rats with no treatment, alloxan-induced diabetic rats treated with an ethanolic extract (200 mg/kg) and alloxan-induced diabetic rats treated with glibenclamide (0.6 mg/kg). All treatments were administered intraperitoneally for 4 weeks. Diabetes induction with alloxan was carried out 2 weeks before starting the treatment. Blood samples were collected three times: before injection of alloxan (Time 0), at 2 weeks (Time 1) and at 6 weeks (Time 2) to determine glycosylated haemoglobin (HbA1C), insulin, glucose, cholesterol, LDL-C, HDL-C, VLDL-C and triglycerides. At Time 1 the levels of blood glucose, cholesterol, VLDL-C and LDL-C were increased ($p<0.05$) and the levels of HDL-C and plasma insulin decreased ($p<0.05$) in all diabetic rats compared to Time 0 and non-diabetic rats. At Time 2, the extract as well as glibenclamide resulted in decreases ($p<0.05$) in blood glucose along with an increase in the insulin level. Serum cholesterol, triglycerides, LDL-C, and VLDL-C levels were decreased in the glibenclamide and the extract groups in comparison with Time 1. In the extract group, the LDL-C level decreased compared to diabetic control ($p<0.05$). The HDL-C level was raised with both the extract and glibenclamide and showed no significant difference when compared with the non-diabetic group and Time 0 values. HbA1C in diabetic control rats was significantly higher ($p<0.05$) compared with non-diabetic rats. The extract and the glibenclamide groups had lower mean HbA1C levels, which were not significantly different compared with the non-diabetic group and Time 0 values. The extract and glibenclamide attenuated the diabetes-induced changes in a comparable manner, bringing values back to near those of the non-diabetic controls. A histological study at Time 2 revealed that the size of islets of Langerhans enlarged to almost normal size in both treated groups as compared with diabetic rats with no treatment [Asgary 2008].

Alloxan-induced diabetic male Wistar rats were treated orally with a 70% methanolic extract (yield 17%) at doses of 250 (I) or 500 mg/kg (II), acarbose 20 mg/kg (III) or vehicle (IV) and normal rats treated with vehicle alone (V). The postprandial blood glucose level was examined after a single dose and after three weeks of daily treatment. The extract had a significant hypoglycaemic effect in both short- and long-term models. Post-prandial glucose levels were at their lowest 8 hours after the single oral dose, with a significant ($p<0.0001$) reduction in glucose of 53% in goup III, 40% in group I and 29% in group II as compared to group IV. After 3 weeks of treatment, post-prandial glucose levels had significantly ($p<0.0001$) decreased by 27% in group I and 43.7% in group III as compared to group IV. There was no change in the insulin and glut-4 gene expression [Teimori 2010].

The antidiabetic effect of a 90% ethanolic extract (DER 8.7:1) was studied in diabetic Wistar rats. Group I were healthy controls fed a normal diet, while diabetes was induced with streptozotocin in groups II, III and IV. The extract was administered orally to groups III and IV at 200 and 400 mg/kg b.w. respectively for 28 days. Treatment with the extract resulted in significant ($p<0.05$) and dose-dependent reductions in blood glucose, glycosylated haemoglobin, LDL, triglycerides and total cholesterol as compared to the diabetic control group (II), with the higher dose reducing levels to those comparable with the healthy control group. Only at the higher dose were insulin and HDL significantly ($p<0.05$) increased compared to group II [Mohammadi 2011].

The effects on blood glucose and lipids of oral administration of a 90% ethanolic extract (DER 8.7:1) were investigated in rats with diabetes induced by streptozotocin-nicotinamide. The rats were divided into six groups, treatments commenced one week after induction of type II diabetes and continued for 4 weeks. Group I: healthy control; II: healthy control receiving 200 mg/kg extract; III: diabetic control; IV: diabetic animals receiving 200 mg/kg extract; V: diabetic animals receiving 400 mg/kg extract; VI: diabetic animals receiving 500 mg/kg metformin). Dose-dependent reductions of fasting blood glucose levels, HbA1c (I: 6.46%; II: 6.20%; III: 13.58%; IV: 11.61%; V: 8.29%; VI: 6.38), total cholesterol (63.11; 61.36; 115.73; 71.33; 61.28; 64.27 mg/dL)

and serum triglycerides (75.28; 74.21; 159.66; 135.32; 89.30; 81.36 mg/dL) were detected after 4 weeks in rats treated with the extract and metformin as compared to the diabetic controls ($p<0.05$). No differences between groups I and II were observed in these parameters [Mohammadi 2012].

The effects of cyclohexane and ethanol extracts (not further specified) on blood glucose and sorbitol dehydrogenase activity were investigated in male Sprague-Dawley rats with diabetes induced with streptozotocin. The rats were divided into seven groups and treated orally for 30 days as follows: control and diabetic control, control cyclohexane and control ethanol (250 mg/kg b.w.), diabetic cyclohexane (250 mg/kg cyclohexane extract), diabetic ethanol 150 and diabetic ethanol 250 (150 and 250 mg/kg ethanolic extract respectively). The cyclohexane extract improved the blood glucose level compared to the other diabetic groups. Both extracts significantly ($p<0.05$) reduced the activity of sorbitol dehydrogenase compared to diabetic controls [Abbasi 2017].

The effects of a methanolic extract (200 mg/kg/day p.o.) were studied in rats with experimentally-induced diabetic nephropathy. One week after induction with streptozotocin the first group began treatment with the extract ("preventive") and at 5 weeks treatment with the extract started for a further group ("curative"). Rats received the extract or saline until the end of the 8th week. Both the "preventive" and the "curative" treatments attenuated the diabetes-induced histopathological changes in the kidney and significantly ($p<0.008$) decreased fasting blood sugar levels compared to the diabetic control group. Increased caspase-3, COX-2, PARP and iNOS expressions observed in diabetic rats were also significantly ($p<0.009$) attenuated by the treatment [Nasiry 2019].

Type II diabetes was induced in rats by administering streptozotocin and nicotinamide. One group was left untreated, a second group received 200 mg/kg/day p.o. of an ethanolic extract (DER 3.43:1), a third group was treated with 1000 mg/kg/day p.o. of metformin, while a fourth group served as a non-diabetic healthy control group. Fasting blood glucose levels decreased significantly ($p<0.05$) in the groups treated with the extract and metformin for 28 days compared to the untreated diabetic group. Average body weight decreased significantly ($p<0.001$) in the untreated diabetic group compared to those in the treated groups. Adiponectin and Fibronectin Type III Domain Containing 5 levels were significantly ($p<0.01$) increased in the treated groups as compared to diabetic controls [Uslu 2019].

Anti-inflammatory and antinociceptive effect
Ethanolic (DER: 4.8:1) and aqueous (DER: 4:1) extracts were evaluated for anti-inflammatory and antinociceptive activities in Swiss albino mice. Single oral doses of the extracts (500 mg/kg) demonstrated significant antinociceptive activity against p-benzo-quinone-induced abdominal contractions, with a 29.5% ($p<0.01$) and 67.9% ($p<0.001$) decrease in the number of writhings for the aqueous and ethanolic extracts respectively. Using the same dose, the ethanolic extract exhibited significant ($p<0.01$) anti-inflammatory activity against carrageenan-induced hind paw oedema without inducing any gastric damage [Erdemoglu 2003].

The effect of concurrent administration of an extract (not further specified) and morphine on analgesia was investigated in rats divided into seven groups (control, sham and five experimental groups). The first experimental group received 2 mg/kg of morphine, 3 further groups received 2 mg/kg of morphine concurrently with the extract at doses of 0.5, 1 and 1.5 mg/kg and the last group received only 1.5 mg/kg of the extract, all i.p. 15 minutes before the formalin test. Effects at minutes 0-5 and 15-60 were assessed for the acute and chronic phases of pain respectively. The extract at the dose of 1.5 mg/kg showed a dose-dependent nociceptive effect in the acute phase of the formalin test. Moreover, the extract significantly ($p<0.05$) enhanced the antinociceptive effect of morphine, especially in the acute phase of the formalin test [Mokhtari 2008].

In mice, antinociceptive activity was studied using hot-plate and writhing tests, and anti-inflammatory effects were studied using xylene-induced ear edema and cotton pellet tests. The aqueous (2.87 and 1.64 g/kg i.p.; $p<0.001$) and ethanolic (extracting solvent 85% ethanol, after defatting with petroleum ether, not further specified; 2.44 and 1.17 g/kg i.p.; $p<0.001$ and $p<0.01$ respectively) extracts showed significant antinociceptive activity in the hot-plate test. Pretreatment with naloxone (2 mg/kg, s.c.) did not inhibit the effect. The extracts (aqueous: 0.41 and 1.64 g/kg; ethanol: 0.292-2.044 g/kg) exhibited significant ($p<0.001$) antinociceptive activities in the writhing test, which were also not blocked by naloxone. In the xylene test, both extracts showed anti-inflammatory activity in distinct doses. The extracts showed anti-inflammatory activity against chronic inflammation in the cotton pellet test [Hosseinzadeh 2011].

Hepatoprotective effect
The hepatoprotective effect of a 40% ethanolic extract (not further specified) against carbon tetrachloride-induced liver damage was assessed in rats randomly divided into seven groups: control, CCl_4 (0.5 mL/kg b.w. i.p.), extract alone (0.2 g/kg b.w./day p.o.), and CCl_4 plus extract (0.05, 0.1, 0.2 and 0.4 g/kg b.w./day p.o.). After 28 days, liver damage was assessed by serum biochemical parameters (ALAT, ASAT, ALP and albumin), antioxidant enzymes (SOD and CAT) and histopathological parameters. Administration of the extract at 0.2 and 0.4 g/kg significantly ($p<0.05$) lowered ALAT, ASAT and ALP levels in CCl_4-treated rats. The extract increased SOD and CAT and reduced fatty degeneration of the liver, cytoplasmic vacuolization and necrosis in CCl_4-treated rats [Eidi 2013].

Sedative effect
Oral administration of a chloroform extract (DER 131.8:1) of fresh leaves and of juglone induced sedative activity in two mouse models (actimeter and sleep potentiation). Significant ($p<0.005$) increases in pentobarbital-induced sleep duration compared to control (olive oil) were observed in mice treated with the extract (2.89- and 1.64-fold for 6.5 and 3.25 mg/kg doses respectively) and juglone (2.47- and 1.5-fold for 0.125 and 0.0625 mg/kg respectively), as well as for diazepam (2 mg/kg p.o.; 3.71-fold). In the actimeter test, both the chloroform extract (3.25-26 mg/kg) and juglone (0.0625-0.5 mg/kg) significantly ($p<0.05$ to $p<0.005$) decreased motor activity compared to control [Girzu 1998].

Anthelmintic effect
Petroleum ether, methanol and water extracts (not further specified) were evaluated for their anthelmintic activity against adult Indian earthworms (*Pheretima posthuma*). Groups of worms were tested with extract concentrations of 10, 25 and 50 mg/mL, distilled water (control) or piperazine citrate (10 mg/mL in distilled water, positive control). All of the extracts showed dose-dependent activity. The methanolic extract demonstrated the greatest anthelmintic activity, and even at the lowest concentration (10 mg/mL) was comparable to piperazine citrate (time taken for paralysis 18.16 vs. 19.36 min) [Das 2011].

Clinical studies
The hypoglycaemic effect of a dry aqueous extract (not further specified) was investigated in a randomized, double-blind, placebo-controlled study involving 58 type II diabetes patients (40-65 years; inclusion criteria: HbA1C > 7%, fasting blood glucose level<250 mg/L, taking maximum dose of metformin and glibenclamide). One group (n=30; 11 M/19 F) received 2 × 200 mg extract daily while the other group (n=28; 12 M/16 F)

received placebo. Fasting blood samples were collected at the beginning of the study and after two months of treatment. The levels of HbA1c and blood glucose were determined as the main outcomes and insulin, SGOT, SGPT and ALP levels as secondary outcomes. Compared to baseline, there were significant decreases in serum fasting HbA1C (from 8.5% to 7.6%; p=0.000) and fasting blood glucose levels (from 165 mg/dl to 144 mg/dL; p<0.017) in the extract group. HbA1C but not glucose was also significantly (p=0.042) decreased in the placebo group. Insulin was significantly (p<0.007) increased in the extract group (from 8.6 U/l to 10.4 U/l) but not in the placebo group [Hosseini 2014a].

In a randomized, double-blind, placebo-controlled study, the effects of an extract (70% ethanol, DER 40:1) on hyperglycaemia and lipid profiles were investigated in 61 type II diabetic patients. Patients with fasting blood glucose between 150 and 200 mg/dL, glycated haemoglobin (HbA1c) between 7% and 9%, and aged between 40 and 60 years, were randomly divided into two groups. The first group (n=32; 15 M/17 F) received 100 mg dry extract two times a day for 3 months while the other group (n=29; 13 M/16 F) received placebo. The standard anti-diabetic therapy (metformin, glibenclamide, nutritional regimen) was continued in both groups. The extract group exhibited significant (p<0.05) decreases compared to baseline in levels of fasting blood glucose (165 mg/dL. vs. 143 mg/dL), HbA1c (8.51% vs. 7.52%), total cholesterol (192.15 mg/dL vs. 179.68 mg/dL) and triglyceride levels (162.53 mg/dL vs.146.38 mg/dL); the decreases were also significantly (p<0.05) greater than for placebo. There was no significant change in insulin level in either group. Patients in the verum group were significantly (p<0.05) more satisfied with the treatment, with 84.5% finding the treatment to be moderately, potently or very potently effective, compared with 3.4% of the placebo group [Hosseini 2014b].

In a randomized, double-blind, placebo-controlled trial, 40 diabetic patients received either 100 mg of a 70% ethanolic (DER 6.45:1) extract (n=20; 1 M/19 F) or placebo (n=20; 2 M/18 F) 2 times daily for 8 weeks. Blood glucose, HbA1c level, body weight, body mass index, blood pressure, lipid profile, serum insulin and insulin resistance were compared at the beginning and at the end of the study. Only body weight, body mass index and systolic blood pressure were significantly (p<0.05) decreased compared to baseline in the extract group. In the placebo (microcrystalline cellulose) group, postprandial glucose and HbA1c levels decreased significantly (p<0.05) compared to baseline. At the end of the study, there were no significant differences between the two groups in the observed end points [Rabiei 2018].

Pharmacokinetic properties
No data available.

Preclinical safety data

Acute toxicity
The role of cell-to-cell interactions in herbal-induced liver toxicity was investigated in monocultures of human hepatocytes (HepG2) and in co-cultures of HepG2 cells and human monocytes (THP1). Cells were treated with various concentrations (1–500 µg/mL) of an aqueous extract for 24 h. In the MTT test, the extract reduced MTT formation by about 60% at a concentration of 500 µg/mL in HepG2 cells. There were no negative effects observed in the co-culture system for all concentrations of the extract. There were no significant changes of LDH levels in the culture medium seen after 24 h of exposure to the extract [Saad 2006].

The acute toxicity of an aqueous and an 85% ethanolic (not further specified) extract was evaluated after i.p. administration. The LD_{50} values were 5.5 and 3.3 g/kg b.w. respectively [Hosseinzadeh 2011].

A methanolic extract and a decoction (not further specified) did not exhibit cytotoxicity on non-tumour liver primary PLP2 cells (GI_{50} > 400 µg/mL) [Santos 2013].

Clinical safety data
In a randomized, double-blind, placebo-controlled study involving type II diabetic patients taking 2 × 200 mg/day of a dry aqueous extract (not further specified; n=30) or placebo (n=28), no differences in SGOT and SGPT levels were observed after 2 months of treatment and no gastrointestinal side effects were reported [Hosseini 2014a].

In a randomized, double-blind, placebo-controlled study involving type II diabetic patients receiving 2 × 100 mg dry extract (70% ethanol, DER 40:1; n=32) or placebo (n=29) daily for 3 months, only mild adverse effects occurred. At least one gastrointestinal event (especially mild diarrhoea at the beginning of the treatment) was experienced by 39% of the patients in the extract group versus 6% in the placebo group. Eleven patients in the extract group complained of mild adverse events at the beginning of the study, such as diarrhoea, vertigo and anxiety compared with 4 patients in the placebo group; general tolerability of the treatment within the groups was not statistically different. The mean values of the analysed laboratory parameters (ALT, AST, ALP, BUN and creatinine) did not significantly change during the trial [Hosseini 2014b].

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MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Online Series, 2019
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Online Series, 2021
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Online Series, 2019
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Online Series, 2019
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHAMOMILLAE ROMANAE FLOS	Roman Chamomile Flower	Online Series, 2019
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Online Series, 2021
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Online Series, 2021
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EPILOBII HERBA	Willow Herb	Online Series, 2024
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Online Series, 2023
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
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HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
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HELICHRYSI FLOS	Sandy Everlastin Flower	Online Series, 2019
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HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John´s Wort	Online Series, 2018

JUGLANDIS FOLIUM	Walnut leaf	Online Series, 2024
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Online Series, 2020
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MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Online Series, 2021
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passionflower Herb	Online Series, 2023
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Online Series, 2020
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Online Series, 2021
PRUNI AFRICANAE CORTEX	Pygeum Bark	Online Series, 2020
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher´s Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Online Series, 2021
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SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TILIAE FLOS	Lime Flower	Online Series, 2022
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Online Series, 2020
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
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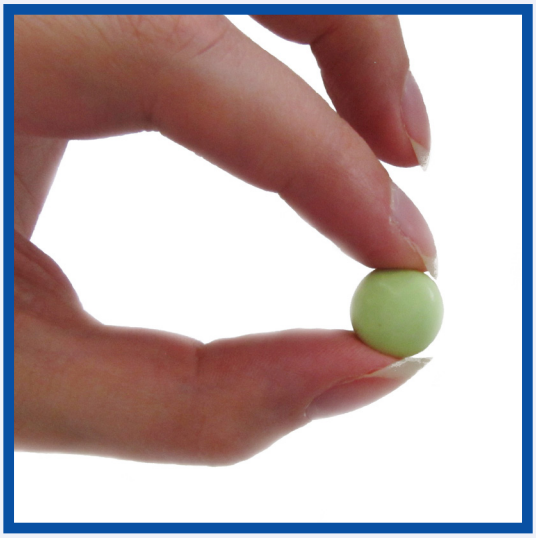

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FOREWORD

It is a great pleasure for me, on behalf of my colleagues in ESCOP, to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

LEONURI CARDIACAE HERBA 2019

Motherwort

DEFINITION

Motherwort consists of the whole or cut flowering aerial parts of *Leonurus cardiaca* L. It contains not less than 0.2% of flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; M_r 464.4) and calculated with respect to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Motherwort].

CONSTITUENTS

The main characteristic constituents are flavonoids including glycosides of apigenin, kaempferol and quercetin [Kartnig 1985; Schulz 1973; Reuter 1970]; a C_{15}-iridoidglucoside, leonuride [Hänsel 1993; Weinges 1973; Schilling 1975], diterpenes of the labdane and clerodane types [Malakov 1985; Brieskorn 1979; Papanov 1998; Knöss 1998], caffeic acid-4-rutinoside [Tschesche 1980] and the phenylpropanoid lavandulifolioside [Milkowska-Leyck 2002].

Other constituents include the alkaloids stachydrine [van Eijk 1952] and leonurine [Gulubov 1970]. In a more recent study using a new HPLC method leonurine could not be detected [Kuchta 2012]. Triterpenes mainly ursolic acid [Schulz 1973; Kartnig 1985; Ali 2007], labdane type diterpenes [Agnihotri 2008], sterols [Senatore 1991] and volatile oil 0.01-0.2% [Mockute 2006; Morteza-Semnani 2008]. The main components in the oil are germacrene D, caryophyllene, α-humulene, epi-cedrole and dehydro-1,8-cineole. The composition depends on the chemotype and provenance [Morteza-Semnani 2008; Mockute 2006; Lawrence 1972].

CLINICAL PARTICULARS

Therapeutic indications
Mild cardiac complaints of nervous origin [Nahrstedt 1985; Wichtl 1990; Milkowska-Leyck 2002; Brayfield 2014, Kartnig 2014]. For this indication, the efficacy is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Adults and children above 12 years
Dried drug: 4.5 g as an infusion (daily dose) [Czygan 2002, Kartnig 2014]; 2-4 g as dried drug or as an infusion three times daily [Bradley 1992].
Liquid extract (1:1, 25% ethanol): 2-4 ml three times daily [Bradley 1992; Czygan 2002].
Tincture (1:5, 25% ethanol): 4-10 ml three times daily [Bradley 1992].
Tincture (1:5, 34% ethanol): 2-6 ml three times daily [Czygan 2002].

Method of administration
For oral administration

Duration of administration
No restriction. If symptoms persist or worsen, medical advice should be sought.

Contra-indications
None known.

Special warnings and special precautions for use

Special warnings
None required.

Precautions for use
As with all cardiac complaints, consultation with a medical practitioner is essential before treatment.

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Interaction with other medicaments and other forms of interaction
None known.

Pregnancy and lactation
Leonurine showed a uterotonic effect on isolated rat uterus in concentrations from 4 µg/ml medium and above [Yeung 1977].

The product should not be used during pregnancy [Bradley 1992].

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No toxic effects reported

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antioxidant effects
The free radical scavenging and antioxidant activities of a methanolic dry extract of motherwort were demonstrated using the DPPH assay, the phosphomolybdenum assay and inhibition of linoleic acid peroxidation. In the DPPH assay, after 5 minutes the EC_{50} value was 0.80 µg/ml. In the phosphomolybdenum assay the activity was concentration dependent. At 40ºC the reducing capacity of the extract at 1 µg/ml and 20 µg/ml was equivalent to 1.35 µg/ml and 43.14 µg/ml of ascorbic acid respectively. In the linoleic acid peroxidation assay the extract at 100 µg/ml reduced lipid peroxidation by 73.3% [Matkowski 2006].

A hydroethanolic (70%; DER 1:5) fluid extract demonstrated an antioxidant and radical scavenging effect using DDPA and ABTS reaction systems. In the DDPA system, after 10 minutes the value was 84.89%. In the ABTS assay it was 88.28% [Bernatoniene 2009].

The same extract, with concentrations of 113.5 ng/mL - 2.3 µg/mL phenolic compounds, increased the state 2 respiration rate (10-38%) but did not affect the state 3 or uncoupled mitochondrial rate. The highest extract concentration, 18.2 µg/mL of phenolic compounds, induced an 85% increase in the state 2 respiration rate and a decrease in the state 3 respiration rate of 44% [Bernatoniene 2014].

The antioxidant effects of a dry acetone-water (30:70 v/v; DER 1:10) extract were assessed by the ABTS radical cation decolorization assay, the DPPH assay and the ferric reducing antioxidant power (FRAP) assay. Distilled water was used as a blank, Trolox as a standard and ascorbic acid as a positive control. The results expressed as Trolox equivalents ranged between 350-455 µM Trolox/g for the extract in the 3 assays. The results for ascorbic acid were 4896, 7180 and 4793 µM Trolox/g for the ABTS, DPPH and FRAP assays respectively [Sadowska 2017].

Effect on isolated rat heart
In the Langendorff model, a butanolic dry fraction from a methanolic dry extract significantly reduced coronary output of isolated rat heart at doses of 50-2000 µg ($p<0.05$ to 0.01). The heart rate was also significantly reduced at doses of 50-2000 µg ($p<0.05$ to 0.001). The electrocardiogram showed a marked prolongation of P-Q and Q-T intervals by 31-78% and 12-29% respectively [Milkowska-Leyck 2002].

Antimicrobial effects
A dry water fraction of an acetone-water (30:70 v/v; DER 1:10) extract was investigated for its antimicrobial, anti-adhesive and antibiofilm properties against *Staphylococcus aureus*. The MIC value of the extract was 6 mg/mL. The anti-adherence activity of the extract at a concentration of 75% MIC was 23.2-32.4%. There was no effect on biofilm formation [Micota 2014].

The same extract at concentrations of 75% MIC and 50% MIC resulted in the reduction of some important *S. aureus* characteristics: formatting of aggregates in plasma, adherence to a fibrin-coated surface, staphylocoagulase dependent plasma clotting, survival in human blood, α-toxin release and surface protein A expression. Tolerance to the exogene hydrogen peroxide was enhanced after pre-incubation with the extract [Micota 2016].

Other effects
In the $GABA_A$ receptor binding assay, a hydroethanolic (45%) dry extract bound to the GABA site and to the benzodiazepine site of rat $GABA_A$ receptors with IC_{50} values of 21 and 28 µg/mL (two independent measurements) for the GABA site and 646 and 372µg/mL for the benzodiazepine site [Rauwald 2015].

Various extracts of motherwort, a dry ethanolic 70% v/v extract, an aqueous dry extract refined by extraction with ethyl acetate and ethyl ether, and the ether phase of a methanolic extract, were tested on the oxidation of 5-thio-(2-nitrobenzoic acid) (TNB) to hypothiocyanite (OSCN). For the ethanol extract, the maximum OSCN production was observed at a concentration of 2 mg/ml, for the refined aqueous extract at 1 mg/ml and for the ether phase of the methanolic extract at 0.25 mg/ml [Flemmig 2015].

A dry acetone-water (30:70 v/v; DER 1:10) extract significantly ($p<0.01$) reduced arachidonic acid-induced platelet aggregation by 15% at a concentration of 100 µg/mL compared to control (no extract). The same extract at concentrations of 50, 100 and 350 µg/ml significantly ($p<0.05$) increased NO production in human umbilical vein endothelial cells (HUVECs). The extract at concentrations of 50 and 100 µg/ml reduced PAF secretion from HUVECs in the presence of *S. aureus* peptidoglycan (a PAF secretion stimulator) by 1.2-1.4 fold. No effects were observed at 350 µg/ml [Sadowska 2017].

In vivo experiments

Sedative effect
In mice, an undefined hydroalcoholic extract significantly ($p<0.05$) prolonged sleeping time induced by hexobarbital [Weischer 1994].

A butanolic dry fraction of a methanolic extract, administered to mice intragastrically at 800 mg/kg b.w., significantly ($p<0.01$) depressed spontaneous locomotor activity by 65% when compared to control animals receiving corresponding volumes of saline [Milkowska-Leyck 2002].

Analgesic effects
An undefined hydroethanolic extract, given to mice at i.p. doses of 500, 250 and 125 mg/kg b.w., demonstrated analgesic effects in various models. Saline at 0.5 ml and morphine at 10 mg/kg were used as negative and positive controls respectively. In the formalin test, the results were only significant compared to negative control at 500 mg/kg ($p<0.01$) in the early phase and at 250 mg/kg ($p<0.05$) in the late phase, with both results

comparable to morphine at 10 mg/kg. In the tail flick test, pre-treatment with the extract demonstrated a dose dependant and significant effect: 500mg/kg significantly ($p<0.01$) increased the antinociceptive activity 30-70 min after the injection; at 250 mg/kg and 125 mg/kg the effect was significant ($p<0.01$) after 45 and 60 minutes. In the hot plate test, the extract at 500 mg/kg significantly increased the latency response at 60 min ($p<0.05$) and at 75 and 90 minutes ($p<0.01$) [Rezaee-Asl 2014].

Clinical Studies
In an open study, 50 patients (male and female) with arterial hypertension stages 1 and 2, accompanied by anxiety and sleep disorders, were treated daily for 28 days with 4 capsules each containing 300 mg of a soybean oil extract of motherwort (1:10 w/v). Psychoneurological symptoms were rated on a 5 point SCAG scale; fatigue, reaction time and emotional condition on a 7 point numerical rating scale and a SAM (state-activity-mood) scale based on the answers of the patients to a questionnaire with 30 pairs of words. The symptoms, together with systolic and diastolic blood pressure, heart rate and EEG, were documented after 7, 14, 21 and 28 days. In the group of patients with stage 1 hypertension all parameters with the exception of heart rate were significantly ($p<0.05$) improved. The patients with stage 2 hypertension did not respond significantly to the treatment [Shikov 2011].

Pharmacokinetic properties
No data available.

Preclinical safety data

Acute toxicity
A butanolic fraction from a methanolic extract showed low toxicity. The intravenous LD_{50} in mice was > 400 mg/kg b.w. and the oral LD_{50} was > 2000 mg/kg b.w. [Milkowska-Leyck 2002].

Mutagenicity and carcinogenicity
A fluid extract, 1:1 in 35% ethanol, showed no mutagenic activity in the Ames mutagenicity test in *Salmonella typhimurium* TA 98 [Schimmer 1994].

Clinical safety data
In an open study of 50 patients with arterial hypertension, treated for 28 days with 1200 mg of an oil extract, side effects were minimal in all groups [Shikov 2011].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2019
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2018
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003

LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2018
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2019
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

E/S/C/O/P Monographs

Online Series

The Scientific Foundation for Herbal Medicinal Products

The second edition of ESCOP Monographs, published as a hardback book in 2003 with a Supplement in 2009, has been widely acclaimed for its authoritative information on the therapeutic uses of herbal medicines. Monographs covering a total of 107 herbal substances include extensive summaries of pharmacological, clinical and toxicological data, and copious references to scientific literature form an important part of each text.

Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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Online Series

The Scientific Foundation for Herbal Medicinal Products

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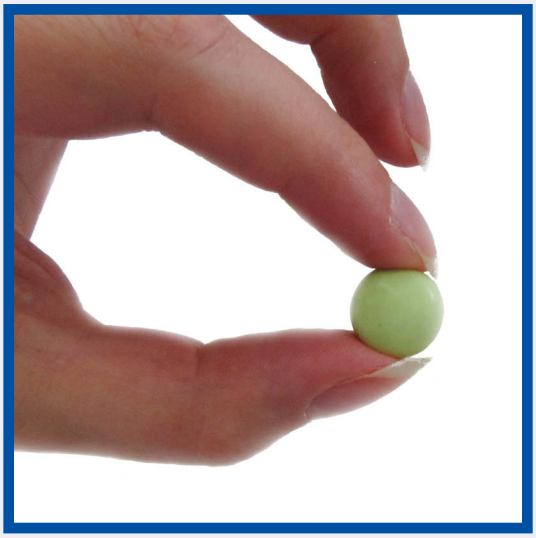

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E/S/C/O/P
MONOGRAPHS

***The* Scientific Foundation for**
Herbal Medicinal Products

LINI SEMEN
Linseed

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
CCl_4	carbon tetrachloride
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase

INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

LINI SEMEN

Linseed

2017

DEFINITION

Linseed consists of the dried ripe seeds of *Linum usitatissimum* L.

The material complies with the monograph of the European Pharmacopoeia [Linseed].

CONSTITUENTS

The seeds contain 3-9% of mucilage polysaccharides composed mainly of galacturonic acid, xylose, galactose and rhamnose units; 30-45% of fixed oil mainly consisting of triglycerides of α-linolenic (40-60%), linoleic and oleic acids; approx. 25% of protein; and 0.1-1.5% of cyanogenic glycosides such as linustatin and neolinustatin (the diglucosides of linamarin and lotaustralin, respectively) [El-Shattory 1976; Smith 1980; Schilcher 1980, 1986a,b; Leng-Peschlow 1993; Fedeniuk 1994; Teuscher 2016].

Glycosides of secoisolariciresinol as the major lignan, together with small amounts of matairesinol, isolariciresinol and pinoresinol [Sicilia 2003; Teuscher 2016]. Secoisolariciresinol and its glycosides are precursors of the mammalian phytoestrogens enterodiol and enterolactone [Axelson 1982; Adlercreutz 1984; Borriello 1985; Johnsson 2000; Teuscher 2016].

CLINICAL PARTICULARS

Therapeutic indications

Internal use

Treatment of habitual constipation or in conditions in which easy defecation with soft stool is desirable [Kurth 1976; Kay 1978; Jens 1981; Schilcher 1986a; Brunton 1990; Weiss 1997; Schilcher 2010; Teuscher 2016].

Supportive treatment of dyslipidaemia [Jenkins 1999; Dodin 2005; Patade 2008; Zhang 2008; Pan 2009b; Fukumitsu 2011; Almario 2013; Khalatbari 2013; Edel 2015] and hypertension [Rodriguez-Leyva 2013; Khalesi 2015; Machado 2015; Ursoniu 2015].

As a demulcent preparation for the symptomatic short-term treatment of gastritis and enteritis [Schilcher 1986a; Grützner 1997; Weiss 1997; Schilcher 2010; Teuscher 2016] and the symptomatic relief of irritable bowel syndrome [Kay 1978; Brunton 1990]. Supportive therapy for symptoms of diverticulosis [Kay 1978; Brunton 1990]. In all of these three indications, efficacy is plausible on the basis of human experience and long-standing use.

External use

Supportive treatment for symptoms of painful skin inflammations [Evans 1996; Weiss 1997]. The efficacy is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Adults and children over 12 years of age

Internal use

As a laxative: 5 g of whole, finely-cracked or freshly crushed seeds, soaked in water and taken with a glassful of liquid three times daily. The effect starts 18-24 hours later [Schilcher 1986a, 1990; Weiss 1997].

Supportive treatment of dyslipidaemia and hypertension: 20-50g daily [Jenkins 1999; Dodin 2005; Patade 2008; Zhang 2008; Pan 2009b; Fukumitsu 2010; Almario 2013; Khalatbari 2013; Rodriguez-Leyva 2013; Edel 2015; Khalesi 2015; Machado 2015; Ursoniu 2015].

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As a demulcent for gastritis and/or enteritis: for a mucilaginous preparation soak 5-10 g of whole linseed in 150 mL water and filter after 20-30 minutes [Weiss 1997; Teuscher 2016].

Topical use
30-50 g of crushed or powdered seed (may be de-fatted) as a warm poultice or warm compress [Weiss 1997].

Children from 6 to 12 years of age: half the adult dose [Kooperation Phytopharmaka 1998].
Children under 6 years of age: to be treated under medical supervision only.

Method of administration
For oral administration or cutaneous application.

Duration of use
Because of the gradual mode of action of bulk-forming laxatives, treatment should be continued for a minimum of 2-3 days to ensure optimum benefit [Reynolds 1993].

If abdominal pain occurs, or if there is no response after 48 hours, use of linseed should be discontinued and medical advice should be sought.

Contraindications
Atonic and obstructive ileus, subileus or conditions likely to lead to intestinal obstruction. Acute abdominal pain of any origin (e.g. appendicitis) [Brunton 1990; Teuscher 2016].

Special warnings and precautions for use
Linseed (whole, finely-cracked or freshly crushed) should be soaked and taken with at least 10 times the amount of fluid, otherwise compaction and intestinal obstruction may occur [Van Olffen 1982; Hardt 1986].

Persons with weight problems should take linseed whole, not cracked, because of its rich energy content of about 470 kcal (1960 kJ)/100 g].

Interaction with other medicinal products and other forms of interaction
The absorption of other medications taken at the same time may be delayed [Jenkins 1978; Lorenc-Kubis 2001].

Diabetics should be aware of a potential delay in glucose absorption [Jenkins 1976, 1978; Holt 1979; Munoz 1979; Vaaler 1980].

Pregnancy and lactation
There is a lack of sufficient data [Basch 2007]. In accordance with general medical practice, the product should not be used during pregnancy without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Reactions of hypersensitivity may occur very rarely [Alonso 1996; Alvarez-Perea 2013].

Overdose
In spite of its content of cyanogenic glycosides, single doses of up to 150-300 g of powdered linseed are not toxic. Health risks are not to be expected [Schilcher 1979, 1986; Anon 1983; Schulz 1983; Czygan 1984; Leng-Peschlow 1993; Weiss 1997; Teuscher 2016].

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Oestrogenic effects
Lignan precursors found in linseed are converted by bacteria present in the colon to metabolites interfering with the metabolism and activity of oestrogens [Borriello 1985]. Oestrogenic effects can be deduced from the effects on oestrogen receptor positive cancer cell lines [Lehraiki 2010; Richter 2010].

α-Amylase and α-glucosidase inhibiting activity
The inhibitory effects of various concentrations of a linseed extract (not further specified), secoisolariciresinol diglucoside (SDG) and secoisolariciresinol (SECO), enterodiol and enterolactone on the activities of α-glucosidase (soluble and immobilised) from rat intestine and α-amylase (soluble) from porcine pancreas were evaluated. Acarbose was used as positive control. The extract as well as SDG, SECO, enterodiol and enterolactone reduced the α-amylase activity. The extract inhibited both soluble α-glucosidase and α-amylase in a dose-dependent manner. The most active compound was enterodiol [Hano 2013].

Anti-proliferative effects
Trophoblast Jeg3 tumour cells were incubated for 48 hours with 2 different concentrations of linseed extract (not specified) and 7 fractions thereof. Cell proliferation as well as the concentrations of human chorionic gonadotropin (hCG) and progesterone produced by the tumour cells were measured. Proliferation of the Jeg3 cells was decreased by four different fractions in a dose-dependent manner, as well as the inhibition of hCG production by four fractions. Two fractions decreased the production of progesterone by 58% to 86%. Other fractions showed a stimulating effect on hormone production and cell proliferation [Waldschläger 2005].

The ability of isolated SECO, anhydrosecoisolariciresinol and of SDG (1 to 100 µM) to modulate the growth of human breast cancer cell lines (MCF-7 and MDA-MB-231) was assessed. Cells were incubated with 100 µL of oestrogen-free medium. After a 24-hour starvation period the cells were exposed to the test compounds for 48 hours. The strongest effect was observed for anhydrosecoisolariciresinol, which significantly decreased cell growth at 50 ($p<0.05$) and 100 pM ($p<0.001$) [Lehraiki 2010].

Enterolactone inhibited actin-based cell motility of MCF-7 and MDA MB 231 cells as evidenced by confocal imaging of a cell migration assay. The results are supported by the observation that the enterolactone down-regulated the metastasis-related metalloproteinases MMP2, MMP9 and MMP14 gene expressions. No significant alteration in the MMP11 gene expression was found [Mali 2012].

After addition of enterodiol or enterolactone, oestradiol-induced VEGF secretion in MCF-7 cells decreased significantly without agonistic effects ($p<0.01$). The elevated VEGF secretion following addition of oestradiol increased the expression of VEGF receptor-2 in umbilical vein endothelial cells, suggesting a pro-angiogenic effect of oestradiol by two different mechanisms, both of which were inhibited by the addition of lignans [Bergman Jungeström 2007].

Radioprotective effect
The radioprotective efficacy of SDG was investigated in murine

lung cells. Cells were pre-treated with SDG (50 μM) at various times (0 h, 2 h, 4 h and 6 h) prior to 2 Gy radiation exposure. Pre-treatment significantly ($p<0.05$) inhibited comet tail length, an indication of decreased DNA damage. Maximum protection was observed at 6 h of SDG treatment. Radiation-induced DNA single- and double strand breaks decreased and the overall cell survival improved. SDG significantly ($p<0.05$) increased gene and protein levels of antioxidant heme oxygenase-1, glutathione S-transferase mu 1 and NAD(P)H dehydrogenase quinone 1 [Velalopoulou 2016].

In vivo experiments

Effect on blood lipid levels

The effect of SDG was investigated in rabbits receiving either their normal diet (control group, n=8), a control diet supplemented with SDG (15 mg/kg b.w./day, n=5), a diet containing cholesterol (1%, n=6) or cholesterol + SDG (1% cholesterol + SDG 15 mg/kg b.w./day, n=5). Blood samples were collected before the experiment, and after 4 and 8 weeks, then the aorta was removed for assessment of atherosclerotic plaques, aortic malondialdehyde (MDA) and aortic tissue chemiluminescence (a marker for antioxidant reserve). Serum total cholesterol (TC), LDL-cholesterol (LDL-C) and the ratios of LDL-C/HDL-cholesterol (HDL-C) andTC/HDL-C increased in the 3rd and 4th groups. SDG significantly reduced TC and LDL-cholesterol by 33 and 35% respectively at week 8 ($p<0.05$), and significantly increased HDL-C by >140% at week 4 ($p<0.05$). It also decreased TC/HDL-C and LDL-C/HDL-C ratios significantly by approximately 64% ($p<0.05$). Comparing atherosclerotic plaques in the 3rd and 4th groups, it was found that SDG significantly reduced hypercholesterolaemic atherosclerosis by 73% ($p<0.05$). The increases in aortic malondialdehyde and chemiluminescence were significantly lower in the 4th group ($p<0.05$) [Prasad 1999].

In a similar study, rabbits were treated slightly differently: Group I, control; Group II, lignan complex (containing 34-38% SDG, 9.6-11% 3-hydroxy-3-methylglutaric acid (HMGA) and 15-21% cinnamic acids; 40 mg/kg b.w. daily p.o.); Group III, 0.5% cholesterol; Group IV, 0.5% cholesterol diet + lignan complex, (40 mg/kg b.w. daily p.o.). Rabbits in Group III developed atherosclerosis: 50.84 ± 6.23% of the intimal surface of the aorta was covered with atherosclerotic changes, associated with an increase in triglycerides, TC, LDL-C, HDL-C, MDA and aortic MDA and antioxidant reserve. The lignan complex reduced the development of atherosclerosis by 34%, with associated decreases in serum TC by 20%, LDL-C by 14%, TC/HDL-C by 34%, serum MDA by 35% and aortic MDA by 58%. HDL-C was increased by 30% in hypercholesterolaemic rabbits and by 25% in normocholesterolaemic rabbits with the lignan complex. The lignan complex did not affect TC, LDL-C and serum MDA, but did increase the aortic MDA, in the normocholesterolaemic rabbits [Prasad 2005].

The effects of ground linseed on plasma and hepatic lipids, and hepatic gene expression, in male and female human apolipoprotein B-100 transgenic (hApoBtg) mice, as well as in mice lacking the LDL receptor and apolipoprotein B mRNA editing enzyme complex 1 ($LDLr^{-/-}$/$apobec^{-/-}$ mice), were examined. The control diet containing 0.1% cholesterol and 30% kcal as fat was fed for 10 days to hApoBtg mice and for 14 days to $LDLr^{-/-}$/$apobec^{-/-}$ mice. Animals from each genetic background were then divided into 2 groups based on gender and mean plasma TC. The hApoBtg and $LDLr^{-/-}$/$apobec^{-/-}$ mice either continued on the control diet for a total of 31 and 35 days respectively, or were fed 20% w/w ground linseed with comparable caloric, macronutrient and fibre content for 21 days. Blood was obtained after a 4 hour fast from all mice prior to feeding both control and linseed diets, and after 10 and 21 days on the linseed diet. The control diet increased TC by >100 mg/dL in the hApoBtg mice with a greater increase in males and by 800 mg/dL in $LDLr^{-/-}$/$apobec^{-/-}$mice. After 3 weeks, the linseed diet significantly reduced plasma TC by 19% and 22% in hApoBtg and $LDLr^{-/-}$/$apobec^{-/-}$ mice respectively and non-HDL cholesterol by 24% in both models (p for all <0.05). Linseed significantly ($p<0.05$) reduced hepatic cholesterol by 32% and 47% in male and female hApoBtg mice respectively and by 66% in $LDLr^{-/-}$/$apobec^{-/-}$ mice. Linseed had no effect on the expression of the hepatic genes: LDLr, 3-hydroxy-3-methylglutaryl (HMG) CoA reductase, phospholipid transfer protein, cholesterol 7α hydroxylase, fatty acid synthase and acyl CoA oxidase in either mouse model [Pellizzon 2007].

Male Wistar rats (n=24) aged 21 days were divided into 3 groups: the control group received a casein-based chow (10% protein; 5% fibre; 7% lipid); the linseed group was fed with the casein-based chow supplemented with 25% linseed (10% protein; 7% fibre; 11% lipid) and the modified control group with the casein-based chow plus soybean oil and cellulose fibre (10% protein; 7% fibre; 11% lipid). After 180 days blood was taken and the vasodilator effect of acetylcholine (1-100 pmol) was evaluated in the preconstricted isolated rat mesenteric arterial beds. The linseed group showed a 47% increase in HDL-C, a 22% reduction in LDL-C, a 23% reduction in triglycerides and an increased ($p<0.05$) vasodilator response to acetylcholine as compared to the control group and modified control group [Daleprane 2010].

For 14 weeks, 140 LDL receptor-deficient mice received one of 14 experimental diets, containing either a regular (pork/soy) or trans fat source at a concentration of either 4 or 8%, with the addition of either dietary cholesterol (2%), whole ground linseed (10%) or one of the components of linseed (linseed oil rich in α-linolenic acid, partially defatted ground linseed (fibre) or lignan (SDG)). Adding ground linseed to the diet partially mitigated the rise in circulating cholesterol levels induced by the cholesterol-enriched diet. Atherosclerosis was stimulated by trans fatty acids and/or cholesterol. Addition of ground linseed to an atherogenic diet significantly ($p<0.05$) reduced atherosclerosis compared with the groups that consumed only the cholesterol and/or trans fatty acids. Of the tested components, only α-linolenic acid inhibited the atherogenic effect of cholesterol and/or trans fatty acids [Bassett 2011].

A linseed lignan concentrate (40mg/g SDG) showed a dose-dependent decrease in TC ($p<0.001$), triglycerides andVHDL in Triton WR 1339-induced hyperlipidaemic rats [Zanwar 2012].

Female Golden Syrian hamsters were either sham-operated or ovariectomised and randomly assigned to one of four treatment groups (n=12) for 90 days. Hamsters received either a semipurified diet or the same diet supplemented with either whole linseed or linseed oil (amount equivalent to the oil content of whole linseed). Whole linseed, but not linseed oil, significantly ($p<0.05$) prevented the ovariectomy-induced increase in serum total cholesterol (12% and 4% reduction by whole linseed and linseed oil respectively). There were no significant differences among groups in serum triglyceride concentration and liver lipids. Both whole linseed and linseed oil more than doubled the hepatic protein levels of 7α-hydroxylase in comparison to the ovariectomised animals receiving the control diet ($p<0.05$) [Lucas 2011].

Effects on β-glucuronidase activity

Six groups of Sprague-Dawley rats were fed one of the following diets for 100 days: a basal high-fat diet (20% fat), the basal diet supplemented with 2.5 or 5% of linseed, the basal diet

supplemented with 2.5 or 5% of defatted linseed, or the basal diet with a daily dose of 1.5 mg of SDG. All rats were injected with a single dose of azoxymethane (15 mg/kg b.w.) one week before treatment. Urinary lignan excretion, an indicator of mammalian lignan production, significantly increased in both the linseed groups and in the low-dose defatted linseed group ($p<0.0003$, $p<0.0001$ and $p<0.0001$ respectively) compared to the control. The total activity of caecal β-glucuronidase significantly increased in a dose-dependent manner in both the defatted linseed groups and in the high-dose linseed group ($p<0.01$, $p<0.047$ and $p<0.0004$ respectively). Compared to control the number of aberrant crypts per focus was significantly reduced ($p<0.01$ to $p<0.04$) in the distal colon of the five groups of treated rats. Four microadenomas and two polyps were observed in the control group but none in the treated groups. The total activity of β-glucuronidase was positively correlated with total urinary lignan excretion ($r=0.280$, $p<0.036$, $n=60$), and negatively with the total number of aberrant crypts ($r=-0.330$, $p<0.010$, $n=57$) and the total number of aberrant crypt foci ($r=-0.310$, $p<0.018$, $n=57$) in the distal colon. There were no significant differences between linseed and corresponding defatted linseed groups [Jenab 1996].

Antidepressant effects
The antidepressant effect of SECO on ovariectomised mice was investigated in the forced swimming test and the tail suspension test. After intragastric administration for 14 consecutive days at doses of 5, 10 and 20 mg/kg, SECO at 10 mg/kg significantly ($p<0.01$) reduced the duration of immobility in these two models as compared to control. The effect was similar to the positive control imipramine. In addition, SECO at doses of 10 and 20 mg/kg substantially increased brain noradrenaline and dopamine levels in ovariectomised mice [Wang 2013].

Effects on BPH
Forty male Wistar rats were divided into five groups: untreated control; treatment with testosterone propionate (TP) to induce prostate enlargement; TP-treated groups fed diets containing either 5% or 10% milled linseed; and TP-treated group fed a diet containing 20 ppm finasteride. Co-administration of linseed or finasteride with TP significantly reduced prostatic VEGF, epithelial cell proliferation and RNA/DNA ratio, and also significantly ($p<0.05$) increased the serum testosterone and testosterone/estradiol ratio compared to treatment with TP alone [Said 2015].

Male Wistar-Unilever rats were randomly divided into four groups of 12 rats each: a negative control, a positive control receiving daily s.c. TP and two verum groups receiving daily s.c. injections of TP and either 0.5% or 1.0% of a lignan-rich extract from linseed hulls in their diet. Treatment started 2 weeks before the induction of BPH and was continued for 5 consecutive weeks. The influence of TP and the lignan-rich extract on body weight, food and water consumption, and enterolactone levels in serum and urine was examined after 5-weeks. In a dose dependent manner, the extract significantly inhibited TP-induced increase in prostate size in comparison with positive controls ($p<0.001$). Higher serum and urine levels of enterolactone correlated with the extract dose [Bisson 2014].

Anti-proliferative effects
The effects of linseed oil fed to female mice as 10% of their diet were compared to corn oil. The respective diets were fed for 3-8 weeks prior to subcutaneous injections of one of two syngeneic mammary tumour cell types (410 and 410.4). The growth of 410.4 mammary tumours was significantly lower in mice given linseed oil than in animals given corn oil ($p<0.05$). Linseed oil also significantly enhanced incorporation of n-3 fatty acids into tumours ($p<0.005$) and significantly reduced tumour prostaglandin E production ($p<0.005$) compared to corn oil [Fritsche 1990].

Supplementation of a high-fat diet (20% corn oil) fed to 70 female rats for 4 weeks with either linseed flour or defatted linseed (5% and 10%) reduced epithelial cell proliferation by 38.8-55.4% and nuclear aberrations by 58.8-65.9% in mammary glands, optimum effects being observed with 5% linseed flour [Serraino 1991].

Five groups of 7 male rats were fed the same diet supplemented in the same manner for 4 weeks following a single injection of azoxymethane at 15 mg/kg b.w.. In the descending colon of the supplemented groups, the total number of aberrant crypts and foci were significantly reduced ($p<0.05$) by 41-53% and 48-57% respectively. The labelling index (the number of labelled cells, or cells undergoing DNA synthesis, per 100 cells in the epithelia of crypts from each section of the rat colon) was 10-22% lower in these groups, except for the 5% linseed meal group [Serraino 1992a].

In a long-term experiment, two groups of 60 female rats were fed the same high-fat diet, in one group the diet was supplemented with 5% linseed flour. After 4 weeks, tumours were induced in 44 rats by a single dose of 5 mg of 7,12-dimethylbenz[a]anthracene. After an additional week, half of the the group fed the basal diet received the supplemented diet for 20 weeks, while half of the group previously supplemented received the basal diet only, in order to differentiate between initiation and promotional effects of supplementation. The group fed a linseed-supplemented diet only during the promotional stage of mammary carcinogenesis had a significantly smaller ($p<0.05$) tumour volume (66.7%) than all other groups, but also an increased tumour burden and number of tumours per group compared with the group fed the supplemented diet throughout the experiment. Feeding the basal diet at the initiation stage of tumour development resulted in a greater number of tumours occurring consistently over time. However, linseed supplementation at initiation and throughout the experimental period tended to reduce the number of tumours per tumour-bearing rat [Serraino 1992b].

After administration of a single dose of 5 mg of 7,12-dimethyl benz[a]anthracene, 5 groups of female rats (n=19-21) received either a basal diet or a diet supplemented with SDG (2200 nmol/day), 1.82% of linseed oil or 2.5 or 5% of linseed. After 7 weeks of treatment, the volume of established tumours was over 50% smaller in all treatment groups ($p<0.08$, $p<0.04$, $p<0.04$, $p<0.04$ respectively) whereas there was no change in the basal diet group. The number and volume of new tumours were lowest in the SDG ($p<0.02$) and 2.5% linseed ($p<0.07$) groups. Combined established and new tumour volumes were smaller in the SDG, 2.5 and 5% linseed groups ($p<0.02$) than in the linseed oil and basal diet groups [Thompson 1996].

White Leghorn hens received a 10% linseed-enriched or standard diet for 1 year. The incidence and severity of ovarian cancer were determined by gross pathology and histology in the two groups. Eggs were collected and analysed to determine omega-3 fatty acid levels. A significant reduction in the number of late stage ovarian tumours was detected in the linseed-fed hens ($p<0.05$). Incidence rates of ovarian cancer were not significantly different between the two groups. Linseed-fed hens' eggs incorporated significantly more omega-3 fatty acids compared to control ($p<0.05$) [Ansenberger 2010].

In a similar study, COX-1 and COX-2 localisation, protein and mRNA expression, and PGE2 and PGE3 concentrations in ovaries were also measured. The results demonstrated a significant reduction in the number of late stage ovarian tumours in the

linseed-fed hens compared to control ($p<0.05$). In correlation with decreased ovarian cancer severity, concentrations of PGE2 and expression of COX-2 were diminished in the ovaries of linseed-fed hens. PGE3 concentrations were below the level of detection [Eilati 2013].

Expression of highly up-regulated genes involved in the embryonic process of branching morphogenesis coincides with expression of E-cadherin in the ovarian tumour epithelium. Levels of expression of these genes in tumours from animals fed with a diet containing 10% flaxseed were reduced by 40-60%. E-cadherin and miR200 were both up-regulated in tumours from control-fed hens, whereas their expression was decreased by 60-75% in tumours from linseed-fed hens. mRNA levels were increased five-fold in tumours, with no significant difference between control-fed and linseed-fed hens [Hales 2014].

Athymic nude mice were orthotopically injected with oestrogen receptor negative breast cancer cells (MDA-MB-435). After 8 weeks mice were randomised and fed either a basal diet or the basal diet supplemented with either 10% linseed, SDG, linseed oil or combined SDG and linseed oil for 6 weeks. The SDG and linseed oil levels were equivalent to the amounts in the 10% linseed. Compared to the basal diet, the tumour growth rate was significantly lower ($p<0.05$) in the linseed, linseed oil and SDG + linseed oil groups, in concordance with decreased cell proliferation and increased apoptosis. Incidence of lung metastasis was reduced (16–70%) by all treatments, significantly ($p<0.05$) in the linseed and SDG + linseed oil groups. Distant lymph node metastasis was significantly ($p<0.05$) decreased by 52% only in the linseed oil group. Although the total metastasis incidence was significantly lowered by 42% ($p<0.05$) only in the SDG + linseed oil group, all treatment groups did not differ significantly from each other [Wang 2005].

Ovariectomised mice were treated with continuous release of 17β-oestradiol by pellets implanted s.c. that provided continuous release of E2 at serum concentrations of approx. 250 pmol/L, which is in the range of physiologic levels seen in mice during the oestrous cycle. MCF-7 tumours were established and mice were fed with basal diet or basal diet supplemented with 10% ground linseed; two groups fed the basal diet received daily injections with enterodiol or enterolactone (15 mg/kg b.w.). Linseed, enterodiol and enterolactone counteracted oestradiol-induced growth and angiogenesis in solid tumours. VEGF was significantly decreased in all intervention groups compared with the basal diet alone ($p<0.001$) [Bergman Jungeström 2007].

Five groups of eight Apc^{Min} mice were fed with control, corn meal, linseed meal, corn oil or linseed oil supplemented diets. Linseed significantly decreased ($p<0.05$) tumour multiplicity and size in the small intestine and colon as compared to control or corn-treated groups. COX-1 and COX-2 expression in the colon samples from the linseed group were significantly lower ($p<0.05$) as compared to the corn group [Bommareddy 2009].

Ovariectomised athymic mice with established MCF-7 tumours were fed a basal diet or basal diet supplemented with linseed oil (40g/kg) for 8 weeks. Compared with pre-treatment, linseed oil reduced tumour size by 33% ($p< 0.05$). Furthermore, linseed oil reduced tumour cell proliferation by 38% ($p<0.05$) and increased apoptosis by 110% ($p<0.01$). It also reduced epidermal growth factor receptor-2 (79%, $p<0.05$) and epidermal growth factor receptor (57%, $p=0.057$) expression. Insulin-like growth factor-1 receptor, vascular endothelial growth factor receptor, MAPK and phosphorylated Akt were not affected. Linseed oil significantly increased ($p<0.001$) serum ALA, eicosapentaenoic acid and docosahexaenoic acid [Truan 2010].

Antihypertensive activity

Thermoase-digested linseed protein hydrolysate samples and peptide fractions were sequentially tested in spontaneously hypertensive rats. The samples produced with 3% thermoase digestion showed the highest ACE- and renin-inhibitory activities. Whereas membrane ultrafiltration resulted in significant ($p<0.05$) increases in ACE inhibition by the <1 and 1–3 kDa peptides, only a marginal improvement in renin-inhibitory activity was observed for almost all samples. The protein hydrolysate samples and peptide fractions also lowered systolic blood pressure with the largest effect occurring after oral administration (200 mg/kg b.w.) of the 1–3 kDa peptide fraction of the 2.5% protein hydrolysate and the 3–5 kDa fraction of the 3% protein hydrolysate [Nwachukwu 2014].

Anti-inflammatory activity

In a murine model of acute lung injury and inflammation, mice were fed with normal or 10% linseed-supplemented diets for at least 3 weeks prior to challenge with hyperoxia (80% oxygen), intratracheal instillation of LPS or acid aspiration. Bronchoalveolar lavage was evaluated for white blood cells, neutrophils and proteins after a 24 h postintratracheal challenge of hydrochloric acid or LPS, or after 6 days of hyperoxia. Following hyperoxia and acid aspiration, bronchoalveolar lavage neutrophils decreased in linseed-supplemented mice ($p=0.012$ and $p=0.027$ respectively). In contrast, neither lung injury nor inflammation were ameliorated by linseed following lipopolysacharide instillation. Lung MDA levels were lower in hyperoxic mice than in unchallenged mice ($p=0.0001$) and decreased with linseed treatment following acid aspiration ($p=0.011$) [Kinniry 2006].

In male rats (n=32) circular, full thickness wounds with 7 mm^2 diameter were caused to both sides of the spine. Two concentrations of linseed oil (0.75 and 1.5%) were applied to the wounds as an ointment. Tissue samples were obtained at the end of 3, 7, 14 and 21 days. Treated animals showed significant reduction of inflammatory cells during re-epithelisation ($p<0.05$). Linseed oil significantly accelerated the wound healing process ($p<0.05$) [Farahpour 2011].

The anti-apoptotic and anti-inflammatory effects of a diet containing 10% linseed were evaluated in a mouse model of lung ischaemia/reperfusion injury (IRI) involving 60 min of ischaemia followed by 180 min of reperfusion. The mice fed linseed had significantly lower levels of caspases and decreased apoptotic activity compared with mice fed without linseed ($p=0.003$). Lung homogenates and bronchoalveolar lavage fluid analysis demonstrated significantly reduced inflammatory infiltrates in mice receiving the linseed diet ($p<0.001$). Additionally, linseed treated mice showed significantly increased expression of antioxidant enzymes and decreased markers of lung injury ($p<0.001$) [Razi 2011].

Male fa/fa and lean Zucker rats were fed with a control diet, and fa/fa rats were fed with an α-linolenic-acid-rich linseed oil supplemented diet for 8 weeks. Adipose tissue and serum were collected and analysed for cytokine (TNF-α, IL-10), haptoglobin, monocyte chemoattractant protein-1 (MCP-1) and adipokine (leptin, adiponectin) levels. Splenocytes were isolated and *ex vivo* mitogen-stimulated cytokine production was measured. Fa/fa rats receiving the linseed oil supplemented diet had 17% smaller adipocytes and 5-fold lower MCP-1 levels in adipose tissue than control. Adipose tissue levels of IL-10 were 72% lower in fa/fa rats fed the oil-supplemented diet compared to baseline, and their TNF-α levels decreased by 80% (equal to levels in lean Zucker rats fed the control diet) compared to fa/fa rats receiving the control diet. There were no changes in *ex vivo* cytokine production by splenocytes between intervention

fa/fa rats and control fa/fa rats. Macrophage infiltration was not different among the groups; however, fa/fa rats receiving the oil had less T-cell infiltration than those receiving control diet [Baranowski 2012].

Photoprotective effect
Sprague-Dawley male albino rats (n=21) were exposed to UV C light for 1 h twice a day for 4 weeks. Rats were divided into three groups as control, UV C and UV C + linseed oil orally (4 mL/kg b.w.). While MDA and protein carbonyl levels of the UV C group increased compared to control, their levels decreased in the UV C + linseed oil group compared with the UV C group in skin, lens and serum. Skin glutathione level decreased significantly ($p<0.05$) in the UV C and UV C + linseed oil groups. Glutathione peroxidase and SOD activities were lower in the UV C group, but higher in the UV C + linseed oil group in skin, lens and serum. While retinal apoptosis increased in both the UV C and the UV C + linseed oil groups, it was lower in the UV C + linseed oil group [Tülüce 2012].

Antioxidant effect
Adult male Wistar rats were pre-fed with a normal PUFA-enriched diet or a diet supplemented with 15% linseed oil for 10 days followed by a single i.p. dose of cisplatin (6 mg/kg b.w.). Serum/urine parameters, enzymes of carbohydrate metabolism and oxidative stress were analysed. The alterations caused by cisplatin treatment were ameliorated in rats fed with the linseed oil supplemented diet [Naqshbandi 2012].

Pharmacological studies in humans

Effect on blood glucose levels
Viscous types of dietary fibre may cause a delay in gastric emptying as shown in two studies with 11 and 7 healthy volunteers [Jenkins 1978; Holt 1979]. In these studies and in one involving 8 non-insulin-dependent diabetic volunteers [Jenkins 1976], it was demonstrated that addition of certain types of dietary fibre to the diet significantly decreased postprandial hyperglycaemia. Thus an improvement in the control of blood-glucose concentration might be expected [Jenkins 1976].

Oestrogenic effects
Quantitative urine assays in 62 women studied 4 times during one year showed a significant positive correlation between the intake of fibre and urinary excretion of lignans and phytoestrogens and the concentration of plasma SHBG [Adlercreutz 1987].

In an open, randomised, cross-over study involving 18 women with normal cycles, the effects of ingestion of linseed powder on the menstrual cycle were investigated. Each subject consumed her usual omnivorous, low fibre (control) diet for 3 cycles and her usual diet supplemented with linseed (10 g/day) for another 3 cycles. The second and third linseed cycles were compared to the corresponding control cycles. During the 36 control cycles, 3 anovulatory cycles occurred, compared to none during the 36 linseed cycles. Compared to ovulatory control cycles, the ovulatory linseed cycles were consistently associated with longer luteal phase (LP) lengths (mean 12.6 vs. 11.4 days; $p=0.002$). There were no significant differences between linseed and control cycles in concentrations of oestradiol or oestrone during the early follicular phase, midfollicular phase or LP. Although linseed ingestion had no significant effect on LP progesterone concentrations, LP progesterone/oestradiol ratios were significantly higher during the linseed cycles. Midfollicular phase testosterone concentrations were slightly higher during the linseed cycles. Linseed ingestion had no effect on early follicular phase concentrations of DHEA-S, PRL or sex hormone-binding globulin [Phipps 1993].

Clinical studies

Effect on gastro-intestinal complaints

Laxative effect
Dietary fibre such as linseed binds with water and swells to form a demulcent gel in the intestine. As water bound to the fibre is prevented from being absorbed in the colon, the faeces are softened and the volume of bowel contents increases [Schilcher 1986a; Brunton 1990; Teuscher 2016].

A decrease in transit time and increase of stool weight by physical stimulation of intestinal peristalsis with linseed have been demonstrated in two multicentric studies (n=108 and n=114) in patients suffering from constipation [Kurth 1976; Jens 1981].

Effect on gastro-intestinal complaints
The mucilage has been reported to have a palliative effect in patients with pain associated with gastro-intestinal problems [Schilcher 1986a; Teuscher 2016].

In an open pilot study 70 patients suffering from various functional upper abdominal complaints such as sensations of pressure and repletion, loss of appetite, nausea, vomiting and heartburn were treated with an aqueous linseed mucilage preparation (1:10) at a dosage of 8 × 25 g (including a small amount of excipients) per day. All except three patients experienced improvements. After 3 days the total symptom score had significantly decreased ($p<0.01$). Each individual symptom score decreased on average, the largest reductions being observed for the sensation of pressure (41.5%) and the sensation of repletion (36.8%). In global assessments by both patients and physicians the efficacy was rated as good or very good in most cases [Grützner 1997].

Effect on blood lipid levels
In a meta-analysis, 28 published studies were assessed according to the Jadad score. Linseed at a daily dose ranging from 20.0 to 50.0 g significantly ($p=0.04$) reduced total cholesterol (TC) and LDL-C by 0.12 mmol/L and 0.15 mmol/L respectively. The cholesterol-lowering effects were more apparent in females (particularly postmenopausal women), individuals with high initial cholesterol levels and in studies with higher Jadad scores. No significant changes were found in the concentrations of HDL-C and triglycerides [Pan 2009b].

In a randomised, cross-over trial, 22 men and 7 postmenopausal women with hyperlipidaemia who followed a National Cholesterol Education Program (NCEP) Step II diet received fibre-rich muffins containing either partially defatted linseed (approx. 50 g daily) or wheat bran as a control, both corresponding to approx. 20 g of fibre daily, for 3 weeks; the treatment phases being separated by at least 2 weeks. Linseed supplementation significantly reduced TC by 4.6% ($p=0.001$), LDL-C by 7.6% ($p<0.001$), apolipoprotein B by 5.4% ($p=0.001$) and apolipoprotein A-I by 5.8% ($p=0.070$) compared to the control, but had no effect on serum lipoprotein ratios. No significant effects were observed on serum HDL-C, serum protein carbonyl content or *ex vivo* androgen and progestin activity [Jenkins 1999].

Menopausal women were randomly assigned to consume 40 g linseed/day (n=101) or wheat germ placebo (n=98) for 12 months. Linseed significantly reduced serum TC by 0.20 ± 0.51 mmol/L ($p=0.012$) and HDL-C by 0.08 ± 0.24 mmol/L ($p=0.031$) compared with wheat germ placebo, whereas the reduction of LDL cholesterol by 0.13 mmol/L was borderline significant ($p=0.086$) [Dodin 2005].

Mild to moderately hypercholesterolaemic (≥5.1 to ≥9.8 mmol/L)

postmenopausal women (n=55) were randomly assigned to control, linseed or linseed + additional oat bran fibre groups. The participants in the linseed and linseed + fibre treatment groups consumed in total 29 g linseed per day. There was a significant decrease of approximately 7% ($p<0.05$) in the mean TC level after 3 months of consumption of linseed or linseed + fibre. No change in TC was observed for women in the control group. In both linseed groups LDL-C was significantly reduced by approximately 10% ($p<0.05$). The levels of HDL-C and triglycerides remained unaltered [Patade 2008].

In an open, randomised clinical trial, 30 haemodialysis patients with dyslipidaemia (triglyceride >200 mg/dL and/or HDL-cholesterol <40 mg/dL) received either 40 g/day ground linseed for 8 weeks or their usual diet without linseed. Serum concentrations of triglyceride ($p<0.01$), TC ($p<0.01$), LDL-C ($p<0.01$) and CRP ($p< 0.05$) decreased significantly in the linseed group at the end of week 8 compared with baseline, whereas serum HDL-C showed a significant increase ($p<0.01$) [Khalatbari 2013].

In a double-blind, randomised, placebo-controlled trial, the effects of a diet for 12 months with foods that contained either 30 g of milled linseed (n=58) or 30 g of whole wheat were examined in patients (n=52) with peripheral artery disease. Plasma lipids were measured at 0, 1, 6 and 12 months. Consuming linseed resulted in a 15% reduction of circulating LDL-C after 1 month ($p=0.05$). The concentration in the linseed group (2.1 ±0.10 mmol/L) tended to be less than in the placebo group (2.5 ±0.2 mmol/L) at 6 months ($p=0.12$), but not at 12 months ($p=0.33$). TC also tended to be lower in the linseed group than in the placebo group after one month (11 %, $p=0.05$) and 6 months (11 %, $p=0.07$), but not at 12 months ($p=0.24$) [Edel 2015].

An 8-week, randomised, double-blind, placebo-controlled study was conducted in 55 hypercholesterolaemic subjects, receiving placebo or the linseed lignan SDG at 300 or 600 mg/day. Significant ($p<0.05$ to <0.001) effects of SDG treatment were found for the decrease of TC, LDL-C and glucose concentrations, when compared to placebo as well as to baseline. At weeks 6 and 8 in the 600 mg SDG group, the decreases of TC and LDL-C concentrations were in the range from 22 to 24.9% respectively (all $p<0.005$ compared with placebo). For the 300 mg SDG group, the only significant differences from baseline were observed for decreases of TC and LDL-C. A substantial lowering of the concentrations of fasting plasma glucose was also noted in the 600 mg SDG group at weeks 6 and 8, especially in the subjects with baseline glucose concentrations ≥5.83 mmol/L (lowered by 25.6 and 25%; $p=0.015$ and $p=0.012$ respectively, compared with placebo). Plasma concentrations of SECO, enterodiol and enterolactone were all significantly raised in the groups supplemented with SDG. The observed cholesterol-lowering effects were correlated with the plasma concentrations of SECO and ED ($r = 0.128$-0.302; $p<0.05$ to <0.001) [Zhang 2008].

In a double-blind, randomised, placebo-controlled study, 30 hypercholesterolaemic men with TC levels of 4.65 to 6.21 mmol/L received SDG (20 or 100 mg/day) or placebo for 12 weeks. Subjects receiving 100 mg of SDG exhibited a significant ($p<0.05$) reduction in the ratio of LDL/HDL-cholesterol at week 12 compared to both baseline and placebo [Fukumitsu 2010].

In a 6-week, randomised, double-blind, placebo-controlled study, 37 subjects (13 men and 24 women; age: 54 ± 7 years; BMI: 29.7 ± 1 kg/m^2) consumed nutrition bars containing 3.0 g of α-linolenic acid and different amount of lignans (0.15 g vs. 0.41 g). The high-lignan bar decreased TC by 12% ($p=0.044$), LDL-C by 15% ($p=0.022$), and oxidised LDL by 25% ($p=0.035$). The lower lignan concentration tended to increase oxidised LDL by 13% ($p=0.051$). The difference between the effects of high-lignan vs. low lignan on oxidised LDL was significant ($p=0.004$) [Almario 2013].

Antihypertensive activity

In a meta-analysis of 15 trials (comprising 19 treatment arms) with 1302 participants following supplementation with various linseed products, significant reductions in both systolic blood pressure (SBP) (weighed mean difference: -2.85mmHg, $p=0.027$) and diastolic blood pressure (DBP) (weighed mean difference: -2.39mmHg, $p=0.001$) were shown. Stratification according to duration showed a greater effect on both SBP and DBP in the subset of trials with ≥12 weeks of duration (weighed mean difference: -3.10mmHg, $p=0.072$ and -2.62mmHg, $p=0.003$ respectively) vs. the subset treated for <12 weeks (weighed mean difference: -1.60mmHg, $p=0.413$, and -1.74mmHg, $p=0.202$, respectively). Reduction of SBP was significant with linseed powder (weighed mean difference: -1.81mmHg, $p<0.001$) but not oil (weighed mean difference: -4.62mmHg, $p=0.211$) or lignan extract (weighed mean difference: 0.28mmHg, $p=0.885$). However, DBP was significantly reduced with powder and oil preparations (weighed mean difference: -1.28mmHg, $p=0.031$, and -4.10mmHg, $p=0.003$, respectively), but not with lignan extract (weighed mean difference: -1.78 mmHg, $p=0.162$) [Ursoniu 2015].

In another meta-analysis including 11 studies (14 trials) involving 1004 participants, similar outcomes were found. Results indicated that linseed supplementation reduced SBP (-1.77mmHg; $p=0.04$) and DBP (-1.58mmHg; $p=0.003$). The results were not influenced by categorisation of participants with a higher baseline blood pressure (≥130mmHg). A significant improvement in DBP was observed in the subgroup consuming whole linseed (-1.93mmHg; $p<0.05$) and in the subgroup of studies with a duration of at least 12 weeks (-2.17mmHg; $p<0.05$) [Khalesi 2015].

In a parallel, single-blind trial, 75 overweight adolescents received 28 g/d of brown linseed, golden linseed or placebo for 11 weeks. The groups consuming brown and golden linseed showed a significant ($p<0.05$) reduction in diastolic blood pressure [Machado 2015].

In a prospective, randomised, double-blind, placebo-controlled trial, 110 patients with peripheral artery disease received 30 g of milled linseed or placebo daily for 6 months. SBP was approx. 10mmHg lower (–6.5% reduction; $p=0.04$), and DBP was approx. 7mmHg lower (–9.8% reduction; $p=0.004$), in the linseed group compared with placebo after 6 months. Patients with a SBP ≥140mmHg at baseline showed a significant reduction of 15mmHg in SBP ($p=0.04$) and 7mmHg in DBP ($p=0.004$) after intake of linseed. The antihypertensive effect was achieved selectively in hypertensive patients [Rodriguez-Leyva 2013].

Anti-inflammatory activity

In a randomised, double-blind, placebo-controlled crossover study, healthy postmenopausal women (n=22) consumed 500 mg/d of SDG or placebo daily for 6 weeks, separated by a 6-week washout period. A significant difference of approximately 15% ($p=0.028$) was observed for CRP concentration between SDG intervention period and placebo. Median CRP concentrations were 0.88 mg/L at baseline and 0.92 mg/L after SDG intervention period compared with 0.80 mg/L at baseline and 1.10 mg/L after placebo. No significant differences in IL-6, TNF-α, soluble intracellular adhesion molecule-1, soluble vascular cell adhesion molecule-1 and monocyte chemoattractant protein-1

were found between the SDG intervention and placebo periods [Hallund 2008].

Diabetic patients (26 men and 44 post-menopausal women) with mild hypercholesterolaemia completed a randomised, double-blind, placebo-controlled, cross-over trial receiving linseed-derived lignan (360 mg/day) or placebo for 12 weeks, separated by an 8-week wash-out period. Baseline to follow-up concentrations of CRP increased significantly within the placebo group (1.42 ± 0.19 vs. 1.96 ± 0.22 mg/L, p<0.001), but were comparatively unchanged in the lignan-supplemented group (1.67 ± 0.19 vs. 1.90 ± 0.26 mg/L, p=0.94); a significant mean difference was observed between treatments of -0.45 mg/L, p=0.021. This effect was confined to women (p=0.016). No between-treatment differences were found with regard to IL-6 or RBP4; although IL-6 concentrations increased significantly from baseline to follow-up in both groups (p=0.004 and p<0.001 following lignan and placebo treatments respectively) [Pan 2009a].

Patients with chronic renal failure undergoing haemodialysis therapy (n=160) were included in a randomised, double-blind, multicentre, placebo-controlled trial. The patients received linseed oil (1 g twice a day) or placebo (mineral oil, 1 g twice a day) for a period of 120 days. Inflammation was observed in 89 patients (61%) at the beginning of the study. There was a correlation between CRP and the body mass index (R_s=0.22; p=0.022) and HDL-cholesterol (R_s=–0.23; p=0.032). The CRP levels decreased significantly in the verum group compared to placebo (p<0.001). In the verum group inflammation disappeared in 33.3% of the patients, but only in 16.9% of the placebo group (p=0.04) [Lemos 2012].

Effect on blood glucose levels
In an 8-week, randomised, double-blind, placebo-controlled study, 55 hypercholesterolaemic subjects received either placebo, or SDG at 300 or 600 mg/day, in order to determine the effect on fasting glucose levels. A significant decrease in fasting plasma glucose concentrations was found in the 600 mg SDG group at weeks 6 and 8 compared with either placebo (p=0.019) or baseline. This decrease was greater in subjects with baseline glucose levels ≥5.8 mmol/L. At weeks 6 and 8 plasma glucose levels had decreased by 25.6% and 25.0% in the 600 mg SDG group compared with placebo (p=0.022 and p=0.021 respectively) and compared with baseline (p=0.003 and p=0.005 respectively) [Zhang 2008].

In a randomised, double-blind, placebo-controlled, cross-over trial, 73 type 2 diabetic patients with mild hypercholesterolaemia received SDG (360 mg per day) or placebo for 12 weeks, separated by an 8-week wash-out period. Of the participants, 68 completed the study. SDG significantly improved glycaemic control as measured by HbA1c (-0.10 ±0.65% vs. 0.09 ±0.52%, p=0.001) compared to placebo. However, no significant changes were observed on fasting glucose and insulin concentrations and resistance [Pan 2007].

In a randomised, cross-over study, overweight or obese men and postmenopausal women (n=25) with pre-diabetes consumed 0, 13 or 26 g ground linseed for 12 weeks. A significant decrease (p=0.036) in plasma glucose was shown for the 13 g treatment group (-2.10 ±1.66 mg/L) as compared to control (+9.22 ±4.44 mg/L). Insulin decreased in the 13 g group (-2.12 ±1.00 mU/L), but not in the 26 g (+0.67 ±0.84 mU/L) (p=0.021) and control (+1.20 ±1.16 mU/L) (p=0.013) groups [Hutchins 2013].

A total of 58 obese postmenopausal women were randomised to a single-blinded, parallel-group intervention with a daily intake of linseed mucilage (10 g) or placebo for 6 weeks. The intake of linseed mucilage led to a reduction in serum C-peptide and insulin release during an oral glucose tolerance test (p<0.05), and improved insulin sensitivity measured by the Matsuda index (p<0.05) [Brahe 2015].

Anti-tumour activity
In a systematic review including 2 randomised controlled trials, 2 uncontrolled trials, one biomarker study and 5 observational studies, linseed (25 g/day) increased the tumour apoptotic index (p<0.05) and decreased HER2 expression (p<0.05) and cell proliferation (Ki-67 index; nonsignificant) among newly diagnosed breast cancer patients when compared with placebo. The uncontrolled and biomarker studies suggested beneficial effects on cell proliferation, atypical cytomorphology and mammographic density, as well as a potential anti-angiogenic activity at doses of 25 g ground linseed or 50 mg SDG daily. Observational data suggest associations between linseed and decreased risk of primary breast cancer (adjusted odds ratio [AOR]=0.82; 95% CI=0.69-0.97), better mental health (AOR=1.76; 95% CI=1.05-2.94), and lower mortality (multivariate hazard ratio = 0.69; 95% CI=0.50-0.95) among breast cancer patients [Flower 2014].

In a multi-centre, randomised, controlled trial, 161 prostate cancer patients were assigned at least 21 days before prostatectomy to one of the following four groups: control (usual diet); linseed-supplemented diet (30 g/day); low-fat diet (<20% total energy); or linseed-supplemented, low-fat diet. Proliferation rates were significantly lower (p<0.002) among men assigned to the linseed groups. Median Ki-67 positive cells/total nuclei ratios (x100) were 1.66 (linseed-supplemented diet) and 1.50 (linseed-supplemented, low-fat diet) vs. 3.23 (control) and 2.56 (low-fat diet). No differences were observed between the groups with regard to side effects, apoptosis and most of the serological endpoints [Demark-Wahnefried 2008].

Pharmacokinetic properties

Pharmacokinetics in humans

Absorption
SDG undergoes metabolism principally to SECO, enterodiol and enterolactone in the human gastrointestinal tract. In the polarised Caco-2 cell system, lignans (100 µM) were added to acceptor or donor compartments and samples were taken at 2 h. Apical-to-basal permeability coefficients for SECO, enterodiol and enterolactone were 8.0 ± 0.4, 7.7 ± 0.2 and 13.7 ± 0.2 (x10-6) cm/s respectively, whereas efflux ratios were 0.8-1.2, consistent with passive diffusion. Permeation of SDG was not detected [Mukker 2014].

Distribution
In digested whole linseed, the bioaccessibility values (free lignan)/(total lignan) of SECO, enterodiol and enterolactone were 0.75%, 1.56% and 1.23% respectively. Conversely, in digested linseed flour, the bioaccessibility values of SDG, enterodiol and enterolactone were 2.06%, 2.72% and 1.04% respectively. The anaerobic count and short-chain fatty acids indicate that bacteria survival and carbohydrate fermentation occurred [Fuentealba 2014].

Metabolism
Three interrelated pharmacokinetic studies were performed in healthy postmenopausal women after oral intake of 25 to 172 mg of SDG. Steady-state lignan concentrations were obtained after daily intake for one week. Blood and urine samples were collected at timed intervals, and SECO, enterodiol and enterolactone concentrations measured. SDG was efficiently hydrolysed and converted to SECO. Serum concentrations increased after oral intake with t_{max} of 5-7 h and $t_{1/2}$ of 4.8 h.

Maximum serum concentrations of enterodiol and enterolactone were established after 12-24 h and 24-36 h respectively, and $t_{1/2}$ was 9.4 h and 13.2 h respectively. A linear dose-response-relationship was observed and SECO bioavailability correlated (r^2=0.835) with cumulative lignan excretion [Setchell 2014].

For metabolism studies, lignans (100 µM) were incubated with Caco-2 cells for a maximum of 48 h. Cell lysates and media were treated with β-glucuronidase/sulfatase, and lignan concentrations were determined. The extent of conjugation after 48 h was <3%, ~95%, ~90%, and >99% for SDG, SECO, enterodiol, and enterolactone respectively [Mukker 2014].

Elimination
The effects of linseed powder on urinary lignan and isoflavonoid concentrations were investigated in 18 premenopausal women in a randomised crossover study. Each woman consumed her usual omnivorous diet, except avoiding foods containing linseed or soy (high isoflavone content) for 3 menstrual cycles and her usual diet supplemented with linseed (10 g/day) for another 3 cycles, or vice versa. Three-day urine samples from follicular and luteal phases were analysed. Excretion of the two major mammalian lignans, enterodiol and enterolactone, increased with linseed supplementation from 1.09 ±1.08 and 3.16 ±1.47 to 19.48 ±1.10 and 27.79 ±1.50 µmol/day respectively (p<0.0002). Enterodiol and enterolactone excretion in response to linseed varied widely among the subjects (3- to 285-fold increase). There were no differences in excretion of isoflavonoids or the lignan matairesinol with linseed. Excretion was not altered by phase of the menstrual cycle or duration of linseed consumption [Lampe 1994].

As a sub-study of the above study, involving 13 women from the original 18 and the same dietary design, faeces were collected on days 7-11 of the last menstrual cycle in each diet period. Excretion of lignans (nmol/day) increased significantly with linseed intake, from 80.0 ± 80.0 to 2560 ± 3100 for enterodiol (p<0.01), from 640 ± 480 to 10,300 ± 7580 for enterolactone (p<0.01), and from 7.33 ±10.0 to 11.9 ±8.06 nmol/day for matairesinol (p<0.05). There were no differences in faecal excretion of isoflavonoids [Kurzer 1995].

In a randomised, cross-over study, 9 healthy young women supplemented their diets with 5, 15 or 25 g of raw or 25 g of processed (as muffin or bread) linseed for 7 days during the follicular phase of their menstrual cycles. Urine samples (24-hours) were collected at baseline and on the final day of supplementation. As an adjunct to the 25 g raw linseed arm, they consumed 25 g for an additional day. Blood and urine samples were collected at specific intervals and analysed for enterolactone and enterodiol. A dose-dependent increase in urinary lignan excretion in response to linseed was observed (r=0.72, p<0.001), and processing did not affect the quantity of lignan excretion. Plasma lignan concentrations were significantly greater than baseline (p<0.001) by 9 hours after linseed ingestion (29.35 and 51.75 nmol/L respectively). The total plasma AUC was higher on the 8th than on the 1st day (1840 and 1027 nmol·h/L, respectively) [Nesbitt 1999].

Preclinical safety data

Acute toxicity
No toxic effects of linseed were observed in a brine shrimp lethality bioassay [Mahmoud 1992].

Mutagenicity and carcinogenicity
Linseed did not show any mutagenic activity in the Ames test using Salmonella typhimurium strains TA 98 and TA 102 [Mahmoud 1992].

Reproductive toxicity
Linseed and linseed meal exerted reproductive changes in the offspring of rats, such as shortening of the anogenital distance and lengthening of oestrous cycles. However, maternal consumption of linseed or SDG during suckling did not affect reproductive indices in male or female offspring [Adolphe 2010].

There were no significant effects of exposing male or female offspring to SDG during suckling on any measured reproductive indices [Imran 2015].

Clinical safety data
No drug-related adverse effects were observed from treatment of 70 patients with functional upper abdominal complaints with an aqueous linseed mucilage preparation (1:10) at a dosage of 8 × 25 g (including a small amount of excipients) daily for 3 days [Grützner 1997].

Natural, heated and extruded fresh linseed were tested using prick-in-prick tests (PIP), SDS PAGE, immunoblots, immunoblot inhibition and Fourier Transform Infrared spectroscopy. PIP tests to linseed were positive in 5.8% of the 1,317 patients. Of the 77 PIP-positive patients, 73 were atopic. There was cross-reactivity with peanut, soybean, rapeseed, lupine and wheat as well as with rape pollen [Fremont 2010].

Participants aged 49-87 years were randomised in a double-blind trial to receive linseed lignan (543 mg/day) or placebo while completing a 6-month walking program. Participants (n=94) who completed the study were stratified by age (<65 years versus >65 years) and treatment category to determine whether older adults were more susceptible to adverse effects. After 6 months of treatment, average plasma glucose level (5.4 ± 0.6 mmol/L), SBP (127 ± 14mmHg), and DBP (80 ± 9mmHg) were within normal clinical range. Controlling for sex and body mass index covariates resulted in no differences between plasma glucose or blood pressure measurements between treatment or age groups (p>0.05). No incidents of hypoglycaemia or hypotension were observed during treatment [Billinsky 2013].

A case of anaphylaxis after intake of linseed was reported. Skin prick tests with linseed yielded positive results [Alonso 1996; Alvarez-Perea 2013].

Investigations in healthy women suggest that linseed may have an oestrogenic effect [Phipps 1993]. There is a lack of sufficient data about the safety of linseed in pregnancy [Basch 2007]. In accordance with general medical practice, the product should not be used during pregnancy without medical advice.

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ALOE CAPENSIS	Cape Aloes	Online Series, 2014
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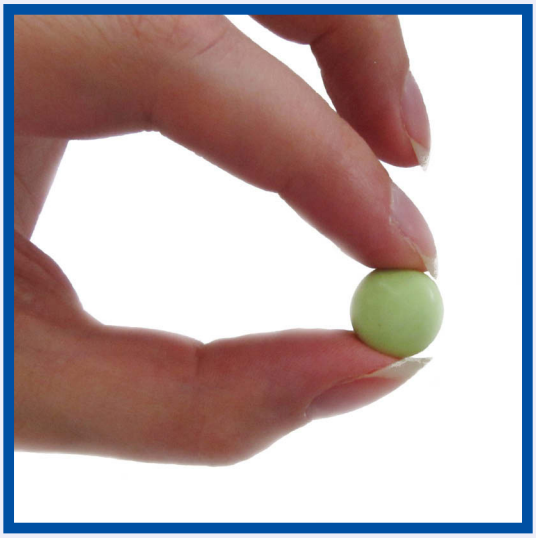

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Plant illustrated on the cover: *Malva sylvestris*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

MALVAE FLOS

Mallow Flower

2016

DEFINITION

Mallow flower consists of the whole or fragmented dried flowers of *Malva sylvestris* L. or its cultivated varieties.

The material complies with the monograph of the European Pharmacopoeia [Malvae flos].

CONSTITUENTS

The main characteristic constituents are:

- Mucilage polysaccharides (6% to more than 10%; mean molecular weight 83,000 D) composed of neutral and acidic monosaccharide residues including galactose, rhamnose, arabinose, glucose and xylose as well as galacturonic acid and glucuronic acid [Classen 1998; Franz 1966; Rosik 1984; Karawya 1971; Blaschek 2014; Paris, Moyse 1967].

- Anthocyanins (6-7%), mainly malvidin 3,5-diglucoside, malvidin 3-glucoside, malvidin 3-(6''-malonylglucoside)-5-glucoside and delphinidin 3-glucoside with traces of petunidin and cyanidin glycosides [Blaschek 2014; Pourrat 1990; Takeda 1989].

- Flavonols such as quercetin-3-*O*-rutinoside (1.5%) and kaempferol-3-*O*-rutinoside (0.084%) [Barros 2012].

Other constituents include scopoletin [Tosi 1995], ursolic acid, phytosterols [Karawya 1979] and total tocopherol (0.017%) [Barros 2010].

CLINICAL PARTICULARS

Therapeutic indications
Dry cough; irritation of the oral, pharyngeal or gastric mucosa [Blaschek 2014; Nosal'ova 1994; Braun 1987].

Posology and method of administration

Dosage

Internal use
Adult dose: 1.5-2 g of the drug as an aqueous cold macerate or hot infusion, repeated if required up to a daily dose equivalent to 5 g of the drug [Blaschek 2014; Saller 1995].

External use
As a gargle, a 5% decoction [Van Hellemont 1986].

Method of administration
For oral administration or local application.

Duration of administration
No restriction. If symptoms persist or worsen, medical advice should be sought.

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported. However, mucilages can reduce the absorption of other drugs taken simultaneously [Consolini 2010].

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Pregnancy and lactation
No human data available. In accordance with general medical practice, the product should not be used during pregnancy or lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None known.

Overdose
No case of overdose reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties
The mucilage from mallow flower covers the epithelium, especially of the mouth and pharynx, affording protection from local irritations [Blaschek 2014; Braun 1987; Saller 1995].

In vitro experiments

Adhesion to buccal epithelium
A polysaccharide fraction (> 95% carbohydrate content) from mallow flower exhibited relatively weak adhesion to isolated porcine buccal membrane in comparison with the stronger bioadhesive effects of similarly-prepared polysaccharide fractions from marshmallow root or calendula flower [Schmidgall 2000].

Antioxidant activity
A methanolic extract showed antioxidant activity in a variety of tests: DPPH radical-scavenging activity, reducing power, inhibition of β-carotene bleaching and inhibition of lipid peroxidation [Barros 2010].

Mallow flower demonstrated dose-dependent increases in radical scavenging activity and inhibition of lipid peroxidation. The highest rate of free radical scavenging (43.5%) and the strongest inhibition of lipid peroxidation (18.8%) were obtained at a concentration of 0.20 mg/mL anthocyanins [Wang 2005].

Antibacterial and antifungal activity
A methanolic extract (not further specified) showed antibacterial activity against *Erwinia carotovora*, *Staphylococcus aureus*, *Streptococcus agalactiae* and *Enterococcus faecalis* with MIC values of 128, 192, 200 and 256 µg/mL, respectively. The extract showed a modest antifungal activity against *Candida kefyr* and *C. albicans* with an MIC range of 640 to 800 µg/mL [Razavi 2011].

In vivo experiments

Antitussive effects
The mucilage isolated from mallow flowers (*Malva sylvestris* ssp. *mauritiana*), and the acidic polysaccharide fraction from it (consisting of a rhamnogalacturonan), were administered orally to non-anaesthetized cats at 50 mg/kg b.w., in order to examine their antitussive effects. A surgically-implanted tracheal cannula served for mechanical stimulation of the airways as well as recording the side tracheal pressure on an electromanometer. Mucous membranes in the laryngopharyngeal and tracheobronchial areas were stimulated five times in succession with a nylon fibre and the effects were monitored 0.5, 1, 2 and 5 hours after administration. Both the mucilage and the rhamnogalacturonan fraction markedly reduced the number of cough efforts and the intensity of cough attacks (in both inspiration and expiration). The mucilage reduced cough frequency mainly from the laryngopharyngeal area, while the rhamnogalacturonan fraction was effective in both areas. Only the rhamnogalacturonan fraction influenced the intensity of maximum cough efforts. The effect of the mucilage and the rhamnogalacturonan fraction in suppressing the number of cough efforts was compared to that of the non-narcotic antitussive drugs prenoxdiazine (30 mg/kg) and dropropizine (100 mg/kg) and the narcotic drug codeine (10 mg/kg, as phosphate). Both herbal substances had a cough-suppressing effect higher than that of prenoxdiazine or dropropizine but lower than that of codeine [Nosal'ova 1994].

Effects on plasma lipids and free radicals
Albino rats fed a lipid rich diet received anthocyanins from mallow flower at 0.03, 0.04 and 0.05 g daily for 14 days. Plasma total cholesterol decreased by 19.7% at an anthocyanin dose of 0.04 g daily and triglycerides by 34.4% at an anthocyanin dose of 0.05 g daily [Wang 2005].

Other effects
A dry 95%-ethanolic extract administered i.p. at 50 mg/kg b.w. increased the survival rate of mice inoculated with *Escherichia coli*. The effect was attributed to stimulation of phagocytic activity of the reticulo-endothelial system [Delaveau 1980].

Pharmacokinetic properties
No data available.

Preclinical safety data
No data available.

Clinical safety data
No data available.

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Supplement 2009
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Second Edition, 2003
FUMARIAE HERBA	Fumitory	Supplement 2009
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Second Edition, 2003
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003

LINI SEMEN	Linseed	Second Edition, 2003
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Second Edition, 2003
RATANHIAE RADIX	Rhatany Root	Supplement 2009
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Second Edition, 2003
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Second Edition, 2003
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Second Edition, 2003
SALICIS CORTEX	Willow Bark	Second Edition, 2003
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

E/S/C/O/P Monographs

Online Series

The Scientific Foundation for Herbal Medicinal Products

The second edition of ESCOP Monographs, published as a hardback book in 2003 with a Supplement in 2009, has been widely acclaimed for its authoritative information on the therapeutic uses of herbal medicines. Monographs covering a total of 107 herbal substances include extensive summaries of pharmacological, clinical and toxicological data, and copious references to scientific literature form an important part of each text.

Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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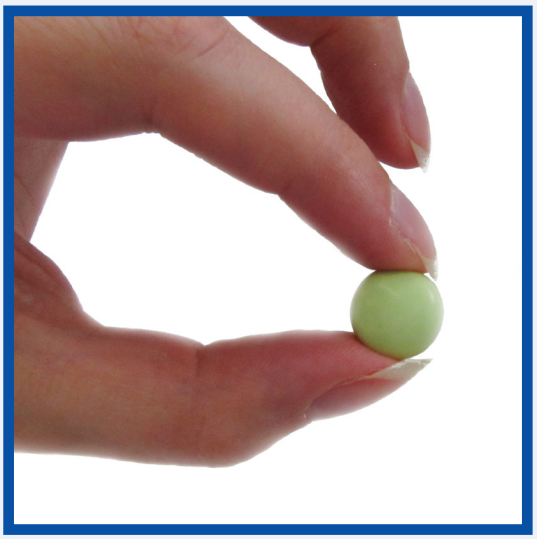
The Scientific Foundation for Herbal Medicinal Products

Marrubii herba
White horehound

2013



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***The* Scientific Foundation for**
Herbal Medicinal Products

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

Edited by Simon Mills and Roberta Hutchins
Cover photograph by Prof. Salvador Cañigueral Folcarà (*Marrubium vulgare*)
Cover and text design by Martin Willoughby
Typeset in Optima by Roberta Hutchins

Plant illustrated on the cover: *Marrubium vulgare*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

MARRUBII HERBA

2013

White horehound

DEFINITION

White horehound consists of the dried, entire or fragmented, flowering aerial parts of *Marrubium vulgare* L. It contains not less than 0.7 per cent of marrubiin ($C_{20}H_{28}O_4$; M_r 332.4) with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [White horehound].

CONSTITUENTS

The characteristic constituents are the labdane-type diterpenes marrubiin (0.12 to 1.0%) and marrubenol, metabolically derived from their precursors premarrubiin (0.13%) and premarrubenol during the growth period, as well as peregrinol or vulgarol; up to 7% tannins; up to 0.1% phenylethanoid esters, including acteoside, ballotetroside, forsythoside B, marruboside and arenarioside; essential oil (0.05 to 0.06%) consisting mainly of phenylpropanoids, mono- and sesquiterpenes; the hydroxycinnamic acids chlorogenic, caffeic, caffeoyl-L-malic acid and 1-caffeoylquinic acid; flavon- and flavonol glycosides, lactoylflavones; the methoxylated flavone ladanein; amines including choline (0.2%) and betonicine (0.3%) [Nawwar 1989; Sahpaz 2002a; Sahpaz 2002b; Martin-Nizard 2003; Belhattab 2006; Knöss 2006; Seitz 2007; Morteza-Semnani 2008; Blaschek 2009; Ahmed 2010; Alkhatib 2010; Zawislak 2011].

CLINICAL PARTICULARS

Therapeutic indications

Loss of appetite; dyspeptic complaints such as bloating and flatulence [Jänicke 2003; Schilcher 2007; Seitz 2007; Blaschek 2009].
Catarrh of the upper respiratory tract [Bradley 1992; Teuscher 2004; Schilcher 2007; Seitz 2007; Blaschek 2009].

Efficacy in these indications is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Adults:
4.5 g of the drug daily, or 1 to 2 g as an infusion three times daily [Bradley 1992; Jänicke 2003; Teuscher 2004; Barnes 2007; Schilcher 2007; Seitz 2007; Blaschek 2009].

Fluid extract (1:1, 20% ethanol): 1 to 4 ml three times daily [Bradley 1992; Jänicke 2003; Barnes 2007; Seitz 2007].

Tincture (1:5; 25% ethanol): 3 to 6 ml daily [Bradley 1992].

Pressed juice: 2 to 6 tablespoons daily [Jänicke 2003; Schilcher 2007; Seitz 2007; Blaschek 2009].

Method of administration
For oral administration.

Duration of administration
No restriction. If symptoms persist or worsen, medical advice should be sought.

Contraindications
None known.

Special warnings and special precautions for use
None required.

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Interaction with other medicaments and other forms of interaction
There are reports from a clinical trial with diabetic patients taking glibenclamide that consumption of aqueous white horehound extract was linked with minor symptoms of nausea, oral dryness or salivation, and dizziness. [Herrera-Arellano 2004].

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
See Interactions above.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro studies

Antioxidant activity
Aqueous, and methanolic extracts and the essential oil of white horehound have been shown to have free radical scavenging and antioxidant activities in various assays, including on ABTS- or DPPH-radicals, determination of ferric reducing/antioxidant power (FRAP) and the inhibition of linoleic acid peroxidation. In some of the studies the effects were attributed to the polyphenol content of the drug [VanderJagt 2002; Berrougui 2006; Katalinic 2006; Matkowski 2006; Wojdylo 2007; Dall´Acqua 2008, Kadri 2011; Pukalskas 2012.].

A 40%-methanolic dry extract (DER 25:1) inhibited the formation of conjugated dienes (CD) in a dose-dependent manner after incubation of human-LDL with $CuSO_4$. The lag phase before CD formation was significantly increased ($p=0.014$) and the maximum rate of oxidation decreased significantly ($p= 0.004$). The degradation of α-tocopherol and the electrophoretic mobility of LDL (both induced by oxidation) were attenuated. HDL-mediated cholesterol efflux from THP-1 macrophages was potentiated in a dose-dependent manner from 50 µg/ml ($p<0.05$) to 100 µg/ml ($p<0.01$) [Berrougui 2006].

Acteoside, forsythoside B, arenarioside, ballotetroside and caffeoyl-malic acid isolated from white horehound inhibited copper-initiated LDL oxidation with ED_{50} values of 0.46, 0.68, 0.64, 1.82 and 2.07 µM, respectively. There were ED_{50} values of 0.39, 0.45, 0.50, 0.72 and 1.04 µM for the inhibition of oxidation induced by 2,2´-azobis(2-aminopropane)dihydrochloride. Extra- and intracellular peroxidation in endothelial cells after incubation with minimally oxidized LDL was measured by the accumulation of thiobarbituric acid reactive substances (TBARS). At a concentration of 10 µM each compound significantly decreased the accumulation of TBARS ($p<0.001$) [Martin-Nizard 2003].

Endothelin-1 secretion in bovine aortic endothelial cells was shown to be significantly increased by treatment with LDL ($p<0.01$) and copper-oxidised LDL ($p<0.001$). Acteoside, forsythoside B, arenarioside and ballotetroside previously isolated from white horehound completely eliminated this effect. The increase in endothelin-1 gene expression after treatment with copper oxidised LDL was also reversed by these compounds ($p<0.05$) [Martin-Nizard 2004].

Vasorelaxant effects
An aqueous extract of white horehound (DER approx 6:1) given to spontaneously hypertensive rats at an oral dose of 80 mg/kg b.w. for five days, reduced KCl-induced contractions in subsequently isolated aorta by 19% ($p<0.05$). Noradrenaline-induced contractions were also decreased.Preincubation of aortic rings with the extract resulted in a dose-dependent inhibition ofKCl-induced contractions. Maximum effects were reached with 0.7 mg/ml and led to an 87% decrease in contraction of aortic rings of spontaneously hypertensive rats (EC50 0.14 mg/ml) and a 73% decrease in those of normotensive rats (EC50 0.19 mg/ml) ($p<0.05$). Calcium-induced contractions in KCl-depolarized aortic rings were markedly decreased by the extract. Contractions induced by noradrenaline were inhibited by 73% and 37% respectively, in aortic rings of spontaneously hypertensive and normotensive rats. [El Bardai 2001].

The contractile tension induced by KCl in isolated rings of the aorta and the mesenteric artery of rats treated orally with an aqueous extract (80mg/kg/day for 10 weeks) was significantly lower when compared to those of untreated rats ($p<0.05$). The effect disappeared in the presence of the NO synthase inhibitor L-NOarginine. Acetylcholine-induced relaxation of the mesenteric artery following contraction induced by noradrenaline was shown to improve [El Bardai 2004].

A dose-dependent inhibition of KCl-induced contractions in isolated rat aorta was observed after preincubation with an aqueous extract of white horehound. After fractionation of the extract the effect was most pronounced in a cyclohexane fraction with 61.2% inhibition at a concentration of 16 µg/ml and 100% inhibition at a concentration of 64 µg/ml. From this fraction marrubiin and marrubenol were isolated, significantly reducing the contractions, with IC_{50} values of 24 µM and 7.7 µM respectively ($p<0.05$) [El Bardai 2003a].

In aortic rings, after removal of the endothelium, a similar relaxant effect was observed for marrubenol with an IC_{50} of 11.8 µM and maximum inhibition of KCl-induced contractions of 93.4%. Marrubenol inhibited contractions induced by noradrenaline to a lesser extent, and was ineffective in the same experiment in the presence of the Ca^{2+} channel blocker nimodipine. In fura-2 loaded aorta, both the cytosolic Ca^{2+} concentration and the contractions were decreased by marrubenol in a dose-dependent manner. The decrease of the quenching rate of the fluorescence of the fluorescent marker fura-2 in the presence of Mn^{2+} suggested that the activity of marrubenol was caused by an inhibition of Ca^{2+} influx. The study demonstrated that marrubenol decreased Ca^{2+} influx in aortic smooth muscle cells and inhibited smooth muscle contraction by blocking L-type calcium channels [El Bardai 2003b].

Other effects
A methanolic dry extract from white horehound leaf significantly suppressed cell growth in the human colon adenocarcinoma cell line HCT-116 by induction of apoptosis at a concentration of 250 µg/ml ($p<0.05$). At 100 µg/ml the extract increased the expression of the pro-apoptotic protein NAG-1 [Yamaguchi 2006].

An ethanolic extract (not further specified) exhibited moderate activity on the viability of a murine neuroblastoma cell line as assessed with resazurin-almar blue indicator dye (LC_{50} 3.64 mg/ml) [Mazzio 2009].

An ethanolic dry extract of white horehound exhibited anti-

microbial activity against *Bacillus subtilis* in the XTT colorimetric assay [Al-Bakri 2007].

A dry acetone extract (yield 6.6%) led to 67.5% inhibition of acetylcholinesterase from the electric eel and 83.5% inhibition of butyrylcholinesterase from horse serum at dosages of 50 µg/mL [Orhan 2010].

A methanolic extract exhibited antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* (MIC 100 mg/ml) as well as *Staphylococcus epidermidis* (MIC 200 mg/ml) [Masoodi 2008].

In an immunomodulation assay an extract prepared with phosphate-buffered saline (10 g drug/100 mL) increased the proliferation of mice splenocytes ($p<0.05$) as compared with concanavalin-A. The protein fraction of this extract reduced the mitogenic effect of concanavalin-A [Daoudi 2012].

Acteoside, forsythoside B and arenarioside isolated from white horehound showed COX-2 inhibition of 63.9%, 72.5% and 67.8% respectively, at a concentration of 1 mM. The IC_{50} values were 0.69, 0.49 and 0.61 mM. The compounds did not inhibit COX-1 [Sahpaz 2002a].

In endothelial cells, acteoside, forsythoside B, arenorioside, ballotetroside and caffeoylmalic acid (10 µM) significantly decreased the cytotoxicity of minimally oxidized LDL ($p<0.001$) [Martin-Nizard 2003].

Ladanein, isolated from white horehound, showed moderate cytotoxicity against the murine leukemia cell line DA1-3b/M2$^{BCR-ABL}$ and the human leukaemia cell lines K562, K562R and 697 with IC_{50} values of 10.4, 25.1, 38.0 and 38.0 µM, respectively. It remained without effect on human acute myeloid leukaemia cells MOLM13 and on human peripheral blood mononuclear cells from healthy volunteers [Alkhatib 2010].

In vivo studies

Gastroprotective effects
A methanolic leaf extract from white horehound (not further specified) and isolated marrubiin were tested in different models of gastric ulcer. Swiss mice received a single oral dose of 25, 50 or 100 mg/kg b.w. of the extract or 25 mg/kg marrubiin. In ulcers induced by ethanol/HCl, the extract at 50 and 100 mg/kg and marrubiin significantly reduced the ulcer lesion index ($p<0.01$). A significant decrease in the total area of lesions and the percentage of lesion area was observed but only at the highest dose of the extract and marrubiin ($p< 0.01$). In indomethacin/bethanecol-induced ulcers all doses of the extract and marrubiin led to a significant reduction of the total area of lesions, the percentage of lesion area and the ulcer lesion index ($p<0.01$). The total area of lesions and the percentage of lesion area were significantly improved by 50 and 100 mg/kg of the extract and marrubiin ($p<0.05$) as compared to the positive control cimetidin. The two higher concentrations of the extract and marrubiin significantly raised gastric pH and decreased the concentration of H^+ ions ($p<0.01$). Free gastric mucus in the tissue was enhanced in all treated groups ($p<0.01$). After pre-treatment with L-NAME it was concluded that the protective effects of the extract and marrubiin were related to NO synthesis [Paula de Oliveira 2011].

Analgesic effects
Marrubiin isolated from white horehound exhibited pronounced antinociceptive effects in different mouse models [De Jesus 1999]:

- Acetic acid-induced writhing was reduced by pretreatment with marrubiin administered intraperitoneally 30 minutes before the challenge in a range of doses. The effect was significant for up to five hours ($p<0.01$ up to four hours, $p<0.05$ at five hours). Co-treatment with naloxone did not affect the activity of marrubiin. The ID_{50} was 2.2 µmol/kg; for aspirin it was 133 µmol/kg and for diclofenac 38 µmol/kg.
- In the formalin-induced pain test, first phase nociception after 5 minutes (representing neurogenic pain) and second phase effects after 15 to 30 minutes (representing inflammatory pain) were registered. Both pain phases were significantly inhibited ($p<0.05$) in a dose-dependent manner following pretreatment of the animals with marrubiin (3 to 90 µmol/kg intraperitoneally or 90 to 900 µmol/kg orally) 60 minutes before the formalin injection.
- Capsaicin-induced neurogenic nociception was inhibited by pretreatment with marrubiin at intraperitoneal doses of 3 to 90 µmol/kg (ID_{50} 28.8 µmol/kg; maximum inhibition 76 %).
- Marrubiin administered intraperitoneally at 180 µmol/kg had no effect in the hot plate test.

Anti-oedematous effects
In a model of microvascular leakage in mouse ears, marrubiin administered intraperitoneally at 1-100 mg/kg exhibited a significant dose-dependent antioedematous effect as determined by extravasated Evans blue. The ID_{50} and maximal inhibition of oedema induced by carrageenan were 13.6 mg/kg and 63% ($p<0.01$) respectively; in histamine-induced oedema 13.8 and 73.7% ($p<0.01$); and in bradykinin-induced oedema 15.8 mg/kg and 70% ($p<0.01$). Oedema after treatment with compound 4880, serotonin or dextran was reduced by up to 46.9%, 49.3% and 32% ($p<0.01$), respectively. To determine the activity in neurogenic inflammation, microvascular extravasation of Evans blue was induced with capsaicin or substance P. For these agents maximal inhibition was 28% and 27.6%. In mice sensitized to ovoalbumin, the allergic oedema after rechallenge was reduced by 67.6% for marrubiin (100 mg/kg i.p.; $p<0.01$) as compared to a 69.8% reduction by dexamethasone (0.5 mg/kg i.p.) [Stulzer 2006].

Antihypertensive effects
An aqueous extract from white horehound (DER appr. 6:1) administered at an oral daily dose of 80 mg/kg b.w. for five days, significantly lowered the systolic blood pressure in spontaneously hypertensive rats ($p<0.05$) but not in normotensive rats. Urine output, as well as the excretion of electrolytes, creatinine and urea, remained almost unchanged in hypertensive and normotensive rats [El Bardai 2001].

Oral treatment of spontaneously hypertensive rats with an aqueous white horehound extract at 80 mg/kg b.w./day for 10 weeks resulted in a significant decrease in systolic blood pressure ($p<0.05$), similar to amlodipine at 10 mg/kg b.w./day. The extract reduced aortic but not mesenteric artery weight ($p<0.05$). [El Bardai 2004].

Hypoglycaemic effects
Oral administration of 300 mg/kg b.w. of an ethanolic extract of white horehound (not further specified) to alloxan-induced diabetic rats led to a significant decrease of the blood glucose level ($p<0.01$) [Novaes 2001].

A methanolic extract (DER approximately 8.3:1) was administered at a daily dose of 500 mg/kg b.w. for 28 days to male Wistar rats. Treatment started 11 days after induction of diabetes with streptozotocin. Blood glucose levels were significantly reduced on treatment days 14, 21 and 28 as compared to diabetic control and baseline (all $p<0.05$), and even slightly better than in the group treated with glibenclamide. Plasma insulin, muscle

glycogen and liver glycogen were significantly increased on day 28 as compared to diabetic control ($p<0.05$). Positive effects on plasma lipid profile after treament with the extract were observed: a 24% decrease in total cholesterol, a 27% reduction in LDL cholesterol and a 27% increase of HDL cholesterol ($p<0.05$ as compared to diabetic control). The treatment improved hepatic enzyme activity and nearly normalized glutathione peroxidase, glutathione reductase, glutathione-S-transferase, reduced glutathione and malondialdehyde levels (all $p<0.05$). In an oral glucose tolerance test the effect of the extract on the total AUC was similar to glibenclamide [Elberry 2011].

Alloxan-induced diabetic Wistar rats were treated with an aqueous extract of white horehound (6 g/25 ml, containing 5.1 mg flavonoids and 14.1 mg cinnamic acid derivatives per 100 mg dry weight; DER 8.3:1). Oral doses of 100, 200 and 300 mg/kg b.w.were administered twice daily for 15 days. From day 5 a significant decrease of blood glucose was observed at all doses as compared to diabetic control ($p<0.001$). The dose-dependent decrease in glycaemia was 50.75%, 61.06% and 62.55% respectively. No significant changes in body weight were observed for the animals treated with white horehound as compared to normal control. The increase in serum glucose, total lipids, triglycerides and total cholesterol in the diabetic rats was significantly reduced by the extract ($p<0.001$) at the end of the experiment. The effects were comparable to the positive control glibenclamide (5 mg/kg b.w.) [Boudjelal 2012].

A slight but not significant hypoglycaemic effect was observed in rats pretreated with an ethanolic dry extract from white horehound leaves (not further specified) at a single oral dose of 100 mg/kg b.w. received 30 min. after a glucose load of 2g/kg [Vergara-Galicia 2012].

Hepatoprotective effects
A terpenoid isolated from white horehound, *p*-menthane-5,6-dihydroxy-3-carboxylic acid, was administered orally to male Wistar rats at a dose of 50 mg/kg b.w. for 7 days after hepatotoxic challenge with CCl_4. The increase in SGOT, SGPT and ALP after intoxication was significantly reduced by the treatment ($p<0.01$, 0.01 and 0.05, respectively). The decreased level in total proteins was significantly increased ($p<0.05$). A recovery of histopathological changes to almost normal architecture of the hepatocytes after treatment with the compound was observed [Ahmed 2010].

Pharmacokinetic properties
No data available.

Preclinical safety data

Acute toxicity
After oral treatment of female rats with 2 g/kg b.w. of a methanolic extract from white horehound no signs of toxicity were observed after 30 minutes, during the first 4 hours after administration and over a follow-up period of 14 days [Paula de Oliveira 2011].

Repeated dose toxicity
Rats treated orally with an aqueous extract of white horehound at a dose of 80 mg/kg/day over a period of 10 weeks did not show any sign of adverse effects [El Bardai 2004].

Male Wistar rats receiving oral doses of 100, 250, 500 and 1000 mg/kg b.w. of a methanolic extract (DER approximately 8.3:1) for 3 weeks did not show any physical signs of toxicity during the experimental period [Elberry 2011].

Mutagenicity
A tincture of white horehound did not cause any mutagenic effect in *Salmonella typhimurium* strain TA98 in the Ames test [Schimmer 1994].

Clinical safety data

In a study with 21 diabetic patients treated with glibenclamide, an infusion of 1g white horehound three times daily was given for 21 days. Only minor side effects such as nausea, oral dryness, hypersalivation or dizziness were observed in five patients [Herrera-Arellano 2004].

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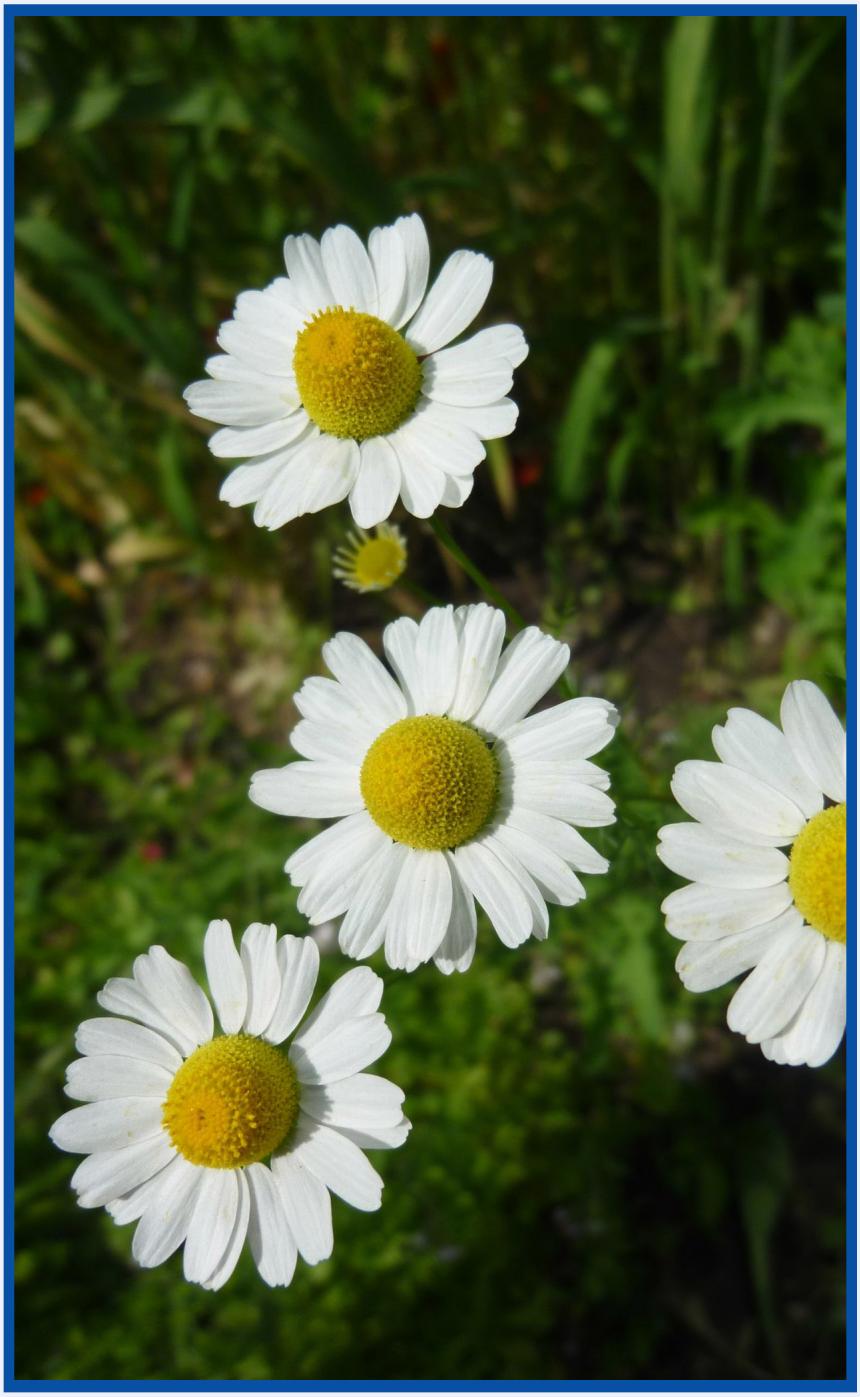

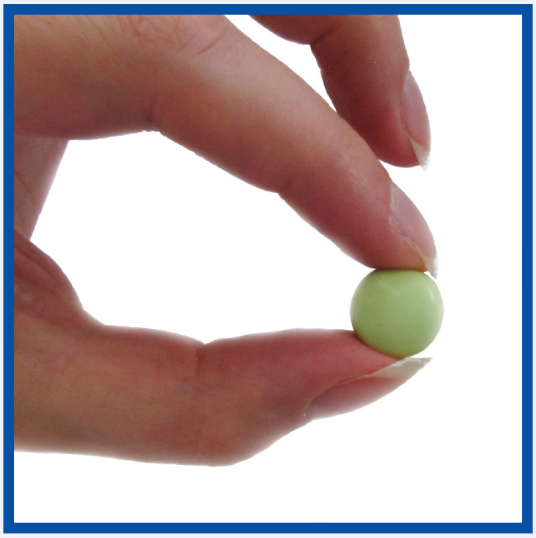

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FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

MATRICARIAE FLOS

2020

Matricaria Flower

DEFINITION

Matricaria flower consists of the dried capitula of *Matricaria recutita* L.[1] [*Chamomilla recutita* (L.) Rauschert]. It contains not less than 4 mL/kg of blue essential oil and not less than 0.25% total apigenin 7-glucoside ($C_{21}H_{20}O_{10}$; M_r 432.4).

The material complies with the European Pharmacopoeia [Matricaria].

Fresh material may also be used provided that when dried it complies with the European Pharmacopoeia.

CONSTITUENTS

The main characteristic constituents are:

- the essential oil (0.3 – 1.5%): up to 50% of the sesquiterpenes (–)-α-bisabolol and its oxides A, B and C, bisabolonoxide A, up to 25% of cis- and trans-en-yne-dicycloethers (or spiroethers), spathulenol and matricin, which is converted to chamazulene on distillation (up to 15%).
- flavonoids (up to 6%), mainly flavone derivatives such as apigenin-7-glucoside and its acetyl esters (approx. 0.5%).

Other constituents include coumarins (herniarin and umbelliferone), phenolic acids and polysaccharides (up to 10%) [Ammon 1992, Zwaving 1982, Schilcher 1987, Ammon 1992, Füller 1993a, Carle 2014, Sticher 2015].

CLINICAL PARTICULARS

Therapeutic indications

Internal use

Symptomatic treatment of gastrointestinal complaints such as minor spasms, epigastric distension, flatulence and belching [Weiß 1982, Bisset 2001, Schulz 2004, Carle 2014].

External use

Minor inflammation and irritations of skin and mucosa, including the oral cavity and the gums (mouth washes), the respiratory tract (inhalations) and the anal and genital area (baths, ointments) [Weiß 1982, Glowania 1987, Saller 1990, Förster 1996, Bisset 2001].

Posology and method of administration

Dosage

Internal use

Adults:
As a tea infusion: 3 g of the drug to 150 mL of hot water, three to four times daily.
Fluid extract (1:2; 50% ethanol as preferred extraction solvent): 3 – 6 mL daily [Mills 2000, Carle 2014].
Dry extract: 50-300 mg three times daily [van Hellemont 1988, Keefe 2016, Mao 2016].
Elderly: Dose as for adults.
Children: Proportion of adult dose according to age or body weight.

External use

For compresses, rinses or gargles: 3-10% m/V infusion or 1% V/V fluid extract or 5% V/V tincture [van Hellemont 1988, Braga 2014, Carle 2010, Seyyedi 2014, Goes 2016].

[1] The correct name is *Matricaria chamomilla* L. (Kew Medicinal Plant Name Services)

E/S/C/O/P MONOGRAPHS

For baths: 5 g of the drug, or 0.8 g of alcoholic extract, per litre of water [Carle 2010].
For solid and semi-solid preparations: hydroalcoholic extracts corresponding to 3-10% m/m of the drug [Zwaving 1982, Schulz 2004].
For vapour inhalation: 10-20 mL of alcoholic extract per litre of hot water [Saller 1990].

Method of administration
For oral administration, local application and inhalation.

Duration of administration
No restriction.

Contra-indications
Sensitivity to matricaria or other members of the Compositae.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No harmful effects reported.

Effects on ability to drive and use machines
None known.

Undesirable effects
Rare cases of contact allergy have been reported in persons with known allergy to *Artemisia* species [Schilcher 1987]. Matricaria flower of the bisabolol oxide B-type can contain traces of the contact allergen anthecotulide [Schilcher 1987, Bisset 2001, Hausen 1992]. Matricaria possesses a much lower allergenic potential than other chamomile species and therefore allergic reactions to matricaria must be considered as extremely rare. Most of the described allergic reactions to matricaria were due to contamination with *Anthemis cotula* or related species, which contain high amounts of anthecotulide. However, in cases where matricaria contact allergy has been acquired, cross-reactions to other sesquiterpene lactone-containing plants are common [Hausen 1992, de Jong 1998].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Anti-inflammatory effects
Ethanolic (48% V/V) and isopropanolic (48% V/V) extracts inhibited 5-lipoxygenase, cyclooxygenase and the oxidation of arachidonic acid with IC_{50} values of 0.05 to 0.3%, while a supercritical carbon dioxide extract had an IC_{50} of 6 to 25 µg/mL for these activities. Investigation of individual constituents revealed that apigenin inhibited 5- and 12-lipoxygenase (IC_{50}: 8 and 90 µM respectively); chamazulene and (–)-α-bisabolol inhibited only 5-lipoxygenase (IC_{50}: 13 and 40 µM respectively); apigenin, cis-en-yne-spiroether and (–)-α-bisabolol inhibited cyclooxygenase (IC_{50}: 70-80 µM); only chamazulene had antioxidative activity (IC_{50}: 2 µM) [Ammon 1996].

Trans-en-yne-dicycloether inhibited the provoked degranulation of rat mast cells in concentrations above 0.1 mM [Miller 1996].

Apigenin markedly inhibited the transcriptional activation of cyclooxygenase (IC_{50}: 8.7 µM) and of nitric oxide synthase (IC_{50}: 3.1 µM) in lipopolysaccharide-activated macrophages [Liang 1999].

Matricin dose-dependently (10 – 75 µM) inhibited TNF-α and LPS-induced cell surface expression of ICAM-1 in HMEC-1 endothelial cells as well as TNF-α-induced NF-κB promotor activity (10 –100 µM) and the cytoplasm to nucleus translocation of NF-κB (10 – 75 µM). In contrast, chamazulene showed no activity [Flemming 2015].

A decoction (1:5) significantly ($p<0.0003$) and dose-dependently (5 – 20 µg/mL) inhibited luminol-amplified chemiluminescence of resting neutrophils and N-formyl methionylleucyl-phenylalanine- or phorbolmyristate acetate-stimulated neutrophils [Jabri 2016].

Antispasmodic effects
A hydroethanolic extract showed antispasmodic activity on isolated guinea pig ileum stimulated by various spasmogens. The ED_{50} (mg/mL) and the strength of activity relative to papaverine (= 1.0) were respectively 1.22 and 0.0011 with barium chloride, 1.15 and 0.0019 with histamine dihydrochloride, 2.24 and 0.00074 with bradykinin, and 2.54 and ca. 0.00062 with serotonin. Pure constituents were also investigated: with barium chloride, (–)-α-bisabolol (ED_{50}: 136 µg/mL) showed activity comparable to papaverine, while apigenin (ED_{50}: 0.8 µg/mL) was more than 3 times as active [Achterrath-Tuckermann 1980].

A methanolic (70%) dry extract demonstrated a dose-dependent (0.3 – 3 mg/mL) relaxation of spontaneous and low potassium-induced contractions in isolated rabbit jejunum. The extract (1 and 3 mg/mL) showed a Ca^{2+} antagonistic effect by shifting the concentration-response curves to the right, with a suppression of the maximum effect comparable to verapamil (0.03 and 0.1 µM) [Mehmood 2015].

Sedative effects
Apigenin competitively inhibited the binding of flunitrazepam to the central benzodiazepine receptor (Ki = 4 µM) but had no effect on muscarinic receptors, α1-adrenoreceptors or on the binding of muscimol to $GABA_A$ receptors [Viola 1995].

HPLC fractions of a methanolic extract were able to displace flunitrazepam from its receptors in rat cerebellar membranes, the ligand Ro 5-4864 from 'peripheral' benzodiazepine receptors in rat adrenal gland membranes and muscimol from GABA receptors in rat cortical membranes. The latter activity was mainly due to GABA present in the fractions [Avallone 1996].

Apigenin inhibited the binding of Ro 15-1788, a specific ligand for central benzodiazepine receptors, with an IC_{50} of 0.25 mM. Apigenin also reduced GABA-activated Cl^- currents on cultured cerebellar granule cells dose-dependently by 15 ± 3% (0.1 µM), 24 ± 2% (1 µM) and 32 ± 4% (10 µM). This effect was blocked by co-application of Ro 15-1788 [Avallone 2000].

Antimicrobial effects
Matricaria flower oil exerted a bactericidal effect against gram-positive bacteria and a fungicidal effect against *Candida albicans* at a concentration of 0.7% V/V. The oil was not active against gram-negative bacteria even in concentrations as high as 8% V/V [Aggag 1972].

An infusion, a hydroethanolic extract and pure herniarin showed

antimicrobial activity against various bacteria and fungi in the presence of near UV light [Ceska 1992, Mares 1993].

Other effects
In the MTT test, cell viability of human gingival fibroblasts treated with an ointment, containing 10% of a liquid extract, was significantly ($p<0.05$) less than that of the control [Martins 2009].

A decoction (1:5) significantly ($p<0.05$) and dose-dependently (1 – 2000 µg/mL) inhibited glucose absorption in isolated rat jejunum [Jabri 2017].

In vivo experiments

Anti-inflammatory effects
The anti-inflammatory effects of orally administered (–)-α-bisabolol have been demonstrated in carrageenan-induced rat paw oedema, adjuvant arthritis of the rat, ultraviolet-induced erythema of the guinea pig and yeast-induced fever of the rat [Jakovlev 1979].

In the carrageenan-induced rat paw oedema test the following ED_{50} values (mmol/kg) were obtained after oral administration: (–)-α-bisabolol 2.69, chamazulene 4.48, guaiazulene 4.59, matricin 2.69 and salicylamide 1.53 [Jakovlev 1983].

A dry extract prepared from infusion of 20 g of matricaria flower in 100 mL of 42% ethanol, applied topically at a dose of 750 µg per ear, inhibited croton oil-induced oedema of mouse ear by 23.4% compared to controls; benzydamine at 450 µg/ear showed a comparable inhibition of 26.6% [Tubaro 1984]. In the same test system, two isolated polysaccharides at a dose of 300 µg/ear inhibited oedema by 14% and 22% respectively [Füller 1993b].

BALB/c mice with experimentally induced atopic dermatitis (sensitized with 2,4-dinitrochlorobenzene) were treated with essential oil (3% in jojoba oil, 70 µL daily on the dorsal skin) for 4 weeks. The verum group showed a significantly ($p<0.05$) lower scratching frequency compared to controls (saline and jojoba oil vehicle). Serum IgE levels were significantly ($p<0.05$) lower at 4 weeks and histamine levels at 2 weeks [Lee 2010].

Hamsters with 5-fluorouracil-induced oral mucositis were treated twice daily for 9 days with an ointment containing 10% of a fluid extract. On day 5 of treatment, the levels of pro-inflammatory cytokines IL-1β ($p<0.05$) and TNF-α were lower than in untreated controls [Curra 2013].

Anti-ulcerogenic and wound healing effect
The development of ulcers induced in rats by indomethacin, stress or ethanol was inhibited by an orally administered extract with an ED_{50} of 1 mL per rat and by (–)-α-bisabolol with an ED_{50} of 3.4 mg/kg body weight. These substances also reduced healing times for ulcers induced in rats by chemical stress (acetic acid) or heat coagulation [Szelenyi 1979].

Rats with experimentally-induced tongue ulcers were treated twice daily with an ointment containing 10% of a fluid extract, or one of three different corticosteroid preparations, for 1, 3, 5, 7 or 14 days. Clinical and histological evaluation of the tongues revealed significantly ($p<0.05$) faster wound healing in the matricaria group when compared to both control (no treatment) and corticosteroid treatment (5 days versus 14 days) [Martins 2009].

Sixty minutes after oral treatment with a 37% ethanolic dry extract (5.65:1; 25-400 mg/kg b.w.), rats were given ethanol to induce gastric ulcers and sacrificed one hour later. The gastric ulcer index was calculated, MDA and GSH in whole blood and gastric tissue were measured. A dose of 200 mg/kg showed the greatest decrease in gastric lesions ($p<0.001$) while a dose of 50 mg/kg showed the strongest prevention of the increase in gastric tissue and blood MDA, and decrease in gastric tissue and blood GSH ($p<0.001$ and $p<0.01$ respectively) [Cemek 2010].

Male Wistar rats with a linear 3 cm incision on their back were treated topically with olive oil (control) or an extract (100 g comminuted flowers in 100 mL olive oil) twice daily for 20 days. Wound healing on days 5, 8 and 11 was significantly ($p<0.05$) better in the verum group. Complete wound closure was 4 days faster in the verum group than in control [Jarrahi 2010].

Rats with experimentally induced tongue ulcers were treated twice daily with 0.04 mL of an ointment (containing 10% of a fluid extract) for 3, 7 or 10 days. The animals were sacrificed and the tongues were examined with respect to the degree of inflammation, fibroblast count and wound size. Histometric analysis of re-epithelialization and percentage of collagen fibres of the lesion were also determined. After 10 days, treated animals showed significantly greater re-epithelialization and a higher percentage of collagen fibres than control ($p=0.008$ and $p=0.022$ respectively) [Duarte 2011].

Male Wistar rats were divided into 4 groups: normoglycaemic rats given verum treatment (ointment containing 10% of a liquid extract) or control (saline solution), and alloxan-induced diabetic rats given verum or control. After induction of oral ulcers by abrasion, the rats were treated twice daily for 10 days. On days 5 and 10 the ulcers were analysed by light microscopy, TUNEL assay and immunohistochemically (TNF-α). The histological score, the ulcer area reduction and the percentage of collagen deposition area were slightly better in the treated diabetic group compared to control [Oliveira 2016].

The wound healing activity of azulene has been demonstrated in studies on the thermally damaged rat tail [Deininger 1956] and of matricaria flower constituents in accelerated healing of experimental injuries [Zita 1955].

Sedative effects
A sedative effect of matricaria flower was demonstrated through prolongation of hexobarbital-induced sleep, reduction of spontaneous mobility and reduction of explorative activity in mice [Della Loggia 1981,1982].

Restriction stress-induced increases in plasma ACTH levels in normal and ovariectomized rats were decreased by administration of diazepam and inhalation of matricaria flower oil vapour. Inhaling the vapour induced greater decreases in plasma ACTH levels in ovariectomized rats than treatment with diazepam; this difference was not observed in normal rats. Furthermore, inhalation of the oil vapour induced a decrease in the plasma ACTH level that was blocked by pretreatment with flumazenil, a potent and specific benzodiazepine receptor antagonist [Yamada 1996].

Apigenin (25 and 50 mg/kg) significantly ($p<0.05$) reduced the time of latency in the onset of picrotoxin-induced (6 and 8 mg/kg) convulsions [Avallone 1996]. Apigenin also reduced locomotor activity after i.p. injection in rats (minimal effective dose: 25 mg/kg), but showed no anxiolytic, myorelaxant or anticonvulsant activity [Zanoli 2000].

Other effects
In an experimental model of polycystic ovary syndrome, virgin adult rats with oestradiol valerate-induced follicular cysts were

treated sixty days after induction with various doses (25, 50, 75 mg/kg i.p.) of a dry 70% ethanolic extract for 10 days. In the rats treated with 50 mg/kg, macroscopic and microscopic morphological examination of the ovarian and uterine tissues revealed a reduction of the cysts and an increase in the number of dominant follicles. Serum concentrations of oestradiol, LH and FSH were significantly ($p<0.05$) decreased in chamomile-treated groups compared to untreated cystic rats [Farideh 2010].

In the sister chromatid exchange assay, daunorubicin-induced damage in mice germ cells was dose-dependently inhibited by an essential oil up to 93.5% (5, 50 and 500 mg/kg b.w. p.o.) [Hernandez-Ceruleos 2010].

Male Wistar rats were fed with paraquat (5 mg/kg/day) with or without a dry 50% ethanolic extract (3.3:1; 50 mg/kg/day) for 7 days and then sacrificed. The extract significantly ($p<0.05$) reversed the paraquat-induced increases in lipid peroxidation and superoxide dismutase and the decrease in total antioxidant power in lung tissue [Ranjbar 2014].

Oral administration of a dry methanolic (70%) extract to mice at 150 and 300 mg/kg b.w. showed significant antidiarrhoeal and antisecretory effects against castor oil-induced diarrhoea and intestinal fluid accumulation ($p<0.01$ and 0.001 respectively), similar to the effects of cromakalim and loperamide. These effects were attenuated in animals pre-treated with K^+-channel antagonists glibenclamide or 4-aminopyridine [Mehmood 2015].

Male Wistar rats were pre-treated with a decoction (1:5; 25, 50 and 100 mg/kg b.w.) for 10 days and then intoxicated with an oral dose of ethanol (4 g/kg b.w.). The decoction significantly ($p<0.005$) inhibited the ethanol-induced increase of white blood cells and platelets. The pre-treatment also significantly ($p<0.005$) improved several ethanol-induced disturbances such as depletion of SOD, catalase, glutathione peroxidase (GPx) and thiol groups; increase of hydrogen peroxide, free iron and calcium levels [Jabri 2016].

Male Wistar rats were fed with a high-fat diet (HFD) with or without a decoction (1:5; 100 mg/kg b.w.) for 6 weeks. The decoction significantly ($p<0.05$) inhibited HFD-induced obesity; the increase in renal, hepatic and abdominal fat; the increase of serum triglycerides, total- and LDL-cholesterol; the increase of AST and ALT activities, and plasma urea and creatinine levels; the increase in renal and hepatic MDA levels; and the decrease of SOD, catalase, GPx, GSH and thiol groups [Jabri 2017].

Pharmacological studies in humans

Anti-inflammatory effects
In a comparative open study involving 20 healthy volunteers with chemically-induced toxic dermatitis, the smoothing effect on the skin of an ointment containing an extract was significantly ($p<0.01$) superior to that of 0.1% hydrocortisone acetate or the ointment base only [Nissen 1988].

In an open study on 12 healthy subjects, a cream containing an extract (20 mg/g) did not suppress UV-induced erythema but did reduce visual scores of skin redness in the adhesive tape stripping test ($p = 0.0625$) [Korting 1993]. In an analogous study, the cream produced 69% of the effect of a hydrocortisone-27-acetate ointment [Albring 1983].

In a randomized, double-blind study, 25 healthy volunteers with UVB light-induced erythema were treated with various matricaria flower preparations, hydrocortisone cream or the respective vehicle. Ranking the preparations according to visual assessment scores and mean values from chromametry, a cream containing a hydroalcoholic extract of matricaria flower gave the best result [Kerscher 1992].

Clinical studies

Anti-inflammatory effects
In a bilateral comparative study 161 patients with inflammatory dermatoses, who had been treated initially with 0.1% diflucortolone valerate, were treated during maintenance therapy with a cream containing 2% of an ethanolic extract (2.75:1; min. 10 mg/g essential oil and 3.5 mg/g α-bisabolol) or one of three alternatives: 0.25% hydrocortisone, 0.75% fluocortin butyl ester or 5% bufexamac. The therapeutic results with the extract were equivalent to those of hydrocortisone and superior to those of fluocortin butyl ester and bufexamac [Aertgeerts 1985].

In an open study on 98 cancer patients, an extract containing 50 mg of α-bisabolol and 150-300 mg of apigenin-7-glucoside per 100 g, applied three times daily, reduced oral mucositis caused by localized irradiation or systemic chemotherapy [Carl 1994].

In a phase III double-blind, placebo-controlled clinical trial involving 164 patients, a mouthwash containing an extract did not decrease 5-fluorouracil-induced stomatitis [Fidler 1996].

In a randomized, partially double-blind, comparison study, a cream containing 2% of an ethanolic extract (2.75:1; min. 10 mg/g essential oil and 3.5 mg/g α-bisabolol) was compared with a 0.5% hydrocortisone cream and placebo. Seventy-two patients with a medium-degree atopic eczema on both arms were allocated to one of the following groups (medication left arm/right arm): matricaria/hydrocortisone, matricaria/placebo, hydrocortisone/matricaria or placebo/matricaria. After 2 weeks of treatment the matricaria cream proved superior to the hydrocortisone cream and marginally superior to the placebo cream with respect to the symptoms pruritus, erythema and desquamation [Patzelt-Wenczler 2000].

In a dose response trial, 25 leukaemia patients (aged 20 to 30 years), with phlebitis resulting from i.v. chemotherapy treatment, were treated 3 times a day, until erythema was resolved, with compresses moistened with an infusion (5, 10, 20 or 40 g dried flowerheads in 400 mL water at 38°C; equivalent to doses of 1.25%, 2.5%, 5% and 10%; total flavonoid content: 0.02 to 0.19 mg/mL) or warm water as control. Phlebitis regression time was shortest in the 2.5% group. Regression time was significantly ($p<0.001$) less in the 1.25, 2.5 and 5% groups compared to control. A within group comparison showed a significantly shorter phlebitis regression time for the 2.5% group compared to the 1.25% ($p<0.01$) and 10% ($p<0.05$) groups [Reis 2011].

In a randomized controlled trial, the incidence and intensity of oral mucositis was assessed in 40 patients undergoing haematopoietic stem cell transplantation who received either routine care plus mouthwash containing a liquid standardised extract (10.7 mg/mL apigenin-7-glucoside) at 0.5%, 1% or 2% twice a day (until oral mucosa was re-established) or standard care alone. The mouthwash at 1% dosage significantly ($p=0.01$) reduced the incidence, intensity and duration of oral mucositis compared to control [Braga 2014].

In a randomized, triple-blind, placebo-controlled trial, 36 patients with recurrent aphthous stomatitis were treated for up to 3 weeks with 10 drops 3 times per day of a mouthwash containing either a tincture (not further specified) or placebo. The healing period (complete healing within 1 week), the number of ulcers (from day 4), as well as pain and burning sensation (from day 2) were significantly ($p<0.001$) lower in the verum group compared to placebo [Seyyedi 2014].

In a randomized, double-blind, placebo-controlled pilot study, 26 patients with severe carpal tunnel syndrome were treated with a night splint and topical application twice daily for 4 weeks with 5 drops of either matricaria oil (dried aqueous extract from 600 g flowers in 1 L sesame oil) or placebo oil. The verum group showed a significant improvement of symptomatic and functional status (p=0.019, p=0.016 respectively) compared to placebo, whereas electrodiagnostic parameters showed no significant changes between the groups [Hashempur 2015].

In a randomized, double-blind, placebo-controlled trial, 86 patients with mild and moderate carpal tunnel syndrome were treated with a wrist splint plus the same preparation or placebo (5 drops topically twice daily) for 4 weeks. Dynamometry, functionality and symptom severity score were significantly improved in the verum group compared to control (p=0.040, p=0.0001, p=0.017 respectively). Compound latency of the median nerve in the verum group was significantly (p=0.035) decreased compared to control [Hashempur 2017].

In a randomized, double-blind, placebo-controlled trial, 84 patients with knee osteoarthritis were treated topically three times daily with either the same oil preparation at a dose of 1.5 mL, a 1% diclofenac gel or placebo for 3 weeks. The oil significantly (p=0.001) reduced the use of paracetamol tablets as compared with diclofenac and placebo groups. There were no significant between group differences in pain, physical function and stiffness as determined by the WOMAC questionnaire [Shoara 2015].

In a randomized, double-blind, placebo-controlled trial, 30 patients with fixed orthodontic appliances suffering from gingival inflammation and plaque formation were treated with 15 ml of a mouthwash containing either 1% of an extract (not further specified), 0.12% chlorhexidine or placebo, twice daily for 15 days. The visible plaque index and gingival bleeding index were significantly ($p<0.05$) decreased in the matricaria group as compared with placebo, and were comparable to chlorhexidine. The matricaria and chlorhexidine groups also exhibited a significant (p=0.0001) reduction in dental biofilm compared to placebo [Goes 2016].

Antispasmodic effects
In an open multicentre study, 104 patients with gastrointestinal complaints such as gastritis, flatulence or minor spasms of the stomach were treated orally for 6 weeks with an extract (standardized to 50 mg α-bisabolol and 150-300 mg apigenin-7-glucoside per 100 g) at a daily dose of 5 mL. Subjectively evaluated symptoms improved in all patients and disappeared in 44.2% of patients [Stiegelmeyer 1978].

Wound healing effects
In an open study, 147 female patients episiotomized during childbirth were treated for 6 days with either an ointment containing 2% of an ethanolic extract (2.75:1; minimum 10 mg/g essential oil and 3.5 mg/g α-bisabolol) or a 5% dexpanthenol cream. The healing effect of the two preparations was comparable [Kaltenbach 1991].

In a randomized, double-blind, placebo-controlled study involving 14 otherwise healthy patients, weeping dermatoses following dermabrasion of tattoos were treated topically with a fluid extract (standardized to 50 mg of α-bisabolol and 3 mg of chamazulene per 100 g). After 14 days, the decrease in weeping wound area and the improvement in drying tendency were both significant ($p<0.05$) in the verum group [Glowania 1987].

In a randomized, open, comparison study, 120 patients with second degree haemorrhoids were treated with rubber band ligature alone, rubber band ligature with anal dilator and vaseline, or rubber band ligature with anal dilator and an ointment containing an extract of matricaria flower (not further specified). The last group showed the best results in amelioration of haemorrhage, itching, burning and oozing [Förster 1996].

Sedative effects
In a randomized, double-blind, placebo-controlled study, 57 patients with mild to moderate generalized anxiety disorder were treated with capsules containing either 220 mg of a standardised dry extract (1.2% apigenin; n=28) or placebo (n=29) once daily for the first week and twice daily for weeks 2 to 8. Patients with less than a 50% reduction in total HAM-A score (versus baseline) received 3 capsules daily in week 3 and 4 capsules daily in week 4, and if necessary 5 capsules from weeks 5 to 8. The verum group showed a significantly (p=0.047) greater reduction in mean total HAM-A score and a favourable change in the secondary outcomes measured [Amsterdam 2009].

In a randomized, double-blind, placebo-controlled study, 34 patients (aged 18 to 65) with a history of at least 6 months of primary insomnia (meeting DSM-IV criteria) were treated with 3 tablets of either a dry hydroethanolic extract (90 mg per tablet; 6:1 v/v; 70% ethanol; standardized to 2.5 mg of (-)-α -bisabolol and ≥ 2.5 mg of apigenin per tablet) or matching placebo, twice daily for 28 days. Sleep diaries were completed for the 7 days prior to treatment and for days 21 to 28, while secondary objectives were measured at baseline and day 28. While there were no significant between group differences, the verum group did show modest improvements in sleep latency, number of night-time awakenings and day-time functioning measures [Zick 2011].

A two-phase study involving 179 patients with moderate to severe generalized anxiety disorder (GAD-7 score ≥10) has been reported in three different publications: study design [Mao 2014], phase one [Keefe 2016] and phase 2 [Mao 2016]. The first phase involved an open study with all patients receiving 3 capsules of an extract daily for 8 weeks. Each capsule contained 500 mg of a dry hydroethanolic (70%; DER 4:1) extract (equivalent to 2 g of flower; corresponding to 6 mg of total apigenin-7-glucoside). The primary outcome was to identify responders at week 8, defined as those with ≥ 50% reduction in baseline GAD-7 score and final CGI-S score of 1 to 3 (normal to mild). Responders were given the extract for a further 4 weeks. Patients who still met the responder criteria at the end of week 12 were entered into the phase 2 randomized, double-blind, placebo-controlled study to assess time to relapse and relapse rate; patients continued to receive treatment with either the extract or placebo for a further 26 weeks. Non-responders were discontinued from the trial.

Of the 151 patients that completed the initial 8 weeks of treatment, 104 (58.1%) met the responder criteria; 23.5% of the subjects responded quickly, meeting the responder criteria after only 2 weeks of treatment. By week 8 there were also significant ($p<0.001$) reductions in GAD-7 and HAM-A scores, and several other secondary outcome scores. Of the 93 responders continuing to the phase 2 trial, 46 continued to receive the extract and 47 were switched to placebo. The extract led to a non-significant reduction in relapse of GAD symptoms compared to placebo: 15.2% of the extract group relapsed compared to 25.5% of the placebo group; mean time to relapse was 11.4±8.4 weeks for the extract compared to 6.3±3.9 weeks for placebo. However, compared to placebo the extract group experienced a significantly smaller increase in GAD-7 scores (p=0.0032) and better psychological well-being (p=0.013) [Mao 2014, 2016, Keefe 2016].

In a randomized, single-blind, placebo-controlled study, 60 nursing home residents over the age of 60, with a Pittsburgh

Sleep Quality Index (PSQI) of ≥ 5, were treated with 200 mg of a dried ethanolic (70%) extract (n=30) or placebo (n=30) twice daily for 28 days. PSQI was assessed immediately before treatment commenced (T1), 2 weeks after beginning treatment (T2), immediately after treatment finished (T3) and 2 weeks after completion of treatment (T4). The mean PSQI score was significantly ($p<0.05$) decreased in the verum group compared to control at T3 and T4, but not at T2 [Adib-Hajbaghery 2017].

Other effects

In a randomized, double-blind, controlled study, 90 students with premenstrual syndrome (PMS) received either 100 mg of an ethanolic extract (not further specified) or 250 mg of mefenamic acid, 3 times daily for 2 cycles (from day 21 of their cycle until the next onset of menstruation). A significant ($p<0.0001$) decrease in mastalgia was observed after the first and second cycles compared to baseline for chamomile (10.5±21.7% and 13.7±201.4%) and mefenamic acid (12.1±24.7% and 13.8±24.0%). There was no significant difference between the chamomile and mefenamic acid groups. The chamomile extract was significantly ($p<0.001$) more effective at reducing psychological symptoms of PMS than mefenamic acid [Sharifi 2014 A&B].

In a randomized, single-blind, placebo-controlled study, 64 patients with type 2 diabetes mellitus (aged 30 to 60 years) were treated with either a tea (3 g/150 mL hot water; n=32) or an equivalent amount of warm water as placebo (n=32), three times daily after meals for 8 weeks. Glycosylated haemoglobin ($p=0.03$), serum insulin ($p<0.001$), homeostasis model assessment index ($p<0.001$), total cholesterol ($p=0.001$), triglycerides ($p<0.001$) and LDL cholesterol ($p=0.05$) were significantly decreased compared to control after the treatment [Rafraf 2014].

The same study showed that total antioxidant capacity, superoxide dismutase, glutathione peroxidase and catalase activities were significantly ($p<0.05$) increased by 6.8%, 26.2%, 36.7% and 45.1% respectively, compared to control. There was also a significant ($p<0.001$) decrease in malondialdehyde adducts (MDA), a marker of hyperglycaemia, compared to baseline and to control [Zemestani 2016].

In a randomized, double-blind, placebo-controlled study, 80 children (aged 6 to 18; mean age for verum and placebo: 9.5±1.9 and 9.9±2.5 years respectively) with monosymptomatic nocturnal or daytime enuresis were treated topically for 6 weeks with either an aqueous extract evaporated in sweet almond oil, or sweet almond oil alone as placebo (6 drops on the perineal and suprapubic area before sleeping). The mean frequency of enuresis was significantly lower in the verum group as compared to placebo after 2, 4 and 6 weeks of treatment ($p<0.001$, $p=0.03$, $p<0.001$ respectively) [Sharifi 2017].

In a randomized, double-blind, placebo-controlled, cross-over study, 72 migraine patients without aura completed treatment with verum and placebo 'oleogels' (oil preparations mixed with colloidal silicon dioxide). The verum oleogel contained a preparation in which 200 g of flowers were combined with 2.5 L of water and the essential oil extracted and saved, the remaining filtered aqueous extract was then boiled in 167.5 mL of sesame oil until all water was evaporated; the remaining oil and essential oil were combined and then formulated into the oleogel [Zargaran 2017]. The placebo used 10% of the chamomile oil preparation in liquid paraffin and subsequently formulated into an 'oleogel'.

Patients applied 2 mL of one of the oleogels topically to the forehead, temporal area and behind the ears when a migraine attack started. After 2 treatments there was a 14-day washout period followed by a change from verum to placebo treatment or vice versa. Results from VAS questionnaires showed that pain, nausea, vomiting, photophobia and phonophobia were significantly ($p<0.001$) decreased after 30 min in the verum group compared to placebo [Zargaran 2018].

Pharmacokinetic properties

After cutaneous administration of [^{14}C](–)-α-bisabolol to mice, 82% of the radioactivity was found in the urine [Hahn 1987, Hölzl 1985].

Apigenin and luteolin were also readily absorbed by the skin. Skin penetration studies applying hydroethanolic solutions of apigenin and luteolin to the upper arms of 9 healthy, female volunteers gave steady state fluxes of 10.31 ng/min/cm² and 6.1 ng/min/cm² respectively [Merfort 1994].

After oral administration of apigenin-7-glucoside to rats, free apigenin was detected in the urine [Griffiths 1972a].

After oral administration of 40 mL of a hydroethanolic extract (containing 225.5 mg of apigenin-7-glucoside, 22.5 mg of apigenin, 15.1 mg of herniarin per 100 mL) to a female volunteer, no flavones could be detected in blood plasma nor in 24-hour urine, while herniarin was found in both (max. plasma concentration of ca. 35 ng/mL; 0.324 mg in 24-hour urine) [Tschiersch 1993].

In germ-free rats, no hydrolysis of flavone glycosides could be observed; obviously intestinal microflora can affect the cleavage of the glycosidic bonds [Griffiths 1972a,b]. Furthermore, orally administered apigenin was detected in the blood serum of animals [Redaelli 1980].

Preclinical safety data

The acute oral LD_{50} of matricaria flower oil in rats and the acute dermal LD_{50} in rabbits exceeded 5 g/kg. No irritant effects of the oil were observed after application to the skin of nude mice [Opdyke 1974].

In a 48-hour patch test in volunteers, matricaria oil neither caused skin irritation nor were there any discernible sensitization reactions or phototoxic effects. Matricaria oil has been granted GRAS status by FEMA and is approved by the FDA for use in food and cosmetics [Opdyke 1974].

The acute oral toxicity of (–)-α-bisabolol in mice and rats was very low, the LD_{50} being about 15 mL/kg. A six-week subacute toxicity study showed that the lowest toxic oral dose of (–)-α-bisabolol in rats and dogs was between 1 and 2 mL/kg. Oral doses up to 1 mL/kg of ((–)-α-bisabolol produced no discernible effects on the prenatal development of rats or rabbits. No malformations were found at any of the tested dose levels [Habersang 1979].

The acute intraperitoneal LD_{50} of *cis*- and *trans*-en-yne-dicycloether is 670 mg/kg [Ammon 1992]. In the Ames test, apigenin and an aqueous matricaria flower extract showed no mutagenic or toxic activity [Rivera 1994, Birt 1986].

No genotoxicity was found in mice after oral administration of essential oil up to 500 mg/kg b.w. [Hernandez-Ceruleos 2010].

Allergenicity

Based on the fact that matricaria flower generally contains no, or only traces of, the sesquiterpene lactone anthecotulide and that millions of people come into contact with matricaria flower daily, allergic reactions due to matricaria flower can be considered to be extremely rare [Hausen 1992, Rudzki 2000]. However, cross-reactions with other sesquiterpene lactone-containing plants are common [Hausen 1992]: 2 reports of a

patient allergic to *Artemisia vulgaris* mention severe anaphylactic reactions following ingestion of matricaria flower infusions and after eye washing with similar infusions [Sánchez 1992, Foti 2000]; 18 of 24 patients with Compositae allergy were also allergic to an ether extract of matricaria flower [Paulsen 1993] ; 11 of 14 patients with Compositae allergy were allergic to an aqueous extract of matricaria flower [de Jong 1998] ; 96 patients from 4800 showed contact hypersensitivity to an ethanolic matricaria flower extract [Dastychová 1992]; 3 case reports mention an allergic reaction to matricaria flower and extract [Rudzki 1998, Jensen-Jarolim 1998, Rodriguez-Serna 1998].

In a study of contact allergy performed with 540 type IV allergic patients, of whom some gave positive reactions to standard phytogenic allergens, none gave a positive reaction to an anthecotulide-free matricaria flower extract [Jablonska 1996].

In a study of 830 patients with contact dermatitis, only 1 patient had a positive reaction to a matricaria flower extract and cream. Even a patient who was highly sensitive to *Anthemis cotula*, and another with oral allergy syndrome and hypersensitive to many plants, tested negative [Rudzki 2000].

These studies demonstrate the importance of using anthecotulide-free matricaria flower.

A 38-year-old Caucasian man developed an episode of severe anaphylaxis with generalized urticaria, angioedema and severe dyspnoea one hour after consuming matricaria tea. Examination showed specific IgE against matricaria. Skin prick test and labial provocation test with matricaria were also positive [Andres 2009].

Clinical safety data
In one clinical trial involving 57 patients with mild to moderate generalised anxiety disorder more adverse events occurred in the placebo arm (22 versus 11 in the verum); 2 discontinued treatment: 1 for allergic reaction (placebo) and 1 for abdominal discomfort (verum) [Amsterdam 2009].

In a study involving 34 patients, no severe adverse events occurred. There was no significant difference between a dry extract and placebo [Zick 2011].

In a study with 40 patients, a mouthwash containing a liquid extract showed no adverse effects [Braga 2014].

In a study with 30 patients, a mouthwash containing 1% of an extract was well tolerated and showed no adverse effects [Goes 2016].

In 2 studies totalling 166 patients, the local application of a dried aqueous extract in sesame oil showed no adverse effects [Shoara 2015; Hashempur 2017].

In a study with 80 children, the local application of an aqueous extract evaporated in almond oil showed no adverse effects [Sharifi 2017].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Online Series, 2019
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Online Series, 2019
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Online Series, 2019
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHAMOMILLAE ROMANAE FLOS	Roman Chamomile Flower	Online Series, 2019
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HELICHRYSI FLOS	Sandy Everlastin Flower	Online Series, 2019
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013

HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Online Series, 2020
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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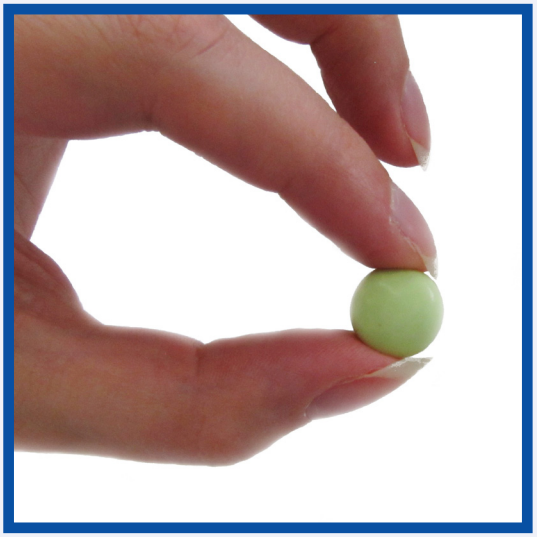

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

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Members of ESCOP
Board of Supervising Editors
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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

MELISSAE FOLIUM

2013

Melissa Leaf

DEFINITION

Melissa leaf consists of the dried leaves of *Melissa officinalis* L. It contains not less than 1.0 per cent of rosmarinic acid ($C_{18}H_{16}O_8$; M_r 360.3), calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Melissa leaf].

Fresh material may be used provided that when dried it complies with the monograph of the European Pharmacopoeia.

CONSTITUENTS

The main constituents are: phenylpropanoids, including hydroxycinnamic acid derivatives such as caffeic and chlorogenic acids, and in particular rosmarinic acid (up to 4%); hydroxybenzoic acids; essential oil (0.05-0.4% V/m) containing monoterpenoid aldehydes, mainly geranial (citral a), neral (citral b) and citronellal; flavonoids including glycosides of luteolin, quercetin, apigenin and kaempferol; monoterpene glycosides; triterpenes including ursolic and oleanolic acids [Pellecuer 1981; Tittel 1982; Enjalbert 1983; Dorner 1985; Mulkens 1985; 1987a; 1987b; Koch-Heitzmann 1988; Mulkens 1988; Schultze 1989; Baerheim-Svendsen 1989; Lamaison 1991a; 1991b; Patora 2002; Karasova 2005; Mencherini 2007; Marques 2009; Mencherini 2009; Hänsel 2010].

CLINICAL PARTICULARS

Therapeutic indications

Internal use
Tenseness, restlessness and irritability; symptomatic treatment of digestive disorders such as minor spasms [Stahl-Biskup 2009; Fintelmann 2009].

External use
Herpes labialis (cold sores) [Wölbling 1984; Vogt 1991; Wölbling 1994; Koytchev 1999].

Posology and method of administration

Dosage

Oral use
1.5-4.5 g of the drug as an infusion, two to three times daily [Stahl-Biskup 2009; Fintelmann 2009]. Tincture (1:5 in 45% ethanol), 2-6 mL three times daily [Wölbling 1994; Brendler 2005]. Fluid extract (1:1 in 45% ethanol quantified to at least 500 µg/mL citral) 60 drops/day [Akhondzadeh 2003]. Other equivalent preparations.

Cutaneous use
Cream containing 1% of a lyophilised aqueous extract (70:1) two to four times daily [Wölbling 1984; Vogt 1991; Wölbling 1994; Koytchev 1999].

Method of administration
For oral or cutaneous use.

Duration of administration

Oral use
No restriction.

Cutaneous use in *Herpes labialis*
From prodromal signs to a few days after the healing of lesions.

E/S/C/O/P MONOGRAPHS

Contraindications
None reported.

Special warnings and special precautions for use
None required.

Interaction with other medicinal products and other forms of interaction
None reported.

Pregnancy and lactation
In accordance with general medical practice the product should not be taken orally during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No case of overdose reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antispasmodic activity
A hydroethanolic extract (30% ethanol; 1:3.5) from melissa leaf significantly inhibited histamine-induced contractions in isolated guinea pig ileum ($p<0.05$). The frequency and maximal force of spontaneous peristaltic movements were also decreased in comparison to solvent control [Heinle 2006].

In contrast, a similar extract (30% ethanol; 1:2.5-3.5) at concentrations of 2.5 mL and 10 mL/L did not show any significant antispasmodic activity when tested on acetylcholine- and histamine-induced contractions of guinea pig ileum [Forster 1980].

A hydroethanolic dry extract (ethanol-free lyophilisate; not further specified) at concentrations of 24 to 188 µg/mL led to minor but significant increased contractions in the fundus and corpus of guinea pig stomach ($p<0.05$). The contractile response in distal stomach motility in the antrum was more pronounced; at 94 µg/mL the mean increase in antral contraction amplitude was 177.3%. This effect was immediate, long-lasting and fully reversible after wash-out [Schemann 2006].

An aqueous dry extract produced a concentration-dependent relaxation in phenylephrine-precontracted thoracic aorta rings with intact endothelium from rats. This effect was abolished by L-N^G-nitroarginine methyl ester (L-NAME) and significantly reduced by glibenclamide and indomethacin ($p<0.05$). No relaxation was observed in de-endothelised phenylephrine-precontracted rings [Ersoy 2008].

Essential oil of melissa leaf showed spasmolytic activity when tested on isolated guinea pig ileum, rat duodenum and vas deferens, and on the jejunum and aorta of rabbits [Debelmas 1967; Wagner 1973]. It also had relaxant effects on guinea pig tracheal muscle (EC_{50}: 22 mg/L) and inhibited phasic contractions of an electro-stimulated ileal myenteric plexus longitudinal muscle preparation (EC_{50}: 7.8 mg/L) [Reiter 1985].

Essential oil of melissa leaf reduced contractions in isolated rat ileum induced by 80 mM KCl, 320 nM acetylcholine or 1.28 µM 5-hydroxytryptamine with IC_{50} values of 29 ng/mL, 20 ng/mL and 20 ng/mL respectively [Sadraei 2003].

Antiviral activity
Aqueous extracts exhibited antiviral activity against Newcastle disease virus, SemLiki forest virus, influenza viruses, myxoviruses, vaccinia and *Herpes simplex* virus [Kucera 1967; May 1978; Vanden Berghe 1985; König 1985].

An aqueous extract demonstrated antiviral activity against *Herpes simplex* virus type 1 (HSV-1; IC_{50} 0.025 µg/mL) and type 2 (HSV-2; IC_{50} 0.027 µg/mL), as well as against an acyclovir–resistant strain, in a plaque reduction assay on RC-37 cells. Treatment of the cells prior to infection resulted in a considerable inhibition of the adsorption of all three virus types. A reduction of intracellular virus replication was not observed [Nolkemper 2006].

An aqueous extract showed anti-HIV-1 activity (ED_{50}: 16 µg/mL). The active components in the extract were found to be polar substances. This extract also inhibited giant cell formation in co-culture of Molt-4 cells with and without HIV-1 infection and showed inhibitory activity against HIV-1 reverse transcriptase [Yamasaki 1998].

An infusion (10 g/100 mL) exhibited a concentration-dependent activity against the infection of HIV-I in T-cells, in primary macrophages and in tonsil histocultures. After incubation of prototypic X4 HIV-1_{NL4-3} with the extract (0.006 to 6%) and addition to human T-lymphoblastoid cell line Sup-T1, productive HIV-1 infection was assessed by the p24 concentration. The IC_{50} for HIV replication was 0.045%, the CC_{50} for Sup-T1 cells was 0.531%. HIV infection was also inhibited in primary human lymphoid aggregate culture and in monocyte-derived macrophages. No antiviral effect on target cells exposed to the extract prior to HIV challenge or on surface-bound virions was observed. A virion-fusion assay demonstrated that HIV-1 entry was impaired after pre-incubation of the virions with the extract [Geuenich 2008].

A methanolic and an aqueous extract (100 mg/mL; not further specified) of melissa leaf were among the most active extracts in an enzymatic assay and inhibited neuroaminidase with IC_{50} values of 0.64 mg/mL and 0.04 mg/mL respectively, as compared to zanamavir with an IC_{50} of 215 µM [Schwerdfeger 2008].

A liquid extract from melissa leaf (1:10; 65 % (V/V) ethanol) was investigated for inhibition of the cytopathogenic effect of *Herpes simplex* virus type 2 (HSV-2) in Vero cells (ATCC CCL-81). Vero cells were infected with the virus and after addition of the extract 48 hours of incubation followed. The extract exhibited a concentration-dependent inhibition of virus replication in infected cells with an IC_{50} between 0.1 and 0.2 mg/mL [Mazzanti 2008].

The infectivity of three different doses of HSV-2 in human larynx epidermoid carcinoma cells (Hep-2 ATCC CCL23) was reduced by the essential oil from melissa leaf (main constituents β-cubebene (15.4%) and β-caryophyllene (14.2%)) at 100 µg/mL [Allahverdiyev 2004].

In a plaque reduction assay in monkey kidney cells, the essential oil of melissa leaf was effective against HSV-1 and HSV-2 and reduced plaque formation when the viruses were pre-treated with the oil. The IC_{50} values were 4 and 0.8 µg/mL respectively. When the oil was applied to the host cells prior to infection, or during intracellular replication, plaque formation was not influenced [Schnitzler 2008].

Antimicrobial activity
Antibacterial activity against several bacteria was shown for aqueous and 50% ethanolic extracts, as well as for petroleum ether, chloroform and ethyl acetate fractions from a 70% methanolic extract [Kwon 2007; Mencherini 2007; Čanadanović-Brunet 2008].

A dichloromethane dry extract displayed antifungal effects against *Rhizopus stolonifer* [López 2007].

Melissa leaf essential oil was active against bacteria, fungi and yeasts [Larrondo 1995; Motiejunaite 2003; Anicic 2005; Mimica-Dukic 2004; Suschke 2007; Chao 2008; Inouye 2009; Hussain 2011].

Receptor-binding activity
Extracts (80% ethanol) of melissa leaf displaced [^{3}H]-(*N*)-nicotine and [^{3}H]-(*N*)-scopolamine from nicotinic and muscarinic receptors in homogenates of human cerebral cortical cell membranes (IC_{50} < 1 mg/mL). Choline, a weak nicotinic ligand (IC_{50}: 3×10^{-4} M), was found in the extracts at concentrations of 10^{-6} to 10^{-5} M. The most potent extract showed a high [^{3}H]-(*N*)-nicotine displacement value with an IC_{50} of 0.08 mg plant material/mL [Wake 2000].

In a similar study in human occipital cortex tissue, the IC_{50} for the displacement of [^{3}H]-(*N*)-nicotine and [^{3}H]-(*N*)-scopolamine from nicotinic and muscarinic receptors by a standardized extract (30% methanol; after evaporation the resulting soft extract was mixed with 10% of inert processing agents) were 11 mg/mL and 4 mg/mL respectively [Kennedy 2002].

In a further assay in human occipital cortex tissue using ethanolic (80%) extracts from different samples of melissa leaf, IC_{50} values of 0.18 to 3.16 mg/mL were determined for the displacement of [^{3}H]-nicotine, and 1.46 to 4.31 mg/mL for the displacement of [^{3}H]-scopolamine. A polar fraction of a methanolic extract containing terpenoids and phenolics gave IC_{50} values of 4.0 to 10.0 µg/mL for the displacement of [^{3}H]-nicotine and a fraction containing ionizable alkaline material provided IC_{50} values of 102.0 to 162.9 µg/mL for the displacement of [^{3}H]-scopolamine [Kennedy 2003].

Neither an aqueous nor a methanolic extract displaced flumenazil from the $GABA_A$ receptor in rat phaeochromocytoma (PC12) cells [López 2009].

The essential oil of melissa leaf inhibited the binding of [^{35}S]-t-butylbicyclophosphorothionate (TBPS) to the rat forebrain $GABA_A$ receptor channel with an IC_{50} of 40 µg/mL, but had no effect on N-methyl-D-aspartate, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate or nicotinic acetylcholine receptors. The oil led to a dose-dependent reversible inhibition of GABA-induced currents in primary cultures of rat cortical neurons [Abuhamdah 2008].

Melissa leaf oil decreased the binding of [^{3}H]-ketanserin to 5-HT_{2A} receptors, of [^{3}H]-8-hydroxy-N,N-dipropyl-2-aminotetralin to 5-HT_{1A} receptors, of [^{3}H]-pirenzepine to muscarinic receptors and of [^{35}S]-TBPS to the $GABA_A$ site channel and of [^{3}H]-clobenprobit to histamine H_3 receptors. The binding of [^{3}H]-muscimol at the $GABA_A$ receptor agonist site was increased as well as the [^{3}H]-flunitrazepam binding to the $GABA_A$ receptor in rat cortex synaptic membrane preparations. Effects were observed at concentrations of 0.1 and/or 1 mg/mL ($p<0.05$ to $p<0.001$) depending on the origin of the oil [Elliott 2007].

Antioxidant activity
Antioxidant and free radical scavenging properties have been reported for aqueous, hydromethanolic, hydroethanolic and methanolic extracts of melissa leaf in numerous different test systems [Van Kessel 1985; Verweij-van Vught 1987; Lamaison 1990; 1991a; 1991b; Ivanova 2005, Germann 2006, Schempp 2006, Speisky 2006, Bouayed 2007, Kwon 2007, López 2007, Mencherini 2007, Woydylo 2007, Dastmalchi 2008, Kirca 2008, Kulišić-Bilušić 2008, López 2009, Orhan 2009, Pereira 2009, Komes 2011, Spiridon 2011, Koksal 2011, Lin 2012]. An extract prepared by supercritical fluid extraction after removal of the essential oil was active against autoxidation and iron- or EDTA-mediated oxidation of linoleic acid [Marongiu 2004].

Rat phaeochromocytoma cells (PC12) were protected against H_2O_2 toxicity by 60 and 80 µg/mL of a methanolic extract as shown in the MTT and the LDH assay ($p<0.05$). A water extract was ineffective. Both extracts reduced ROS formation in the cells after co-incubation with H_2O_2 [López 2009].

Radical scavenging properties were determined in the DPPH assay for the essential oil of melissa [Mimica-Dukic 2004; Chung 2010].

The n-butanol fraction from a 70% methanolic extract of the flowering aerial parts of melissa showed activity in the DPPH assay, in the hydroxyl radical assay and in the lipid peroxidation system [Čanadanović-Brunet 2008].

Cholinesterase inhibitory effects
A 45% hydroethanolic extract inhibited acetylcholinesterase (AChE) in a time- and dose-dependent manner. In a bioactivity-guided fractionation of this extract the activity was attributed to rosmarinic acid derivatives [Dastmalchi 2009].

Inhibition of butyrylcholinesterase (BChE) by approximately 70% was observed with an essential oil from melissa leaf. The same preparation did not show any activity against AChE [Orhan 2008]. For a 75% ethanolic extract from melissa leaf the same group reported a 40% inhibition of AchE at a dose of 2 mg/mL [Orhan 2009].

A concentration of 100 µg/mL of melissa essential oil led to an 82.5% inhibition of AchE and a 96.1% inhibition of BChE [Chaiyana 2012].

In further studies no or very low acetylcholinesterase inhibition was exhibited by different extracts from melissa leaf [Ferreira 2006; Mukherjee 2007].

Insecticidal effects
A methanolic extract (DER 3.6:1) significantly ($p \leq 0.05$) affected larvae of *Spodoptera littoralis* at a dose of 0.5% (w/v) in an artificial diet. Medium insecticidal activity was shown with an LC_{50} of 3.74% (w/v) [Pavela 2004].

Larvicidal activity against West Nile virus mosquito *Culex pipiens* L. (3rd to 4th instar larvae) was shown by melissa essential oil (LC_{50}: 61.25 µg/mL and LC90 88.62 µg/mL) [Koliopoulos 2010].

Other effects
The viability of foetal hippocampal neurons from rats after treatment with a neurotoxic dose of 3,4-methylenedioxymethamphetamine (MDMA; 1200 µM) was significantly increased by co-treatment with 10 µg/mL of an aqueous melissa leaf extract ($p<0.05$). The increased caspase-3 activity and rate of apoptosis/necrosis after MDMA were reduced by the extract ($p<0.05$) [Hassanzadeh 2011].

An aqueous extract showed inhibitory activity (IC_{50} 0.43 mg/mL) against glutamic acid decarboxylase (GAD), whereas an

ethanolic extract was less active (IC_{50} 1.86 mg/mL). The inhibition of GABA transaminase (GABA-T) was more pronounced (IC_{50} 0.35 mg/mL for the aqueous and IC_{50} 0.79 mg/mL for the ethanolic extract) [Awad 2007].

In a bioactivity-guided approach an ethyl acetate, a methanol and a water extract resulted in an inhibition of GABA-T (IC_{50} 2.55, 0.55 and 0.82 mg/mL, respectively). Rosmarinic, ursolic and oleanolic acids were identified as active components [Awad 2009].

Human thyroid stimulating hormone (TSH)-induced adenylate cyclase activity was inhibited by a lyophilized aqueous extract in Chinese hamster ovary cells transfected with recombinant TSH receptor (87.1% inhibition at 500 µg/mL). A 64% inhibition of TSH binding to its receptor and a 23.9% inhibition of antibody binding to TSH were observed at the same concentration. cAMP production induced by thyroid stimulating antibody was also inhibited by the extract (45.4% inhibition) [Santini 2003].

The viability of mouse thymocytes after 18 to 20 hours incubation with three different doses of an aqueous extract was significantly increased to 109.9 ($p<0.001$), 118.6 ($p<0.0001$) and 112.8% ($p<0.05$) as compared to control [Drozd 2003].

Antiproliferative effects on HeLa cells and MCF-7 cells were observed with different fractions (petroleum ether, chloroform, ethyl acetate, *n*-butanol, water) from a 70% methanolic extract with IC_{50} values of 90 to 520 µg/mL for HeLa cells and 100 to 380µg/mL for MCF-7 cells. The water fraction had no effect on MCF-7 cells up to a concentration of 1 mg/mL [Čanadanović-Brunet 2008].

Melissa essential oil was shown to be cytotoxic against human keratinocytes (HaCaT) and human bronchial epithelial (BEAS-2B) cells with a CC_{50} of 0.0096% and 0.0391% (v/v) respectively, after 4 hours of incubation [Suschke 2007].

An essential oil of melissa demonstrated considerable nematicidal activity against *Meloidogyne incognita*, with EC_{50} values of 9.15 and 6.15 µL/mL after exposure for 24 and 96 hours respectively [Ntalli 2010].

In vivo experiments

Sedative effects
The sedative effect of a lyophilised hydroethanolic (30%) extract administered intraperitoneally to mice has been demonstrated by means of familiar (two compartment) and non-familiar (staircase) environment tests. The effect was dose-dependent up to 25 mg/kg body weight, the latter dose producing maximum effects. Low doses (3-6 mg/kg) of the extract induced sleep in mice treated with an infra-hypnotic dose of pentobarbital and also prolonged pentobarbital-induced sleep. At high doses (400 mg/kg) a peripheral analgesic effect in the acetic acid-induced writhing test, but no central analgesic effect was observed [Soulimani 1991; Soulimani1993].

Melissa essential oil administered intraperitoneally to mice had no effect in the staircase test nor was it active in prolonging pentobarbital-induced sleep [Soulimani 1991]. When administered orally to mice it showed sedative and narcotic effects at doses of 3.16 mg/kg and higher [Wagner 1973].

Anti-inflammatory effects
Oral administration of an aqueous extract to mice at doses of 50, 100, 200 and 400 mg/kg b.w. significantly ($p<0.05$) reduced histamine-induced paw oedema by 64.2, 45.7, 57.6 and 88% at three hours after challenge, respectively. In carrageenan-induced oedema the decrease was 47.6, 48.5, 29.4 and 40.3% respectively, significant ($p<0.05$) only for the two highest doses. At these doses the extract also showed significant antinociceptive effects in the writhing test (61.9, 70.3, 67.8 and 98.5% inhibition; $p<0.05$) and the formalin-induced paw licking test (83.1, 67.4, 73.6, 74.0% inhibition; $p<0.05$) [Birdane 2007].

Behavioural effects
A single oral dose (30, 100 or 300 mg/kg b.w.) of an ethanolic extract (yield 13%) administered to mice did not significantly affect indicators of anxiety in the elevated plus maze model. In contrast, repeat oral treatment for ten days resulted in significant effects at all doses in female mice ($p<0.05$) and at the highest dose in male mice ($p<0.05$). In the forced swimming test single dose administration remained without effect. After 10-day treatment the two higher doses in males, and all three doses in females, resulted in a significant reduction in immobility time during the last three minutes of the observation ($p<0.05$). A significant between-gender difference was found in immobility time ($p<0.01$)[Taiwo 2012].

In male albino mice oxidative stress in the brain was induced by addition of manganese to the drinking water for 90 days. This regimen was followed by co-administration of 100 mg/kg b.w./day of an aqueous hot water extract (corresponding to 30 mg plant material in 100 mL water) in the drinking water for a further 90 days. Co-treatment with the extract significantly ($p<0.05$) attenuated the manganese-induced increase in TBARS levels in the hippocampus and the striatum. The manganese-induced decrease in total thiol content in the hippocampus was completely restored by the extract. The increase in SOD activity in all brain structures after manganese was reduced by the extract, significantly only in the hippocampus ($p<0.05$); the increase in CAT activity after manganese was reduced only in the cortex ($p<0.05$) [Martins 2012].

A 30% ethanolic extract (16.62% hydroxycinnamic acids and 9.32% rosmarinic acid) was administered p.o. to mice at doses of 120, 240 and 360 mg/kg b.w. for 15 consecutive days. In the elevated plus maze test the two higher doses significantly increased the activity ratio and the latency ratios ($p<0.03$ and 0.004). The total activity over 24 hours, during the 12-hours light phase and the 12-hours dark phase, showed no significant difference. No changes were observed in the open field test and in the exploratory activity in the four-hole board [Ibarra 2010].

A 75% ethanolic extract did not demonstrate any effect on scopolamine-induced amnesia in mice [Orhan 2009].

An aqueous extract (DER 3:1) at doses of 25, 75, 150, 300 and 900 mg/kg or the essential oil at doses of 10, 25, 75, 150 and 300 mg/kg b.w. were administered i.p. to mice. In the test on spontaneous motor activity the essential oil did not show any activity. In contrast, the aqueous extract at doses of 75, 150 and 300 mg/kg significantly ($p<0.01$) reduced the motor activity by 49.6%, 46.6% and 66.4%, respectively. In the forced swimming test the aqueous extract caused a significant reduction in immobility ($p<0.01$) at all doses and a significant increase in climbing ($p<0.01$) as compared to control. The essential oil significantly reduced immobility ($p<0.01$) and increased climbing ($p<0.01$) at doses of 25, 75, 150 and 300 mg/kg in a dose-dependent manner. The swimming behaviour was increased dose-dependently as well, but significantly only at the highest dose ($p<0.01$) [Emamghoreishi 2009].

Hepatoprotective effects
Hyperlipidaemia induced in rats by a lipogenic diet and 3% ethanol per day for 42 days resulted in a significant increase of serum cholesterol, total lipids, alanine transaminase (ALAT),

aspartate transaminase (ASAT), alkaline phosphatase (ALP) and in the levels of tissue lipid peroxidation (LPO) ($p<0.0001$) as compared to control on a normal diet. The blood glutathione (GSH) levels were significantly decreased in hyperlipidaemic rats ($p<0.0001$). After two weeks on either a lipogenic or a normal diet, two groups received 2 g/kg b.w. p.o. of a dried hot water extract from melissa leaf daily for a further 28 days. The extract decreased the degenerative changes in the hepatocytes of hyperlipidaemic rats such as vacuolization, piknotic nuclei etc. and had no effect on normal rats as observed by light or electron microscopy. Serum cholesterol levels, serum total lipids, blood glutathione levels, serum ALT, AST, ALP and liver glutathione and lipid peroxidation remained unchanged in rats on a normal diet. After administration of the extract in hyperlipidaemic rats, serum cholesterol, total lipids, ALAT, ASAT, ALP and LPO were significantly ($p<0.0001$) reduced and blood GSH ($p<0.017$) as well as liver GSH ($p<0.0001$) increased at day 42 [Bolkent 2005].

In the same experiment several other parameters were investigated: serum triglycerides, serum uric acid and γ-glutamyltransferase were significantly reduced ($p<0.001$, <0.0001 and <0.0001 respectively) in hyperlipidaemic rats. Decreased catalase and paraoxonase activities in the hyperlipidaemic rats were elevated by the extract ($p<0.0001$). Mean skin total lipids, cholesterol, lipid peroxidation and non-enzymatic glycosylation levels, skin catalase, lactate dehydrogenase, glutathione peroxidase and myeloperoxidase elevated after the lipogenic diet were reduced. Skin glutathione and superoxide dismutase, which decreased in hyperlipidaemic rats, were increased by the extract [Sacan 2007].

Gastroprotective effects

An ethanolic liquid extract was tested for its potential anti-ulcerogenic activity against indomethacin-induced gastric ulcers in rats as well as for its antisecretory and cytoprotective activity. It showed dose-dependent anti-ulcerogenic activity at oral doses of 2.5-10 mL/kg associated with reduced acid output and increased mucin secretion, an increase in prostaglandin E_2 release and a decrease in leukotrienes. The effect on pepsin content was rather variable and did not seem to bear a relationship to the anti-ulcerogenic activity. The anti-ulcerogenic activity of the extract was also confirmed histologically. Cytoprotective effects of the extract could be partly due to its flavonoid content and to its free radical scavenging activity [Khayyal 2001].

A 30% ethanolic extract was administered orally to male Wistar rats one hour before challenge with indomethacin. The extract showed a pronounced anti-ulcerogenic effect at 5 and 10 mL/kg. Acidity, acid output and leukotriene content of gastic juice in pyloric-ligated rats were significantly reduced ($p<0.05$), pepsin content was hardly affected, whereas mucin and prostaglandin E_2 content were increased ($p<0.05$) as compared to the indomethacin-treated control group [Khayyal 2006].

Other effects

Topical treatment with a 1% ethanolic solution of the essential oil of melissa leaf applied to the shaved skin of guinea pigs had a repellent activity against *Anopheles stephensi* comparable to a 15% ethanolic solution of *N,N*-diethyl-*meta*-toluamide (Deet) [Oshagi 2003].

Mice were treated orally with an ethanolic extract (yield 13%) or rosmarinic acid in several different assays assessing anti-nociceptive activity. Abdominal contractions induced by i.p. injection of acetic acid were reduced by the extract in a dose-dependent manner, the effect was significant for doses between 30 mg and 1 g/kg b.w. ($p<0.01$ for 30mg/kg and $p<0.001$ for 100 mg, 300 mg and 1 g). Significant inhibition ($p<0.05$) of the neurogenic (0-5 min) and inflammatory (15-30 min) phases of formalin-induced licking were observed with the same dosages of extract. Glutamate-induced nociception was dose-dependently inhibited by the extract, with an ID_{50} of 198.54 mg/kg b.w. and an inhibition of 62% at 1 g/kg; the mean ID_{50} for rosmarinic acid was 2.64 mg/kg, with a 64% inhibition at 3 mg/kg. Locomotor activity was not influenced by the extract. Pretreatment with naloxone reversed the antinociception caused by morphine in the assay of glutamate-induced pain, but not that caused by the extract. In contrast, pretreatment with atropine reversed the antinociception after administration of the extract, and of pilocarpine and nicotine [Guginski 2009].

A dry extract (20% ethanol; 9:1) was administered orally to mice at daily doses of 50 or 200 mg/kg b.w. for 21 days. A control group received only the vehicle (distilled water). Serum corticosterone and GABA-T levels in homogenates of the subgranular zone of the hippocampal dentate gyrus (DG) were significantly decreased in a dose-dependent manner ($p<0.05$) as compared to control. Cell proliferation in the DG as shown by Ki67 immunohistochemistry was increased by 244.1% and 763.9% after 50 and 200 mg/kg respectively, relative to the control. In neuroblast differentiation in DG the treatment led to a dose-dependent and significant increase of doublecortin-immunoreactive neuroblasts with tertiary dendrites (211.4% and 387.4% relative to control; $p<0.05$). BrdU/calbindin D-28 k double labeled cells (integrated neurons into granule cells in DG) increased to 245% in the treatment group relative to the control [Yoo 2011].

In male BALB/c mice i.p. administration of an extract (60% methanol) at doses of 100, 300, 600, 900 and 1200 mg/kg b.w., 30 minutes before challenge with 90 mg/kg pentylenetetrazole i.p., significantly reduced the onset of general clonus ($p<0.01$ to 0.001), of myoclonic convulsion ($p<0.001$ for all doses) and of tonic-clonic convulsion ($p<0.05$ to 0.001) in a dose-dependent manner. The extract exhibited a bell-shaped protective effect against seizures and mortality (increasing dose-dependently up to 600 mg/kg and decreasing at 900 and 1200 mg) [Hariry 2011].

A dry extract (83% ethanol) was administered i.p. to male rats at a dose of 100 mg/kg b.w. daily for seven days, one hour before exposure to repeated restraint stress. Increased plasma levels of cortisol, malonyldialdehyde and protein carbonyl and cerebral nitric oxide exhibited in untreated animals were significantly reduced by the extract ($p<0.05$). The stress-dependent decrease in antioxidant defense systems was improved by the extract as shown by its influence on the cerebral level of reduced glutathione, glutathione-S-transferase and catalase as well as on plasma β-carotene and vitamins A, C and E [Ozkol 2011].

An aqueous extract was administered orally or subcutaneously to BALB/c mice for four days. On day 1 the animals underwent immunization with ram erythrocytes. On day 5 blood samples were taken for an active haemagglutination test in which no immunomodulating activity was observed irrespective of the route of administration. However, the number of splenocytes forming spontaneous E rosettes with ram erythrocytes was significantly increased with both routes of administration ($p<0.0001$) compared to control [Drozd 2003].

Male C57BL/ksJ-*db/db* mice received 0.0125 mg of melissa essential oil with their food for 6 weeks. Blood glucose levels decreased significantly after 3 weeks ($p<0.05$) and by up to 64.6% after 6 weeks ($p<0.001$) as compared to control. Serum insulin levels were significantly increased in comparison to control ($p<0.05$) at weeks 3 and 6. The essential oil also improved glucose tolerance as determined at week 6, with blood glucose levels in the treated animals significantly lower at 0, 15 and 120 min after glucose loading ($p<0.05$ to $p<0.001$). Body weight and

total fat content did not differ from control after 6 weeks. Gene and protein expression of hepatic glucose-regulating enzymes explained the decreased glucose concentrations by stimulation of hepatic GCK activity and a decrease of G6Pase and PEPCK. The mRNA expression of hepatic GLUT4 and SREBP-1c as well as of adipocyte GLUT4, PPAR-α, PPAR-γ and SREBP-1c was increased significantly ($p<0.05$ to $p<0.001$). After 6 weeks, plasma triacylglycerol concentrations were significantly lower ($p<0.05$) as compared to control. No significant differences were observed in plasma total cholesterol or HDL-cholesterol [Chung 2010].

Pharmacological studies in humans

A randomized, double-blind, placebo-controlled, crossover study was carried out in 20 healthy volunteers (mean age 19.2 years). The participants attended 4 days of treatment, receiving a single oral dose of either placebo or 300, 600 or 900 mg of a standardized extract (30% methanol; after evaporation the resulting soft extract was mixed with 10% of inert processing agents). Each treatment day was followed by a 7-day wash-out period. On each treatment day cognitive performance was assessed in a pre-dose testing session (baseline) and 1, 2.5, 4 and 6 hours after treatment using the Cognitive Drug Research computerised test battery and two serial subtraction tasks. Subjective mood was measured by Bond-Lader visual analogue scales. Significant improvement was observed for quality of attention at all times after a dose of 600 mg ($p = 0.0001$ to $p = 0.049$). Significant decreases in the quality of working memory and secondary memory were seen 2.5 and 4 hours after the higher doses ($p = 0.0005$ to $p = 0.05$). Reduction of working memory was more pronounced at 1 and 2.5 hours after the higher doses. Self-rated calmness was elevated significantly after 1 and 2.5 hours by the lowest dose ($p = 0.01$ to $p = 0.05$), while alertness was significantly reduced at all time points ($p = 0.001$ to $p = 0.05$) [Kennedy 2002].

A randomized, double-blind, placebo-controlled, cross-over study was carried out in 20 healthy volunteers (mean age 19.2 years). The participants attended 4 days of treatment, receiving a single oral dose of either placebo or 600, 1000 or 1600 mg of powdered melissa leaf, followed by a 7-day wash-out period. Cognitive performance and subjective mood were assessed by the Defined Intensity Stressor Simulation (DISS), which comprises a set of four concurrent cognitive and psychomotor tasks, on each treatment day in a pre-dose testing session (baseline) and 1, 3 and 6 hours after treatment. Significant improvement was observed for "quality of memory" at the last testing session after a dose of 600 mg ($p = 0.042$) and at 3 and 6 hours after 1600 mg ($p = 0.02$ and $p = 0.003$ respectively). Trends towards an improved performance were observed for the task "secondary memory factor" and some scores in "working memory factor". Significant decreases in the "speed of memory" were seen at all time points after the highest dose ($p = 0.0005$ to $p = 0.05$) and at 3 and 6 hours after treatment with 600 mg ($p = 0.001$ and 0.05 respectively). In the "rapid visual information processing task" significant effects on speed ($p = 0.007$) and accuracy ($p = 0.05$) in terms of false-positive responses were observed. Self-rated calmness was elevated at all time points after the highest dose ($p = 0.05$ to $p = 0.01$) [Kennedy 2003].

In a similar study, 18 healthy volunteers received a dry extract (30% methanol) at single oral doses of 300 or 600 mg extract or placebo. The effects on mood and cognitive performance were assessed by DISS. The three treatments were separated by washout phases of seven days. The higher dose of the extract ameliorated the negative mood effects of the DISS, significantly increased the self-ratings of calmness ($p<0.02$) and reduced those of alertness as compared to placebo. No modulation of the mood effects were seen after the lower dose, but this dose was associated with increased speed and accuracy of mathematical processing [Kennedy 2004].

Improvements in parameters linked to oxidative stress were measured in radiology staff members (occupationally exposed to chronic low-dose ionizing radiation) before and after treatment with an infusion of melissa. The participants (n=55) took the infusion (1.5 g in100 mL) twice daily for 30 days. At the end of treatment plasma superoxide dismutase, glutathione peroxidase and catalase were significantly increased ($p<0.0001$). Significant decreases were observed in plasma myeloperoxidase ($p<0.006$) and lipid peroxidation ($p<0.0001$). Plasma 8-OH-2-deoxyguanosine, as a measure of DNA damage, was significantly reduced ($p<0.001$) [Zeraatpishe 2011].

A 1% ethanolic solution of melissa essential oil protected human skin against *Anopheles stephensi*. The efficacy, based on the number of bites as compared to control, was 60 % [Oshagi 2003].

Clinical studies

In a randomized, double-blind, placebo-controlled, multicentre study, patients (n=42) with mild to moderate Alzheimer´s disease (score of ≥ 12 on the cognitive subscale of Alzheimer´s disease assessment scale (ADAS-cog) and ≤ 2 on the clinical dementia rating CDR) received 60 drops/day of either a 45% ethanolic (1:1) extract from melissa leaf (quantified to at least 500 µg/mL citral) or placebo for 16 weeks. At the endpoint, in the 35 patients who completed the trial (verum n=20, placebo n=15), a significantly better outcome for cognitive function was observed for verum treatment as compared to placebo ($p< 0.001$ for ADAS-cog and CDR) [Akhondzadeh 2003].

In an open study, 20 patients meeting the DSM-IV-TR criteria for primary diagnosis of anxiety disorders and sleep disturbances received 300 mg of a hydroalcoholic dry extract (standardized to more than 15% hydroxycinnamic acid derivatives and more than 7% rosmarinic acid) twice daily for 15 days. The patients were evaluated with the Free Rating Scale for Anxiety (for anxiety and related symptoms) and the Hamilton Rating Scale for Depression (to assess insomnia) at baseline and at the end of the study. The Clinical Global Impression-Improvement (CGI) scale was used for measurement of the overall clinical improvement in anxiety disorders and sleep disturbances. Total anxiety manifestations were reduced significantly by 18% ($p<0.01$). Agitation decreased by 35%, tension by 18% and hyperactivity as well as motor disturbances by 10% each. Anxiety-associated symptoms such as psychosomatic symptoms, eating problems, fatigue, intellectual disturbance and feeling of inferiority, declined in total by 15% ($p<0.01$). Initial insomnia (difficulty falling asleep) decreased by 53%, middle insomnia (waking during the night) by 45% and delayed insomnia (early morning waking) by 28%; the total reduction was 42% ($p<0.01$). Based on the CGI improvement a positive treatment response was experienced by 19 of 20 patients for anxiety disorders and by all patients for insomnia [Cases 2011].

A double-blind, randomized, placebo-controlled, multicentre trial was performed in patients with Alzheimer´s disease (mean age 85.6 years) associated with severe agitation (score ≥ 39 on the Cohen Mansfield Agitation Inventory). The patients were randomized to one of 3 groups: verum oil and placebo tablets (n=35), donepezil (5 mg/day increasing to 10 mg/day after one month orally) and placebo oil (n=35), or placebo oil and placebo tablets (n=36). A lotion containing either 200 mg of melissa essential oil (verum) or sunflower oil (placebo) was massaged into the skin of the hands and upper arms of the patients twice daily. No signficant differences in the Pittsburgh

Agitation Scale, the Barthel Scale of Activities in Daily Living or the Neuropsychiatric Inventory were observed compared to baseline after 4 and 12 weeks of treatment and between the three groups. A significant difference between melissa and donepezil was seen in quality of life measured with the Blau QOL Scale: a significantly lower QOL occurred in the donepezil group (p=0.033) at week 12 [Burns 2011].

A 4-week multicentre, double-blind, placebo-controlled study involved 72 patients of mean age 78.5 years with clinically significant agitation in the context of severe dementia. The patients were treated topically twice daily with a lotion containing 10% of melissa essential oil, providing a daily total of 200 mg of the oil (n = 36), or a placebo lotion (n = 36). Lotion was gently applied to the patient's face and both arms as an aromatherapy treatment. Changes in agitation were determined by the Cohen-Mansfield Agitation Inventory (CMAI) score. Improvements in the CMAI total score (35% reduction in the verum group and 11% in the placebo group) were significantly greater in the verum group ($p<0.0001$). A 30% improvement in CMAI score was attained by 21 subjects in the verum group compared to only 5 in the placebo group ($p<0.0001$). Quality of life indices measured by Dementia Care Mapping also improved significantly in the verum group; compared to the placebo group the percentage of time spent socially withdrawn was reduced ($p<0.005$) and time engaged in constructive activities increased ($p<0.001$) [Ballard 2002].

In a multicentre, open, controlled study involving 115 patients, a cream containing 1% of a lyophilised aqueous extract from melissa leaf (70:1) significantly reduced the healing time of cutaneous *Herpes simplex* lesions ($p<0.01$). It also significantly extended the intervals between recurrences of infection compared to other external virustatic preparations containing idoxuridine and tromantidine hydrochloride ($p<0.01$) [Wölbling 1984; Wölbling 1994]. These effects, particularly a significant reduction in the size of lesions within 5 days ($p = 0.01$), were confirmed in a multicentre, double-blind, placebo-controlled study on 116 patients [Vogt 1991; Wölbling 1994].

A randomized, double-blind, placebo-controlled study was carried out using a cream containing 1% of a melissa leaf dry extract (70:1) standardized in terms of antiviral potency. Sixty-six patients with a history of recurrent *Herpes simplex* labialis (at least four episodes per year) were treated topically; 34 of them with verum cream and 32 with placebo. The cream was applied to the affected area 4 times daily over 5 days. A symptom score (ranging between 0 and 9), derived by combination of the severity ratings for complaints, size of affected area and number of blisters on day 2 of therapy, was used as the primary target parameter. There was a significant difference ($p<0.05$) in scores for the primary target parameter between treatment groups: verum 4.03 ± 0.33 (3.0); placebo 4.94 ± 0.40 (5.0); values given are the mean ± SEM (median) of the symptom scores on day 2. The significant difference in symptom scores on the second day of treatment is of particular importance because the complaints in patients suffering from *Herpes labialis* are usually most intensive at that time [Koytchev 1999].

Pharmacokinetic properties

After incubation with an extract, a dose-dependent absorption of rosmarinic acid as a marker for melissa leaf was shown *ex vivo* using the everted gut sac technique, and *in vitro* in the Caco-2 cell model [Kelber 2006].

Preclinical safety data

A hydroethanolic melissa extract (50% ethanol), a juice of fresh melissa, rosmarinic acid and a sulphated triterpene, in a solution of phosphate buffer (PBS), were tested on reconstituted human epidermis, a model validated to classify skin irritants. Compared to the control (PBS) group, after 15 min exposure to the test solutions and a period of 42 hours of recovery, no decrease in epidermis viability was observed as measured in the MTT assay or in trans-epithelial electrical resistance, a measure for the barrier function of the epithelium. No increased release of IL-1α occurred as compared to control [Mencherini 2009].

Melissa essential oil did not cause any symptoms of irritation at a concentration of 25% when applied to chorioallantoic membrane. The endpoint of evident haemorrhage was seen after 5 min. incubation with 35% of the oil [Suschke 2007].

Mutagenicity
A tincture (ethanol 70%, 1:5) of melissa leaf gave negative results in the Ames test using *Salmonella typhimurium* TA 98 and TA 100 strains with or without metabolic activation [Schimmer 1994]. No genotoxic effects from a 20% tincture of melissa leaf were detected in a somatic segregation assay using the diploid strain *Aspergillus nidulans* D-30 [Ramos Ruiz 1996].

Male CF-1 mice were treated orally with either an extract (45% ethanol; 1:5) at 250 and 500 mg/kg b.w. per day, or an infusion (0.6 g of the drug in 20 mL) at 100 mg solid matter/kg b.w. per day for 14 days prior to an i.p. injection of saline or the alkylating agent methylmethansulfonate (MMS; 40 mg/kg b.w.). Neither genotoxic nor mutagenic effects were demonstrated in the blood and bone-marrow of animals treated with the melissa extracts plus saline compared to the negative control, as evaluated in the comet assay and the micronucleus test . After both doses of the extract a significant decrease in MMS-induced DNA damage was observed ($p<0.05$ to 0.001) in the comet assay. In the micronucleus test the higher dose of the extract significantly decreased the number of polychromatic erythrocytes after challenge ($p<0.05$), thus suggesting an antimutagenic effect [Cassettari de Carvalho 2011].

Clinical safety data

After single doses of 300 and 600 mg of a 30% methanolic extract, administered to healthy volunteers (n=18), no adverse effects were observed [Kennedy 2004].

No significant differences in side effects were observed between verum (n = 21; 60 drops per day of a 45% ethanolic (1:1) extract) and placebo during a 16 weeks trial. Only agitation was more common in the placebo group (p=0.03) [Akhondzadeh 2003].

In a double-blind, placebo-controlled, randomized trial in Alzheimer patients, after topical application of a lotion containing melissa essential oil (n = 35; 200 mg of the oil twice daily) two serious adverse events (not further specified) were reported [Burns 2011].

No adverse effects on the skin of four volunteers were observed during the study and for one month after the topical application of a 1% ethanolic solution of melissa essential oil [Oshagi 2003].

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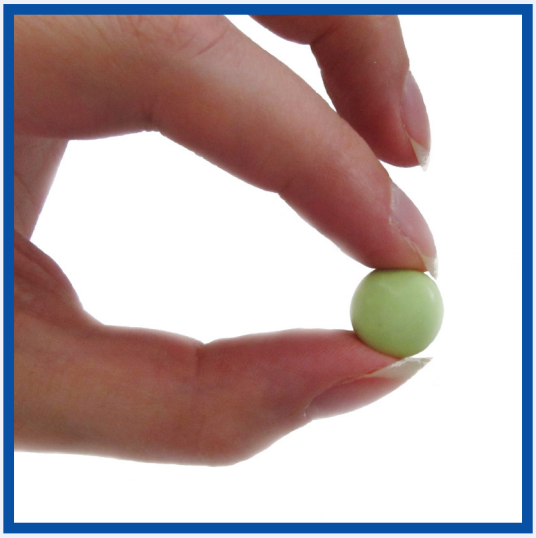

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MONOGRAPHS

***The* Scientific Foundation for**
Herbal Medicinal Products

MENTHAE PIPERITAE FOLIUM
Peppermint Leaf

2019

E/S/C/O/P
European Scientific Cooperative
On Phytotherapy

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Plant illustrated on the cover: *Mentha × piperita*

FOREWORD

It is a great pleasure for me, on behalf of my colleagues in ESCOP, to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

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Notes for the Reader
Abbreviations
The monograph text
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Information on the member organizations and people involved in the production of ESCOP monographs and other activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

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Peppermint Leaf

DEFINITION

Peppermint leaf consists of the whole or cut dried leaves of *Mentha × piperita* L. The whole drug contains not less than 12 mL/kg of essential oil. The cut drug contains not less than 9 mL/kg of essential oil.

The material complies with the monograph of the European Pharmacopoeia [Peppermint Leaf].

Fresh material may also be used, provided that when dried it complies with the monograph of the European Pharmacopoeia.

CONSTITUENTS

The main active component is essential oil (0.5-4%), of which the principal constituent is usually menthol, in the form of (-)-menthol (30.0-55.0%) with smaller amounts of stereoisomers such as (+)-neomenthol (2.5-3.5%) and (+)-isomenthol (approx. 3%), together with menthone (14.0-32.0%), isomenthone (1.5-10.0%), menthyl acetate (2.8-10.0%), 1,8-cineole (3.5-8.0%), limonene (1.0-3.5%), menthofuran (1.0-8.0%), pulegone (max 3.0%) and other monoterpenes. Small amounts of sesquiterpenes, notably β-caryophyllene, germacrene D and viridoflorol [Lawrence 1993; Peppermint Oil; Stahl-Biskup 2011, 2016].

Various flavonoids including luteolin and its 7-glycosides, rutin, hesperidin, eriocitrin and highly oxygenated flavones; phenolic acids such as rosmarinic acid and lithospermic acid and small amounts of triterpenes [Croteau 1973; Hoffmann 1984; Litvinenko 1975; Jullien 1984; Barberan 1986; Duband 1992; Hänsel 2010; Guédon 1994; Fecka 2007].

CLINICAL PARTICULARS

Therapeutic indications

Symptomatic treatment of digestive disorders such as dyspepsia, flatulence and gastritis [Demling 1969; Forster 1980; Forster 1983; Bradley 1992; Hänsel 2010; Stahl-Biskup 2016]. In these indications, the efficacy is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Adults: As an infusion, 1.5-3 g of the drug to 150 mL of water, three times daily [Bradley 1992; Stahl-Biskup 2016]. Tincture (1:5, 45% ethanol), 2-3 mL, three times daily [Bradley 1992].
Elderly: Dose as for adults.
Children from 4 years of age, daily dose as infusions only: *4-10 years*: 3-5 g; *10-16 years*: 3-6 g [Dorsch 2002].

Method of administration
For oral administration.

Duration of administration
If symptoms persist or worsen, medical advice should be sought.

Contra-indications
Known hypersensitivity to peppermint or to menthol.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

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Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties
The pharmacological actions of peppermint leaf are largely, but not exclusively, attributable to the essential oil; other components such as flavonoids also appear to play a role [Bradley 1992; Stahl-Biskup 2016]. Pharmacodynamic data relating to the essential oil are given in the monograph on Peppermint Oil.

In vitro experiments

Antispasmodic activity
An extract (ethanol 30%; not further specified) exhibited antispasmodic activity at concentrations of 2.5 and 10.0 mL/L, causing significant and dose-dependent increases in ED_{50} values for acetylcholine- and histamine-induced contractions of isolated guinea pig ileum ($p<0.01$ and $p<0.0005$ respectively for histamine-induced contractions), and a significant decrease in the maximum possible contractility ($p<0.05$ and $p<0.001$ respectively for histamine-induced contractions). The effect of the extract at 10.0 mL/L corresponded to that of 1.6 µg/L of atropine [Forster 1980]. A similar peppermint leaf extract inhibited carbachol-induced contractions of isolated guinea pig ileum [Forster 1983].

A total flavonoid fraction from peppermint leaf, dissolved in water so that 1 mL corresponded to about 0.5 g of dried leaf, inhibited barium chloride-induced contractions of isolated guinea pig ileum [Lallement-Guilbert 1970].

Antimicrobial activity
A methanolic extract (not further specified) inhibited growth of *Candida albicans* with a MIC value of 7 µg/mL [Höfling 2010].

A dichloromethane fraction of a methanolic extract (not further specified) showed inhibitory activity against *Giardia lamblia* trophozoites with an IC_{50} of 0.75 µg/mL after 48 hours of incubation compared to metronidazole with an IC_{50} of 0.8 µg/mL after 24 hours of incubation. In an adherence inhibition assay the same fraction at a concentration of 1 µg/mL significantly ($p<0.01$) reduced adherence of trophozoites on coverslips after 48h [Vidal 2007].

Three aqueous extracts (not further specified) significantly ($p<0.01$) reduced the growth of *Chlamydia pneumoniae* in the range of 51.2-69.5% of rifampicin activity (at a dose of 9 ng/sample). The IC_{50} values ranged between 98 und 224 µg/mL [Kapp 2013].

Antiviral activity
An aqueous extract (not further specified) exhibited activity against HSV-1 and HSV-2 with IC_{50} values of 0.041 µg/mL and 0.227 µg/mL respectively, in a plaque reduction assay using RC-37 cells. Plaques were reduced by > 90% for HSV-1 and HSV-2 and > 85% for an acyclovir-resistant strain of HSV-1. Effects were time-dependent and best results were observed after two hours of incubation prior to the infection. The effective concentrations were far below cytotoxic concentrations (EC50 =107 µg/mL) [Nolkemper 2006].

An infusion (10 g/100 mL) added to HIV-1 virions prior to infection of cells led to a concentration-dependent reduction of HIV-1 virion replication in Sup-T1 T-cells ($IC_{50}=0.190 \pm 0.124\%$ [1%=1mL stock-solution/100mL culture medium]), C8166 T-cells ($IC_{50}=0.026 \pm 0.016\%$) and primary human lymphoid aggregate culture (HLAC) from tonsil tissue ($IC_{50}=0.666 \pm 0.156\%$) monitored by p24 ELISA. These concentrations did not affect cell viability. Extracts were active against infection of virions carrying diverse envelopes (X4 and R5 HIV-1), but not against non-enveloped adenovirus. The extract induced an increase of the virion's density [Geuenich 2008].

Other effects
Acetonitrile and methanolic extracts (not further specified) showed radical-scavenging activity in DPPH assay with IC_{50} values of 0.24 mg/mL and 0.21 mg/mL respectively [Dorman 2009].

A chloroform and an ethyl acetate extract (not further specified) were tested for their cytotoxicity against cervical adenocarcinoma (HeLa), breast adenocarcinoma (MCF-7), T-cell lymphoblast (Jurkat), urinary bladder carcinoma (T24), colon adenocarcinoma (HT-29), pancreatic adenocarcinoma (MIAPaCa-2), normal lung fibroblast (IMR-90), and kidney epithelial (HEK-293) cells using the Annexin-V-FITC/PI assay. Both extracts showed significant increases ($p<0.01$) in the apoptotic index within all cancer cell lines. No effect was observed in normal IMR-90 and HEK-293 cells [Jain 2011].

In vivo experiments

Choleretic effects
In experiments with cannulated dogs, peppermint tea (0.4 g/kg b.w.) increased the secretion of bile [Steinmetzer 1926]. Flavonoids, as well as the essential oil, contribute to this action [Pasechnik 1966; Pasechnik 1966b; Hänsel 2010].

Mixed flavonoids from peppermint leaf (optimum dose 2 mg/kg) showed choleretic activity in dogs [Pasechnik 1966]. Flavomentin, a flavonoid preparation from peppermint leaf, stimulated bile secretion and the synthesis of bile acids in dogs at doses of 0.5-6 mg/kg (optimum 2 mg/kg) [Pasechnik 1966b].

In experiments with cannulated rats, intravenous injection of a peppermint tea at 0.5 mL/rat or a flavonoid preparation (corresponding to a dose of 3.3 g of peppermint leaf per kg b.w.) proved effective in increasing the amount of bile acids [Lallement-Guilbert 1970].

Anti-ulcerogenic effect
Oral pre-treatment of rats with an ethanolic liquid extract (not further specified) at 2.5-10 mL/kg b.w. dose-dependently protected against oral indometacin-induced gastric ulcers (80% protection at 10 mL/kg); this was confirmed histologically. The extract also had gastric antisecretory and cytoprotective effects; compared to rats treated intraperitoneally with indometacin (10 mg/kg), analysis of the gastric contents of animals pre-treated orally with the extract indicated reduced acid output, an increase in PGE_2 release and a decrease in leukotrienes (all $p<0.05$ at 2.5 mL/kg) [Khayyal 2001].

Antinociceptive effects
An aqueous extract (not further specified) administered i.p. at doses of 200 mg/kg and 400 mg/kg b.w. to albino mice

significantly ($p<0.01$) lowered acetic acid-induced abdominal writhing. There was no significant difference between the two doses. In a hot-plate test, the extract at both doses significantly ($p<0.001$) increased the latency to a response to thermal stimulation compared to controls (20.2% and 17.1% protection respectively) [Taher 2012].

In a similar experiment, a dry extract (ethanol 80%; not further specified) administered orally at 200 mg/kg or 400 mg/kg b.w. to mice significantly reduced acetic acid-induced writhing ($p<0.01$ and $p<0.001$ respectively) as well. The response time of mice to thermal stimulation in the hot-plate test also increased significantly 45 and 60 minutes after i.p. administration of the extract at 400 mg/kg ($p<0.01$ and $p<0.001$ respectively) [Atta 1998].

Anti-inflammatory activity
A dry extract (ethanol 80%; not further specified) showed anti-inflammatory activity against acute and chronic inflammation in rodents. After oral administration, it reduced xylene-induced ear oedema in mice (acute model) by 49% at 200 mg/kg and 50% at 400 mg/kg b.w. (both $p<0.05$). After i.p. administration in the cotton pellet granuloma test in rats (chronic model), only the higher dose of 400 mg/kg had a significant inhibitory effect ($p<0.01$) [Atta 1998].

Sedative effects
A dry aqueous extract (not further specified), administered orally to mice at single doses of 300 or 1000 mg/kg b.w., caused weak sedative effects in several tests: hexobarbital-induced sleep, exploratory behaviour, spontaneous motility and motor coordination. The same extract had a significant diuretic effect in mice at 100 and 300 mg/kg ($p<0.05$), but not at 1000 mg/kg [Della Loggia 1990].

Effects on blood lipid profile
An aqueous extract (not further specified) at a dose of 250 mg/kg b.w. administered orally to rats with fructose-induced hyperlipidaemia significantly ($p<0.05$) decreased elevated body weight and levels of glucose, serum cholesterol, triglycerides, VLDL, LDL as well as the atherogenic index and increased the level of serum HDL. The extract also significantly ($p<0.05$) increased the activity of SOD as well as the concentration of GSH and significantly ($p<0.05$) decreased lipid peroxidation, as shown by a reduced level of TBARS [Badal 2011].

Antiallergic effects
An extract of leaves and stems previously depleted of essential oils by steam distillation (ethanol 50%; not further specified) reduced experimentally increased histamine release by compound 48/80 from peritoneal mast cells of male Wistar rats with an IC_{50} value of 4.72 µg/mL (95% confidence interval 2.54 – 7.65 µg/mL). A fraction of the extract, administered orally at doses of 300 and 1000 mg/kg, significantly ($p<0.01$) decreased sneezing, nasal rubbing and dye leakage induced by an antigen. No significant effect was seen in sneezing and nasal rubbing induced by histamine [Inoue 2001].

Tumour-preventive effects
Male albino mice were divided into 3 groups. The first group (n=8) received no treatment; in the second and third groups (n=36 each) the mice had the ventral and dorsal surfaces of their tongues painted 3 days a week for 9 weeks with the carcinogens 7,12-dimethylbenz[a]anthracene (DMBA) and formaldehyde (starting on day 9); the third group were additionally treated at the same time with an aqueous extract (not further specified) at an oral dose of 1 g/kg b.w. The extract non-significantly reduced dysplastic cellular changes in the tongue epithelium by 61% and inhibited tumour incidence by 100% as compared to group 2 in histological analysis. Immunohistochemical evaluation after 6 weeks showed significantly ($p\leq0.001$) reduced expression of caspase 3 in the extract treated group compared to group 2 [Kasem 2014].

Protective effects against irradiation
Adult male Swiss albino mice were treated orally with an aqueous extract (not further specified) at a dose of 1 g/kg b.w. per day for three days and subsequently exposed to whole-body gamma-irradiation (8 Gy). Ten days after irradiation, pretreatment with the extract showed significant increases ($p<0.001$) in total erythrocyte and leucocyte counts, haemoglobin concentration and haematocrit value compared to irradiation only. Glutathione levels in blood and liver were significantly elevated ($p<0.005$ and $p<0.001$ respectively) in the extract + irradiation group in contrast to the irradiation only group [Samarth 2003].

Irradiated mice showed significantly ($p<0.001$) decreased levels of pronormoblasts and normoblasts of the erythroid series as well as increased levels of leucoblasts and myelocytes compared to normal values, whereas these irradiation-caused changes in blood values were significantly ($p<0.001$) reduced by pre-treatment with the above-mentioned extract [Samarth 2007].

Under the same conditions, the testicles of extract-pretreated, irradiated mice showed normal testicular morphology with regular arrangement of germ cells and a slight degeneration of seminiferous epithelium. There was a significant decrease in the testicular weight of mice exposed to radiation ($p<0.001$) compared to the group treated with distilled water only. Extract pre-treatment plus irradiation led to significantly ($p<0.001$) elevated testicular weight compared to the irradiation only group, 14 days after irradiation. A significant decrease of lipid peroxidation ($p<0.001$) and of acid phosphatase ($p<0.01$) and a significant increase of alkaline phosphatase ($p<0.001$) were also observed in testicular tissue fourteen days after irradiation as compared to irradiated mice without pre-treatment [Samarth 2009].

Swiss albino mice treated orally with an aqueous extract (not further specified) at a dose of 1 g/kg b.w. per day for seven days and subsequently exposed to whole-body gamma-irradiation (6 Gy) showed significantly ($p<0.05$) reduced expression of p53 and up-regulation of Bcl2, as well as significantly ($p<0.05$) reduced levels of G2/M and G0/G1- Phase, and a significantly ($p<0.05$) elevated level of S-phase in CNS cells, compared to an irradiation only group. Concentration of brain DNA, RNA, GSH and SOD significantly ($p<0.05$) increased in the extract-treated irradiated group in contrast to irradiated mice [Hassan 2013].

Other effects
An aqueous extract (not further specified) was administered orally to albino mice at a dose of 1 g/kg b.w. per day for 10 days prior to sodium arsenite intoxication and continuously for 30 days afterwards. Treatment with the extract significantly ($p<0.001$) ameliorated the sodium arsenite-induced decrease in body and liver weight after 30 days. The extract significantly ($p<0.001$) lowered the sodium arsenite-induced enhancement of ALP, acid phosphatase (ACP), SGOT, SGPT and lipid peroxidation. Sodium arsenite-induced declines in LDH activity and GSH level were raised significantly ($p<0.001$) by the extract [Sharma 2007].

An extract (ethanol 70% (V/V); not further specified) at a dose of 100 mg/kg b.w. per day administered orally for seven weeks to female Balb/c mice previously infected with *Schistosoma mansoni* caused significantly ($p<0.05$) increased serum levels of IL-10, INF-γ, IgG2a and IgE and a significant ($p<0.05$) decrease in egg counts in the faeces and intestine, compared to control [Dejani 2014].

Male rabbits were divided into 4 groups (n=6 each) and treated daily for 21 days with either saline (i.m.), gentamicin (80 mg/kg b.w. i.m.), an ethanolic extract (not further specified; 200 mg/kg b.w. p.o.) or the extract and gentamicin together. The significant increases ($p<0.05$ to $p<0.0001$) in the levels of serum creatinine, blood urea nitrogen, serum uric acid and urinary protein in the gentamicin group compared to control were significantly ($p<0.05$ to $p<0.0001$) decreased in the groups receiving the extract. Creatinine clearance, serum potassium and serum calcium levels were significantly ($p<0.05$ to $p<0.0001$) increased in both extract groups compared to the group treated with gentamicin only. Additionally, the extract did not reduce the antimicrobial activity of gentamicin [Ullah 2014].

An aqueous infusion (not further specified) showed antimutagenic activities against hydrogen peroxide in the Somatic Mutation and Recombination Test (SMART) in wings of *Drosophila melanogaster* [Romero-Jimenez 2005].

Pharmacological studies in humans

The carminative action of extracts (not further specified) is due to a reduction in tonus of the oesophageal sphincter, enabling release of entrapped air [Demling 1969].

The metabolic ratio of N-acetyltransferase-2 was significantly ($p<0.05$) reduced after six days by an aqueous extract (2 g dry leaves/200 mL water; not further specified) administered twice daily to healthy volunteers. The extract had no significant influence on the activity of CYP 1A2, CYP 2A6, xanthine oxidase and UDP-glucuronosyltransferase-1A1 and -1A6 [Begas 2017].

Pharmacokinetic properties

Pharmacokinetic data relating to the essential oil are given in the monograph on Peppermint Oil.

Preclinical safety data

After oral administration of an extract (not further specified) to mice (n=12) as a single dose at 4000 mg/kg b.w., none of the animals died and none showed macroscopic signs of toxicity over a 7-day period of observation [Della Loggia 1990].

An infusion (20 g/L) was provided to male Wistar albino rats (n=12) instead of drinking water for 30 days. Histological analysis of kidney tissue showed tubular epithelial cells with slight hydropic degeneration and epithelial cells with pycnotic nuclei and eosinophilic cytoplasm. Moderate tubular dilatation and enlargements in Bowman capsules were observed [Akdogan 2003].

In the same experimental setup, the infusion caused significantly ($p<0.01$) decreased plasma testosterone levels, whereas LH and FSH levels significantly ($p<0.05$) increased. Histological analysis showed changes in germinal epithelium and spermatogenesis arrest. Johnsen testicular biopsy score significantly decreased ($p<0.01$) [Akdogan 2004a].

In a similar trial, the same infusion caused a significant ($p<0.05$) decrease in serum iron and ferritin levels and a significant ($p<0.01$) increase in unsaturated iron-binding capacity [Akdogan 2004b].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2019
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003

LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2019
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
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ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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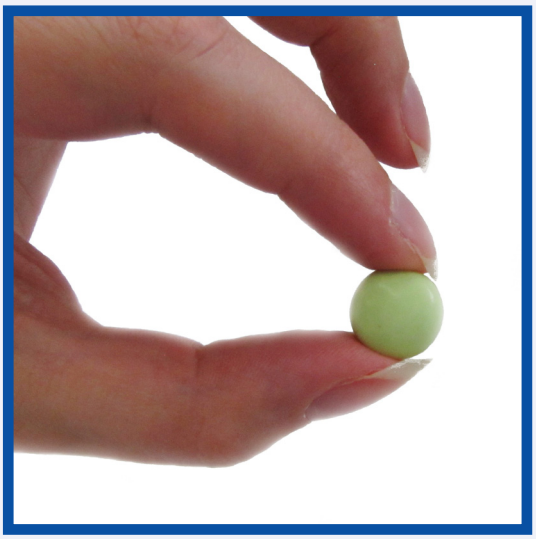

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

MENYANTHIDIS TRIFOLIATAE FOLIUM 2013

Bogbean Leaf

DEFINITION

Bogbean leaf consists of the dried, entire or fragmented leaves of *Menyanthes trifoliata* L. The material complies with the monograph of the European Pharmacopoeia [Bogbean Leaf].

CONSTITUENTS

The main characteristic constituents are iridoid glycosides - approx. 1% of bitter substances including the iridoid loganin and the seco-iridoids foliamenthin, dihydrofoliamenthin, menthiafolin and sweroside. Maximum concentrations of bitter substances were recorded during flower-bud initiation [Hänsel 1966; Battersby 1968; Gessner 1974; Maksimova 1980; Steinegger 1988; Junior 1989; Wagner 1996; Martz 2009]. Flavonol glycosides such as isoquercitrin, hyperoside and rutin [Krebs 1957; Lebreton 1973; Mel'chakova 1976; Bohm 1986; Wagner 1996], as well as trifolin [Krebs 1958]. Chlorogenic acid and other phenolic acids such as caffeic, ferulic, sinapic, vanillic and protocatechuic acids [Herrmann 1959; Ciaceri 1972; Swiatek 1986]. Coumarins - scopoletin (6-methoxy-7-hydroxycoumarin), scoparone (6,7-dimethoxycoumarin) and braylin [Ciaceri 1972; Adamczyk 1990].

Other constituents include ascorbic acid, tannins [Pamakstyte-Jukneviciene 1971; Maksimova 1980], polysaccharides [Kuduk-Jaworska 2004], polypeptides [Lindholm 2002] and phytosterols [Popov 1969]. The alkaloid gentianine has been reported [Steinegger 1951; Rulko 1969] but may be an artefact [Cordell 1981].

CLINICAL PARTICULARS

Therapeutic indications

As a bitter [BHP 1996; Bruneton 1999] in loss of appetite, peptic discomfort and atonic dyspepsia [Schilcher 2007], as well as for gall bladder and biliary tract disorders [Sweetman 2007].

Posology and method of administration

Dosage

Dried powdered leaf
0.5-2.0 g two to three times daily [BHP 1976; Steinegger 1988; Blaschek 2007].

Cut drug
0.5-1 g as an infusion in 150 mL boiling water taken half-an-hour before meals [Steinegger 1988; Bisset 1994; Blaschek 2007; Wichtl 2009].

Liquid extract (1:1; 25% ethanol)
1-2 mL; two to three times daily [BHP 1976; Steinegger 1988].

Tincture (1:5; 45% ethanol)
1-3 mL; three times daily [BHP 1976; Steinegger 1988; Blaschek 2007].

Method of administration
For oral administration.

Duration of administration
No restriction. If symptoms persist or worsen, medical advice should be sought.

Contraindications
None known.
Not recommended in cases of diarrhoea, dysentery or colitis [BHP 1976; Steinegger 1988; Blaschek 2007].

Special warnings and special precautions for use
None required.

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Interaction with other medicaments and other forms of interaction
None reported. Due to their structure and low content the coumarins are presumed not to interact with anticoagulants [Williamson 2009].

Pregnancy and lactation
In accordance with general medical practice, the product should not be used during pregnancy or lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
Can irritate the stomach and induce vomiting in sensitive individuals [Steinegger 1988; Blaschek 2007].

Overdose
May lead to nausea, vomiting and diarrhoea [Blaschek 2007].

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties
Bogbean leaf is required to have a minimum bitterness value of 3000.

In vitro experiments

Antibacterial activity
A 70%-ethanolic extract (1:5) [Moskalenko 1986] and an aqueous extract [Brantner 1994] of bogbean leaf showed activity against various microorganisms including strains of *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli*. Using the microdilution broth method MIC values (mg/mL, calculated as the dry residue) were 25.7, 29.4 and 25.7 respectively [Brantner 1994].

Anti-inflammatory activity
Aqueous extracts (0.2 mg/mL) from bogbean leaf produced a low percentage inhibition of prostaglandin biosynthesis (22%) in comparison with 1 µg/mL of indomethacin (88%) and 76 µg/mL of quercetin (96%). In the PAF-exocytosis assay the aqueous extracts (0.25 mg/mL) also produced a low inhibition (35%) when compared to 100 µM of the PAF-inhibitor BN 52021 [Tunón 1995].

Enzymatic activation of iridoid glycosides with β-glucosidase yield an active hydrolysed-iridoid product (H-iridoid). H-loganin prepared from loganin (1mM) preincubated with enzyme (0.5 mM) (iridoid:enzyme=1.0:0.5V/V) showed significant inhibition of thromboxane B_2 formation in intact HEL cells at 10, 50 and 100 µM ($p<0.05$) compared to vehicle only. Significant inhibitory activities were recorded on COX-1 (IC_{50} value of 3.55 µM; $p<0.05$) compared to aspirin (7.23 µM), against PGE_2 at 50 and 100 µM ($p<0.05$) and suppression of TNFα (IC_{50} value of 154.6 µ M; $p<0.05$) compared to vehicle (DMSO) [Park 2010].

Immunomodulatory activity
Tests using human blood-derived lymphocytes and granulocytes showed that some polysaccharide fractions isolated from bogbean leaf were strongly immunostimulatory, while other fractions were potent immunosuppressive and anti-inflammatory agents [Kuduk-Jaworska 2004].

Maturation of human dendritic cells (DCs) in the presence of a lyophilised aqueous extract of *Menyanthes trifoliata* (50 µg/mL) did not affect expression of the surface molecules but reduced the ratio of secreted IL-12p40/IL-10, compared with cells matured in the absence of extracts. Allogeneic $CD4^+$ T cells co-cultured with DCs matured in the presence of an aqueous extract secreted less IFN-γ, IL-10 and IL-17 than $CD4^+$ T cells co-cultured with DCs matured without an extract ($p<0.05$) [Jonsdottir 2011].

In vivo experiments

Anti-inflammatory activity
When applied topically, some of the phenolic acids present in bogbean leaf have shown anti-inflammatory activity against tetradecanoylphorbol acetate (TPA)-induced mouse ear oedema (protocatechuic acid, 71.6% inhibition, $p<0.001$; ferulic acid, 71.0% inhibition, $p<0.001$) [Fernandez 1998].

Loganin applied topically (in acetone or 80% aqueous ethanol) at 1 mg/ear inhibited TPA-induced mouse ear oedema by 76.8%. The effect of oral administration at 100 mg/kg b.w. was minimal and short-lived, with maximum activity at 3 hours (n = 6) [Recio 1994].

Oral administration of loganin to type 2 diabetic *db/db* mice (n=6 or 10) at a dose of 100 mg/kg b.w. daily for 8 weeks significantly reduced liver weight ($p<0.01$), hepatic glucose ($p<0.05$), triglycerides ($p<0.01$) and total cholesterol levels ($p<0.01$) compared to control mice receiving vehicle only; 20 mg/kg had no significant effect. Levels of biomarkers (TBARS and ROS) associated with oxidative stress in the liver and kidney were also evaluated. Both 20 and 100 mg/kg loganin significantly reduced TBARS and ROS ($p<0.01$) in liver, while in the kidneys both dose levels reduced ROS ($p<0.01$) but only the higher dose reduced TBARS ($p<0.05$) [[Yamabe 2010].

In a continuation of the experiment the effects on diabetic *db/db* mice receiving loganin (20 or 100 mg/kg b.w./day p.o.) were compared with vehicle-treated *db/db* and non-diabetic *m/m* mice. Administration of loganin at both dose levels led to a reduced food intake (g/day) by *db/db* mice compared to vehicle-only controls ($p<0.05$). With 100 mg/kg b.w. a lowering in serum glucose ($p<0.01$) and an elevation of leptin ($p<0.05$) were observed. Diabetic oxidative stress was significantly up-regulated in the liver of *db/db* mice and loganin (100 mg/kg) administration decreased expressions of hepatic proteins Nox-4 ($p<0.01$) and $p22^{phox}$ ($p<0.05$). Hepatic levels of ROS and TBARS were higher in *db/db* mice in comparison to *m/m* mice. Loganin administration at both dose-levels down-regulated hepatic protein expression of NF-κB, COX-2 and iNOS. At a dose of 100 mg/kg loganin led to a significantly reduced expression ($p<0.05$) of the anti-inflammatory factor Nrf-2 [Park 2011].

Pharmacokinetic properties
No data available for bogbean leaf.

A study using rats (n = 5) examined the tissue distribution of loganin. After a single oral dose of 20 mg/kg b.w. the highest level was found in kidney tissue, followed by stomach, lung and small intestine. Peak levels in most tissues were attained at 90 minutes. No long-term accumulation of loganin was found in rat tissues. The results indicated that the kidney was the major distribution tissue of loganin in rats [Li 2006] and can have a nephroprotective effect [Jiang 2011].

Preclinical safety data

Mutagenicity
Extracts from bogbean leaf have been evaluated in the Ames mutagenicity test using *Salmonella typhimurium* strains TA98 and TA100 (with and without activation by S9 mix). Tinctures

(1:5, ethanol 60% and 70%) and a fluid extract (1:1, ethanol 25%) gave negative results with TA100 and also with TA98 (without activation), but showed weak mutagenicity with TA98 activated by S9 mix. An undefined dry extract showed weak mutagenicity with TA98, both without and with activation [Schimmer 1994].

Clinical safety data
No data available.

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Supplement 2009
ALOE CAPENSIS	Cape Aloes	Second Edition, 2003
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Second Edition, 2003
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
BALLOTAE NIGRAE HERBA	Black Horehound	Supplement 2009
BETULAE FOLIUM	Birch Leaf	Second Edition, 2003
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Second Edition, 2003
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Supplement 2009
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Second Edition, 2003
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Second Edition, 2003
FUMARIAE HERBA	Fumitory	Supplement 2009
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Supplement 2009
GRINDELIAE HERBA	Grindelia	Supplement 2009
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Second Edition, 2003
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Second Edition, 2003
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003

LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Supplement 2009
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Second Edition, 2003
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Second Edition, 2003
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Second Edition, 2003
RATANHIAE RADIX	Rhatany Root	Supplement 2009
RHAMNI PURSHIANI CORTEX	Cascara	Second Edition, 2003
RHEI RADIX	Rhubarb	Second Edition, 2003
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Second Edition, 2003
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Second Edition, 2003
SALICIS CORTEX	Willow Bark	Second Edition, 2003
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Second Edition, 2003
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VIOLAE HERBA CUM FLORE	Wild Pansy	Supplement 2009
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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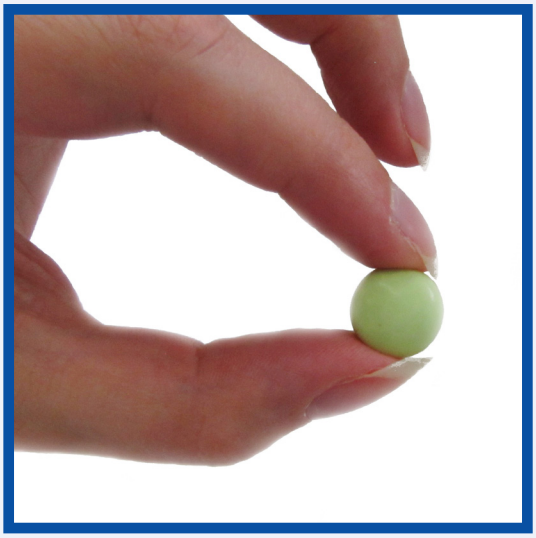
The Scientific Foundation for Herbal Medicinal Products

Millefolii herba

Yarrow

2021



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***The* Scientific Foundation for**
Herbal Medicinal Products

MILLEFOLII HERBA
Yarrow

2021

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

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Plant illustrated on the cover: *Achillea millefolium*

FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
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Verso
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Notes for the Reader
Abbreviations
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Information on the member organizations and people involved in the production of ESCOP monographs and other activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

MILLEFOLII HERBA

2021

Yarrow

DEFINITION

Yarrow consists of the whole or cut, dried flowering tops of *Achillea millefolium* L.s.l. It contains not less than 2 mL/kg of essential oil and not less than 0.02 per cent of proazulenes, expressed as chamazulene ($C_{14}H_{16}$: M_r 184.3), both calculated with reference to the dried drug.

Fresh material may also be used, provided that when dried it complies with the above definition.

Note: The above definition differs slightly from the current monograph for Yarrow/Millefolii herba in the European Pharmacopoeia [Yarrow, Ph. Eur], which defines the botanical source as *Achillea millefolium* L. (without "s.l.", i.e. sensu latiore). Since flowering tops of the hexaploid plant "*Achillea millefolium* L." do not contain proazulenes [Kubelka 1999], a requirement for a minimum content of proazulenes would be unattainable from that particular species.

Various taxa from the *A. millefolium* group are used for medicinal purposes [Jurenitsch 2013]. Extensive pharmacobotanical and phytochemical investigations have led to greater understanding of the *A. millefolium* group and shown that only a few species fulfil the quantitative requirements of the Pharmacopoeia, the most important one being the tetraploid *A. collina* [Saukel 1992a,b; Kubelka 1999; Rauchensteiner 2002].

CONSTITUENTS

The main characteristic constituents (depending on the particular species within the *A. millefolium* group) are:

- Essential oil (0.2-5.9%) containing variable amounts of the main components: α- and β-pinene, 1,8-cineole, sabinene, camphor, borneol, β-caryophyllene and germacrene D [Lawrence 1984, Héthelyi 1989, Hachey 1990, Kokkalou 1992, Figueiredo 1992, Hofmann 1992, Michler 1992, Rohloff 2000, Orth 2000, Rauchensteiner 2002, Benedek 2008, Raal 2012, Chou 2013, Stevanovic 2015, Soltani Howyzeh 2019, Farhadi 2020].

- Sesquiterpenes (appr. 0.5%) [Kastner 1995, Glasl 1999a]:

  Proazulene sesquiterpenes (up to 0.2%) of the guaianolide type [Benedek 2008], which degrade to chamazulene, e.g. 8α-tigloxy-, 8α-angeloxy- and 8α-acetoxy-artabsin, which dominate in *A. asplenifolia* [Kastner 1992, Todorova 2006], *A. roseo-alba* [Kastner 1991], *A. collina* [Schröder 1994, Trendafilova 2006], *A. ceretanica* [Glasl 1999b] and *A. asiatica* [Narantuya 1999, Glasl 2001], but are not present in *A. millefolium* or *A. pannonica* [Kubelka 1999].

  Chamazulene carboxylic acid [Stahl 1954, Imming 2001, Ramadan 2006].

  Non-azulogenic sesquiterpenes of the guaianolide type, e.g. rupicolins [Zitterl-Eglseer 1991], 3-oxa-guaianolides [Schröder 1994], achillinin-type guaianolides [Li 2011, 2012a], 1,10-secoguaianolides [Li 2012b], matricarin derivatives (e.g. achillin, leucodin) [Glasl 2002, Farooq 2012], 1,4-endoperoxides [Rücker 1992, 1994], eudesmanolides [Glasl 1999a, Zhang 2015], longipinanes [Kubelka 1999], germacranes [Kubelka 1999, Ulubelen 1990, Mustakerova 2002, Todorova 2007], achimillic acids [Tozyo 1994].

- Flavonoids (up to 0.8%), mainly flavone- and flavonol-*O*-glycosides, e.g. apigenin 7-*O*-glucoside, luteolin 7-*O*-glucoside, rutin, as well as flavone-*C*-glycosides, e.g. schaftoside and isoschaftoside; polymethoxy derivatives of 6-hydroxy-flavones and -flavonols in leaf exudates [Guédon 1993, Valant-Vetschera 1988, 1994, Kasaj 2001a,b,c, Benedek 2007a; 2008; Benetis 2008].

- Phenolic acids, mainly dicaffeoylquinic acids (DCQA; up to 1.5%) such as 3,5- DCQA (0.3 to 0.8%), 4,5-DCQA, 1,5-DCQA and 3,4-DCQA [Tunón 1994, Benedek 2007a, 2008].

Other constituents include betaines, e.g. stachydrine and betaine [Mehlführer 1997]; amino acids [Goldberg 1969, Petlevski 2002]; protein-carbohydrate complexes [Goldberg 1969]; triterpenes and sterols [Chandler 1982]; tannins [Ivancheva 2000]; thiophenes [Tosi 1991]; carotenoids (mainly lutein) [Kukui 2001]; coumarins (0.35%); polyacetylenes [Teuscher 2016]; tocopherols [Dias 2013]; lignans and terpenoids [Liu 2017]; N-alkylamides [Veryser 2017]; polysaccharides [Freysdottir 2016].

CLINICAL PARTICULARS

Therapeutic indications

Internal use
Loss of appetite; dyspeptic disorders such as mild spasmodic complaints in the abdominal region [Teuscher 2016].

Supportive treatment of spasm associated with dysmenorrhea [Jenabi 2015].

External use
Supportive treatment of small wounds; mild inflammations of the skin and mucous membranes [Jurenitsch 2013, Miranzadeh 2014; 2015, Teuscher 2016, Hajhashemi 2018]; as an insect repellent [Tunón 1994, Thorsell 1998, Jaenson 2006].
In the form of hip baths: spasms of the small pelvis (pelvic congestion) [Jurenitsch 2013, Teuscher 2016].
In these indications, the efficacy is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Internal use
Adults: Three to four times daily, 2-4 g of the drug as an infusion; other corresponding preparations [Jurenitsch 2013, Teuscher 2016].

External use
2-4 mL of tincture (1:5, 45% ethanol); 2-4 mL of fluid extract (1:1, 25% ethanol); as a hip bath, 100 g of the drug to 20 litres of water [Jurenitsch 2013].

Elderly: dose as for adults.

Children from 3 to 12 years under medical supervision only: proportion of adult dose according to body weight.

Method of administration
For oral administration and local application.

Duration of administration
No restriction.
If symptoms persist or worsen, medical advice should be sought.

Contra-indications
Known allergy to plants of the Asteraceae family.

Special warnings and special precautions for use
Several cases of yarrow-induced allergic contact dermatitis have been reported [Diepgen 1989, Stingeni 1999, Paulsen 2001].

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available.
In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
Several cases of contact allergy have been reported [Diepgen 1989, Paulsen 2001, Compes 2006].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antimicrobial activity
A 95% methanolic extract inhibited 15 different strains of *Helicobacter pylori* with MICs in the range 1.56-100 µg/mL [Mahady 2005].

A lipophilic extract (ether-hexane-methanol 1:1:1, DER approx. 11:1) exhibited bactericidal and fungicidal activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis, Aspergillus niger* and *Candida albicans* [Stojanovic 2005].

Extracts from different origin (Sardinia, Portugal) obtained by supercritical CO_2 extraction were tested for their antifungal activities against yeasts, dermatophytes and *Aspergillus* species. The extracts showed the highest activity against dermatophytes such as *Trichophyton rubrum, T. mentagrophytes, Microsporum gypseum* and *Epidermophyton floccosum*, with MIC values ranging from 0.32-1.25 µL/mL [Falconieri 2011].

In a study on cariogenic bacteria, a 50% ethanolic extract demonstrated an MIC value of 12.5 µg/mL for *Lactobacillus rhamnosus* [Kermanshah 2014].

Significant ($p<0.05$) antifungal activity of essential oil from flowers against *Aspergillus nidulans* A757//UT448 was observed at 0.25 and 0.5 µL/mL [de Sant´Anna 2009].

The growth of nine different Gram positive and Gram negative bacteria was inhibited by essential oils from two different locations with MICs between 12.6 and 112 µg/mL. The best effect was observed against *Staphylococcus epidermidis* [Mazandarani 2013].

The essential oil (5 µL/well) of *A. millefolium* subsp. *millefolium* Afan. exhibited activity against 5 different bacteria in the agar well diffusion assay with the best effect against MRSA [Sevindik 2016].

Chamazulene-free oils of yarrow (*A. millefolium* ssp. *millefolium* and *A. millefolium* ssp. *pannonica*) at a concentration of 5% V/V showed antimicrobial activity against *C. albicans, Proteus vulgaris* and *Salmonella typhimurium* [Karamenderes 2002].

Anti-inflammatory activity
A 20% methanolic extract (DER 2.8:1) as well as the flavonoid and the dicaffeoyl-quinic acid fractions thereof inhibited human neutrophil elastase with IC_{50} values of 23, 20 and 72 µg/mL respectively. The activity on matrix metalloproteinase-2 and -9 was insignificant (IC_{50} values between 600 and 900 µg/mL) [Benedek 2007b].

At 50 µg/mL, a hydroethanolic extract (not further specified) moderately inhibited COX-2 and leukotriene biosynthesis [Kopeinig 2010].

A methanolic extract (yield 21%, containing 9.7% flavonoids and 11.8% dicaffeoylquinic acids) at 100 µg/mL significantly ($p<0.05$) attenuated the effect of IL-1β on NFκB activation in human umbilical vein endothelial cells [Dall'Acqua 2011].

Dendritic cells (DCs) matured under addition of an aqueous extract (yield 3%) at 1, 10 or 50 µg/ml secreted significantly ($p<0.05$) less IL-12p40 as compared to DCs matured without extract. Addition of the extract at 50 or 100 µg/mL during maturation significantly ($p<0.05$) increased secretion of IL-10. The ratio of IL-12p40/IL-10 was reduced, while secretion of IL-6 and IL-23 remained unchanged. Co-culture of DCs matured in the presence of the extract with allogeneic CD4+ T cells decreased IL-17 secretion but did not affect IFN-γ and IL-10 secretion by the CD4+ T cells [Jonsdottir 2011].

Lactone fractions from chloroform extracts of *A. asplenifolia*, *A. collina* and *A. distans* at 500 µg/mL caused 98%, 91.6% and 94.5% inhibition, respectively, of the respiratory burst activity of human neutrophils, which was higher than the inhibition by indomethacin of 72.2% [Choudhary 2007].

In LPS-stimulated THP-1 monocytes, a polysaccharide fraction resulted in a dose-dependent significant increase of the secretion of IL-1β, IL-8, IL-10, IL-12p40, IL-23 and TNF-α (all $p<0.001$) at 100 µg/mL. A tendency to an increase was observed for IL-6 and IL-27. In unstimulated THP-1 cells the compounds had no effect on these cytokines. The LPS-induced nuclear concentration of NF-κB in THP-cells was decreased, but IκBα remained unchanged [Freysdottir 2016].

Dilutions of an essential oil from leaves at 1:50, 1:100 and 1:200 moderately stimulated H_2O_2 and TNF-α production in peritoneal macrophages ($p<0.05$) without causing an overproduction of these compounds [Lopes 2005].

In LPS-stimulated RAW 264.7 macrophages, steam-distilled essential oil significantly ($p<0.05$) decreased cellular NO concentration at 20, 40 and 80 µg/mL in a dose-dependent manner. At these doses, superoxide anion production, lipid peroxidation and GSH concentration were reduced as well (all $p<0.05$). The oil protected the macrophages against LPS-induced DNA damage. Decreased activities of SOD, CAT and glutathione peroxidase (GPx) showed that these enzymes were not involved in this effect. In contrast, the down-regulation of iNOS, COX-2, TNF-α, IL-6 and heme oxygenase-1 expression was shown to contribute to the effects [Chou 2013].

A 78% inhibition of LPS-induced NO production in RAW 264.7 macrophages was observed with essential oil at 45 µg/mL [Ladan Moghadam 2017].

Chamazulene demonstrated dose-dependent inhibition of 5-LOX-mediated leukotriene B_4 synthesis in rat peritoneal nucleophilic granulocytes (IC_{50}: 15 µM) and in a cell-free system (IC_{50}: 10 µM). Inhibition of chemical peroxidation of arachidonic acid metabolites in a cell-free peroxidation system was also demonstrated (IC_{50}: 2 µM) [Safayhi 1994].

Chamazulene carboxylic acid inhibited COX-2 by 43.5% at 50 µM, comparable to the effect of the COX-2 inhibitor nimesulide (50% at 50 µM), whereas COX-1 was only minimally inhibited (17.4% at 50 µM) [Ramadan 2006].

Gastrointestinal effects
Bile flow in the isolated perfused rat liver was dose-dependently increased by a fraction containing 48.8% of dicaffeoylquinic acids and 3.4% of luteolin 7-*O*-glucuronide at concentrations of 10-40 mg/L. The choleretic effect was two- to threefold greater than that produced by cynarin ($p<0.01$) [Benedek 2006].

The aqueous fraction from a 50% methanolic extract (yield 7.8%) was tested for contractile activity on isolated gastric antrum strips from mice and humans. The fraction caused a dose-dependent effect with an EC_{50} of 617.5 µg/mL and a maximal contractile effect of 89.2% at 30 mg/mL in the mouse model and of 581.4 µg/mL and 97.7% respectively in the human model. The contractions remained unaffected by tetrodotoxin and hexamethonium, but were abolished by atropine. Choline was shown to be responsible for the effect [Borrelli 2012].

Radical scavenging and antioxidant effects
Infusions of various *Achillea* species protected human erythrocytes and leucocytes against hydrogen peroxide-induced oxidative damage. This was shown by increased CAT, SOD and GPx activities as well as by reduced glutathione content of the cells and a decrease in lipid peroxidation [Konyalioglu 2005].

A macerate (40% ethanol) at 5.5 and 11 µg/mL significantly ($p<0.05$) suppressed H_2O_2 production in isolated rat heart mitochondria as compared to control [Trumbeckaite 2011].

In human gastric epithelial AGS cells, pre-incubation with a 70% ethanolic extract at 100 µg/mL significantly ($p<0.01$) suppressed ROS generation induced by *Helicobacter pylori* infection [Zaidi 2012].

The activity of an 80% ethanolic extract corresponded to 12 g and 11.7 g rosmarinic acid equivalents/L in the ferric reducing antioxidant power assay and in the iron (III) to iron (II) reducing assay respectively [Mekinić 2013].

Chloroform and methanol extracts from aerial parts and inflorescences resulted in IC_{50} values between 2.7 and 3.1 µg/mL in the DPPH assay and between 2.3 and 3.0 µg/mL in the ABTS assay [Bhat 2014].

Lipid peroxidation induced by sodium nitroprusside in mice brain homogenates was determined by the increase of MDA, which was significantly ($p<0.001$) inhibited in a dose-dependent manner by a methanolic and an ethanolic extract at 250, 500 and 1000 µg/mL [Barut 2017].

The EC_{50} values of DPPH scavenging were 43.4 µg/mL for an ethanolic extract and 40.3 µg/mL for a 50% ethanolic extract [García-Risco 2017].

An aqueous and an ethanolic extract inhibited DPPH radicals with IC_{50} values of 20.4 and 18.1 µg/mL respectively [Guz 2019].

Steam-distilled and non-distilled plant material from yarrow was extracted with solvents of different polarity and the resulting fractions were tested for radical scavenging activity (NBT/hypoxanthine superoxide, DPPH$^{\bullet}$ and $^{\bullet}$OH/luminol chemiluminescence methods) and antioxidant activity (β-carotene bleaching test). Most of the fractions had radical

scavenging properties, whereas antioxidant activity of all the extracts was very low or undetectable [Parejo 2002].

In human platelets, a protective effect on proteins against damage induced by peroxynitrite was observed with a purified fraction containing carbohydrates and polyphenols from an alkaline extract (yield 6.6%). The fraction inhibited the tyrosine nitration and reduced the level of carbonyl groups with IC_{50} values of 115 and 100 µg/mL respectively [Saluk-Juszczak 2010].

An ethylacetate fraction prepared from a methanolic extract exhibited radical scavenging effects with 55.1% inhibition at 40 µg/mL, 97% at 20 µg/mL and 85.5% at 100 µg/mL against DPPH, ABTS and superoxide anion radicals respectively. The reduction of lipid peroxidation was 36.6% at 10 µg/mL [Sevindik 2015].

An essential oil (*A. millefolium* subsp. *millefolium* Afan.) strongly reduced the DPPH$^{\bullet}$ radical (IC_{50}: 1.56 µg/mL) and had a hydroxyl radical scavenging effect in the Fe^{3+}-EDTA-H_2O_2 deoxyribose system (IC_{50}: 2.7 µg/mL). It also inhibited non-enzymatic lipid peroxidation of rat liver homogenate (IC_{50}: 13.5 µg/mL) [Candan 2003].

B16 melanoma cells were stimulated with melanocyte stimulating hormone (MSH). The essential oil restored the MSH-induced changes in lipid peroxidation and the levels of superoxide anion, GSH, SOD, CAT and GPx in a dose-dependent manner. The changes were significant ($p<0.05$) for all parameters at 10 and 20 µg/mL [Peng 2014].

An essential oil demonstrated an IC_{50} value of 12.5 µg/mL in the carotene bleaching assay and an EC_{50} of 13 µg/mL in the ferric reducing power assay. Lipid peroxidation was inhibited with an IC_{50} of 78.5 µg/mL [Ladan Moghadam 2017].

Spasmolytic activity
An extract (5.5:1, 70% methanol) inhibited both spontaneous and K^+-induced contractions in isolated rabbit jejunum. Incubation with the extract shifted the concentration-response curves of Ca^{++} to higher concentrations. The effects were comparable to those of verapamil [Yaeesh 2006].

Extracts of different polarity showed dose-dependent relaxant activity on carbachol-induced contractions of rat tracheal rings. The hexane extract was most active (EC_{50}=412.5 µg/mL; positive control theophylline EC_{50}=51 µg/mL) and acted as a non-competitive antagonist on muscarinic receptors. The NO/cGMP pathway contributed to the relaxation of the tracheal smooth muscle and the Ca^{2+} influx blockade into the smooth muscle cells was identified as the most important mechanism. The sesquiterpene lactones leucodin and achillin were shown to be responsible for the observed activities [Arias-Durán 2020].

A flavonoid fraction (10.2% total flavonoids) dose-dependently decreased the force of contractions in isolated guinea-pig ileum. Apigenin and luteolin were much more potent than their respective glycosides. Contractions caused by histamine were significantly ($p<0.05$) decreased by the flavonoid fraction. The spasmolytic effect was caused mainly by inhibition of calcium influx [Lemmens-Gruber 2006].

Oestrogenic effects
A methanolic extract and a fraction thereof showed oestrogenic activity in recombinant MCF-7 cells expressing oestrogen receptor (ER) α and transfected with luciferase transporter gene. The lowest effective concentrations were 8.57×10^{-5} mg/mL and 2.8×10^{-4} mg/mL respectively ($p<0.01$). In a test for α or β ER preference using SK-NBE cells, the maximum activity of the 10% methanolic extract was achieved at 6.19×10^{-5} mg/mL on ER α and 2.48 × 10–4 mg/mL on ER β [Innocenti 2007].

A significant ($p<0.05$) increase in the proliferation of vascular smooth muscle cells was observed with 20 µg/mL of a methanolic extract (yield 21%, containing 9.7% flavonoids and 11.8% dicaffeoylquinic acids). The effect was abolished by the ER antagonist ICI 182.780 [Dall'Acqua 2011].

In a human ER α yeast assay, an essential oil showed antioestrogenic activity and at 0.1 µl/mL reduced the oestradiol-mediated activity by 77.2% [Contini 2020].

Antiproliferative and cytotoxic effects
At 50 µg/mL, chloroform and methanolic extracts from aerial parts and inflorescences showed cytotoxicity against cancer cell lines: THP leukaemia cells were inhibited by 58-99%, PC-3 prostate cancer cells by 20-77%, MCF-7 breast cancer cells by 46-98% and OVCAR-5 ovary cancer cells by 19-99%. The chloroform extracts were more active than the respective methanol extracts [Bhat 2014].

Antiproliferative effects on MIA PaCa-2 human pancreatic cancer cells were shown for an extract obtained by supercritical fluid extraction (SFE; IC_{50}: 31.5 µg/mL) and an ethanolic extract (IC_{50}: 65.0 µg/mL) [García-Risco 2017].

An extract (80% ethanol, not further specified) inhibited the growth of the human cancer lines NCI-H460 (non-small cell lung cancer) and HCT-15 (human colorectal adenocarcinoma) with GI50 values of 187.3 and 70.8 µg/mL respectively. At 100 µg/mL, the extract caused significant ($p<0.05$) reductions in cell viability of 55% and 82% respectively. The extract led to alterations in the cell cycle and increased apoptosis levels in both cell lines. The p53 and p21 expression in NCI-H460 cells (which have wild type p53) increased, while XIAP levels in HCT-15 cells (with mutant p53) were reduced [Pereira 2018].

In a study comparing different extraction methods, a supercritical fluid extract from leaves was most active on MIA PaCa-2 cell viability with an IC_{50} of 31.4 µg/mL and an LC_{50} of 70.6 µg/mL. A significant ($p<0.001$) induction of late apoptosis was observed at 70 and 100 µg/mL of the extract. In a three-dimensional model, the extract at 50 and 70 µg/mL significantly reduced size ($p<0.0001$) and numbers ($p<0.001$) of cancer spheroids. Pretreatment with the extract at 25 µg/mL potentiated the effect of various concentrations of 5-flourouracil ($p<0.05$ to 0.001). The activity against PANC-1 human pancreatic cancer cells was less pronounced [Mouhid 2018].
An additional study showed that this extract significantly ($p<0.05$) downregulated Sterol Regulatory Element Binding Transcription Factor (SREBF1) at 70 µg/mL in MIA PaCa-2 cells. The expression levels of two downstream targets of SREBF1, namely fatty acid synthase and stearoyl-CoA desaturase were significantly inhibited in MIA PaCa-2 and PANC-1 cells ($p<0.001$ to 0.0001) [Mouhid 2019].

The water-soluble fraction from a hydroalcoholic extract had a cytotoxic effect on B16 mouse melanoma cells, inhibiting cell proliferation at 0.05-0.1 mg/mL [Trouillas 2003].

A chloroform fraction from a methanolic extract at 10 µg/mL exerted an 88.9%, 53.9% and 21.4% inhibition of proliferation of HeLa, MCF-7 and A-431 carcinoma cell lines respectively. In an activity-guided approach, centaureidin was isolated as the most active component with IC_{50} values of 0.082 µM for HeLa, 0.125 µM for MCF-7 and 0.354 for A-431 cells [Csupor-Löffler 2009].

Cholinesterase inhibiting effects
A hexane macerate (yield 1.96%) from *A. millefolium* L. subsp. *pannonica* inhibited acetylcholinesterase and butyrylcholinesterase with IC_{50} values of 27.2 and 6.4 µg/mL respectively. An ethanolic macerate (yield 3.8%) showed only butyrylcholinesterase inhibition (IC_{50} 49.6 µg/mL) [Kurt 2018].

An n-butanol fraction from a methanolic extract demonstrated inhibitory activity against acetylcholinesterase (51.3% at 25 µg/mL). The inhibition of butyrylcholinesterase was less pronounced (23.5%) [Sevindik 2015].

Other effects
A 5% m/V hot water infusion significantly ($p<0.001$) shortened recalcification time (a test of blood coagulation) in human plasma to 43% of that of 0.9% sodium chloride. The flowering herb had the highest haemostyptic activity, whereas pressed juice significantly ($p<0.05$ to $p<0.001$) prolonged blood coagulation [Sellerberg 2000].

An ethanolic extract moderately inhibited the human cytochrome P450 enzymes CYP3A4, CYP19 and CYP2C19 by 25%, 43% and 63% respectively [Scott 2006].

An extract (70% ethanol, yield 18%) caused a concentration-dependent inhibition of atrial force and rate in spontaneously beating guinea-pig atrial tissues (EC_{50} 1.4 and 1.5 mg/mL respectively). In isolated rabbit aortic rings, the extract relaxed contractions induced by phenylephrine and K^+ with EC_{50} values of 2.8 and 3.1 mg/mL respectively. In guinea-pig tracheal strips, carbachol- and K^+-induced contractions were inhibited (EC_{50} 2.7 and 2.6 mg/mL respectively) [Khan 2011].

Punch biopsies from full-thickness abdominal skin from a 60-year-old female donor were treated with a water extract (not further specified) for 72 hours. Changes in the expression pattern of three epidermal differentiation markers were visualized by immunostaining. The markers were significantly improved by addition of 0.5% extract to the cultivation medium as shown by the staining intensity: filaggrin from 56.9 to 62.7 ($p<0.05$), cytokeratin 10 from 34.7 to 70.8 ($p<0.001$), transglutaminase-1 from 35.6 to 22.6 ($p<0.001$). A significant difference ($p<0.05$) was also measured in the average depth of staining between control (68.5 µm) and biopsies treated with the extract (52 µm), which was closer to the distribution usually observed in young skin biopsies. As compared to control, a significantly thicker epidermis was observed in treated biopsies (97.9 vs. 86 µm; $p<0.05$) [Pain 2011].

A methanolic extract (not further specified) showed anti-plasmodial activity against the chloroquine-resistant *Plasmodium falciparum* strain W2 with an IC_{50} of 44.6 µg/mL [Vitalini 2011].

In isolated rat heart mitochondria, a macerate (40% ethanol) induced a dose-dependent reduction in State 3 respiration rate without any changes in the integrity of the inner mitochondrial membrane. Under use of pyruvate+malate as substrate this decrease was significant ($p<0.05$) at the highest dose of 33 µg/mL. With succinate as substrate, significant ($p<0.05$) reduction was observed at 11, 22 and 33 µg/mL of the extract. State 2 and State 4 respiration rate remained unchanged upon treatment with the extract [Trumbeckaite 2011].

Pretreatment of 3T3-L1 adipocytes with a 70% ethanolic extract at 100 µg/mL significantly ($p<0.05$) increased PPARγ mRNA relative expression fivefold compared with control. The increase was also approximately fivefold compared to the expression induced by pioglitazone as positive control. The effect correlated with overexpression of GLUT4 (about two-fold; $p<0.05$ with respect to control), resembling the one of pioglitazone. In RINm5F cells, the extract (200 µg/mL) significantly increased insulin secretion and promoted intracellular $[Ca^{2+}]$ rise ($p<0.05$ as compared to control), both to a similar extent as glibenclamide (400 µM) [Chávez-Silva 2018].

Matrix metalloproteinases (MMP)-1, -8 and -13 were inhibited by a methanolic extract at 2, 5, 10, 25 and 50 µg/mL. The inhibition was 67.2 to 81.2% for MMP-1, 72.0-73.5% for MMP-8 and 77.9 to 89.2% for MMP-13 [Gonulalan 2019].

A methanolic extract at 100 µg/mL resulted in a significant ($p<0.0001$) activation of the gene expression mediated by the electrophile response element [Papadi 2019].

MSH-induced melanin production and cellular tyrosinase activity in B16 melanoma cells were significantly ($p<0.05$) reduced by steam-distilled essential oil at 5, 10 and 20 µg/mL. The decrease in melanin production by the oil was regulated by restoration of MHS-induced changes of protein levels of several kinases of the MAPK family [Peng 2014].

In vivo experiments

Anti-inflammatory effects
A dry 80% ethanolic extract, administered orally at 100 mg/kg, inhibited oedema in the carrageenan-induced rat paw oedema test by 29% ($p<0.05$) compared to 45% by indomethacin at 5 mg/kg ($p<0.01$) [Mascolo 1987].

Infusions of *A. aspleniifolia* (diploid, rich in proazulenes) and *A. pratensis* (tetraploid, free from proazulenes) were extracted with dichloromethane and ethyl acetate to yield dry fractions of low (terpenoids), intermediate (flavonoids) and high polarity. When applied topically in the croton oil ear test in mice, the lipophilic fractions and the dry aqueous infusion from *A. asplenifolia* showed higher activity than those from *A. pratensis* [Della Loggia 1992].

In similar experiments with infusions and fractions from *A. asplenifolia*, *A. collina*, *A. x roseoalba*, *A. setacea* and *A. pratensis*, lipophilic fractions of the proazulene-containing taxa showed higher anti-oedematous activity (≥ 50% reduction at concentrations of 270-485 µg/ear) than the proazulene-free taxa [Kastner 1993a]. Several main sesquiterpene lactones of these taxa produced inhibitory effects comparable to that of matricin [Kastner 1993b; Rohloff 2000].

After removal of chloroform-soluble components from yarrow, the water-soluble components were extracted and fractionated for evaluation in the yeast-induced mouse paw oedema test. At 40 mg/kg b.w. various fractions reduced inflammation by 10-35%, compared to 44% and 26% respectively, for the same doses of indomethacin and phenylbutazone [Goldberg 1969].

In another mouse paw oedema test, oral administration of dichloromethane-soluble fractions from an infusion (250 mg/kg) and a decoction (500 mg/kg) (*A. collina*) inhibited inflammation by 21% and 40% respectively [Pavlescu 1995].

A germacrane sesquiterpene from *A. pannonica* dose-dependently inhibited croton oil-induced ear oedema in mice (ID_{50}: 0.40 µmol/cm^2). At a concentration of 0.75 µmol/cm^2 it inhibited the oedematous response to an extent (61%) greater than that of an equimolar dose of indomethacin (43%). Its effect on granulocyte recruitment at the site of inflammation (61% inhibition) was also greater than that of equimolar indomethacin (51%) [Sosa 2001].

In the mouse ear inflammation test, topical application of chamazulene carboxylic acid at 1 µM per ear resulted in 38% inhibition of oedema after 6 hours ($p<0.05$). After oral administration at 300 mg/kg b.w. only 17% inhibition was observed in the carrageenan-induced rat paw oedema test [Ramadan 2006].

Spasmolytic effects
Spasmolytic activity of an ethanolic extract from *A. collina* was demonstrated in mice after oral administration at 500 mg/kg b.w.; it delayed intestinal transit by 43% [Pavlescu 1995].

Gastroprotective effects
Seven days after induction of chronic gastric lesions in rats by acetic acid, a hot water extract (approx. 3:1) was administered orally at 100 or 300 mg/kg/day for 7 days. Compared to controls a significant and dose-dependent healing effect was observed ($p<0.05$, ED_{50}: 32.4 mg/kg). When the same treatment started 1 day after injection of acetic acid, it did not prevent the formation of gastric ulcers. Oral pre-treatment with the extract one hour before induction of acute gastric lesions by ethanol had a dose-dependent protective effect ($p<0.05$, ED_{50}: 936 mg/kg). Gastric lesions induced by indomethacin one hour after subcutaneous administration of the extract were significantly reduced ($p<0.05$) only by 2000 mg/kg [Cavalcanti 2006].

A 90% ethanolic macerate (yield 17.4%) was administered orally to female Wistar rats at 30, 100 and 300 mg/kg one hour before induction of acute gastric lesions with ethanol. The extract at all doses significantly ($p<0.001$) reduced the lesion area by 35%, 56% and 81% respectively. The treatment restored decreased GSH levels (300 mg/kg; $p<0.05$) and SOD activity (all doses; $p<0.05$) in the gastric mucosa. Oral treatment with 1 and 10 mg/kg twice daily for 7 days significantly reduced the volume of gastric ulcers induced by acetic acid by 43% ($p<0.05$) and 65% ($p<0.001$) respectively. In histological analyses, regeneration of the gastric mucosa was observed. GSH levels were restored to baseline ($p<0.001$) at 10 mg/kg, and SOD activity was improved ($p<0.05$ at both doses). Increased myeloperoxidase activity was also decreased by 68% at 10 mg/kg [Potrich 2010].

An aqueous fraction from a 50% methanolic extract (yield 7.8%) dose-dependently stimulated gastric emptying in male Swiss mice at 10 mg/kg i.p. ($p<0.01$ vs. control) and 30 mg/kg i.p. ($p<0.001$) with an EC_{50} value of 6.2 mg/kg. Delayed gastric emptying in cisplatin-treated mice was significantly improved at all doses (1, 3, 10 and 30 mg/kg i.p.; $p<0.01$ to $p<0.001$ compared to cisplatin; EC_{50} 1.6 mg/kg) [Borrelli 2012].

Antidiabetic effects
Streptozotocin-induced diabetic Wistar rats received an extract (80% ethanol, not further specified; 100 mg/kg/d i.p.) for 14 days. The extract improved the decreased body weight and serum insulin as well as increased blood glucose levels in diabetic animals. Serum insulin levels were significantly ($p<0.05$) higher at days 10 and 14 and reached normal values. Blood glucose levels were significantly ($p<0.05$) reduced at days 10 and 14. A significant ($p<0.05$) increase in body weight was observed at the end of the study (day 14). Elevated iNOS and IL-1β expression in pancreatic tissues of diabetic rats were significantly ($p<0.05$) reduced by the extract. No differences between normal control rats and normal rats receiving the extracts were observed in any of the parameters at all time points (days 3, 7, 10 and 14) [Zolghadri 2014].

Normoglycaemic CD1 mice were treated orally with 100 µg/kg b.w. of an extract (70% ethanol; yield 18.6%) 30 min before receiving 2 g/kg glucose in an oral glucose tolerance test. The extract significantly ($p<0.05$) decreased the percentage variation of glycaemia at 0.5 and 1 h as compared to control. In a similar sucrose tolerance test, compared to control the reduction was significant ($p<0.05$) over three hours and slightly better than the positive control acarbose. In mice with streptozotocin (STZ)-induced Type-2 diabetes, the extract at 33, 100 and 330 mg/kg significantly decreased the glucose levels starting at 30 min. This reduction sustained for 7 h ($p<0.05$ for all doses at all time points compared to control) and was similar to glibenclamide (3 mg/kg) as positive control [Chávez-Silva 2018].

An aqueous dry extract (1:10) was administered orally at doses of 10, 30, 90 and 270 mg/kg b.w. to STZ-induced diabetic Balb/c mice for 20 days. Increased fasting blood glucose levels after STZ were significantly ($p<0.05$) reduced at days 7, 13 and 20, with the two highest doses being more effective than glibenclamide. The same effects were observed for creatinine and urea levels, where the two highest concentrations restored the levels to normal control. Decreased red blood cell parameters were significantly ($p<0.05$) improved by all treatments. Increased platelets and white blood cell counts in STZ mice were significantly ($p<0.05$) reduced by all extract doses. The improvements in red and white blood cell counts reached normal values with the two highest doses. Histopathological signs of renal hypertrophy were ameliorated by the extract [Zangeneh 2018].

Streptozotocin-induced diabetic rats were treated with an extract (70% ethanol, not further specified; 25 and 100 mg/kg b.w./day p.o.) for 28 days. The extract at both doses alleviated the decreased body weight gain of diabetic rats. Significant dose-dependent improvements of the increased levels of fasting blood glucose, AST, ALT, LDL-, total cholesterol and triglycerides were observed ($p<0.001$ to $p<0.013$). The reduced HDL levels in diabetic rats were elevated significantly at both doses ($p<0.05$ and $p<0.006$, respectively). All effects of the higher dose were comparable to 250 mg/kg/day metformin as positive control [Rezaei 2020].

Hepatoprotective effects
The anti-hepatotoxic activity of dry extracts of varying polarity was evaluated in rats treated with carbon tetrachloride or paracetamol. Liver function was assessed by the levels of ALT and AST. Intraperitoneal administration of the extracts at 50 mg/kg reduced ALT/AST levels by 50-96% in carbon tetrachloride-treated animals and 41-91% in paracetamol-treated animals ($p<0.05$) [Graf 1995].

An extract (5.5:1, 70% methanol) at 150, 300 and 600 mg/kg b.w. i.p. exerted a protective effect against D-galactosamine + LPS-induced hepatitis in mice, significantly ($p<0.05$) and dose-dependently reducing plasma ALT and AST levels in treated animals compared to controls. Assessment of liver histopathology revealed an absence of congestion and focal necrosis in treated animals, with a dose-dependent improvement in cellular swelling and the number of apoptotic cells. Pre-treatment with the extract reduced mortality from 100% to 40% [Yaeesh 2006].

Effects on blood pressure
Intravenous administration of an extract (70% ethanol, yield 18%) to normotensive rats under anaesthesia at 1, 3, 10, 30 and 100 mg/kg b.w. caused a dose-dependent decrease of mean arterial blood pressure (MAP) between 8.3 and 45.0% [Khan 2011].

A 90% ethanolic extract (yield 17.39%) was administered orally to male Wistar rats at various time points before the measurement of MAP. At 3 hours after administration of 100 and 300 mg/kg, significant reductions (13 and 14 mm Hg respectively; $p<0.01$) in MAP were observed. The dichloromethane fraction from this extract at 20 mg/kg led to a reduction of 10 mm Hg at the same

time point. Artemetin, as a major compound in this fraction, dose-dependently decreased MAP by up to 11.5 mm Hg at 1.5 mg/kg i.v. At 0.75 mg/kg i.v. it significantly ($p<0.01$) reduced the hypertensive response to angiotensin I whereas it increased the average duration of bradykinin-induced hypotension. The compound (1.5 mg/kg p.o.) decreased plasma angiotensin converting enzyme (ACE) by about 37% and vascular ACE by up to 63% as compared to control [de Souza 2011].

Other effects

Addition of 0.5%, 1% and 1.5% yarrow to the diet of broilers resulted in significantly better feed conversion rates ($p<0.01$ compared to control). No significant differences were observed in the final body weight. The gastrointestinal tract weight, relative to body weight, significantly ($p<0.01$) increased [Norouzi 2015].

No changes were observed in relative uterus weights of immature female rats after oral administration of an aqueous extract (1:2.5) at 0.6 and 1.2 g/kg daily for 3 days in comparison with vehicle-treated animals. In oestrogen-treated rats the extract (1.2 g/kg/day p.o.) was unable to block the uterotrophic action [Dalsenter 2004].

A decoction (corresponding to 6 to 12 mg drug/kg i.p.) was administered to female Wistar rats during late proestrus or diestrus. Doses of 8, 10 or 12 mg/kg reduced conflict behaviour during late proestrus ($p<0.05$ at all doses). During diestrus, 12 mg/kg showed a significant reduction of conflict behaviour ($p<0.05$) [Molina-Hernandez 2004].

Cutaneous lesions were induced in female Balb/c mice by intradermal injection of *Leishmania major* promastigotes. Topical treatment with an extract (80% ethanol, not further specified) twice daily for 6 weeks started 35 days after infection. A control group received meglumine antimoniate (0.02 mL/g i.p. once daily for 20 days). The mean ulcer size at the end of the study was significantly ($p<0.006$) reduced by 43.3% with the extract when compared to the control group that exhibited a 22.6% increase in ulcer size [Nilforoushzadeh 2008].

A hydroalcoholic extract (not further specified) was evaluated for antinociceptive effects in male Swiss mice. In animals treated orally with 500 and 1000 mg/kg b.w. significant ($p<0.001$) inhibition of abdominal contortions, by 65% and 23% respectively, was observed in the writhing test. No activity of the extract was seen in the hot plate test or in immediate or late responses in the formalin test. Motor activity, motor coordination and intestinal transit of charcoal remained unchanged [Pires 2009].

An extract (90% ethanol, yield 17.4%) was tested for anxiolytic effects in male albino Swiss mice. In the elevated plus-maze test, the extract at single oral doses of 300 or 600 mg/kg resulted in significantly longer time spent in the open arms and a higher number of entries into the open arms ($p<0.05$ to $p<0.01$ as compared to vehicle). The effects were slightly stronger than those of 0.75 mg/kg diazepam. Doses of 30 and 100 mg/kg remained without effect. After treatment for 25 days similar effects were observed ($p<0.01$ to $p<0.001$). In the marble-burying test acute ($p<0.05$) and chronic treatment ($p<0.01$ and $p<0.001$) at all doses reduced the number of marbles buried compared to the vehicle group. Neither treatment regimen demonstrated any effect in locomotor activity in the elevated maze assay. The extract did not induce changes in [3H]-flunitrazepam binding [Baretta 2012].

Male NMARI mice received an aqueous extract (not further specified) at doses of 50, 250, 500 or 1000 mg/kg i.p. daily for 20 days. The treatment did not affect activity levels in the novel object recognition test. The extract did not decrease body weight or cause death [Ayoobi 2013].

Wistar rats received an infusion (35 g fresh leaves/L) at 1 mL/100 g via gavage 2 hours before, simultaneously or 2 hours after i.p. administration of cyclophosphamide. Chromosome aberrations induced by cyclophosphamide were reduced by 68.1% by pretreatment with the extract, by 68.5% under simultaneous administration and by 67.1% with post-treatment [Düsman 2013].

The diuretic activity of extracts and fractions and the underlying mechanisms of activity were evaluated in male Wistar rats. An aqueous extract (AEAM) at 125, 250 and 500 mg/kg, a 90% ethanolic extract (HEAM, yield 17.4%) at 30, 100 and 300 mg/kg as well as a dichloromethane subfraction (DCM) at 10 and 30 mg/kg were administered orally, hydrochlorothiazide (HCTZ; 10 mg/kg) served as a positive control. Conductivity, pH and density of the urine remained unchanged with all treatments. AEAM did not affect urinary volume or electrolyte excretion. Similar to HCTZ, increased diuresis was observed with HEAM at 300 mg/kg from 4 to 8 hours (between 30 and 60%; $p<0.05$ to 0.001) and with DCM at 30 mg/kg at 8 hours ($p<0.01$) as compared to control (vehicle only). Both treatments increased the excretion of Na^+ and K^+. The diuretic effect of DCM was abolished by the bradykinin B2 receptor antagonist HOE-140 and by indomethacin, a COX inhibitor ($p<0.001$) [de Souza 2013].

In male Wistar rats, urolithiasis was induced by ethylene glycol (EG) for 30 days. An 80% ethanolic extract (not further specified) was administered orally from day 15 until day 30 at 200 mg/kg (group C) and 400 mg/kg (group D). Group E was treated with the extract at 200 mg/kg from day 1 to day 30. Group A received tap water and group B EG only. At day 30, the increased urinary oxalate concentration found in group B was significantly reduced in groups C and D ($p<0.04$) and in group E ($p<0.02$ compared to groups C and D). The improvement of the decreased urinary citrate concentration after EG was significant in groups D and E ($p<0.03$ and 0.015 respectively, compared to group B). The mean number of calcium oxalate deposits was significantly higher in all EG-treated groups ($p<0.05$ as compared to group A) and was above 40 in group B only. Treatment with the extract resulted in a significant reduction to 20.1 ($p<0.001$, group C), 16.4 ($p<0.01$, group D) and 14.3 ($p<0.004$, group E) as compared to group B [Bafrani 2014].

An aqueous extract (not further specified) was administered to male C57BL/6 mice 24 hours after induction of experimental autoimmune encephalomyelitis (EAE) at 40, 200 and 400 mg/kg daily for 21 days. Behavioural disabilities were determined by a score system for different stages of paralysis and started in the EAE group on day 13.2 with the maximum score of 3.4 on day 17 after induction. In the groups receiving the low or the high dose of the extract, first disabilities were seen on day 18 and day 17, respectively, with significantly lower scores compared to the EAE group ($p<0.014$ and $p<0.021$). With the medium dose, no disabilities were observed until day 21. The body weight was significantly decreased in EAE on day 21 as compared to day 1 ($p<0.003$), but unchanged in any other group. In the brain, the extract caused decreases of demyelinated lesions at all doses and of inflammatory cells with the two higher doses ($p<0.013$ and 0.01). Decreased serum levels of the cytokine TGF-β in EAE were improved with the extract, significant ($p<0.026$) at the highest dose [Vazirinejad 2014].

The mean daily energy intake of male Wistar rats was recorded over ten days. The animals then received a 70% methanolic extract at 50 mg/kg (group 1), 100 mg/kg (group 2) and 150 mg/kg (group 3) daily for 7 days by gavage. In groups 1 and

2 the mean energy intake significantly ($p<0.001$) increased as compared to that during the observation period. In contrast, in group 3 the intake decreased significantly ($p<0.001$). The total energy intake during 24 hrs after extract administration at doses of 50 and 100 mg/kg also displayed a significant ($p<0.05$) increase compared to control. In a second experiment, no significant changes of plasma ghrelin levels were measured at 0, 0.5, 1, 2 and 4 hours after administration of 100 mg/kg extract [Nematy 2017].

Male Wistar rats were treated for 48 days with nicotine alone (0.2 and 0.4 mg/kg/day i.p.) or combined with oral administration of an extract (70% methanol, not further specified, 120 mg/kg/day). The decreased sperm count and motility as well as the percentage of immature sperm following nicotine administration were improved by the extract. The percentage of dead sperm after high dose nicotine (HNic) was diminished significantly ($p<0.05$). In the serum, the extract reduced the adverse effects of HNic on FSH, LH and testosterone concentrations and on SOD levels. LDH levels were significantly ($p<0.05$) decreased by the extract as compared to both nicotine groups and control. Total NO and MDA in testes tissues were improved with the extract. The tubule differentiation index as well as oedema and atrophy in seminiferous tubules after nicotine were almost restored to control, and other histological parameters were improved by the extract [Hallaj Salahipour 2017].

In a very similar experiment with widely consistent results, the extract also significantly ($p<0.05$) reduced the increased epididymal sperm apoptosis after nicotine. In this study the extract alone was administered as well and did not cause changes in any of the measured parameters as compared to control [Hasanzadeh Khosh 2017].

In a xenocraft model, male athymic Nude-Foxn1nu mice were subcutaneously injected with MIA PaCa-2 cells. After 15 days at an average tumour volume of 100 mm^3, intragastric treatment with a supercritical fluid extract (1 g/kg 3 times per week for 15 days) started. A significant reduction in tumour growth compared to control was observed from day 10 ($p<0.05$ to $p<0.001$). At the end of the study, tumour weight was significantly ($p<0.05$) lower and tumour volume decreased by 28.7%. No differences were seen in body or liver weights. The number of Ki-67 positive cells and the expression levels of SREBF1 in the tumours of mice treated with the extract decreased compared to control, indicating a reduction in proliferation of tumour cells [Mouhid 2019].

Fractions from an ethanolic extract and several constituents of the extract showed repelling properties against the mosquito *Aedes aegypti*. A solution or suspension of each test substance in 70% ethanol was applied to 42 cm2 of the test person's skin. All fractions of the extract as well as stachydrine and homostachydrine, caffeic, chlorogenic and salicylic acids, pyrocatechol and other compounds had distance- and contact-repelling effects comparable to that of *N,N*-diethyl-*m*-toluamide (DEET). Mixtures of effective single substances showed additive effects [Tunón 1994, Thorsell 1998].

Pharmacological studies in humans

A randomized, double-blind, placebo-controlled trial was performed in women (n=20; 47 to 68 years) with skin phototype I to III. After browning of the skin with dihydroxy-acetone on four areas on the forearm, treatment started twice daily for seven consecutive days with I) a water-in-oil preparation (pH 5.5; placebo), II) placebo with addition of 2% extract (pH 5.5; not further specified), III) placebo with addition of 3% glycolic acid (pH 3.5; positive control), IV) no treatment (negative control). The mean epidermal turnover time was 19.5 ± 14.4 days for the negative control, 16.4 ± 13.9 days for the placebo, 12.9 ± 6.1 days for the extract and 12.3 ± 4.3 days for glycolic acid.

In a second randomized, double-blind, placebo-controlled, split-face trial with the same preparations, 63 women aged 48–67 with crow's feet grade 3 to 6 were enrolled for a treatment twice daily for 56 days. Group 1 applied the preparation with 2% extract to one half of the face and the preparation with 3% glycolic acid to the other side. Group 2 used the preparation with 2% extract and placebo on the two halves of the face respectively. In group 1, the wrinkle grade with the extract was significantly reduced by 14% and with glycolic acid by 12% as compared to baseline (both $p<0.001$). In group 2, wrinkle grade decreased with the extract compared to baseline and to placebo ($p<0.01$ and 0.05 respectively). A reduction in pore appearance (21% with the extract, 19% with glycolic acid) was observed in group 1 (both $p<0.001$, compared to baseline). In group 2 the same reduction was seen, also significant compared to placebo ($p<0.05$). Skin softness was significantly improved in group 1 by 31% with the extract and by 28% with glycolic acid (both $p<0.001$) and in group 2 by 24% compared with baseline ($p<0.001$) and by 19% compared with placebo [Pain 2011].

Four different extracts (DER 1:5) were prepared: I) by maceration of yarrow with 96% ethanol followed by addition of olive oil or sunflower oil under removal of ethanol (E1 and E2) or II) by maceration with olive oil or sunflower oil (E3 and E4). In a double-blind study in 23 volunteers, skin irritation was induced by treatment of the forearm with 8% sodium lauryl sulphate. The participants applied the preparations twice daily for 7 days on specified areas of the irritated skin. The decrease in skin hydration after irritation was improved to basal values by E3 and E4 after 3 days, and with E1 and E2 it took 7 days. The lauryl sulphate-induced increase in skin surface pH was restored to basal values by all preparations after 3 days and after 7 days. A significant ($p<0.05$) improvement compared to untreated irritated skin was observed with E3 and E4. The erythema index (EI) as a measure of skin surface inflammation significantly declined with E1 and E2 after 3 days ($p<0.05$ as compared to untreated, irritated skin). E3 and E4 improved the EI after 7 days [Tadić 2017].

In a field experiment, an extract (ethylacetate, DER 1:4) showed a significant repellent effect ($p<0.03$) against various mosquitos [Jaenson 2006].

Clinical studies

Thirty-one patients with chronic kidney disease were included in a randomized, placebo-controlled trial and received either 1.5 g of powdered yarrow flowers 3 days a week for 2 months (n=16) or placebo (n=15). At the end of the study, plasma levels of nitrite and nitrate were decreased in the verum group from 0.82 ± 0.51 µmol/L to 0.63 ± 0.42 µmol/L and 50.55 ± 17.92 µmol/L to 44.09 ± 17.49 µmol/L respectively, but increased in the placebo group [Vahid 2012].

In a randomized, double-blind, controlled study, cancer patients with chemotherapy-induced oral mucositis (OM; n=56) gargled four times daily for 14 days with either 15 mL of a solution (1.4 g lidocaine, 224 mg dexamethasone, 35g sucralfate/L diphenhydramine solution; control) or a 50/50 mixture of this solution combined with a distillate (for 20 L distillate, 10 kg of yarrow flowers were boiled with 50 L water). The severity of OM was assessed three times before and at days 7 and 14 after the intervention. The mean severity score of OM was 2.39 ± 0.875 in both groups at the start of the study. In the yarrow group, severity was significantly ($p<0.001$) decreased to 1.07 ± 0.85 and 0.32 ± 0.54 at days 7 and 14 respectively. In contrast, the severity significantly ($p<0.001$) increased to 2.75 ± 0.87 and 2.89 ± 0.956 respectively in the control group [Miranzadeh 2014; 2015].

A randomized, double-blind, placebo-controlled study assessed the effectiveness of a tea (4 g/300 mL water three times daily) on the relief of primary dysmenorrhea in women aged 19 to 23 years. The participants took verum (n=45) or placebo (n=46; teabags contained starch) for 3 days from the first menstrual day to the third menstrual day, for 2 cycles, and rated the severity of their menstrual pain using a VAS. The mean change in pain score was significantly greater after verum as compared to placebo after 1 month ($p<0.001$) and after 2 months ($p<0.0001$) [Jenabi 2015].

The efficacy of an ointment containing 5% of a dried extract (90% ethanol) on episiotomy wound healing was studied in primiparous women (n=35) in a randomized, double-blind, controlled trial. Control groups received either no treatment (n=35) or a placebo ointment (n=35). The ointments were applied to the episiotomy wound twice a day for 10 days from day 2 after delivery. The healing process (redness, ecchymosis, oedema, discharge and wound dehiscence) as well as the pain level (assessed by a VAS) were investigated on days 7, 10 and 14 after delivery. A significant improvement in pain level was observed on day 7 when treatment was compared with no intervention ($p<0.05$) and on day 10 when compared to placebo and no intervention ($p<0.05$ and $p<0.01$ respectively). Significant ($p<0.05$) differences between the groups were seen in reduction of redness at days 7, 10 and 14, and of ecchymosis at days 7 and 10. Oedema was significantly ($p<0.05$) reduced on days 7 and 10 as compared to no intervention [Hajhashemi 2018].

In a triple-blind, randomized, placebo-controlled study, 75 patients with multiple sclerosis received either a dried aqueous extract (250 mg corresponding to 2 g drug or 500 mg corresponding to 4 g per day) or placebo for one year as add-on therapy. As compared with placebo, the annualized mean relapse rate was significantly lower ($p=0.003$ for 250 mg and $p=0.013$ for 500 mg), the time to a first relapse significantly longer ($p=0.013$ for 250 mg and $p=0.039$ for 500 mg), and the paced auditory serial addition task was significantly increased ($p=0.025$ and $p=0.009$ for 250 and 500 mg respectively). In the group receiving the higher dose, the changes from baseline to 12 months of the expanded disability status scale, of the multiple sclerosis functional composite and of the volume of T2 lesions were significantly better ($p=0.001$, 0.003 and 0.004 respectively) than placebo. The mean score of Word-pair learning test decreased ($p=0.037$) after 1 year compared to placebo. A significant decrease in depression was observed ($p=0.033$ at month 6 and $p=0.046$ at month 12) after the lower dose [Ayoobi 2019].

Pharmacokinetic properties

Proazulenic sequiterpene lactones, such as matricin and others, have been shown to be precursors of chamazulene carboxylic acid. When matricin was taken by 3 healthy volunteers as a single oral dose of 500 mg in aqueous suspension, plasma concentrations of chamazulene carboxylic acid, determined 75-90 minutes after ingestion, ranged from 0.9 to 2.2 µg/mL [Ramadan 2006].

Preclinical safety data

Single dose toxicity

Yarrow dry extracts of varying polarity (chloroform, methanol or water) were non-toxic in mice; the intraperitoneal LD_{50} was determined as 1.5 g/kg b.w. [Graf 1995].

According to a safety assessment for its use in cosmetics, the oral and subcutaneous LD_{50} values in mice of a yarrow extract (2% yarrow in propylene glycol and water) were both determined as 1 g/kg b.w. Alcoholic extracts of dried leaves and stalks of yarrow did not produce a phototoxic response [Anonymus 2001].

Single doses of a hot water extract (approx. 3:1) at up to 10 g/kg orally or 3 g/kg b.w. i.p. did not cause deaths, changes in behaviour or manifestations of toxic symptoms in Wistar rats over the observation period of 14 days [Cavalcanti 2006].

After i.p. treatment of mice with an extract (5.5:1, methanol 70%) at 3 g/kg b.w. no changes in behaviour were apparent during a 6-hour observation period and there was no mortality after 24 hours [Yaeesh 2006].

No signs of toxicity were observed in male Swiss mice following administration of a hydroalcoholic extract (not further specified) at i.p. doses of 1 and 10 mg/kg and oral doses of 10, 100, 500 and 1000 mg/kg. After an i.p. dose of 100 mg/kg, abdominal contortion without any other signs occurred [Pires 2009].

Strong sensitizing effects in guinea pigs were found to be caused by the sesquiterpene alpha-peroxyachifolide due to the presence of an alpha-methylene-lactone group [Hausen 1991, Rücker 1991].

Repeated dose toxicity

Oral treatment of rats with a hot water extract (approx. 3:1) at 0.3, 0.6 or 1.2 g/kg/day for 28 and 90 days did not result in differences in mobility, reflexes, muscular tonus, breathing patterns or gain in body weight or organ weight compared to control animals, with the exception of a decrease in liver weight in female animals at 0.3 g/kg for 90 days and in male animals at 0.6 g/kg for 28 days and 1.2 g/kg for 28 and 90 days. Histopathological evaluation did not reveal any changes in the stomach, jejunum, ileum, colon, liver, pancreas, adrenals, lung, spleen or kidneys. Slight changes in serum biochemistry were observed, e.g. in cholesterol, HDL-cholesterol and glucose, but were not correlated with dose or time of exposure. Changes in haematological parameters were found only in female rats after 90 days of treatment and were restricted to lymphocyte and neutrophil numbers [Cavalcanti 2006].

An extract (70% ethanol, not further specified) was administered orally to Sprague-Dawley rats at 25 or 100 mg/kg/day for 28 days. No differences in body weight gain, fasting blood glucose, ALT, AST, LDL-, HDL- and total cholesterol as well as triglycerides were observed as compared to saline treated control [Rezaei 2020].

Reproductive toxicity

A liquid extract of aerial parts excluding flowers (1:2, ethanol 45%, corresponding to 500 mg of crude drug per mL) was administered orally to female rats (n=5) at a dose level corresponding to 2.8 g of crude drug per kg b.w. daily (i.e. 56 times the human dose) on either gestation days (GD) 1-8 or 8-15. Control groups were treated with water or the amount of ethanol in the extract. From evaluation performed on GD 20 the dosage used was not materno-toxic. No significant differences were observed between groups in pre- or post-implantation losses. Compared to those of water control animals, placental weights were significantly higher in both yarrow-treated groups (GD 1-8, $p<0.05$; GD 8-15, $p<0.01$) without any histological signs of pathology, while foetal weights were significantly ($p<0.01$) lower in the yarrow GD 8-15 group. No significant differences were observed in the incidence of external or internal malformations [Boswell-Ruys 2003].

Following oral administration of an aqueous extract (1:2.5) from yarrow leaf to male Wistar rats at 0.3, 0.6 and 1.2 g/kg b.w. daily for 90 days, no significant differences in weights of

testis, epididymis, prostate or seminal vesicles were observed compared to controls. Daily sperm production, sperm number and sperm transit rate also remained unaffected. At the highest dose, the percentage of abnormal sperm was significantly higher than in control animals (3.1 versus 1.6%; $p<0.05$) but remained within the expected range (up to 5%) [Dalsenter 2004].

An aqueous extract (1:10) was added at 1.0, 5.0 and 10.0 mL to 100 mL of standard Drosophila medium used for the development of F1 eggs from Oregon-R strain of *Drosophila melanogaster.* After emergence of the flies, no significant changes in the number of offspring or the percentage of malformations were observed as compared to control [Uysal 2007].

Male albino Wistar rats received an ethanolic extract (1:10) for 22 days at 200, 400 or 800 mg/kg daily, i.p or orally. No changes in body and testis weight gain or in behaviour were observed. The doses of 200 mg/kg i.p. and 200 and 400 mg/kg p.o. did not cause significant histological differences of the testes. After 400 mg/kg i.p. scattered immature cells on the basal membrane and a decrease in cell accumulation and vacuolization in seminiferous tubules was observed. Administration of 800 mg/kg i.p. resulted in thickened seminiferous tubules on the basal membrane, decreased cell accumulation in seminiferous tubules, distinct disarrangement, degenerative cells and a strong decrease in sperm count.

After the oral dose of 800 mg/kg/day, the basal membrane was partially thickened and disarrangement of cells was observed. After 40-days of recovery, normal physiology was restored in the group which had received 400 mg/kg i.p. compared to control. The changes induced by 800 mg/kg were not reversed [Takzare 2010].

Mutagenicity

In an early study, a tea (20 g/100 mL) showed weak mutagenic effects in the Drosophila wing spot assay [Graf 1994].

An infusion at a concentration equivalent to 0.35 mg drug/mL of culture medium did not cause clastogenic effects in human lymphocytes. However, after pretreatment of the cells with the alkylating agent mitomycin C the infusion significantly increased the number of chromatid and isochromatid breaks compared to controls treated with mitomycin C only [Roncada 2004].

In the Ames test an aqueous extract (0.06-5 mL) was not mutagenic with or without metabolic activation in *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 [Vivotecnia Research SL 2009; cited according to Becker 2016].

In 2 micronucleus tests in V79 cells, an aqueous extract (up to 15,000 mg/mL) was not clastogenic or aneugenic with or without metabolic activation [Anonymus 2012; cited according to Becker 2016].

A dried aqueous extract (DER: 6-9:1) did not show any mutagenic effect at concentrations from 70, 221, 700, 2212, 7000 and 8536 µg/plate in the same *Salmonella typhimurium* strains with or without metabolic activation in an incorporation test or a preincubation test [LPT report 2015].

The protective effect of a methanolic extract (not further specified) against radiation-induced genotoxicity was investigated in human lymphocytes. Blood samples from volunteers were incubated with the extract at 10, 50, 100, and 200 µg/mL for 2 hours before exposure to 2.5 Gy X-ray radiation. Then lymphocytes were cultured under mitogenic stimulation. The extract resulted in a significant ($p<0.01$) decrease in the incidence of micronuclei in binucleated cells compared with untreated, irradiated lymphocytes by 49%, 66%, 79% and 86%, respectively. The extract at 200 µg/mL did not exhibit genotoxicity in non-irradiated lymphocytes and the frequency of micronuclei was lower than in the control ($p<0.01$) [Shahani 2015].

An essential oil from flowers at 0.19 and 0.25 µL/mL increased the number of diploid segregants of *Aspergillus nidulans* A757// UT448 in a dose dependent manner. The mitotic segregation index raised significantly ($p<0.05$) from 1.0 in control to 2.0 and 3.7 respectively [de Sant´Anna 2009].

In phytohaemagglutinin-stimulated human peripheral lymphocytes, treatment with an an essential oil for 3 and 48 hours resulted in a dose-dependent, significant ($p<0.01$) decrease in the proliferation index. The effect was less pronounced in the short-term treatment with and without metabolic activation. The presence of micronuclei in mononucleated and binucleated cells increased dose-dependently in both assays, significant only in binucleated cells at dosages above 0.1 µl/mL [Contini 2020].

Clinical safety data

Product formulations containing 0.002% to 0.01% of yarrow extract were generally not irritating. A formulation containing 0.1% of an extract (2% in propylene glycol and water) was not a sensitizer in a maximization test, and alcoholic extracts of dried leaves and stalks did not produce a phototoxic response [Anonymus 2001].

One case of occupational asthma attributed to yarrow has been reported in a 44-year-old woman. The patient began to experience rhinitis, asthma und urticaria symptoms in the workplace when handling dried flowers. Skin prick tests with aqueous extracts from dried flowers of *A. millefolium* gave positive results and a Specific Inhalation Bronchial Challenge with an aqueous extract (1.25 mg/mL) elicited an asthmatic response with a fall in FEV_1 of 31%. Specific IgE (EAST) was 0.9 kU7l and immunoblotting revealed several IgE binding bands of 51, 21 and 18 kDa. The data strongly suggested that an IgE-mediated immunologic mechanism was responsible for the patient's symptoms [Compes 2006].

In a patch test involving 20 patients with known contact allergy to sesquiterpene lactones, two aqueous extracts (residue of 1.5 g drug/50 mL boiling water dissolved in 1 mL water = 100%) caused positive reactions in 8 and 11 participants respectively. Eighteen reactions were observed with the 100% solution, 4 with a 10% dilution and 1 with a 1% dilution. Only one reaction was strong, 3 were weak and all others medium. As compared to chamomile, goldenrod, wormwood and dandelion teas tested in parallel, yarrow resulted in the largest number of late reactions with 3 cases occurring on day 7 and another 3 cases on day 10 [Lundh 2006].

A study performed for the chemical validation of an Asteraceae mix used for patch tests included a yarrow extract among the 5 tested extracts. The data were cross-validated with those of patch tests in 1,778 patients, of which 125 had reacted positively to one of the applied Asteraceae extracts. A positive reaction against yarrow was observed in 28% of the 125 sesquiterpene-sensitive patients [Salapovic 2013].

No significant side effects or adverse events were observed in 75 patients with multiple sclerosis who received a dried aqueous extract (250 mg corresponding to 2 g drug or 500 mg corresponding to 4 g per day) for one year [Ayoobi 2019].

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MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Online Series, 2019
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Online Series, 2021
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Online Series, 2019
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Online Series, 2019
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHAMOMILLAE ROMANAE FLOS	Roman Chamomile Flower	Online Series, 2019
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Online Series, 2021
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Online Series, 2021
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HELICHRYSI FLOS	Sandy Everlastin Flower	Online Series, 2019
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013

HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Online Series, 2020
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Online Series, 2021
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Online Series, 2020
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Online Series, 2021
PRUNI AFRICANAE CORTEX	Pygeum Bark	Online Series, 2020
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Online Series, 2021
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Online Series, 2020
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
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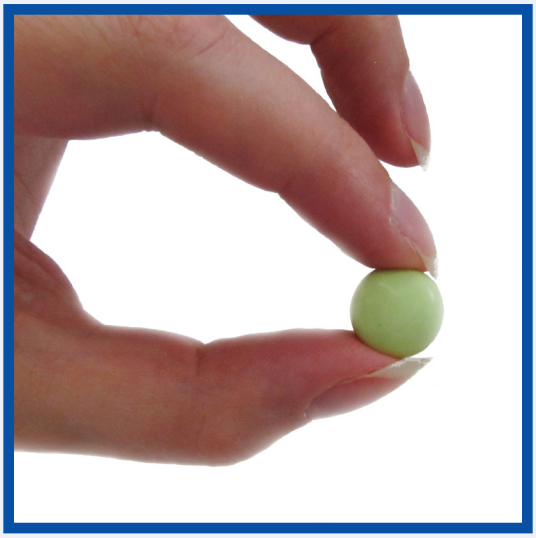

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

MYRRHA

Myrrh

2014

DEFINITION

Myrrh consists of a gum-resin, hardened in air, obtained by incision or produced by spontaneous exudation from the stem and branches of *Commiphora molmol* Engler and/or other species of *Commiphora*.

The material complies with the monograph of the European Pharmacopoeia [Myrrh].

Species other than *Commiphora molmol* Engler [synonym: *C. myrrha* (Nees) Engler var. *molmol*] which may be acceptable sources of medicinal myrrh include *Commiphora abyssinica* (Berg) Engler and *C. schimperi* (Berg) Engler [Wichtl 1999].

CONSTITUENTS

Myrrh contains three major groups of compounds: volatile oil (2-10%), resin (25-40%) and gum (30-60%) [Wiendl 1994].

The main constituents of the volatile oil are furanosesquiterpenes of various structural types including furanoeudesma-1,3-diene (principal component), furanoeudesma-1,4-diene-6-one, lindestrene, curzerenone, furanodiene, 2-methoxyfuranodiene and 4,5-dihydrofuranodiene-6-one, together with sesquiterpenes such as α-copaene, elemene and bourbonene [Brieskorn 1982, Brieskorn 1983, Martinetz 1988, Hänsel 1992].

Characteristic constituents of the resin are α-, β- and γ-commiphoric acids, α- and β-heerabomyrrhols, heeraboresene and burseracin [7, 8]; also various terpenes [Mincione 1972;Zhu 2001;Shen 2009;Su 2009;Xu 2011a; Xu 2011b] and a sesquiterpene lactone, commiferin [Mincione 1972].

The water soluble gum is composed of a heterodisperse mixture of proteoglycans in which chains of alternating galactose and 4-*O*-methylglucuronic acid, and separate chains of arabinose, are attached to the protein moieties via hydroxyproline linkages [Wiendl 1994, Hough 1952, Jones 1955].

CLINICAL PARTICULARS

Therapeutic indications

Topical treatment of gingivitis, stomatitis (aphthous ulcers), minor skin inflammations, minor wounds and abrasions; supportive treatment for pharyngitis, tonsillitis [Moeck 2007, Martinetz 1989, Bradley 1992, Hiller 2009, Sweetman 2011, Barnes 2007].

Posology and method of administration

Dosage

Adults: As a gargle or mouthwash, 1-5 ml of tincture (1:5, ethanol 90% V/V) in a glass of water several times daily [Hager, Bradley 1992, Barnes 2007]. For use on skin, dab 2-3 times daily with diluted or undiluted tincture (1:5, ethanol 90% V/V) [Moeck 2007, Bradley 1992, Hiller 2009, Sweetman 2011, Martinetz 1989].

Elderly: as for adults.

Children: as for adults except using only diluted tincture on skin.

Method of administration
For topical application.

Duration of use
No restriction.

E/S/C/O/P MONOGRAPHS

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Because of the alcohol content, a transient burning sensation on the skin may be experienced depending on the level of dilution of the tincture.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice the product should not be used during pregnancy or lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
Very rare cases of allergic contact dermatitis have been reported [Gallo 1999, Al-Suwaidan 1997].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antibacterial and antifungal effects
Various sesquiterpene-containing fractions from myrrh inhibited *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* with minimum inhibitory concentrations of 0.18-2.8 µg/mL [Dolara 2000].

Essential oil from myrrh inhibited the growth of a variety of Gram-positive and Gram- negative bacteria with MIC values from 0.1-0.4 % [Wanner 2010].

Antiproliferative effect
An aqueous dry extract (7.5 g herbal drug/125 mL water) showed an antiproliferative activity in eight different murine and human cancer cell lines with IC_{50} values of 208-419 µg/mL [Shoemaker 2005].

Myrrh showed an effect on cell viability in a murine neuroblastoma cell line with LC_{50} of 0.158 mg/mL [Mazzio 2009].

Aromatase inhibiting effect
Diterpenes isolated from myrrh showed aromatase inhibiting activity with IC_{50} values of 0.21 µM and 0.32 µM, and significant ($p<0.01$) inhibition of human umbilical vein endothelial cell (HUVEC) growth [Su 2009].

In vivo experiments

Anti-inflammatory effects
In carrageenan-induced paw oedema in rats, a petroleum ether dry extract of myrrh, at an oral dose of 500 mg/kg b.w. given 1h before carrageenan injection, exerted anti-inflammatory activity comparable to the effect of the anti-inflammatory agent oxyphenbutazone 100mg/kg b.w. In the cotton pellet granuloma test the same extract, at an oral daily dose of 500mg/kg b.w. for four consecutive days, demonstrated a 26.49% inhibition of the resulting increase in pellet weight compared to control (saline) [Tariq 1985].

An ethanolic dry extract of myrrh (approximately 6:1), administered intraperitoneally to mice, exerted a significant anti-inflammatory effect ($p<0.05$) at 400 mg/kg b.w. in the xylene-induced ear swelling model. The same extract significantly inhibited cotton pellet granuloma ($p<0.05$) in rats at an oral dose of 400 mg/kg [Atta 1998].

An ethanolic (85%) dry extract and a petroleum ether fraction from the extract at doses of 100 mg/kg b.w. given orally to mice significantly ($p<0.05$) inhibited formalin-induced paw swelling [Su 2011].

Antipyretic effect
Mice with hyperpyrexia induced by administration of a 20% aqueous suspension of brewer's yeast were then administered either an ethanolic (95%) dry extract or a petroleum ether extract (25:1) of myrrh at a dose of 500 mg/kg b.w. A significant antipyretic effect ($p<0.001$ to 0.05) was demonstrated [Mohsin 1989, Tariq 1985].

Stimulation of phagocytosis
Male mice (19±1g) inoculated with *Escherichia coli* were treated intraperitoneally with either a dried ethanolic extract or the unsaponifiable fraction of myrrh, as solutions in aqueous ethanol (10% V/V) at 50 mg/kg b.w. Both treatments stimulated phagocytosis in 80% of the mice compared to controls [Delaveau 1980].

Analgesic effects
In the hot plate test in mice a significant analgesic effect ($p<0.01$) was demonstrated after oral administration of myrrh at 1 mg/kg b.w. [Dolara 1996].

Furanoeudesma-1,3-diene isolated from myrrh showed significant analgesic properties in mice when administered by intracerebroventricular injection at 1.25 mg/kg b.w.($p<0.01$) or orally at 50 mg/kg in the hot plate test, and also at 50 mg/kg in the writhing test. The analgesic effects were reversed by naloxone at 1 mg/kg, indicating an interaction with brain opioid receptors. Furanoeudesma-1,3-diene concentration-dependently displaced the specific binding of [^{3}H]diprenorphine to rat brain membrane *in vitro* [Dolara 1996].

A fraction from myrrh composed of furanodiene-6-one and methoxyfuranoguaia-9-ene-8-one, administered as eye drops at a concentration of 280 µg/mL into the conjunctival sac of rabbits, had a strong local anaesthetic effect ($p<0.01$ compared to the vehicle as control) of about half that of procaine at 100 µg/mL [Dolara 2000].

An ethanolic dry extract of myrrh (approximately 6:1), administered orally to mice, exerted a significant and dose-dependent analgesic effect in the acetic acid-induced writhing test at 200 mg/kg ($p<0.05$) and 400 mg/kg ($p<0.01$) [Atta 1998].

Cytoprotective effect
Oral administration of myrrh to male Wistar albino rats (aged 7-8 weeks) at 250, 500 and 1000 mg/kg b.w. provided significant and dose-dependent protection to the gastric mucosa against the ulcerogenic effects of various necrotizing agents: 80% ethanol, 25% sodium chloride, 0.2 M sodium hydroxide, indometacin 30 mg/kg and combined ethanol 80%-indometacin 2.5 mg/kg ($p<0.05$ to $p<0.001$, depending on the dose). The same suspension significantly and dose-dependently protected against

ethanol-induced depletion of gastric wall mucus ($p<0.05$ at 500 mg/kg; $p<0.001$ at 1000 mg/kg) [Al-Harbi 1997].

Antitumour and cytotoxic effects
After oral treatment of Ehrlich solid tumour (EST)-bearing mice with an aqueous suspension of myrrh at daily doses of 250 or 500 mg/kg b.w., the higher dose produced significant decreases ($p<0.05$) after 25 days and 50 days, in tumour weight, in the viability of EST cells and in levels of DNA, RNA and protein in EST cells. The antitumour potential of myrrh was found to be comparable to that of the cytotoxic drug cyclophosphamide 10mg/kg b.w. [Al-Harbi 1994].

The antitumour activity of an aqueous suspension of myrrh, equivalent to that of cyclophosphamide, has also been demonstrated in Ehrlich ascites carcinoma (EAC) cell-bearing mice. At an oral dose of 500 mg/kg, significant reductions in the DNA ($p<0.05$), RNA ($p<0.01$) and protein ($p<0.01$) contents of EAC cells, and in their viability ($p<0.05$), were observed together with an increased survival rate of the animals (25.1 days) compared to negative control (16.7 days)[Qureshi 1993].

Oral administration of an extract of myrrh (not further specified) demonstrated no anti-tumour activity against Ehrlich ascites carcinoma in mice when given at doses of 100 mg/kg b.w. daily for 6 days or 250 mg/kg b.w. every other day for 6 days, as evaluated by total ascetic volume. The treatment caused a 5 day increase in the median survival time compared to control [El-Naggar 2011].

Hypoglycaemic effects
Intragastric treatment of normal and streptozotocin-induced diabetic rats with a 5% m/V aqueous extract of myrrh (prepared with boiling water and filtration) daily for one week at 10 ml/kg b.w. significantly lowered fasting blood glucose levels ($p<0.05$ for both groups) and, in the oral glucose tolerance test, significantly increased glucose tolerance in both normal ($p<0.02$) and diabetic animals ($p<0.05$) [Al-Awadi 1987].

Oral administration of two fractions (200-250 mg/kg b.w.) and two pure furanosesquiterpenes (150-175 mg/kg) from myrrh (*C. myrrha*) to obese diabetic mice produced significant reductions in blood glucose at 27 hours post-dose ($p<0.005$ in all cases). One active fraction at 200 mg/kg reduced blood glucose by 50% ($p<0.0001$), compared to a 41% reduction with the oral antidiabetic metformin at 250 mg/kg [Ubillas 1999].

Anthelmintic effects
Mice infected with *Schistosoma mansoni* were treated intragastrically with 10 mg/kg b.w. per day of an extract of myrrh for three successive days 6 weeks after infection. Three weeks after treatment, the serum antischistosomal antibody level was significantly decreased ($p<0.0001$). The result was comparable to praziquantel at 250 mg/kg b.w. per day. The level of IL-2 was decreased significantly ($p<0.01$); the level of gamma interferon was not affected [Abdel-Aziz 2006].

Mice infected with 60 cercariae of *Schistosoma mansoni* were orally treated with an undefined myrrh extract (250 mg or 500 mg/kg b.w.), praziquantel as a positive control (250 mg/kg b.w.) or vehicle only, twice a day for three consecutive days. The treatment with the extract and praziquantel caused a significant ($p<0.001$) reduction in the worm burden of the mice, it induced the separation of male-female coupled worms and shifted female worms from their normal habitat to the liver [Badria 2001].

Antischistosomal activity of different myrrh preparations were tested at dosages from 180-10000 mg/kg b.w. in mice and hamsters infected with Schistosoma mansoni strains of different geographical origin. No signs of antibilharzial activity were observed [Botros 2004].

Other effects
Powdered myrrh, administered orally at 100 mg/kg b.w., provided 86% protection against experimental thrombosis in mice ($p<0.05$), comparable to the effect of acetylsalicylic acid at 20 mg/kg [Olajide 1999].

To assess the effect of myrrh on the genotoxic effects and liver oxidising effects of lead acetate, male albino mice (4 months old) received either normal diet (negative control), normal diet with 0.5% lead acetate (positive control) or normal diet with 1% myrrh powder and 0.5% lead acetate for 8 weeks (n=30/group). Addition of myrrh to the diet significantly ($p<0.05$) decreased the level of GSH, increased the activity of GSH-transferase and reduced lipid peroxidation determined in liver homogenate compared to negative control. It significantly ($p<0.05$) reduced genotoxicity of lead acetate, the number of aberrant cells as well as the increased frequency of chromosomal aberrations [El-Ashmawy 2006].

Clinical studies

Anthelmintic effects
Oral treatment of 204 patients suffering from schistosomiasis with myrrh at 10 mg/kg b.w. per day for 3 days in an open study produced a cure rate of 91.7%. Non-responding patients treated for 6 further days with the same dose gave a cure rate of 76.5%, increasing the overall rate to 98%. 20 patients provided biopsy specimens 6 months after treatment and none of them showed living ova [Opdyke1976].

In an open study, 65 patients infected with *Schistosoma mansoni* (n=26) or *S. haematobium* (n=39) were treated with two capsules (600 mg) of a myrrh preparation (not further specified) for six consecutive days. The cure rates after three months were 96.2% and 97.4% respectively [Abo-Madyan 2004].

In an open study, 104 patients with schistosomiasis were treated with 600 mg of an undefined myrrh extract or praziquantel (positive control) at 40 mg/kg b.w. for 3 consecutive days. The treatments were given twice with a 3 week interval. After the first treatment phase the cure rate for the extract was 15.6% compared to 73.7% for praziquantel. After the second treatment phase the rates were 8.9% and 76.3% respectively [Baracat 2005].

Note: The studies above all point to clinical evidence that myrrh is effective against schistosomiasis; however in all cases the administered preparations are not sufficiently characterized.

In a preliminary open study 7 patients with fascioliasis (infection with parasitic liver flukes) were treated orally with a preparation consisting of 8 parts of myrrh resin and 3.5 parts of myrrh volatile oil at 12 mg/kg b.w./day for 6 days. The therapy proved to be effective, with pronounced improvement in the general condition of the patients and amelioration of all symptoms and signs. By the end of treatment a dramatic drop in the egg count was observed and eggs were no longer detectable in the faeces after 3 weeks or after a follow-up period of 3 months. High eosinophilic counts, elevated liver enzymes and *Fasciola* antibody titres returned to nearly normal [Rao 2001].

In an open study, 18 patients infected with *Dicrocoelium dendriticum* (a liver fluke) were given two capsules (600mg) of an unspecified myrrh extract for six successive days. Sixteen of the eighteen patients were followed up for two months and showed clinical and parasitological cure [Al-Mathal 2004].

Antimicrobial effects
In an open study, 13 patients infected with metronidazole- and tinidazole-resistant *Trichomonas vaginalis* were treated with 600 mg of an unspecified myrrh preparation for six to eight successive days. 11 of the 13 patients were reported to be cured by the treatment [El-Sharbini 2009].

Pharmacokinetic properties
No data available.

Preclinical safety data
The acute oral LD_{50} for rats of myrrh oil has been determined as 1.65 g/kg b.w. [Opdyke1976].

The LD_{50} for rats of an extract from myrrh given intragastrically was determined to be 2 g/kg b.w. [Badria 2001].

LD_{50} in mice for an undefined myrrh extract were 3139 mg/kg b.w. given by gastric intubation and 1740 mg/kg b.w. given orally [Botros 2004, El-Naggar 2011].

In the open mouse ear assay, the essential oil, a chloroform dry extract and some isolated constituents from myrrh exhibited irritant potential [Saeed 2004].

Myrrh and the volatile oil of myrrh are reported to be non-irritating, non-sensitizing and non-phototoxic when applied to animal or human skin [Martinetz 1989,Al-Harbi 1994].

Oral toxicity studies of myrrh were carried out in mice using acute doses of 0.5, 1.0 and 3.0 g/kg b.w. and chronic doses of 100 mg/kg/day for 90 days. Compared to controls, no significant differences in mortality, weight gain or biochemical parameters were observed after acute or chronic treatment. After chronic treatment there were significant increases in weight of testes and seminal vesicles ($p<0.05$) and of caudae epididymis ($p<0.01$), and a significant increase in red blood cell count and haemoglobin ($p<0.05$). The toxicity studies supported the safe medicinal use of myrrh [Rao 2001].

Genotoxicity and cytotoxicity
Myrrh administered orally to normal mice for 7 days at 125-500 mg/kg b.w. per day as an aqueous suspension showed no mutagenicity in the micronucleus test. It caused a significant, dose-dependent reduction in the RNA content of hepatic cells ($p<0.01$ at 250 mg/kg), but not the DNA or protein content, and a highly significant, dose-dependent, mitosis-depressant effect in femoral cells ($p<0.001$) [Qureshi 1993, Al-Harbi 1994].

Clinical safety data
Myrrh was well tolerated with only mild and transient side effects when administered orally to 204 patients with schistosomiasis at 10 mg/kg b.w./day for 3-9 days [Sheir 2001].

No signs of toxicity or adverse effects were observed from treatment of 7 patients with fascioliasis with myrrh resin/volatile oil at 12 mg/kg b.w. for 6 days [Massoud 2001].

Ethnopharmacological evidence suggests that myrrh has been extensively used both internally and externally without serious adverse effects [Martinetz 1989].

No side effects were reported in an open study involving 18 dicrocoeliasis patients treated for six days with a dose of 600 mg of an unspecified myrrh extract [Al-Mathal 2004].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Supplement 2009
ALOE CAPENSIS	Cape Aloes	Second Edition, 2003
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Second Edition, 2003
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
BALLOTAE NIGRAE HERBA	Black Horehound	Supplement 2009
BETULAE FOLIUM	Birch Leaf	Second Edition, 2003
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Second Edition, 2003
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
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ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Supplement 2009
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Second Edition, 2003
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Second Edition, 2003
FUMARIAE HERBA	Fumitory	Supplement 2009
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Supplement 2009
GRINDELIAE HERBA	Grindelia	Supplement 2009
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Second Edition, 2003
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LINI SEMEN	Linseed	Second Edition, 2003
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003

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MALVAE FLOS	Mallow Flower	Supplement 2009
MARRUBII HERBA	White horehound	Online Series, 2013
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MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
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MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Second Edition, 2003
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Second Edition, 2003
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
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SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
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SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
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TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
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URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Second Edition, 2003
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VIOLAE HERBA CUM FLORE	Wild Pansy	Supplement 2009
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
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The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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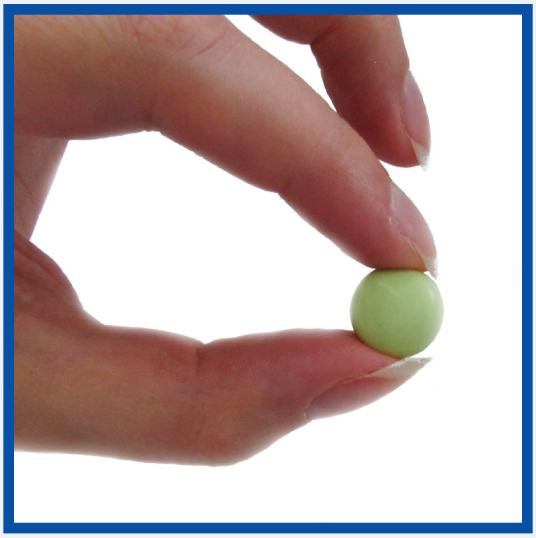

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

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Abbreviations
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Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

MYRTILLI FRUCTUS

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Bilberry Fruit

DEFINITION

Bilberry fruit, dried consists of the dried ripe fruit of *Vaccinium myrtillus* L., containing a minimum of 1.0 percent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) calculated with reference to the dried drug.

Bilberry fruit, fresh consists of the fresh or frozen ripe fruit of *Vaccinium myrtillus* L., containing a minimum of 0.30 per cent of anthocyanins, expressed as cyanidin-3-*O*-glucoside chloride (chrysanthemin, $C_{21}H_{21}ClO_{11}$; M_r 484.8) calculated with reference to the dried drug.

Fresh bilberry fruit dry extract, refined and standardised, produced from Bilberry fruit, fresh using ethanol (96 per cent V/V) or methanol (minimum 60 per cent V/V). It contains 32.4 per cent to 39.6 per cent of anthocyanins, expressed as cyanidin 3-*O*-glucoside chloride [chrysanthemin ($C_{21}H_{21}ClO_{11}$); M_r 484.8) calculated with reference to the dried drug*.

The materials comply with the respective monographs of the European Pharmacopoeia [Bilberry fruit, dried; Bilberry fruit, fresh; Fresh bilberry fruit dry extract, refined and standardised].

CONSTITUENTS

Bilberry fruit, dried

Tannins and tannin precursors: up to 4%, mainly catechol tannins, also catechin and epicatechin; Procyanidins: B-1, B-2, B-3 and B-4; Anthocyanins: 0.5% glycosides of mainly delphinidin, cyanidin and petunidin; Flavonoids: including hyperoside, isoquercitrin, quercetin, astragalin; Iridoids: asperuloside; Caffeic acid derivatives: chlorogenic and other caffeoylquinic acids; Other constituents: resveratrol, pectins, vitamin C, β-carotene [Friedrich 1973; Baj 1983; Jänicke 2003; Wichtl 2009].

Bilberry fruit, fresh
Fresh bilberry fruit dry extract, refined and standardised

Anthocyanins: mono- and di-saccharides of delphinidin, cyanidin, petunidin, peonidin and malvidin, highest amounts occurring in berry skins; Anthocyanidins: delphinidin, cyanidin, petunidin, peonidin, malvidin; Catechins: (+)-catechin, (-)-epicatechin, epigallocatechin gallate; Flavonols: quercetin, myricetin; Phenolic acids: ferulic, p-coumaric, m-coumaric, caffeic, chlorogenic, protocatechuic, syringic and gallic; Procyanidins: dimers and trimers A-type derived from (-)-epicatechin; Stilbenes: trans-resveratrol; Other constituents: triterpenes, scopoletin, aliphatic organic alcohols [Jaakola 2002; Roy 2002; Du 2004; Viljanen 2004; Ehala 2005; Faria 2005; Määttä-Riihinen 2005; Burdulis 2007; Cassinese 2007; Milbury 2007; Bao 2008a; Bao 2008b; Latti 2008; Ogawa 2008; Burdulis 2009; Thomasset 2009; Akerström 2010; Karlsen 2010].

CLINICAL PARTICULARS

Therapeutic indications

Internal use
Extracts of bilberry fruit enriched with anthocyanins: supportive treatment of problems related to varicose veins such as painful and heavy legs, peripheral vascular insufficiency and microcirculatory disorders of the eye [Ghiringhelli 1978; Passariello 1979; Grismondi 1980; Tori 1980; Nuti 1980; Scharrer 1981; Allegra 1982; Signorini 1983a; Signorini 1983b; Corsi 1985; Gatta 1988; Morazzoni 1996].

Dried bilberry fruit: supportive treatment of acute non-specific diarrhoea. In this indication the efficacy is plausible due to long-standing use [Schilcher 1997;

1*The HPLC-based assay defines anthocyanins (Syn. anthocyanosides) content of the Fresh bilberry fruit dry extract as 36%, which corresponds to the 25% specification determined by spectrophotometry based on anthocyanidins [Penman 2006; Cassinese 2007; Ulbricht 2009].

Jänicke 2003; Wichtl 2009; Schilcher 2010].

External use
Topical treatment of mild inflammation of mucous membranes of the mouth and throat and for superficial wounds. In this indication the efficacy is plausible due to long-standing use [Jänicke 2003; Wichtl 2009].

Posology and method of administration

Internal use
Dried bilberry fruit: For adults and children from 10 years, 20 to 60 g daily, taken with water [Dorsch 2002; Jänicke 2003; Wichtl 2009; Schilcher 2010].

Decoction: 5-10 g of dried fruit boiled for 10 minutes in 150 mL water and drunk cold several times daily [Jänicke 2003; Blaschek 2007; Wichtl 2009].

Cold macerate: 5-10 g dried fruit soaked in 150 ml water for 2 hours and drunk several times daily [Jänicke 2003; Blaschek 2007; Wichtl 2009].

Fluid extract [1:1]: 3 to 6 ml daily [Mills 2005].

Dry standardized extract containing 36% of anthocyanins: 60 to 160 mg three times daily (equivalent to 65 to 173 mg anthocyanins daily) [Alfieri 1964; Bonacina 1973; Lietti 1976; Robert 1977; Detre 1986; Virno 1986; Bertuglia 1995; Sweetman 2008].

Topical use
A 10% decoction as a gargle or mouthwash [Blumenthal 2003; Sweetman 2008; Wichtl 2009].

Method of administration
For oral administration or local application.

Duration of use
No restriction.
If diarrhoea persists for more than 3-4 days medical advice should be sought. In microcirculatory disorders a use of at least 4 to 8 weeks is recommended [Blumenthal 2003].

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
Due to the potential for bilberries to enhance the antiplatelet effect of acetylsalicylic acid and other NSAIDs, bilberries should be used with caution by patients with haemorrhagic disorders and those taking warfarin or antiplatelet drugs [Morazzoni 1990; Rasetti 1996; Laplaud 1997; Abebe 2002; Mills 2005].

Pregnancy and lactation
The dry extract is well tolerated in pregnancy. Bilberry has been used safely in high doses in pregnant women with varicose veins and haemorrhoids [Grismondi 1980; Teglio 1987; De Smet 1993].

Scientific evidence for the safe use of bilberry during lactation is not available.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antioxidant activity
An anthocyanin-rich extract of bilberry fruit was reported to be a potent scavenger of free radicals, behaving both as a scavenger against superoxide anion [Salvayre 1981; Meunier 1989; Martin- Aragón 1998; Martin- Aragón 1999] and as an inhibitor of lipid peroxidation induced by adenosine diphosphate (ADP)/Fe^{2+} and ascorbate in liver microsomes [Salvayre 1981; Martin- Aragón 1998; Martin- Aragón 1999].

The K^+ loss induced by free radicals in human erythrocytes, as well as the cellular damage caused by oxidant compounds such as daunomycin and paraquat, was inhibited by an anthocyanin-rich extract [Maridonneau 1982; Mavelli 1984].

An aqueous extract of bilberry fruit was shown to have a potent protective action on human low density lipoprotein (LDL) during copper-mediated oxidation [Laplaud 1997] and a standardized extract containing 37% of anthocyanins prevented photo-induced oxidation of human LDL and fragmentation of apoprotein [Rasetti 1996/7].

The peroxy-radical scavenging capacity of berry extracts was studied using the Oxygen Radical Absorbing Capacity (ORAC) assay. The ORAC value (expressed using Trolox equivalents per g) for bilberry extract was 44 [Roy 2002].

The antioxidant activity of a bilberry extract (573 mg total phenolic compounds / g dry weight containing 94.8% anthocyanins) was investigated in a lactalbumin-liposome system at concentrations of 1.4, 4.2 and 8.4 µg / mL of liposome sample. The extracts showed an effective dose-dependent antioxidant activity towards protein and lipid oxidation. Anthocyanins were the major contributors to the antioxidant effect by inhibiting both hexanal and protein carbonyl formation [Viljanen 2004].

An ethanolic extract of fresh berries (258 mg/L total phenolics) had an antiradical activity (assessed by DPPH method) of 5.37 (µM Trolox equivalents). The extract provided protection of a liposomal membrane against peroxyl radicals by increasing the induction time of oxidation [Faria 2005].

To study structure-activity relationships and potential synergisms, antioxidant activities of 15 bilberry phenolics were evaluated. The potency of activity towards the superoxide radical was in the order: delphinidin > petunidin > malvidin=cyanidin > (+)-catechin > peonidin > pelargonidin. The activity towards the peroxynitrite radical (ONOO-) was: delphinidin > cyanidin=petunidin > malvidin = (+)-catechin > peonidin > pelargonidins [Rahman 2006].

In an assay using cytochrome *C*-enhanced 6-hydroxydopamine oxidation reaction a 50% inhibition was observed with an aqueous bilberry extract (7 µM of total anthocyanin) [Yao 2007].

Effects on vision
Pyridinium bisretinoid A2E, an autofluorescent pigment

accumulating in human adult retinal pigment (ARPE) cells with age and other retinal disorders, can mediate a detergent-like perturbation of cell membranes and light-induced damage to cells. Light-exposed A2E-laden ARPE cells exhibit a propensity for apoptosis via generation of epoxides which then interact with DNA. Incubation of the cells with a bilberry extract (40mM) reduced A2E-epoxidation, incidence of DNA damage and cell death [Sparrow 2003].

A water-soluble fraction of a bilberry extract was found to reduce A2E photooxidation. Separated single anthocyanins (100 µM) suppressed the photooxidation of A2E. The ARPE cells that had been incubated with anthocyanins (100 µM) exhibited an increased resistance to A2E-induced membrane damage [Jang 2005].

A bilberry extract (total anthocyanin content 28.1% cyanidin-3-glucoside equivalent; total phenol content 61.8% catechin equivalent) was found to upregulate the oxidative stress defence enzymes haeme oxygenase-1 and glutathione *S*-transferase-pi in retinal pigment epithelial cells [Milbury 2007].

A bilberry extract (10 µg/mL) and anthocyanidins (cyanidin at 10 µM, delphinidin at 30 µM, malvidin at 10 µM) significantly inhibited 3-(4-morpholinyl) sydnonimine hydrochloride-induced retinal ganglion cell death and decreased intracellular radical formation. Each inhibited the production of thiobarbituric acid reactive substance (TBARS) in a concentration-dependent manner, the IC_{50} values being 1.0 µg/mL, 0.7, 1.9 and 8.3 µM respectively versus vehicle ($p<0.05$) [Matsunaga 2009].

A study examined the effects of anthocyanins on the regeneration of the photoreceptor rhodopsin using frog rod outer segment membranes. Cyanidin-3-glycosides stimulated the regeneration, but the corresponding delphinidin glycosides showed no significant effect [Matsumoto 2003].

In frog rod outer segment membranes cyanidin-3-glucoside [C3G] was found to bind directly with rhodopsin depending on pH [Yanamala 2009]. Further work confirmed the C3G-enhanced regeneration [Tirupula 2009].

Anti-angiogenic activity
The antiangiogenic activity of a standardised bilberry extract (25% anthocyanins) was evaluated using the chick chorio-allantoic membrane (CAM) assay. A 100 ng/50 µl concentration resulted in inhibition (Score -2) compared to control (Score +2) [Ozgurtas 2008].

A bilberry extract (50 µg polyphenols/mL) showed a 96% inhibition of H_2O_2- induced vascular endothelial growth factor (VEGF) expression by human HaCaT keratinocytes, compared to control (76%) ($p<0.05$) [Roy 2002].

A bilberry extract (25% anthocyanins) at 0.3-30 µg/ml inhibited both tube formation and migration of human umbilical vein endothelial cells (HUVECs) induced by vascular endothelial growth factor-A (VEGF-A). In addition the bilberry extract inhibited VEGF-A-induced proliferation of HUVECs and VEGF-A-induced phosphorylations of extracellular signal-regulated kinase 1/2 and serine/threonine protein kinase family protein kinase B, but not that of phospholipase C-gamma [Matsunaga 2007].

In a second study the inhibitory effects induced by individual anthocyanidins (delphinidin, cyanidin and malvidin) at 3 and 10µM significantly inhibited VEGF-induced tube formation ($p<0.01$) in a co-culture of HUVECs and fibroblasts. Each anthocyanidin (0.3 – 10µM) also scavenged the DPPH radical in a dose-dependent manner. The effects were weaker than those produced by the bilberry extract (25% anthocyanins) at 30 µg/mL [Matsunaga 2010].

Effect on cardiovascular tissues
An anthocyanin-rich extract of bilberry fruit inhibited proteolytic enzymes such as elastase [Jonadet 1983] and interacted with collagen metabolism by cross-linking collagen fibres, making them more resistant to collagenase action [Robert 1977] and reducing the biosynthesis of polymeric collagen [Boniface 1982].

An anthocyanin-rich standardized extract (corresponding to 25% of anthocyanidins) had a slightly relaxing effect on various isolated vascular smooth muscle preparations and reduced the response to contraction inducers such as serotonin and barium [Bettini 1984a; Bettini 1984b; Bettini 1984c; Bettini 1985].

Application of a bilberry extract (12.1% total anthocyanins and 35.7% total phenolics) directly to pig coronary arterial rings resulted in a dose- and endothelium-dependent vasorelaxation (% maximal relaxation at 5 mg total anthocyanins /L was 59 ± 10) [Bell 2006].

A bilberry extract (standardized for 25% anthocyanidin) was evaluated for effects on ACE in cultured endothelial cells. Doses of the extract (6.25, 12.5, 25, 50 and 100 µg/mL) resulted in a significant and dose-dependent inhibition of the ACE activity ($p<0.001$). Reference anthocyanidins had no significant effect [Persson 2009].

Bilberry anthocyanins isolated from fresh ripe berries were evaluated for their intracellular capacity to inhibit peroxy radical formation on endothelial cells (EA.hy926). Anthocyanins were significantly active ($p<0.001$) at low concentrations (<0.5mg/L) with a saturable effect being observed >0.5 mg/L. Coronary flow (CF) of isolated rat hearts in experiments with 40-min ischaemia followed by reperfusion indicated a concentration-dependent bi-phasic response: vasodilation at ≤1mg/L ($p<0.05$) and vasoconstriction at ≥10 mg/L up to 50 mg/L. Perfusion of isolated rat hearts with low anthocyanin concentrations (0.01 – 1 mg/L) significantly attenuated the ischaemia-reperfusion (I-R) injury as evidenced by decrease in the release rate of lactate dehydrogenase (LDH), increase in postischaemic coronary flow and decrease in the incidence and duration of reperfusion arrhythmias (heart arrest, ventricular fibrillation, ventricular tachycardia, ventricular premature complexes). High concentrations (5 - 50 mg/L) diminished cardioprotection and showed cardiotoxic activity [Ziberna 2010].

Antiaggregatory effects
A standardized extract of bilberry fruit showed activity against aggregation induced by ADP, collagen and sodium arachidonate in rabbit platelet-rich plasma [Morazzoni 1990]. This was confirmed *ex vivo* on ADP- and collagen-induced aggregation of platelets obtained from the blood of 30 healthy volunteers given 480 mg/day of the standardized extract orally for 30-60 days [Pulliero 1989].

An anthocyanin extract of bilberry fruit also inhibited *in vitro* platelet aggregation induced by ADP or adrenaline in human plasma [Serranillos 1983].

Wound healing effects
Pre-treatment (1 h) with a bilberry extract (25% anthocyanins) increased the viability of UV-A irradiated (20 J/cm^2) keratinocytes (HaCaT cells) in a dose-dependent manner, with 100 mg/L providing nearly 50% protection ($p<0.05$). Pre- and post-treatment (4 h) of the cells with the bilberry extract (25–100

mg/L) attenuated the UVA-induced reactive oxygen species (ROS) generation ($p<0.05$) [Svobodova 2008].

Further evaluation of the photoprotective effects of bilberry fruit extract (25% w/w anthocyanins) on UVB-induced phototoxic stress (200 mJ/cm^2) showed that pre-treatment (1 h) of HaCaT cells with the extract (5 – 50 mg/L) and post-treatment (4 h) with 5 and 50 mg/L, reduced the extent of DNA breakage ($p<0.05$), and laddering, together with caspase-3 and -9 activity ($p<0.05$). Pretreatment with the extract before UVB exposure also significantly ($p<0.05$) decreased reactive oxygen or nitrogen species (RONS) generation and partially diminished IL-expression [Svobodova 2009].

Treatment of fibroblasts with anthocyanins (cyanidin-3-glucoside (72%), delphinidin-3-glucoside (20%) and petunidin-3-glucoside (6%)) after 24 h showed a significant increase in migration at 100 µg/ml ($p<0.05$), while the migration of keratinocytes increased significantly at 50 and 100 µg/mL compared to control ($p<0.05$). Treatment with anthocyanins for 48 h significantly stimulated the migration of both human dermal fibroblasts and keratinocytes at 50 and 100 µg/mL ($p<0.001$). Treatment of cells with anthocyanins stimulated wound-induced VEGF production in fibroblasts and keratinocytes. However, anthocyanins inhibited ROS accumulation and VEGF production in TNF-α-stimulated endothelial cells. Anthocyanin treatment also reduced, in a dose-dependent manner, the adhesion of inflammatory monocytes to endothelial cells. Anthocyanins also blocked both the translocation of NF-κB p65 into the nucleus and the phosphorylation of the inhibitory factor κBα [Nizamutdinova 2009].

Anti-inflammatory activity
The effects of a standardised bilberry extract (36% anthocyanins) on genome-wide gene expression were investigated in an inflammatory cell model (RAW264). Cells were pretreated with or without 75 µg/mL bilberry extracts for 30 mins and then exposed to 40 ng/mL lipopolysaccharide (LPS) for another 6 hrs. The induction of pro-inflammatory genes, including TNF, IL-1β, IL-6, TNC, PTGS2 and COX-2 were significantly reduced by bilberry ($p<0.001$ vs LPS only) [Chen 2008].

In cultured human monocytes (U937-3XκB-LUC cells) anthocyanins (100 mg/L) isolated from bilberries and black-currants efficiently suppressed LPS-induced activation of NF-κB [Karlsen 2007].

Human monocytic cells (ATCC CRL-1593.2) were co-incubated with 13 polyphenols to evaluate their ability to modulate LPS-induced NF-κB activity. Quercetin inhibited the NF-κB activation in a dose-dependent manner as compared with control cells (77% and 95% at 25 and 50 µmol/L (respectively, both $p<0.001$). Resveratrol (50 µmol/L) and epicatechin (25 µmol/L) inhibited the LPS-induced NF-κB activation by 79% ($p=0.020$) and 32% ($p<0.003$) respectively. Petunidin (50 µmol/L) and delphinidin (50 µmol/L) increased LPS-induced NF-κB to respectively 245 and 146% of controls ($p= 0.003$ and $p=0.007$) [Karlsen 2010].

Antimicrobial / gastroprotective activity
A bilberry extract (0.25%) significantly ($p<0.05$) inhibited the growth of *Helicobacter pylori* (ATCC strain 49503) compared to controls and increased susceptibility of *H. pylori* to clarithromycin (15 µg/mL). With a 1% concentration of the extract, >90% inhibition was observed, with and without clarithromycin [Chatterjee 2004].

Bilberry extract (1mg/mL) showed strong inhibition against *Escherichia coli* (strains 50 and CM871) [Puupponen-Pimiä 2001]. Phenolic extracts have also been shown to be effective against *Bacillus cereus*, *Salmonella enterica* sv. *Typhimurium* and sv. *Infantis*, *Staphylococcus aureus*, *Clostridium perfringens* and *Helicobacter pylori*. Activity is associated with phenolics, mainly anthocyanins [Puupponen-Pimiä 2001; Puupponen-Pimiä 2005; Nohynek 2006; Puupponen-Pimiä 2008].

Sugars and low molecular weight phenolic compounds of bilberry juice were found to inhibit *Streptococcus pneumoniae* binding to human bronchial cells (Calu-3). Adhesion inhibition was 52% with a fraction concentration of 9 mg/g [Huttunen 2011].

Fractions of bilberry juice (mainly anthocyanins, proanthocyanidins and flavonol glycosides) inhibited and reversed co-aggregation of pairs of *Streptococcus mutans* with *Fusobacterium nucleatum* or *Actinomyces naeslundii*, species which are common in dental plaque [Riihinen 2011].

Polyphenolic fractions of bilberry juice containing mainly anthocyanins and proanthocyanidins, between concentrations of 5-50 µg/mL, were found to significantly inhibit adhesion of *Neisseria meningitides* to HEC-1B human epithelial cells when compared to control ($p<0.05$) [Toivanen 2011].

Antiproliferative and apoptotic activity
A lyophilized ethanol extract of frozen bilberries (55.1 mg/g of total phenolics, 26.3 mg/g of total anthocyanins) showed a DPPH radical scavenging activity of 288 (µmol of Trolox/g). The extract decreased the number of viable human promyelocytic leukaemia (HL 60) cells by 84 and 88% at a concentration of 4 and 6 mg/mL respectively and the number of viable human colon carcinoma (HCT116) cells by 66% and 97% at 2 and 4 mg/mL respectively. HL60 cell growth was inhibited when incubated with extract concentrations from 0.5 to 4 mg/mL for 24 and 48 h. In contrast HCT116 cell growth showed inhibition at 1-4 mg/mL only after 48 h incubation. The bilberry extract induced apoptotic cell bodies in HL60 cells and nucleosomal DNA fragmentation in HL60 cells but not in HCT116 cells [Katsube 2003].

Pretreatment of cultured mouse JB6 cells with cyanidin-3-glucoside (C3G) inhibited UVB- and TPA-induced transactivation of NF-κB and AP-1 as well as expression of COX-2 and TNF-α. These inhibitory effects appeared to be mediated through the inhibition of MAPK activity. C3G (10 - 40µM) significantly inhibited, and at a concentration of 80 µM completely abolished, TPA-induced neoplastic transformation in JB6 cells ($p\leq0.05$). In addition C3G (10 - 40µM) inhibited proliferation of a human lung carcinoma cell line A549 [Ding 2006].

A methanolic extract from fresh bilberries (2:1) contained anthocyanins (12.8 mg/mL) and flavonols (0.29 mg/mL). For the determination of cell proliferation, HT-29 colon cancer cells were exposed to 200 µL of culture medium containing 0-60 mg/mL of berry extract or pure compounds (0-100 µg/mL) for 24 h. Bilberry extracts showed significant ($p<0.001$) growth inhibition (30% cell reduction) at 10 mg/mL. The bilberry extract had no effect on DNA fragmentation after 48 h at concentrations up to 10 mg/mL. However, exposure of the HT-29 cells to higher concentrations (20-60 mg/mL) resulted in pronounced fragmentation [Wu 2007].

A bilberry extract was tested for growth inhibition (IC_{50}) using a human colorectal adenocarcinoma (HT29) cell line. The sulforhodamine B assay was applied to determine cell growth. The IC_{50} for bilberry extract was found to be 32.2 µg/mL of cyanidin-3-glucoside equivalent [Jing 2008].

A standardised anthocyanin-rich bilberry extract (36%

anthocyanins) reduced receptor tyrosine kinase (RTK) domains of epidermal growth factor (EGFR, ErbB1) and ErbB2-4 receptors and the vascular endothelial growth factor receptors (VEGFR-2 and VEGFR-3), with preferential inhibition of VEGFR-2 and EGFR (≤ 3.4 µg/mL). Ligand-induced autophosphorylation of human vulva carcinoma or porcine aortic endothelial cells was suppressed, with ErbB3 and VEGFR-3 being preferentially inhibited. The extract completely abolished VEGFR-3 phosphorylation at concentrations of ≥50 µg/mL [Teller 2009].

In human breast cancer cells (MCF7) a bilberry extract inhibited proliferation in a concentration-dependent manner, without inhibiting microtubule polymerization or mitosis. Significant inhibition of proliferation ($p<0.05$) occurred with bilberry extract concentrations >0.25 mg/mL, with an IC_{50} of 0.43 mg/mL. At higher concentrations (0.5-1 mg/mL) inhibition of polymerization (~30%) did occur with an increase in the fraction of cells at the G_2/M phase, together with destruction of microtubules and formation of punctate tubulin aggregates in the cells [Nguyen 2010].

Various anthocyanins and anthocyanidins were exposed to the human efflux transporters-multidrug resistance protein 1 (MDR1) and breast cancer resistance protein (BCRP) using dye efflux, ATPase and vesicular transport assays. Malvidin, petunidin, malvidin-3-galactoside, cyanidin-3-galactoside, peonidin-3-glucoside and cyanidin-3-glucoside emerged as potential BCRP substrates, while others (including cyanidin, peonidin, pelargonidin, delphinidin) emerged as potential inhibitors of BCRP. Some exhibited bimodal activities, with malvidin, malvidin-3-galactoside and petunidin serving as BCRP substrates at low concentrations and BCRP inhibitors at higher concentrations. Aglycones exerted only mild inhibitory activity on MDR1 [Dreiseitel 2009].

Other effects
Pre- and co-incubation of human colon adenocarcinoma cells (HT29) with a standardized bilberry extract (36% anthocyanins) at concentrations ≥1µg/mL significantly suppressed ($p\leq0.001$) the strand-breaking effects of camptothecin and significantly diminished doxorubicin-mediated DNA strand breaks at concentrations ≥ 1µg/mL ($p\leq0.001$) [Esselen 2011].

Human HepG2 cells were incubated for 24 hrs with varying C3G concentrations (5–100 µmol/L) and assayed for cholesteryl ester transfer protein (CETP) activity. Concentrations of 5 µmol and above significantly suppressed CETP activity ($p<0.001$) compared to the untreated control [Qin 2009].

In vivo experiments

Antioxidant activity
Oral pre-treatment of mice with 250 or 500 mg/kg of an anthocyanin-rich extract of bilberry fruit inhibited liver peroxidation stimulated by $FeCl_2$, ascorbic acid and ADP. The malondialdehyde content in the liver was significantly reduced ($p<0.05$) [Martin-Aragon 1999].

Oral administration of an anthocyanin-enriched bilberry extract (42% anthocyanins) to mice (n=10) at 50, 100 and 200 mg/kg/day for 5 days showed significant decreases ($p<0.001$) in plasma alanine aminotransferase (ALAT) levels at all doses, with the 200 mg/kg/day dose having an ALAT level of 17.23 ± 2.49 U/L compared to 107.68 ± 3.19 U/L 18 h in restraint stressed mice and 18.08 ± 1.46 U/L in starved controls. Extracts also significantly ($p<0.001$) increased GSH and Vitamin C levels and significantly decreased MDA and nitric oxide (NO) levels in liver tissues at the highest dose [Bao 2008a].

In another experiment the same anthocyanin-enriched bilberry extract (42% anthocyanins) was given orally to mice at 50, 100 and 200 mg/kg/day for 5 days. After the final treatment, potassium bromate ($KBrO_3$), an oxidizing agent and potent nephrotoxin, was given as a single intraperitoneal injection to mice (n=10) at a dose of 200mg/kg. Serum blood urea nitrogen (BUN) and creatinine levels were significantly higher in the $KBrO_3$-treated animals when compared with control (247.31 ± 3.22 mg/L vs 215.10 ± 2.82 mg/L; 22.26 ± 1.75 µg/mL vs 9.91 ± 0.86 µg/mL respectively). The 5-day oral intake of the extract significantly ($p<0.001$) reduced levels of BUN and serum creatinine to normal levels and decreased kidney MDA, NO and xanthine oxidase levels. Improved ORAC levels in kidney tissue were also observed indicating a reduced degree of oxidative stress and kidney damage [Bao 2008b]. Administration of the extract also elevated the Na^+/K^+-ATPase activity and mitochondrial potential which was reduced in the stressed group. Bilberry extract had a protective effect on the liver by scavenging free radicals and attenuating mitochondrial dysfunction [Bao 2010].

A standardized bilberry fruit extract (36% anthocyanins) was evaluated in carbon tetrachloride (CCl_4)-induced hepatic fibrosis. Balb/C mice were treated i.p. with CCl_4 dissolved in olive oil (20% V/V; 2ml/kg) twice a week for 7 weeks. Control animals received the vehicle only. The bilberry extract in saline (1, 5 and 10 mg/kg to groups 4, 5 and 6 respectively) was then given daily for 15 days prior to sacrifice. Results showed that bilberry extract, in a dose-dependent manner, reduced hepatic hydroxyproline (an indicator of hepatic collagen) from 945 µg/g liver (CCl_4 + saline) to 248 µg/g liver (CCl_4 + BE 10 mg/kg; $p<0.05$) vs 198 µg/g liver (control). The extract (10 mg/kg) markedly attenuated oxidative stress, decreased TNF-α, TGF-β1 and α-smooth muscle actin (α-SMA) expression and eliminated hepatic collagen deposits. The extract reduced liver fibrosis by decreasing oxidative stress and inactivating hepatic stellate cells via down-regulation of the fibrogenic cytokines TNF-α and TGF-β1 [Domitrovic 2011].

Effects on vision / ophthalmic activity
An anthocyanin-rich extract of bilberry fruit, administered intravenously to rabbits at 3.2 mg/kg, reduced the permeability of vessels of the ciliary body, which had been increased by ocular puncture [Virno 1986].

A mixture of anthocyanins administered intravenously to rabbits at 160 mg/kg promoted dark adaptation after dazzling [Alfieri 1964].

Senescence-accelerated OXYS rats with early senile cataract and macular degeneration were given control diets or supplementation with 25% bilberry extract (20 mg/kg b.w. = 4.5 mg anthocyanidin) or vitamin E (40 mg/kg) for comparison. After 3 months 70% of control rats had cataracts and macular degeneration. Supplementation with vitamin E showed no significant effects. In contrast the extract completely prevented impairments in the lenses and retina. Both antioxidants decreased lipid peroxides in the retina and serum [Fursova 2005].

Administration of an ethanolic dry extract of bilberry to mice by intravitreal injection (300 ng per eye) inhibited the formation of neovascular tufts during oxygen-induced retinopathy, in comparison to saline [Matsunaga 2007].

This extract was further evaluated for the effects on *N*-methyl-D-aspartic acid (NMDA)-induced retinal damage using haematoxylin-eosin and terminal deoxynucleotidyl transfer-mediated dUTP nick-end labelling (TUNEL) staining. Intravitreal injection of bilberry extract (100 µg per eye co-injected with NMDA at 5 nmol) significantly inhibited the morphological

retinal damage and increase in TUNEL-positive cells in the ganglion cell layer (both the GCL cell count and the IPL thickness) [Matsunaga 2009].

Ocular inflammation (uveitis) was induced in mice by a foot-pad injection of lipopolysaccharide (100 mg/mouse). Oral administration of a bilberry extract (42% anthocyanins) at doses of 50, 100 and 200 mg/kg/day for 5 days before challenge significantly reduced the ocular nitric oxide levels, especially at the highest dose ($p<0.001$). Also the malondialdehyde level was decreased, while increased levels were found in ORAC, glutathione and vitamin C, as well as increased activities of superoxide dismutase and glutathione peroxidase. Increased expressions of Cu/Zn superoxide dismutase, Mn superoxide dismutase and glutathione peroxidase were also found [Yao 2010].

In a similar experiment an anthocyanin-rich bilberry extract (39% anthocyanins; 500 mg/kg b.w.) or PBS as control, were administered orally to mice for 4 days. Three hours after the last administration animals received a single intraperitoneal injection of lipopolysaccharide (6 mg/kg b.w.) to induce uveitis and retinal inflammation (EIU). Pretreatment with the bilberry extract had a protective effect on visual functioning during retinal inflammation. Anthocyanin treatment was found to preserve rhodopsin levels ($p<0.05$), suppress an increase of the pro-inflammatory cytokine IL-6 ($p<0.05$), reduce oxidative stress ($p<0.01$), significantly suppress a-wave and b-wave amplitudes ($p<0.05$ and $p<0.01$ respectively) and to suppress NF-κB ($p<0.01$) in the inflamed retina [Miyake 2012].

Effects on glucose and lipid metabolism (anti-diabetic activity)
Bilberry extract (375 g anthocyanins/kg) included in the diet (10 g anthocyanin/kg diet) of type 2 diabetic mice (male KK-A^y) for 5 weeks, significantly reduced blood glucose concentration and enhanced insulin sensitivity ($p<0.05$) via activation of AMP-activated protein kinase (AMPK). Activation occurred in white adipose tissue, skeletal muscle and the liver and was accompanied by upregulation of glucose transporter 4 in white adipose tissue and skeletal muscle, and suppression of glucose production and lipid content in the liver. At the same time acetyl-CoA carboxylase was inactivated and PPARα, acyl-CoA oxidase and carnitine palmitoyltransferase-1A were upregulated in the liver. After 5 weeks there was no difference in body weight gain or adipose tissue weight between the test and control groups. However, liver weight was significantly lower in the group receiving the bilberry extract ($p<0.05$). Compared with the control group total lipid, triglyceride and cholesterol contents of the liver were significantly reduced in the test group, while triglyceride and total cholesterol concentrations in serum were also significantly lowered ($p<0.05$) [Takikawa 2010].

KK-A^y mice were fed control or control + 0.2% of C3G diet for 5 weeks. Dietary C3G significantly reduced blood glucose concentration and enhanced insulin sensitivity ($p<0.05$). C3G upregulated the glucose transporter 4 and downregulated retinol binding protein 4 in the white adipose tissue, which was accompanied by downregulation of the inflammatory adipocytokines (monocyte chemoattractant protein-1 and TNF-α) ($p<0.05$ for both) [Sasaki 2007].

Vasoprotective activity
Anthocyanin-rich extracts of bilberry fruit exert modulating effects on capillary resistance and permeability, as was demonstrated in various animal models [Lietti 1976; Robert 1977; Detre 1986]. For example, an extract administered orally to rabbits at 200-400 mg/kg protected against capillary permeability induced by topical application of chloroform [Lietti 1976].

Treatment of rats with bilberry anthocyanins for 12 days before the induction of hypertension kept the blood-brain barrier permeability normal and limited the increase in vascular permeability in the skin and the aorta wall [Detre 1986].

When administered orally to rats at 100-200 mg/kg, or intra-peritoneally or intramuscularly at 25-100 mg/kg, the extract protected against capillary lesions induced by intradermal injection of bradykinin [Lietti 1976].

The same extract was found to antagonise damage induced by ischaemia-reperfusion in the hamster cheek-pouch micro-circulation model after oral administration at 100 mg/kg for 4 weeks [Bertuglia 1995].

Effects of a bilberry extract on arteriolar vasomotion were assessed in cheek pouch microcirculation of anaesthetised hamsters and in skeletal muscle microvasculature of unanaesthetised hamster skin fold window preparations. Intravenous injection of the extract induced vasomotion in cheek pouch arterioles and terminal arterioles with higher frequency in smaller vessels. In the skeletal muscle arteriolar networks the extract increased vasomotion frequency and amplitude in all vessel types. [Colantuoni 1991].

The effects of anthocyanins on skeletal muscle vascular permeabililty were evaluated in streptozotocin-induced diabetic rats. Animals (n=11) received bilberry extract (40 mg/kg) daily in drinking water or water only (n=10). Changes in vascular permeability were determined by measuring the capillary filtration of intravenously administered ^{99m}technetium-labelled albumin (CFA), inducing venous compression in a hindquarter with a tourniquet and measuring the radioactivity externally on the limb before (time 0), during (after 6 weeks) and after removal of compression (after 12 weeks). Interstitial albumin retention (AR) was significantly higher in the diabetic rats than the controls. In the groups receiving the extract AR decreased significantly ($p=0.015$) after 6 and 12 weeks [Cohen-Boulakia 2000].

Antiatherogenic (atheroprotective) activity
An anthocyanin-rich extract of bilberry fruit, administered intraperitoneally at 100 mg/kg for 45 days to rabbits fed on a cholesterol-rich diet, reduced the proliferation of intima and calcium and lipid deposition on the aortic wall [Kadar 1979].

Two bilberry extracts, one rich in anthocyanins extracted from bilberries (52% total anthocyanins) and one extracted from yeast-fermented bilberries (almost devoid of typical anthocyanins but rich in complex polymers) were evaluated for their effects on the development of atherosclerosis in apolipoprotein E-deficient mice. For 16 weeks mice received a diet supplemented with 0.02% of either extract. Both bilberry extracts caused a significant reduction in lipid deposits. The atherosclerotic lesions area was decreased in the anthocyanin-rich group by 15% ($p<0.05$) and by 36% ($p<0.0001$) in the yeast-fermented group compared to control. No significant differences in triacylglycerol and total cholesterol levels in either plasma or liver were observed between the groups [Mauray 2009].

In a continuation of the experiment, apo E-deficient mice (n=40) were fed *ad libitum* for two weeks either a control diet or the same diet supplemented with 0.02% of the anthocyanin-rich extract. After two weeks plasma lipid and antioxidant capacity were evaluated and the global genomic analysis carried out using pangenomic microarrays. Anthocyanin supplementation significantly ($p<0.05$) reduced total-cholesterol (mmol /L) from 7.0 (control) to 5.6, whereas plasmatic antioxidant status remained unchanged. Genomic analysis identified 1,261 genes

in the aorta whose expression was modulated by the bilberry extract [Mauray 2012].

Further investigation of the anti-atherogenic effect of a bilberry extract (52% anthocyanins) on gene expression in the liver of apo E-deficient mice was carried out. Mice received for 2 weeks a standard diet supplemented with an anthocyanin-rich bilberry extract (0.02%). After a 2-week supplementation plasma total cholesterol (mmol /L) and hepatic triglyceride levels (mg /g) were significantly reduced ($p<0.05$ and $p<0.01$ respectively), while plasmatic antioxidant status remained unchanged. Transcriptional analysis showed that the expression of 2,289 genes was significantly altered, with over-expression of genes involved in bile acid synthesis and cholesterol uptake into the liver, but down-regulation of expression of pro-inflammatory genes [Mauray 2010].

Wound healing effects
Topical application of 5-10 mg of an anthocyanin-rich extract of bilberry fruit accelerated the healing of experimental wounds in rats [Curri 1976]. In another experimental model (healing delayed by prednisone) the same extract applied topically at concentrations of 0.5-2% for 3 days to skin wounds in rats promoted healing activity in comparison to prednisone-treated controls [Cristoni 1987].

Anti-inflammatory activity
The vasoprotective effect could also be responsible for anti-inflammatory activity exhibited by anthocyanins (50-500 mg/kg given orally) against paw oedema induced by irritant agents such as carrageenan, histamine or hyaluronidase [Bonacina 1973; Lietti 1976].

Mice sensitized by an epicutaneous application of 2,4,6-trinitro-1-chlorobenzene (TNCB; 100 µL of 1.1% v/v) to their shaved abdomen, were challenged with 10 µL/ ear of TNCB solution on day 0. TNCB was reapplied to each ear 3 times per week until day 16. Daily oral administration (0.4 or 2 g/kg b.w.) of a standardized bilberry extract (>25% anthocyanidins and >36% anthocyanins) significantly decreased ear swelling ($p<0.001$ vs control) and suppressed bouts of scratching associated with progressive and chronic allergic contact dermatitis ($p<0.001$ vs control). The lower dose of bilberry was more effective [Yamaura 2011].

In a further experiment using the same methodology the standardized bilberry extract (400 mg/kg b.w.) was administered orally for 4 weeks to BALB/c mice with allergic contact dermatitis induced by application of TNCB. Treatment with the anthocyanin-containing extract (non-heated) significantly attenuated the TNCB-induced increase in scratching behaviour compared to the vehicle ($p<0.05$). The anthocyanidin-rich (2-h heated) extract had no effect. The non-heated extract (300 µg/mL) resulted in significant inhibition of degranulation in RBL-2H3 mast cells ($p<0.05$) [Yamaura 2012].

Anti-ulcer/ gastroprotective activity
A standardized extract (corresponding to 25% of anthocyanidins) administered orally to rats at doses ranging from 25 to 200 mg/kg, showed antiulcer activity on ulcers induced by pyloric ligation, non-steroidal-antiinflammatory drugs (NSAIDs) and reserpine [Cristoni 1987].

Oral pretreatment of rats with C3G (4 and 8 mg/kg body weight) resulted in a 36 and 80% inhibition respectively ($P<0.05$) of ethanol-induced gastric lesions in comparison to control rats receiving only 80% ethanol. Pretreatment with C3G also inhibited elevation of lipid peroxide levels, and in gastric tissues significantly ($p<0.05$) increased glutathione levels and the activities of superoxide dismutase, catalase and glutathione peroxidase [Li 2008].

Pretreatment of mice (male ddY) with a bilberry extract (25% anthocyanidins) at doses of 10, 30 and 100 mg/kg, 1 h before HCl / ethanol administration, significantly inhibited gastric erosions in a dose-dependent manner ($p<0.01$ vs vehicle-treated mice) [Ogawa 2011].

Neuroprotection / Memory
Rats given intraperitoneal injections of bilberry anthocyanins (200 mg/kg/day) for 5 days had significantly more triiodothyronine (T3) in their brains than rats given the solvent (26% alcohol). Bilberry-treated animals showed superior memory, better vision and better control of sensory input [Saija 1990].

An aqueous suspension of lyophilised bilberries (equivalent to 3.2 mg anthocyanin/ kg/day) was given orally to rats for 30 days before first training (n=10 per group). Bilberries significantly enhanced short-term memory ($p<0.05$), but not long-term memory in the inhibitory avoidance task, and induced an increase in the number of crossings in the first exposure to the open field. In the radial arm maze test, anthocyanin-treated rats showed significant choice accuracy improvement ($p<0.05$) in the early sessions, an effect which became attenuated in later sessions [Ramirez 2005].

The effects of long-term supplementation with bilberry extract (2 g/kg of diet = 0.35 g anthocyanins/kg of food) and vitamin E (140 mg/kg of diet) were studied in young senescent-accelerated OXYS and Wistar rats. The role of oxidative stress on brain biomolecules such as protein carbonyls, lipid peroxides, reduced glutathione, α-tocopherol and superoxide dismutase as well as on learning ability was investigated. Bilberry extract and vitamin E improved cognitive deficits (tested by passive avoidance reaction retrieval) in OXYS rats. Supplementation with both vitamin E ($p= 0.008$) and bilberry extract ($p=0.002$) decreased lipid peroxides in rat brains. Bilberry extract significantly decreased MDA level only in OXYS rat's brain ($p=0.048$), while vitamin E significantly decreased MDA levels in both Wistar and OXYS rats, when compared with their respective controls [Kolosova 2006].

A bilberry extract was administered orally to mice (n=9; 100 mg/kg b.w.) for 7 days. Psychological stress was assessed by whisker removal which is known to increase both protein carbonyl formation and lipid peroxidation in the brain, heart, kidney and liver. Treated mice showed less lipid peroxidation in all these tissues. The stress-induced shift of dopamine in the brain was almost completely suppressed. [Rahman 2008].

Chemopreventive activity
Bilberry extracts were assessed for their effects on multiple biomarkers of azomethane-induced colon cancer in male rats which were fed *ad libitum* for 14 weeks on a control diet or one supplemented with bilberry (containing 3.85 g monomeric anthocyanins / kg of diet). Total colonic aberrant crypt foci (ACF) were reduced ($p<0.05$) compared with the control. The number of large ACF was also reduced ($p<0.05$) and colonic cellular proliferation was decreased. Bilberry-fed rats had lower COX-2 mRNA gene expression. High levels of faecal anthocyanins and increased faecal mass and moisture were found, as well as a significant reduction ($p<0.05$) in faecal bile acids [Lala 2006].

Apc^{Min} mice, a genetic model of human familial adenomatous polyposis, ingested C3G or a standardised bilberry extract (40% anthocyanins) at 0.03, 0.1 or 0.3% in the diet for 12 weeks. Ingestion of the highest doses of C3G and the bilberry extract showed a significant decrease in intestinal adenomas by 45%

($p<0.001$) or 30% ($p<0.05$) respectively compared to controls [Cooke 2006].

Multiple Intestinal Neoplasia/+ mice were fed modified high fat diets containing 10% freeze-dried bilberries (5.53 g total anthocyanins, 0.104 g total flavonols /kg diet) or a similar berry-free diet as control for 10 weeks. The berry diet significantly inhibited the formation of intestinal adenomas in the distal small intestine, as indicated by a 15-30% reduction in tumour cell number ($p<0.05$) [Misikangas 2007].

The effect of C3G on xenograft growth of human lung carcinoma cell line A549 was evaluated. Nude mice were treated intraperitoneally with either PBS or C3G (9.5 mg/kg) three times per week beginning 2 days after s/c tumour cell implantation. C3G-treated mice showed a ~50% reduction in tumour size, compared to control animals ($p<0.02$) and inhibited tumour metastasis [Ding 2006].

Immunostimulation / myelo- and cyto-protective activity
A single injection of 5-fluorouracil (5-FU) at 200 mg/kg into C57BL/6 mice induced severe peripheral erythrocytopenia, thrombocytopenia and leucopenia as well as hypocellularity of the spleen and bone marrow. Oral administration of 500 mg/kg of an anthocyanin-rich bilberry extract for 10 days significantly increased the number of red blood cells, neutrophils and monocytes in peripheral blood to 1.2-fold, 9-fold and 6-fold respectively, compared to 5-FU alone ($p<0.05$ - 0.001). The hypocellularity of the spleen and bone marrow caused by 5-FU was also distinctly alleviated [Choi 2007].

Pharmacological studies in humans

In a double-blind, placebo-controlled study involving 40 healthy subjects the activity of an anthocyanin-rich extract, administered orally at 240 mg daily for 3 months, was evaluated for pupillary movements through examination of the direct photomotor reflex. The study demonstrated a more efficient pupillary photomotor response after administration of the extract compared to placebo [Vannini 1986].

Young males with good vision, selected for a study using a double-blind, placebo-controlled, cross-over design, received 160 mg of bilberry extract (25% anthocyanins) (n=7) or placebo (n=8) three times daily for 21 days, followed by a one-month washout period and the second three-week treatment period. After the three-month study no difference in night visual acuity or night contrast sensitivity were found between placebo and test subjects [Muth 2000].

In a parallel-design, placebo-controlled clinical trial the effect of a purified anthocyanin preparation (150 mg, equivalent to 100 g fresh bilberries, twice daily for 3 weeks) was studied in healthy adults (n=120). In the anthocyanin group, changes were observed in (i) the NF-κB-controlled pro-inflammatory chemokine IL-8, (ii) RANTES (regulated upon activation, normal T cell expressed and secreted) and (iii) IFNα (an inducer of NF-κB activation) (45, 15 and 40% decreases from baseline, respectively) relative to those in the placebo group (20, 0 and 15% decreases from baseline, respectively) ($p<0.05$). Similarly, changes in IL-4 and IL-13, cytokines that mediate pro-inflammatory responses and induce NF-κB activation, were found in the test group (60 and 38% decreases from baseline respectively) compared with those in the placebo group (4 and 6% decreases) ($p=0.056$ and $p=0.089$, respectively) [Karlsen 2007].

Platelet aggregation was inhibited *ex vivo* in blood taken from healthy volunteers after oral administration of bilberry extract (480 mg/day; standardized to 36% anthocyanins) for 30-60 days [Pulliero 1989]. Purified anthocyanins and *in vivo* metabolites of polyphenols were also found to have anti-thrombotic properties [Rechner 2005].

Clinical Studies

Ophthalmic disorders

A systematic review of 30 relevant trials with bilberry anthocyanins on night vision, 12 of which were placebo-controlled, concluded that "negative outcome was associated with more rigorous methodology but also with lower dose level and extracts from geographically distinct sources that may differ in anthocyanoside composition. The hypothesis that bilberry anthocyanosides improve normal night vision is not supported by evidence from rigorous clinical studies. However when all studies are taken together (controlled and un-controlled trials with bilberry anthocyanins, controlled trials with related anthocyanins and evidence from animal studies), a sizeable body of positive auxiliary evidence exists for the potential therapeutic role of bilberry" [Canter 2004; Lee 2005].

Daily treatment of 14 patients suffering from tapetoretinal degeneration with 3 x 150 mg of a bilberry fruit extract resulted in an improvement in light sensitivity of the retina starting from the second day of treatment and remaining almost constant during the 3-month treatment period [Zavarise 1968].

Patients with glaucoma (n=8) were given a single dose (800 mg) of a standardized bilberry extract (25% anthocyanins). Evaluation using electroretinography showed improvements [Caselli 1985].

The efficacy of a standardized extract (320 mg/day) was evaluated in 40 patients with refractory defects. Accurate ocular and electrofunctional examinations showed a significant increase in the flash electroretinogram amplitude in medium ($p=0.002$) and high myopia ($p=0.008$), indicating an improvement in retinal sensitivity [Spadea 1991].

In another study, daily administration of 320 mg of the extract for 3 months to 26 myopic patients resulted in improvement of electrophysiological functions [Contestabile 1991].

Oral administration of a standardized extract of bilberry fruit at 480 mg/day for 180 days to 10 patients with type II diabetes mellitus and non-proliferative retinopathy resulted in improvement of the diabetic retinopathy, with marked reduction or disappearance of retinal haemorrhages [Orsucci 1983].

In a double-blind, placebo-controlled study, 40 patients with diabetic or hypertensive retinopathy were treated with 320 mg of a standardized extract of bilberry fruit or placebo daily for 30 days. An improvement in ophthalmoscopic and angiographic patterns was observed in 77-90% of the verum patients [Perossini 1987].

In another placebo-controlled study, involving 40 patients with diabetic retinopathy at a relatively early phase, a standardized extract of bilberry fruit administered at 320 mg/day for 12 months promoted the regression of hard exudates, which is considered a reliable index of altered permeability [Repossi 1987].

An open trial of bilberry extract (standardized to 25% anthocyanins; 80-160 mg three times daily for 4 weeks) was conducted on 31 patients with retinal pathology from various causes; diabetic (n=20); retinitis pigmentosa (n=5); macular degeneration (n=4) and haemorrhagic retinopathy from anticoagulant therapy (n=2). Reduced vascular permeability

and incidence of haemorrhage was reported in all patients, especially those with diabetic retinopathy [Scharrer 1981].

In a double-blind study, 14 diabetic and/or hypertensive outpatients with vascular retinopathy underwent therapy with bilberry anthocyanidins (160 mg twice daily) or placebo (n=20) for 1 month. At the end of the month placebo patients received the active for an additional month. Ophthalmoscopic and fluoroangiographic findings obtained before and after treatments showed improvements ranging from 77 to 90% in anthocyanin-treated patients [Bravetti 1989].

Twenty-two subjects (14 males, 8 females; age range 23-59) with self-reported symptoms of dry eye were assessed at baseline by the Ocular Surface Disease Index (OSDI), Tear Break-up Time and Schirmer's test. Patients were assigned to either bilberry (80 mg orally, twice daily; no other details given) or placebo for 30 days. The bilberry group showed a statistically significant improvement ($p<0.01$). The mean OSDI baseline for bilberry patients was 22.8 ±11.0, with post-treatment values of 13.7 ± 8.5 ($p<0.01$), while baseline for placebo was 27.9 ± 26.8 with post treatment values of 19.6 ± 20.0 (p=n.s.) [Anderson 2011].

Peripheral vascular diseases
In 47 patients with lower limb varicose syndrome, oral treatment with bilberry fruit extract at 480 mg/day for 30 days led to improvements in objective symptoms such as limb oedema and dyschromic skin phenomena, and subjective symptoms such as heaviness, paraesthesia and pain [Ghiringhelli 1977].

Improvements in vessel fragility and objective symptoms, after administration of bilberry fruit extract at 480 mg/day for 30-180 days, have been confirmed in trials performed on 22 diabetic and dyslipidaemic patients [Passariello 1979], on 97 patients with complaints induced by stasis of the lower extremities such as prevaricose syndrome, primary varicosis and post-phlebitic syndrome [Tori 1980], and on 42 patients with severe arterio-sclerotic vascular disease of the lower limbs [Nuti 1980].

In a double-blind, placebo-controlled study performed on 47 patients with peripheral vascular disorders of various origins, a standardized extract of bilberry fruit (480 mg/day for 30 days) reduced subjective symptoms such as heaviness, paraesthesia and pain, and improved oedema and mobility of finger joints [Allegra 1982].

The efficacy of a standardized extract of bilberry fruit has been evaluated in two clinical studies, each in 15 patients with venous insufficiency who were treated orally with 480 mg/day for 2-4 months. Significant improvements in plethysmographic ($p<0.05$) and duoregional rheographic ($p<0.001$) observations were reported [Signorini 1983a, 1983b].

In 24 patients with chronic venous insufficiency, oral administration of 480 mg of an anthocyanin-rich extract of bilberry fruit daily for 60 days induced a decrease in the total time of drainage after reactive hyperaemia evaluated by the strain gauge technique [Corsi 1985].

The efficacy of the same extract (480 mg/day for 30 days) was further demonstrated in a single-blind, placebo-controlled study carried out in 60 patients with different stages of venous insufficiency; this study showed significant activity of the extract ($p<0.01$ to $p<0.001$) on subjective parameters, namely feeling of pressure in the lower limbs and muscle cramps as well as oedema and leg and ankle girth [Gatta 1988].

Significant improvements in subjective symptoms ($p<0.01$) and reductions in oedema and capillary fragility were observed in 54 cases of phlebopathies induced by stasis during pregnancy after oral administration of 320 mg of extract daily for 60-90 days [Grismondi 1980].

Cardiovascular health
Unmedicated subjects with cardiovascular risk factors consumed berry (n = 35) or control products (n=36) for 8 weeks in a single-blind, randomized, placebo-controlled intervention trial. Mean daily intake from berry products was 837 mg polyphenols, of which 275 mg anthocyanins were from fresh bilberries (100 g). Berry consumption inhibited platelet function as measured with a platelet function analyser (using collagen and ADP as platelet activator) [changes: 11% and -1.4% in the berry and control groups, respectively; p=0.018, analysis of covariance]. Serum HDL-cholesterol concentrations increased significantly more (p=0.006, analysis of covariance) in the berry than in the control group (5.2% and 0.6%, respectively), but total cholesterol and triacylglycerol concentrations remained unchanged. Systolic blood pressure decreased significantly (p=0.050, analysis of covariance); the decrease mostly occurred in subjects with high baseline blood pressure (7.3 mm Hg in highest tertile; p=0.024, analysis of covariance). Polyphenol and vitamin C concentrations in plasma increased, whereas e.g. folate, tocopherols, sodium and potassium were unaffected [Erlund 2008].

Intake of anthocyanins (160 mg twice daily; cyanidin glycosides 33% with delphinidin glycosides 58%) for 12 weeks by subjects with elevated blood lipids (n=120, age 40-65 years) was compared with placebo in a double-blind, randomised, placebo-controlled trial. Anthocyanin consumption increased HDL-cholesterol concentrations (13.7% and 2.8% in the anthocyanin and placebo groups, respectively; $p<0.001$) and decreased LDL-cholesterol concentrations (13.6% and -0.6% in the anthocyanin and placebo groups, respectively; $p<0.001$). Cellular cholesterol efflux to serum increased more in the anthocyanin group than in the placebo group (20% and 0.2%, respectively; $p<0.001$). Anthocyanin intake decreased the mass and activity of plasma cholesteryl ester transfer protein (CETP) by 10.4% and 6.3% respectively, in the anthocyanin group and by -3.5% and 1.1% respectively in the placebo group ($p<0.001$). In the anthocyanin group, the change in HDL cholesterol was negatively correlated with the change in CETP activity (r_s=-0.33). The change in LDL cholesterol was positively correlated with the change in CETP mass activity (r_s=0.354). The change in cellular cholesterol efflux to serum was positively correlated with the change in HDL cholesterol activity (r_s=0.485) [Qin 2009].

Anti-inflammatory activities
In a randomized controlled trial, subjects at elevated risk of cardiovascular disease consumed either fresh bilberry juice diluted to 1 litre with water (n=31) or 1 litre of water (n=31) for 4 weeks. Participants in the test group consumed 330 mL bilberry juice /day. At the end of the intervention period plasma concentrations of inflammatory mediators showed C-reactive protein (CRP), interleukin (IL)-6, IL-15 and monokine induced by INF-γ (MIG) were significantly decreased in the bilberry group (p=0.027, p=0.037, p=0.008 and p=0.047, respectively [Karlsen 2010].

In another randomized controlled trial, subjects with features of metabolic syndrome were given either a diet with added bilberries equivalent to 400g fresh berries per day (n=15) or their normal diet alone (control group; n=12) for 8 weeks. Bilberry supplementation decreased serum high-sensitivity C-reactive protein, IL-6, IL-12 and LPS concentrations. An "inflammation score" based on changes of hsCRP, IL-6, IL-12 and LPS was significantly different between the groups (p=0.024) with the bilberry group showing decreased inflammation [Kolehmainen 2012].

Other effects
Colorectal cancer patients (n=25) scheduled to undergo resection of primary tumour or liver metastases each received 1.4, 2.8 or 5.6 g daily of a standardised bilberry extract (36% anthocyanins; containing 0.5, 1.0 or 2.0 g anthocyanins respectively) for 7 days before surgery. Colorectal tumour sections, obtained before and after intake of the bilberry extract, were stained for Ki-67 (mouse anti-human monoclonal antibody) or cleaved caspase-3 to determine proliferation of the tumour. In tumours from all patients receiving the bilberry extract the proliferation index (reflected by Ki-67 staining) showed a significant 7% reduction (p=0.003) compared with preintervention values. No significant decrease in tumour tissue proliferation was found in patients receiving 1.0 g and 2.0 g anthocyanins, while a significant 9% decrease (p=0.021) occurred in patients receiving 0.5 g anthocyanins [Thomasset 2009].

Overweight and obese women (n=110) were recruited into a study of randomized cross-over design to examine the effects of berries on variables of obesity-related diseases. Bilberries (100g daily, whole, frozen) were given for 33-35 days followed by a wash-out period of 30-39 days. Consumption of bilberries resulted in a statistically significant (p=0.041) decrease in waist circumference and a small decrease in weight (p=0.028). Significant decreases in vascular cell adhesion molecule (p=0.002) and TNF-α values (p=0.031) were also recorded [Lehtonen 2011].

Pharmacokinetic properties

Pharmacokinetics in animals

Absorption, distribution, metabolism and elimination
After intravenous or intraperitoneal administration of an anthocyanin-rich extract (equivalent to 25% of anthocyanidins) to rats at doses of 20-40 mg/kg or 25 mg/kg, respectively, anthocyanins were rapidly distributed to the tissues. Elimination occurred mainly in the urine (25-30% of the dose within 24 hours after administration) and to a lesser extent (15-20%) in bile [Lietti 1976].

After oral administration to rats of a single dose of 400 mg/kg, a standardized extract of bilberry fruit was rapidly absorbed from the gastrointestinal tract, reached a plasma concentration of C_{max} of 2.47 µg/mL after 15 min, and rapidly declined within 2 h [Morazzoni 1991].

A bilberry extract (containing 153.2 mg total anthocyanins/kg), with delphinidin galactoside as the main constituent (19.6 mg/kg), was administered to rats orally (400 mg/kg b.w.) or intravenously (5 mg/kg b.w.). After oral administration most anthocyanins rapidly appeared in the plasma and reached their maximum level at 15 min, except galactosides of petunidin, peonidin and malvidin that reached their maximum at 30 min. The bioavailability of individual anthocyanins was in the range 0.6 to 1.8%, and 0.93% as the anthocyanin mixture. After intravenous administration anthocyanins recovered in urine and bile during the first 4 h were only 30.8 and 13.4% respectively [Ichiyanagi 2006].

Oral administration of a bilberry extract to mice (dried, prepared from fresh berries; 100 mg/kg b.w.) resulted in a maximum plasma anthocyanin concentration (1.18 µM) after 15 min. Urinary excretion ceased by 24 h after a maximum between 0 and 6 h after administration. Total amount of anthocyanins excreted into the urine represented 1.88%. Malvidin-3-glucoside and -3-galactoside were the principal anthocyanins in the plasma 60 mins after administration. Maintenance of the mice for 2 weeks on a diet containing 0.5% of bilberry extracts resulted in a maximum anthocyanin plasma concentration of 0.26 µM. Anthocyanins were detected in the liver (605 pmol/g), kidneys (207 pmol/g), testes (149 pmol/g) and lungs (116 pmol/g), with the malvidin glycosides being the predominant anthocyanins. No anthocyanins were detected in the spleen, thymus, heart, muscle, brain, white fat or eyes [Sakakibara 2009].

Apc^{Min} mice ingested cyanidin-3-glucoside (C3G) or a bilberry extract (standardised to contain 40% anthocyanins) at 0.03, 0.1 or 0.3% in the diet (equivalent to 45, 150 or 450 mg/kg b.w./day) for 12 weeks. Anthocyanins were found in the plasma, the intestinal mucosa and urine. At 0.3% in the diet, anthocyanin levels were at the limit of detection in plasma, were 43 ng/g tissue for C3G and 8.1 ng/g tissue for the bilberry extract in the intestinal mucosa and 7.2 and 12.3 µg/mL (C3G and extract respectively) in the urine. Anthocyanin glucuronide and methyl metabolites were identified in the intestine and urine [Cooke 2006].

Fifteen minutes after oral supplementation of a mixture of 320 mg/kg b.w. C3G and 40 mg/kg b.w. cyanidin-3,5-diglucoside (Cy-dg), rats showed an increase to a maximum of 1563 µg (3490 nmol) of C3G/L and 195 µg (320 nmol) of Cy-dg/L in plasma and 0.067 µg (0.15 nmol) of C3G/g and a trace of Cy-dg together with methylated metabolites in the liver [Miyazawa 1999].

Pharmacokinetics in humans

A review of the data from human studies of cyanidins bio-availability shows that single doses of 188 – 3,570 mg total anthocyanins produced very low maximal plasma concentrations, of the range of 2.3 - 96 nM. The mean time to reach these concentrations was around 1.5 h. Most studies reported very low relative anthocyanin urinary excretions, ranging from 0.018 to 0.37%. Maximal urinary excretion was usually achieved in less than 4 h. Methylated derivatives, anthocyanidin glucuronide conjugates as well as anthocyanin glycoside glucuronides have been identified in urine or plasma [Galvano 2007].

All studies have shown that absorption is very low, with often less than 0.15% of the ingested dose appearing in the urine [McGhie 2003; McGhie 2007; Dreiseitel 2009].

Colorectal cancer patients (n=25) received 1.4, 2.8 or 5.6 g daily of a standardised bilberry extract containing 36 % w/w anthocyanins for 7 days. Anthocyanins, as well as methyl- and glucuronide metabolites were identified in plasma, colorectal tissue and urine, but not the liver [Thomasset 2009].

Thirty minutes after receiving a single oral dose of a mixture of 2.7 mg/kg b.w. C3G and 0.25 mg/kg b.w. cyanidin-3,5-diglucoside (Cy-dg), human adults (n=12, 20-29 years, 40-70 kg) had an average of 24 nM of C3G and traces of Cy-dg in plasma. Neither cyanidin aglycone, nor conjugated or methylated metabolites were found [Miyazawa 1999].

Healthy elderly women (n=4) having fasted overnight were given a single oral dose of 720 mg anthocyanins (cyanidin-3-glucoside and cyanidin-3-sambubioside). Maximum plasma concentrations of total anthocyanins varied from 55.3 to 168.3 nM, with an average of 97.4 nM, and were reached within 71.3 min (t_{max}). The elimination of plasma anthocyanins appeared to follow first-order kinetics. Half-life of cyanidin-3-glucoside is 97.3 min and cyanidin-3-sambubioside is 168.9 min. No anthocyanin glucuronides or sulphates were detected. The anthocyanins were excreted as glycosides in urine mostly during the first 4h (77.2 µg/h during the first 4 h and 13.4 during the second 4 h) [Cao 2001].

Adult males (n=3) received a dose of 721 mg cyanidin-3-glycosides. The cumulative concentration of total anthocyanins (parent and metabolites) detected in the serum (0-7 h) was 376.7 (nmol.h)/L, reaching a maximum concentration of 96.1 nM/L within 2.8 h. Parent anthocyanin glycosides represented only 32.0% of the total anthocyanins detected with 68% identified as glucuronidated and methylated derivatives. Total urinary excretion of anthocyanins over 24 h was 1072 µg, reaching a maximal excretion rate (203 µg/h) at 3.7 h [Kay 2005].

Preclinical safety data

Acute toxicity
Intraperitoneal and intravenous LD_{50} values of an anthocyanin-rich extract from bilberry fruit containing about 70% of anthocyanins were determined as 4.11 g/kg and 0.84 g/kg in the mouse, and 2.35 g/kg and 0.24 g/kg in the rat, respectively. No deaths were observed following oral doses of up to 25 g/kg in the mouse and 20 g/kg in the rat [Pourrat 1967].

Acute oral LD_{50} of an anthocyanin-rich berry extract (containing cyanidin, delphinidin, petunidin, malvidin) was greater than 5 g/kg in rats. Acute dermal LD_{50} was greater than 2 g/kg [Bagchi 2006].

Repeated dose and chronic toxicity studies
Treatment of rats for 90 days with the same extract at a daily dose corresponding to approximately five times the human clinical dose (i.e. 600 mg/day) did not produce any toxic effects [Pourrat 1967].

Mutagenicity and carcinogenicity
A bilberry extract (36% anthocyanins) did not show mutagenic activity in various tests of mutagenesis, with and without metabolic activation, and showed no teratogenic effect or influence on rat fertility [Eandi 1987].

Cytotoxicity to HaCaT keratinocytes was determined using a lactate dehydrogenase (LDH) leakage assay. Berry extracts (50 µg/mL) were added to the cells and after 24 h media were collected for assay. No significant cytotoxicity was noted with the bilberry extract (50 µg/mL) when compared to control [Yasmin 2003].

The effects of different concentrations of bilberry extract (containing 25% total anthocyanins) were studied using human corneal limbal epithelial cells (HCLEC). The extract (10^{-5} M) promoted cell growth to 120% compared with the control group ($p<0.05$) after 24 h and 48 h incubation. Cytotoxicity was not noted [Song 2010].

Clinical safety data

The safety of the bilberry extract (36% anthocyanins; mostly 160 mg twice daily for 1-2 months) was confirmed in a post-marketing surveillance study on 2295 subjects affected by: lower limb venous insufficiency (24.23%), states of fragility and altered permeability of the blood capillaries (21.51%), functional changes in retinal microcirculation (10.15%) and haemorrhoids (9.93%) [Eandi 1987].

Daily intake of 100 - 335 mg of anthocyanins was considered safe [Karlsen 2007]. Long-term oral administration in humans in doses equivalent to 180 mg/kg anthocyanins per day for six months produced no toxic effects [Eandi 1987].

In colorectal cancer patients, daily consumption of 5.6 g of a standardised bilberry extract (36% anthocyanins) for 7 days was well tolerated [Thomasset 2009].

Asymptomatic subjects with intraocular hypertension were given either a combination of two phenolic extracts from bilberry (80 mg, standardised to 36% anthocyanins) and French maritime bark (40 mg, standardised to 70% procyanidins) (n=20) twice daily or were not treated (n=18). During 2 months of treatment, no side effects were observed [Steigerwalt 2008]. In a further trial with this product, in patients (n=33) who received one tablet daily, no side effects occurred [Steigerwalt 2010].

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E/S/C/O/P
MONOGRAPHS

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SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Second Edition, 2003
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VIOLAE HERBA CUM FLORE	Wild Pansy	Supplement 2009
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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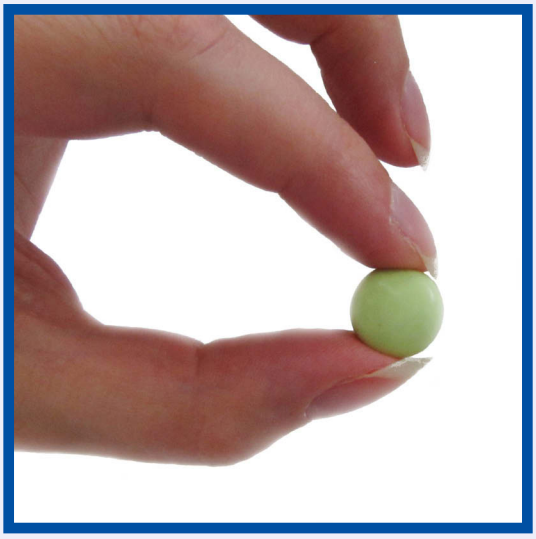
2015



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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

Edited by Simon Mills and Roberta Hutchins
Cover photographs by Prof Salvador Cañigueral Folcara (*Ononis spinosa*)
and Martin Willoughby
Cover and text design by Martin Willoughby
Typeset in Optima by Roberta Hutchins

Plant illustrated on the cover: *Ononis spinosa*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
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Notes for the Reader
Abbreviations
The monograph text
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Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

ONONIDIS RADIX

2015

Restharrow Root

DEFINITION

Restharrow root consists of the dried roots of *Ononis spinosa* L.

The material complies with the monograph of the European Pharmacopoeia [Restharrow root].

CONSTITUENTS

Isoflavones including trifolirhizin, formononetin together with its 7-O-glucoside-6''-malonate and 7-O-glucoside (= ononin) [Háznagy 1978; Köster 1983], biochanin A 7-O-glucoside, medicarpin and related compounds [Fujise 1965; Háznagy 1978; Köster 1983; Pietta 1983; Dannhardt 1992]. Triterpenes, notably α-onocerin [Háznagy 1978; Rowan 1972; Spilková 1982; Pauli 2000, Daruhazy 2008]; phytosterols, especially β-sitosterol [Köster 1983; Rowan 1972, Daruhazy 2008]; deoxybenzoines, especially ononetin [Straub 2013]; phenolic acids [Luczak 1991]; tannins [Dedio 1977a]; minerals [Steinegger 1992] and about 0.02% of essential oil containing mainly *trans*-anethole, carvone, menthol and aromatic hydrocarbons [Hilp 1975; Dedio 1977b; Hesse 1977].

CLINICAL PARTICULARS

Therapeutic indications
Irrigation of the urinary tract, especially in cases of inflammation and renal gravel, and as an adjuvant in treatment of bacterial infections of the urinary tract [Schilcher 1987, 1990; Kartnig 2007; Weiß 2009].

Posology and method of administration

Dosage

Adults: An infusion of 2-3 g of dried material (the mixture being strained after 20-30 minutes) two to three times per day; equivalent preparations [Wichtl 2009].

Method of administration
For oral administration.

Duration of administration
No restriction. If symptoms persist or worsen, medical advice should be sought.

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

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PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

Diuretic activity

In vivo experiments
Four preparations of restharrow (presumed to be from the root) were administered intragastrically to different groups of 4 rats, each dose being equivalent to 0.3 g of root and including 20 mL of water. The controls were water or theophylline (5 mg/kg), administered to the same rats several days later. The volumes of urine collected over a 5-hour period following administration of the respective preparations were: dried methanolic extract (rich in flavonoids) 19.9 mL, ash (rich in minerals, especially potassium) 18.7 mL, a mixture of methanolic extract and ash 20.9 mL, and aqueous infusion 21.4 mL, compared to 15.1 mL for water and 17.9 mL for theophylline. Corresponding amounts of sodium, determined by atomic absorption spectrophotometry in the urine, were 20, 32, 21 and 24 mg, compared to 6 and 16 mg with water and theophylline respectively. The potassium figures were 95, 79, 66 and 62 mg, compared to 44 and 61 mg with water and theophylline. These results demonstrated moderate diuretic ($p<0.001$) and saluretic activity, particularly natriuretic, in all cases higher than that of theophylline at 5 mg/kg. It was concluded that the diuretic activity of restharrow root was caused by its content of potassium salts and flavonoid glycosides [Rebuelta 1981].

An ethanolic extract (not defined) at a dose corresponding to 2 g/kg b.w. significantly increased urinary volume in rats by 103% ($p<0.05$) compared to saline. No influence was observed on sodium or potassium elimination. Intraperitoneal administration did not show any diuretic effect at doses of drug up to 500 mg/kg [Bolle 1993].

Older studies gave the following results: an infusion from restharrow root administered orally to rabbits increased the urinary output by approx. 26% [Vollmer 1937a]; in mice an increase of urinary output and chloride excretion was demonstrated [Vollmer 1937a], while in rats the excretion of urea and chloride increased after oral administration [Vollmer 1937b].

After oral administration to rats, infusions of the root caused slight diuresis (average of 12%) and decoctions an antidiuretic effect of 7-20%. In these studies the aqueous residue after steam distillation showed an antidiuretic effect of 7-16 % depending on the duration of distillation, whereas 0.5-1.0 mL of the essential oil obtained by steam distillation (2-4 hours) produced a diuretic effect [Hilp 1976].

Other effects

In vitro experiments
A methanolic restharrow root extract (6:1) was found to inhibit 5-lipoxygenase selectively with an IC_{50} of 7.8 µg/mL. Medicarpin, isolated from the extract, inhibited leukotriene B_4 formation with an IC_{50} of 6.7 µM [Dannhardt 1992].

Ononetin showed a high potency in blocking the melastin-related Transient Receptor Potential cation channel, subfamily M, member 3 (TRMP3 channels) expressed in neuronal and non-neuronal tissues of the human body [Straub 2013].

In vivo experiments
In the phenylquinone writhing test in mice, an ethanolic restharrow root extract (not further defined) reduced reaction to pain by up to 80% at doses of 100 and 500 mg/kg b.w. after intraperitoneal administration, while no effect was observed after oral administration. No analgesic effects were observed in the hot plate test in mice after oral or intraperitoneal administration of the extract [Bolle 1993].

In the carrageenan-induced rat paw oedema test, the same extract significantly reduced oedema by a maximum of 46% after 3 hours ($p<0.05$) at an intraperitoneal dose corresponding to 500 mg of restharrow root per kg b.w., while no significant effects were observed at 100 mg/kg [Bolle 1993].

Pharmacokinetic properties
No data available.

Preclinical safety data
An ethanolic extract (not further defined), administered orally or intraperitoneally at a daily dose corresponding to 2 g/kg b.w. for 14 days to rats or mice did not cause any visible toxic effects [Bolle 1993].

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MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Second Edition, 2003
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
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ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Second Edition, 2003
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Second Edition, 2003
FUMARIAE HERBA	Fumitory	Supplement 2009
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Supplement 2009
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Second Edition, 2003
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Second Edition, 2003

LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Supplement 2009
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Second Edition, 2003
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Second Edition, 2003
RATANHIAE RADIX	Rhatany Root	Supplement 2009
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Second Edition, 2003
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Second Edition, 2003
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Second Edition, 2003
SALICIS CORTEX	Willow Bark	Second Edition, 2003
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
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SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Second Edition, 2003
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Supplement 2009
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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The second edition of ESCOP Monographs, published as a hardback book in 2003 with a Supplement in 2009, has been widely acclaimed for its authoritative information on the therapeutic uses of herbal medicines. Monographs covering a total of 107 herbal substances include extensive summaries of pharmacological, clinical and toxicological data, and copious references to scientific literature form an important part of each text.

Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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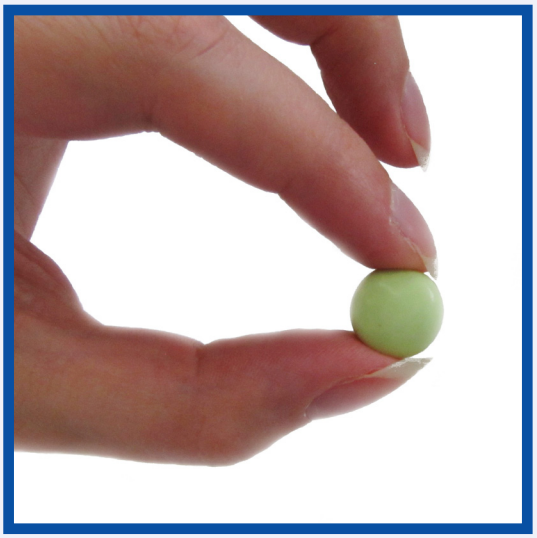
The Scientific Foundation for Herbal Medicinal Products

Orthosiphonis folium

Java Tea

2014



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ORTHOSIPHONIS FOLIUM
Java Tea

2014

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

Edited by Simon Mills and Roberta Hutchins
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Plant illustrated on the cover: *Orthosiphon stamineus*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

ORTHOSIPHONIS FOLIUM 2014

Java Tea

DEFINITION

Java tea consists of the fragmented, dried leaves and tops of stems of *Orthosiphon stamineus* Benth. (*O. aristatus* Miq.; *O. spicatus* Bak.). It contains not less than 0.05% of sinensetin ($C_{20}H_{20}O_7$; M_r 372.4) (dried drug).

The material complies with the monograph of the European Pharmacopoeia [Java tea].

CONSTITUENTS

Up to 12% of minerals with approx. 3% of potassium [Van der Veen 1979; Fintelmann 2002; Schilcher 2010].
Approx. 0.2% of lipophilic flavones including sinensetin and isosinensetin [Bombardelli 1972; Schneider 1973; Wollenweber 1985; Malterud 1989; Pietta 1991; Sumaryono 1991; Proksch 1992; Takeda 1993; Olah 2003; Merfort 2007; Wichtl 2009], flavonol glycosides [Sumaryono 1991; Proksch 1992].
Rosmarinic acid (0.1-0.5%) [Gracza 1984; Lamaison 1990; Proksch 1992; Takeda 1993] and other caffeic acid depsides [Sumaryono 1991; Proksch 1992; Olah 2003], inositol [Van der Veen 1979] and up to 0.7% of essential oil with mainly sesquiterpenes [Schut 1986; Merfort 2007].
Pimarane, isopimarane and staminane diterpenes, e.g. the highly oxygenated orthosiphols [Masuda 1992a, 1992b; Takeda 1993; Shibuya 1999a; Stampoulis 1999a, 1999b; Ohashi 2000a, 2000b; Tezuka 2000, Awale 2001, 2002, 2003a, 2003b, 2003c; Nguyen 2004], triterpenes such as ursolic and oleanolic acid [Proksch 1992; Tezuka 2000] and chromenes such as methylripariochromene A [Guérin 1989; Shibuya 1999b; Ohashi 2000b].

CLINICAL PARTICULARS

Therapeutic indications

Irrigation of the urinary tract, especially in cases of inflammation and renal gravel, and as an adjuvant in the treatment of bacterial infections of the urinary tract [Proksch 1992; Premgamone 2001; Fintelmann 2002; Jänicke 2003; Bradley 2006; Merfort 2007; Premgamone 2007; Kieley 2008; Schilcher 2010].

Efficacy in these indications is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Adults: An infusion of 2-3 g of dried material in 150 mL of water two to three times per day; equivalent preparations [Van Hellemont 1988; Premgamone 2001; Fintelmann 2002; Jänicke 2003; Bradley 2006; Premgamone 2007; Wichtl 2009; Schilcher 2010].

Method of administration
For oral administration.

Duration of administration
If symptoms persist or worsen, medical advice should be sought.

Contraindications
None known.

Special warnings and special precautions for use
Java tea should not be used in patients with oedema due to impaired heart and kidney function [Wichtl 2009].

Interaction with other medicaments and other forms of interaction
None reported.

E/S/C/O/P MONOGRAPHS

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antibacterial activity
Bacteriostatic [Van der Veen 1979; Chen 1989] and fungistatic [Wolters 1966] activity has been demonstrated, and the bacteriostatic activity has been attributed to caffeic acid derivatives, particularly rosmarinic acid [Proksch 1992].

Receptor-binding activity
An aqueous (1g/100 ml) and a methanolic (5:1) extract showed dose-dependent inhibitory activity on ^{125}I-TGF-β_1 binding to its receptor in Balb/c 3T3 cells. The inhibition was 42.3% at a concentration of 10 µL/mL of the aqueous extract. Complete inhibition was observed with 100 µL/mL of the aqueous and 200 µL/mL of the methanolic extract. Ursolic and oleanolic acid inhibited binding of ^{125}I-TGF-β_1 to its receptor with IC_{50} values of 6.9 ± 0.8 and 21.0.± 2.3 µM, respectively [Yoshimura 2003].

Antagonistic effects on the adenosin A_1 receptor (which might cause diuretic and saluretic effects) have been shown for seven flavonoids obtained by bioassay-guided fractionation of an 80% methanol extract [Yuliana 2009].

Enzyme-inhibiting effects
A hexane extract (not further specified) inhibited ouabain-sensitive activity of Na^+/K^+-ATPase from rat brain with an IC50 of 8.5 µg/mL (compared to ouabain with an IC_{50} of 1.3 µg/mL) indicating a diuretic potential [Ngamrojanavanich 2006].

Effects on lipoxygenase activity
Various isolated lipophilic flavones inhibited 15-lipoxygenase [Lyckander 1992]. In another study, flavonoids isolated from Java tea prevented inactivation of soybean 15-lipoxygenase. The compounds with the strongest enzyme-stabilizing effects, 5,7,4′-trimethylapigenin, eupatorin and 5,7,3′,4′-tetramethylluteolin, gave 50% protection at concentrations of 2.0, 2.4 and 4.3 µM, respectively [Lyckander 1996].

Antioxidant effects
Aqueous leaf extracts (1:10) containing total phenols between 6.54 and 9.04 mg/g dry weight showed antioxidant activity in the β-carotene/linoleic acid system; butylated hydroxytoluene and quercetin were used as references. The relative inhibition at a dose of 0.2 mL of the extracts as compared to control (distilled water) varied between 41.29% and 70.13% depending on the origin of the plant material, it was 58.42% for BHT and 72.27% for quercetin, respectively [Akowuah 2003].

A lyophilised extract (16:1; 50% methanol) showed a concentration-dependent antioxidant activity by scavenging DPPH radicals with an IC_{50} value of 0.28 mg/mL. The total antioxidant activity of 1 mg/ml of the extract was 2.73 mM Trolox equivalents. The same extract inhibited Fe^{3+}-induced lipid peroxidation in rat liver homogenate in a dose-dependent manner with an IC_{50} of 0.17 mg/ml [Yam 2007].

An ethanolic extract (10:1) demonstrated marked inhibitory effects on LPS-stimulated NO production (28.7 ± 2.0 nM) at a dose of 25 µg/mL, and on PGE_2 production in LPS-stimulated RAW 264.7 cells (6.35 ± 0.76 ng/mL) at the same dose [Hsu 2010].

A 60% methanolic extract and its fractions (hexane, chloroform, ethyl acetate, *n*-butanol and water) revealed DPPH scavenging activities with IC_{50} values of 16.7 ± 1.5 µg/mL, 126.2 ± 23 µg/mL, 31.3 ± 1.2 µg/mL, 15.3 ± 2.3 µg/mL, 13.6 ± 1.9 µg/mL and 23.0 ± 3.2 µg/mL, respectively [Abdelwahab 2011].

Nitric oxide inhibiting effect
Highly oxygenated isopimarane-type diterpenes (e.g. orthosiphols, deacetylorthosiphols and siphonols) isolated from a methanolic extract inhibited NO production in LPS-activated macrophage-like J774.1 cells. Some of them were more potent than L-NMMA (N^{γ}-monomethyl-L-arginine) used as positive control [Awale 2003a, 2003b, 2003c, 2003d; Nguyen 2004].

Cytoprotective effects
A 60% methanolic extract and its fractions (hexane, chloroform, ethyl acetate, *n*-butanol and water) showed significant protective effects against hydrogen peroxide-induced apoptosis on MDA-M231 epithelial cells at concentrations between 62.5 µg/mL and 500 µg/mL ($p<0.05$ and 0.01, respectively). IC_{50} values ranged between 23.1 ± 3.2 µg/mL and 56.4 ± 6.0 µg/mL at a concentration of 62.5 µg/mL, between 20.6 ± 0.9 µg/mL and 84,8 ± 4.0 µg/mL at a concentration of 125 µg/mL, between 22.47 ± 1.6 µg/mL and 100.0 ± 16.5 µg/mL at a concentration of 250 µg/mL and between 25.9 ± 1.0 µg/mL and 100.0 ± 27.8 µg/mL at a concentration of 500 µg/mL [Abdelwahab 2011].

Antiproliferative effects
Isolated highly oxygenated diterpenes (orthosiphols A, B, K, L, M and N as well as norstaminone A and neoorthosiphol A) displayed mild antiproliferative activity on liver metastatic murine colon 26-L5 carcinoma and human HT-1080 fibrosarcoma cell lines (ED_{50} between 12.8 and 63.1 µg/mL and between 18.6 and <100 µg/mL, respectively). Isolated flavones showed more potent activity on these cell lines, with the strongest being 5,6-dihydroxy-4′7-dimethoxyflavone with an ED_{50} value of 2.3 and 3.0 µg/mL, respectively [Awale 2001]. In a similar experiment, orthosiphols D, E, O, P and Q as well as nororthosiphonolide A and orthosiphonone A showed the same effects [Awale 2002]. Isolated diterpenes and flavonoids showed weak cytotoxicity towards murine colon carcinoma 26-L5 cells [Tezuka 2000].

In the enzyme-linked immunosorbent assay a 50% ethanolic extract significantly reduced the vascular endothelial growth factor level (211 ± 0.26 pg/mL cell lysate) compared to control (378 ± 5 pg/mL). In various angiogenesis models the extract caused inhibition of human umbilical vein endothelial cell proliferation at concentrations between 6.25 and 100 µg/mL with an IC_{50} of 48.23 µg/mL compared to vincristine with an IC_{50} 0.13 µg/mL [Ahamed 2011].

Effects on metabolic enzymes
The inhibitory effects of a 50% methanolic extract on various isoforms of UDP-glucuronyl transferases (UGT) was examined using 4-methylumbelliferone as substrate. At doses between 0.01 and 50 µg/mL, the extract inhibited the activities of most

of the isoforms in a dose-dependent manner [Ismail 2010].

A petroleum ether extract competitively inhibited CYP2C19 activity with an IC_{50} of 67.1 µg/mL (K_i = 41.5 µg/mL), eupatorin gave a mixed type inhibition with an IC_{50} of 12.1 µg/mL (K_i = 7.1 µg/mL) [Pan 2011a]. The effects of four different extracts (petroleum ether, dichloromethane, methanol, water) as well as three constituents (sinensetin, eupatorin, rosmarinic acid) were examined in a further experiment. While CYP2C9 was not inhibited at all, CYP2D6 was inhibited only by eupatorin (IC_{50} = 3.8 µg/mL; K_i = 3.5 µg/mL). All extracts as well as rosmarinic acid and sinensetin were found to not significantly inhibit CYP2D6 activity. With regard to CYP3A4, the aqueous and methanolic extracts and sinensetin did not exhibit significant modulatory effects. However, CYP3A4 was non-competitively inhibited by the petroleum ether extract and eupatorin (IC_{50} = of 46.3 and 5.0 µg/mL, respectively; K_i = 44.9 and 3.2µg/mL, respectively). Additionally, CYP3A4 was moderately inhibited by the dichloromethane extract in a mixed-type mode (IC_{50} = 96.5 µg/mL, K_i = 93.7 µg/mL). The effect of rosmarinic acid was also moderate with an IC_{50} of 86.9 µg/mL and a K_i of 118.3 µg/mL [Pan 2011b].

Ex vivo experiments

Suppression of contractile force
Various substances isolated from the leaves of Java tea had a concentration-dependent suppressive effect on contractile responses in endothelium-denuded rat thoracic aorta strips. IC_{50} values were between 1.01×10^{-1} µmol/ml for acetovanillochromene and 8.08×10^{-3} µmol/ml for tetramethylscutellarein; nifedipine as a positive control gave a value of 1.79×10^{-5} µmol/ml [Ohashi 2000a, 2000b]. Methylripariochromene A at doses of 1.1×10^{-2} mM, 3.8×10^{-2} mM and 1.1×10^{-1} mM decreased the maximum contractions caused by Ca^{2+} at 30 mM to 73.8%, 47.0% and 21.0% respectively [Ohashi 2000b; Matsubara 1999].

After cumulative applications of 3.8×10^{-2} mM and 1.2×10^{-1} mM to spontaneously beating isolated guinea pig atria, methylripariochromene A significantly suppressed the contractile force by 18.8% ($p<0.05$) and 54.7% ($p<0.01$) respectively, without significantly reducing the beating rate [Ohashi 2000b; Matsubara 1999].

Hypoglycaemic effects
In perfused rat pancreas, the extract did not increase insulin secretion in the presence of 5.5 mM glucose, but 100µg/ml extract did potentiate glucose-induced insulin secretion [Sriplang 2007].

Gastroprotective effects
Oral administration of a 50% methanolic extract to rats at doses between 250 and 1000 mg/kg exhibited dose-dependent inhibition of ethanol-induced lipid peroxidation in the isolated stomach between 16.3 and 52.9 % ($p<0.1$ to <0.001), and at doses between 0.0625 and 1 mg/mL inhibited $FeCl_2$-induced lipid peroxidation ($p<0.05$) with an ED_{50} of 0.157 mg/mL [Yam 2009].

In vivo experiments

Diuretic activity
The volume of urine and excretion of electrolytes (K^+, Na^+, Cl^-) were increased by intravenous infusion into dogs of a 50% ethanolic extract at 18.8 mg/kg/min [Chow 1979]. Oral administration to rats of a lyophilized aqueous extract at 750 mg/kg b.w. enhanced ion excretion (K^+, Na^+, Cl^-) to a level comparable to that obtained with 100 mg/kg furosemide, but no aquaretic effect was observed [Englert 1992].

Although it is not yet clear which are the active compounds [Proksch 1992], the diuretic effect could be partially due to the potassium content of Java tea [Van der Veen 1979; Englert 1992] as well as to the flavones sinensetin and 3'-hydroxy-5,6,7,4'-tetramethoxyflavone, which exhibited diuretic activity in rats after intravenous administration of 10 mg/kg b.w. [Schut 1993].

Aqueous (5:1) and ethanolic (7:1, ethanol 70%) spray-dried extracts of Java tea were administered by gavage to male rats as single doses at two levels: the aqueous extract at 18.0 mg/kg and 180 mg/kg, the ethanolic extract at 13.5 mg/kg and 135 mg/kg. Each extract was given to 5 rats, a further 5 rats receiving water as controls, and urine was collected over a period of 6 hours. After all doses the urine volume was between 2.99 and 3.36 ml/100 g rat, significantly higher ($p \leq 0.05$) than that of controls (2.15 mL). With both doses of the aqueous extract, a significant increase ($p=0.009$) in sodium elimination was observed (0.1-0.12 mEq/ 100 g rat) compared to controls (0.05 mEq). The higher doses of aqueous and ethanolic extracts significantly ($p=0.009$ and $p=0.02$ respectively) increased chloride elimination (0.11 and 0.09 mEq/100g rat) compared to controls (0.07 mEq). No significant changes were observed in elimination of potassium or urea [Casadebaig-Lafon 1989].

A hydroethanolic extract was administered intraperitoneally to 13 male rats as a single dose of 50 mg/kg b.w.; a control group of 28 rats received hypotonic saline solution. Another group of 10 rats received hydrochlorothiazide at 10 mg/kg b.w. After 8 hours the urine volume from rats treated with the extract was significantly higher ($p<0.001$) than that of the controls and was comparable to the volume after hydrochlorothiazide treatment [Beaux 1998].

Two tinctures (1:5; ethanol 50% and 70%) were tested in male Wistar rats having a minimal diuresis of 40 % in 2 hours after 24 hours abstinence of water. The tinctures at a dose of 700 mg/kg b.w. each were compared to furosemide 30 mg/kg b.w. and control (water), urine was collected for 24 hours. Urine output was found to be 41 and 29 ml/24h/kg rat, respectively, for the tinctures compared to furosemide (81 mL) and water (32 mL). Sodium saluresis was 5.7 and 4.6 mEq/24h/kg b.w. for the tinctures compared to furosemide (4.5 mEq/24h/kg b.w.) and water (3.6 mEq/24h/kg b.w.); potassium saluresis 1.1 and 0.9 mEq/24h/kg b.w. for the tinctures compared to furosemide (2.5 mEq/24h/kg b.w.) and water (0.6 mEq/24h/kg b.w.) The quantity of uric acid was 7.0 and 5.0 mg/24h/kg b.w. for the tinctures as compared to water (3.7 mg/24h/kg b.w.) [Olah 2003].

After oral administration of two different extracts (3.3:1, methanol 100% and 2.8:1, methanol 50%) to Sprague-Dawley rats at 2 g/kg b.w., sodium and potassium excretion increased significantly ($p<0.05$ and <0.01) in the first 8 hours of treatment. The effect was comparable to hydrochlorothiazide (10 mg/kg). Repeated administration of the extracts for 7 days at a dose 0.5 g/kg b.w. led to a significant increase in urine volume ($p<0.01$) and electrolyte excretion ($p<0.05$ and <0.01). Administration of 0.5, 1 and 2 g/kg b.w. of the extracts as well as allopurinol (50 mg/kg b.w.) significantly reduced ($p<0.05$) the serum urate levels in hyperuricaemic rats after 6 hours [Arafat 2008].

An aqueous extract (9:1) at doses of 5 and 10 mg/kg b.w. was administered orally to Sprague-Dawley rats. Furosemide and hydrochlorothiazide (each at a dose of 10 mg/kg b.w.) were used as controls. Urine volume, pH, density and electrolytes were determined every hour for 4 hours. The total urine volume after 4 hours was 0.85 and 1.87 mL for the extract doses, and 3.06 and 2.55 mL for furosemide and hydrochlorothiazide respectively.

The extract increased potassium excretion considerably more than the reference substances. No significant changes in urine pH and density were observed after administration of the extract [Adam 2009].

Methylripariochromene A isolated from Java tea was suspended in 0.5% Tween 80 and administered orally to 5 rats at three dose levels (25, 50 and 100 mg/kg b.w.) followed by oral administration of saline at 20 mL/kg. The highest dose produced a significant increase ($p<0.01$) in urinary volume over a period of 3 hours, comparable to that of hydrochlorothiazide at 25 mg/kg. Excretion of K^+, Na^+ and Cl^- increased significantly at 100 mg/kg b.w. compared to controls ($p<0.05$, $p<0.01$ and $p<0.01$ respectively), but less than with hydrochlorothiazide [Ohashi 2000b; Matsubara 1999].

Anti-inflammatory and analgesic effects
A lyophilised extract (16:1, methanol 50%) at oral doses of 500 and 1000 mg/kg b.w. significantly reduced hind paw oedema in rats at 3 and 5 hours after carrageenan administration ($p<0.01$ and $p<0.01$; $p<0.01$ and $p<0.05$, respectively). The extract at a dose of 1000 mg/kg b.w. produced significant ($p< 0.05$) analgesic activity in both the acetic acid-induced writhing test and the formalin-induced licking test (late phase) in mice and rats. The extract showed no effect on the tail flick test and hot plate test in mice [Yam 2008].

Oral administration of a flavonoid-rich chloroform extract fraction of the leaves at a dose of 1000 mg/kg b.w. significantly reduced carrageenan-induced rat paw oedema after 3 and 5 hours by 41 and 57% ($p<0.05$). This fraction, at oral doses of 500 and 1000 mg/kg b.w., significantly ($p<0.01$ and $p<0.001$) prevented dye leakage into the peritoneal cavity and inhibited the carrageenan-induced oedema; the higher dose showed an effect comparable to 10 mg/kg indomethacin [Yam 2010].

Orthosiphols A and B, applied topically at 200 µg/ear, inhibited the inflammation of mouse ears induced by 2 µg of 12-*O*-tetradecanoylphorbol-13-acetate by 42% and 50% respectively [Masuda 1992b].

Antipyretic effects
A lyophilised extract (16:1, methanol 50%) at oral doses of 500 and 1000 mg/kg b.w. showed no effect on normal body temperature but did significantly ($p<0.05$ and $p<0.01$) reduce its yeast-induced elevation, with the effect persisting up to 4 hours after administration and comparable to that of 150 mg/kg b.w. paracetamol p.o. [Yam 2009].

Hypoglycaemic and other metabolic effects
A dried aqueous extract (yield 3.3%) dissolved in saline was administered to rats by gavage at doses of 0.5 and 1.0 g/kg b.w.; the control group received saline at 5 mL/kg b.w. In rats treated with an oral glucose load or streptozotocin, the extract produced a hypoglycaemic effect (no values given). The effect of the extract on streptozotocin-induced diabetes was comparable to that of 10 mg/kg of glibenclamide [Mariam 1996].

An aqueous extract (25:1) at an oral dose of 0.2 to 1.0 g/kg b.w. significantly ($p<0.05$) decreased plasma glucose concentration comparable to glibenclamide (5 mg/kg b.w.) in streptozotocin-induced diabetic rats. After repeated daily administration of 0.5 g/kg b.w. for 14 days, it significantly ($p<0.05$) reduced plasma glucose on days 7 and 14. At the end of the study, plasma triglyceride concentration was significantly ($p<0.05$) lower in the treatment group compared to control. Plasma HDL cholesterol was significantly ($p<0.05$) increased in animals treated with the extract [Sriplang 2007].

Blood glucose, urea nitrogen and creatinine were slightly increased after oral administration of an aqueous extract (9:1) at doses of 5 and 10 mg/kg b.w. to Sprague-Dawley rats [Adam 2009].

A subfraction of a chloroform extract significantly ($p<0.05$) reduced blood glucose levels in the subcutaneous glucose tolerance test at a dose of 1 g/kg b.w. in rats loaded subcutaneously with 150 mg/kg b.w. glucose. In streptozotocin-induced diabetic rats this fraction did not exhibit a hypoglycaemic effect up to 7 hours after treatment [Mohamed 2011].

Effects on blood pressure and heart rate
Methylripariochromene A at doses of 50 and 100 mg/kg b.w. was administered subcutaneously to conscious, stroke-prone, spontaneously hypertensive male rats. A decrease of 15-30 mm Hg in mean systolic blood pressure was observed from 3.5 to 24 hours with the higher dose ($p<0.05$ and $p<0.01$ respectively), whereas no changes were observed in the control group. The lower dose caused a significant decrease ($p<0.05$) only at 8 hours. The substance also significantly ($p<0.01$) reduced the heart rate (75 and 45 beats/min at 6.0 and 8.5 hours, respectively, after administration of the higher dose), initial values in the treatment groups being 334 (at 50 mg/kg) and 340 (at 100 mg/kg) beats/min, and in the control group 345 beats/min. A slight decrease in heart rate was observed 6 hours after administration of the lower dose; the heart rate returned to baseline after 24 hours [Ohashi 2000b; Matsubara 1999].

Hepatoprotective effects
Pretreatment of rats with a lyophilised extract (16:1, methanol 50%) at oral doses of 125, 250, 500 and 1000 mg/kg dose-dependently reduced CCl_4-induced necrotic changes in rat liver. The increase of serum ALT and AST activity was inhibited significantly by doses of 250, 500 and 1000 mg/kg p.o. ($p<0.05$, $p<0.01$ and $p<0.01$ respectively) [Yam 2007].

Two groups of 8 male Sprague Dawley rats received a 95% ethanolic extract (yield 8.1 %) at a dose of 100 or 200 mg/kg b.w. orally for two months as well as a parenteral dose of thioacetamide three times weekly at a dose of 200 mg/kg b.w.. Treatment with the extract, as well as with silymarin (50 mg/kg b.w. orally), significantly ($p<0.05$) reduced the level of liver function biomarkers (ALT, AST, ALP, bilirubin) and malondialdehyde as antioxidant parameter. At a dose of 100 mg/kg b.w. the effect was only marginal, whereas at 200 mg/kg b.w. the extract prevented liver damage induced by thioacetamide as could be shown by 7 different parameters [Alshawsh 2011].

Gastroprotective effects
Oral administration of a 50% methanolic extract at doses between 125 and 1000 mg/kg administered together with absolute ethanol significantly decreased the ulcer index from 9.0 ± 0.5 in the control group to values between 6.7 ± 0.6 and 1.2 ± 0.3 ($p<0.01$ and 0.001 respectively); as compared to omeprazole (30 mg/kg b.w., ulcer index 1.8 ± 0.5). Histological studies of the rat stomach also showed a marked prevention of mucosal damage in groups which received the mentioned doses of the extract together with omeprazole [Yam 2009].

Antiproliferative and antiangiogenic effects
A 50% ethanolic extract demonstrated a 47.6 ± 6.4 % suppression ($p<0.05$) of HCT 116 tumour growth in athymic mice at an oral daily dose of 100 mg/kg b.w., and an 83.4 ± 4.1 % suppression ($p<0.01$) at a dose of 200 mg/kg b.w. for 4 weeks. In the enzyme-linked immunosorbent assay the extract at 200 mg/kg significantly ($p<0.05$) reduced the vascular endothelial growth factor level (91 ± 2 pg/mL tissue homogenate) compared to control (136 ± 4 pg/mL) [Ahamed 2011].

Pharmacological studies in humans

Diuretic activity
In a placebo-controlled, double-blind crossover study, no influence on 12- or 24-hour urine output or sodium excretion was observed in 40 healthy volunteers after administration of 600 mL (3 times 200 mL at 4-hour intervals) of a decoction equivalent to 10 g of dried leaf [Doan 1992].

Six healthy male volunteers drank 4 times 250 mL of a decoction of Java tea at 6-hourly intervals during one day, for comparison they drank the same amounts of water on a separate control day. The acidity of the urine increased 6 hours after ingestion of the tea. There were no changes in urine volume or electrolytes [Nirdnoy 1991].

From much earlier observations in healthy volunteers, however, increased diuresis was reported after oral administration of aqueous extracts of Java tea (400 mL/day of a 3.75% extract, 400 mL/day of a 15% extract, 500 mL/day of a 3.3% extract) [Schumann 1927; Gürber 1927; Westing 1928].

Clinical studies
An open study in 67 patients suffering from uratic diathesis did not reveal any influence of Java tea on diuresis, glomerular filtration, osmotic concentration, urinary pH, plasma content or excretion of calcium, inorganic phosphorus and uric acid during 3 months of treatment [Tiktinsky 1983].

Forty-eight patients with nephrolithiasis identified by ultrasonography were recruited and randomly assigned to two groups. For a period up to 18 months they received 5g of a related species *O. grandiflorus* daily (as an infusion) or 5-10 g of a sodium potassium citrate solution as a control. Every 5 to 7 weeks the patients were interviewed, given a kidney ultrasound, and urine samples were collected. From the recorded ultrasound images, rates of stone size reduction per year were calculated. The mean value was 28.6 ± 16.0% and 33.8 ± 23.6% for verum and control respectively, with no significant difference [Premgamone 2001.

In a placebo-controlled study, patients suffering from nephrolithiasis with multiple health complaints (at least two active symptoms), and negative for urine white blood cells, received an aqueous dry extract at a daily dose corresponding to 3.2 to 3.6 g dried leaves (n=36) or placebo (n=40) for 14 days. Neither group was permitted to consume any of 25 purine-rich foods during treatment. The primary measure was the reduced sum of active severity of symptoms as recorded using the visual analogue scale before and after therapy (i.e. on day 7 and 14). The mean of the total symptom scores (95% CI) was decreased significantly ($p<0.001$) in both groups; from 185.6 to 94.7 in the verum group and from 196.1 to 89.6 in the placebo group [Premgamone 2009].

Pharmacokinetic properties

Six adult male Sprague-Dawley rats were administered either a Java tea extract (containing 15 mg/kg sinensetin, 21 mg/kg eupatorin and 5 mg/kg 3′-hydroxy-5,6,7,4′-tetramethoxyflavone) orally or a mixture containing 2.5 mg/kg of each of these flavonoids intravenously, in a cross-over study with a wash out period of one week. The calculated mean absolute oral bioavailability for sinensetin was 9.4%, for eupatorin 1.0% and for 3′-hydroxy-5,6,7,4′-tetramethoxyflavone 1.5% [Loon 2005].

One hour after oral administration of an extract (not further specified) to rats, the maximum plasma concentration of betulinic acid was 1.2 ± 0.3 µg/mL [Akowuah 2008].

Preclinical safety data

In vitro experiments
In a somatic segregation assay using the diploid strain *Aspergillus nidulans* D-30, no genotoxic effects (mitotic crossover, chromosome malsegregation, clastogenic damage) were detected after plate incorporation of an ethanolic extract of Java tea (7.4% dry matter) at 1.4 mg/mL [Ramos Ruiz 1996].

In the Ames test, with and without metabolic activation, an aqueous extract (yield 4.8%) at a dose of up to 5000 µg/plate did not increase the number of revertant colonies. In the mouse bone marrow assay the extract did not alter the polychromatic: normochromatic erythrocytes ratio, nor did it increase the incidence of micronucleated polychromatic erythrocytes. No overt toxicity and no change in CYP1A and 2B9/10 activities were observed [Muhammad 2011].

In vivo experiments
The LD_{50} of a lyophilised extract (16:1, methanol 50 %) after oral administration to rats was higher than 5 g/kg b.w. [Yam 2008].

Acute toxicity
The acute toxicity of an extract (containing 0.15 % sinensetin, 0.21 % eupatorin and 0.05 % 3′-hydroxy-5,6,7,4′-tetramethoxyflavone) was studied in Sprague Dawley rats which had received a single oral dose of 5 g/kg b.w. and been observed for 14 days. The animals did not show any signs of toxicity. General behaviour, body weight, food and water intake, relative organ weight, haematology and clinical biochemistry were unaffected as compared to control. The LD_{50} was estimated to be more than 5 g/kg b.w. [Abdullah 2009].

A dry aqueous extract re-dissolved in saline was administered to rats by gavage at 0.5 and 1.0 g/kg b.w., while a control group received saline only (5 ml/kg). Treatment of 6 normoglycaemic rats with the extract at 0.5 mg/kg b.w. had no significant effect on fasting blood glucose levels during a 7-hour period. At 1.0 mg/kg, however, a significant decrease ($p<0.05$) in blood glucose concentration was observed in comparison with the control group [Mariam 1996].

A single dose of 5 g/kg of a 50% ethanol extract was given orally to 5 healthy Sprague-Dawley female adult rats. The rats were observed for clinical signs for 3 h and then periodically for 14 days. The extract caused neither visible signs of toxicity nor mortality until the end of observation period [Mohamed 2010].

Subchronic toxicity
Possible toxic effects following 14 days of oral administration of a methanolic extract (not further specified) were investigated in female Sprague Dawley rats. Control groups were given distilled water (vehicle) orally while the four test groups received 0.5 g/kg, 1 g/kg, 3 g/kg and 5 g/kg b.w. of the extract daily. Toxicity of the extract was evaluated by the incidence of lethality and blood serum biochemical parameters. Neither lethality nor toxic effects were seen in any group. A significant ($p< 0.05$) decrease in several serum biochemical parameters i.e. AST and ALT and increase in liver weight was observed in young female rats. No delayed toxic effects or lethality were observed in all animals during the following 14 days [Chin 2008].

In a study in female and male Sprague Dawley rats, a 50% ethanolic extract was administered orally at doses of 1.25, 2.5 and 5 mg/kg b.w. per day for 28 days. There were no signs of mortality and no significant differences in the general condition, growth or haematological parameters. The animals were sacrificed with subsequent examination of their organ weights, and gross as well as microscopic appearance of the

organs. There was no difference between treatment groups and a control group [Mohamed 2010].

Clinical safety data
In a study involving 48 patients suffering from nephrolithiasis, no adverse effects were reported in those who had consumed 5 g of Java tea daily for 18 months [Premgamone 2001].

In a study with nephrolithiasis patients receiving an aqueous dry extract at a daily dose corresponding to 3.2 – 3.6 g dried leaves (n=36) or placebo (n=40) for 14 days, adverse effects including musculo-skeletal and gastrointestinal complaints, as well as headache, occurred mainly during the first week in the verum group [Premgamone 2009].

One case of hepatitis after intake of a product containing powdered Green tea and Java tea was reported but a causal relationship between the hepatitis and the intake of Java tea could not be established [Garcia-Moran 2004].

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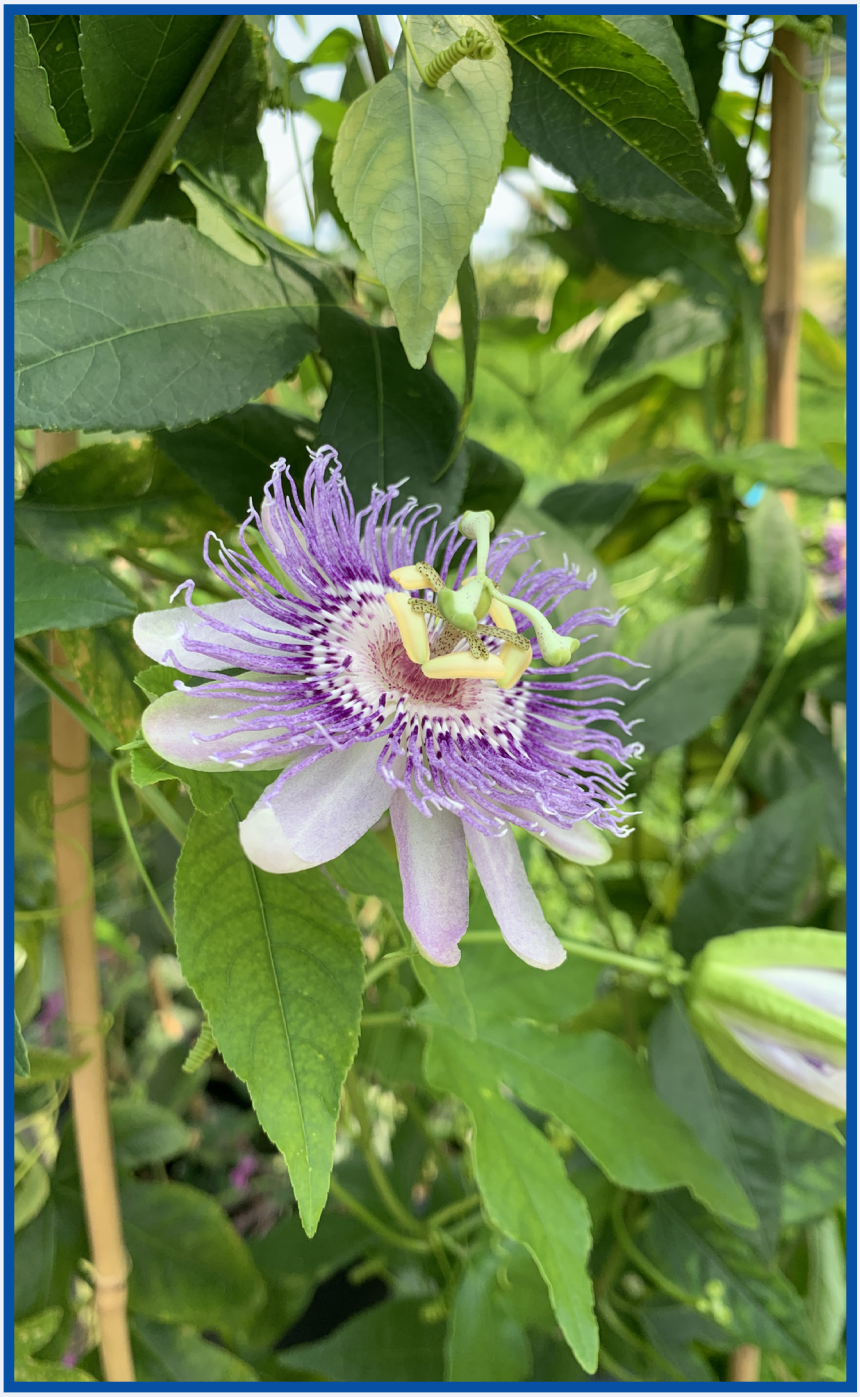

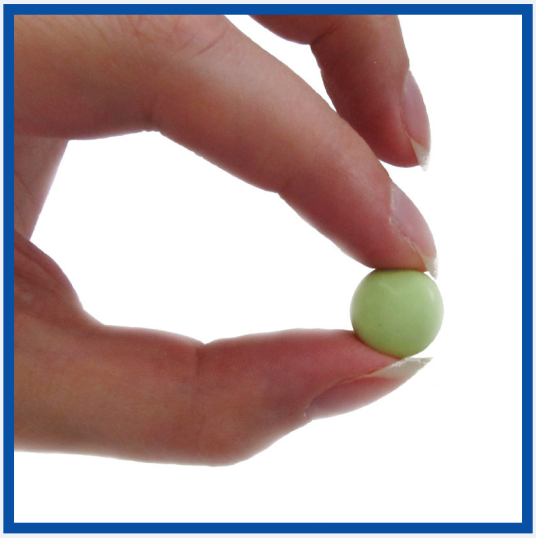

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FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

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i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-kB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-b	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

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PASSIFLORAE HERBA

2023

Passionflower herb

DEFINITION

Passionflower herb consists of the fragmented or cut, dried aerial parts of *Passiflora incarnata* L. of the swertisin chemotype or the isovitexin chemotype or a mixture of both. It may also contain flowers and/or fruits. It contains not less than 1.0 per cent of total flavonoids, expressed as isovitexin ($C_{21}H_{20}O_{10}$, M_r 432.4), calculated with reference to the dried drug [Passionflower herb].

The material complies with the European Pharmacopoeia [Passionflower herb].

Fresh material may also be used provided that when dried it complies with the European Pharmacopoeia.

CONSTITUENTS

Flavonoids, mainly glycosylflavonoids of apigenin and luteolin, e.g. isovitexin, isoorientin and their 2"-*O*-β-D-glucosides, schaftoside, isoschaftoside, vicenin-2 and swertisin, with considerable variation in qualitative and quantitative composition according to source [Glotzbach 1968, Schilcher 1968, Geiger 1986, Qimin 1991, Schmidt 1993, Rehwald 1994, Chimichi 1998, Rahman 1997, Raffaelli 1997, Voirin 2000, Grice 2001, Ferreres 2007, Wohlmuth 2010, Avula 2012]; traces of essential oil containing more than 150 components [Buchbauer 1992]; a cyanogenic glycoside, gynocardin [Spencer 1984]; amino acids with GABA as dominant component [Meier 1995, Elsas 2010].

Traces of β-carboline alkaloids (e.g. harmol, harmalol, harman) may be present, depending on the source, the maturity of the plant and the extraction method used [Löhdefink 1974, Lutomski 1981, Brasseur 1984, Rehwald 1995, Tsuchiya 1999a, 1999b, Grice 2001, Abourashed 2003, Frye 2007], but investigations using highly sensitive UPLC-UV-MS detected no β-carboline alkaloids in a number of validated samples of *Passiflora incarnata* [Avula 2012].

The structure of a tri-substituted derivative of α-naphthoflavone (7,8-benzoflavone), isolated from a methanolic leaf extract [Dhawan 2001a; 2004], has never been disclosed and attempts to re-isolate the compound were not successful [Holbik 2010].

CLINICAL PARTICULARS

Therapeutic indications
Tension, restlessness and irritability with difficulty in falling asleep [Bradley 1992, Akhondzadeh 2001a, Movafegh 2008, Meier 2013, Krenn 2016, Gibbert 2017, Lee 2020].

Posology and method of administration

Dosage

Adult single dose, three to four times daily: 0.5-2 g of the drug; 2.5 g of drug as infusion; 1-4 mL of tincture (1:8); other equivalent preparations [Bradley 1992, Schilcher 1995, Meier 2013, Krenn 2016].

Children from 3 to 12 years under medical supervision only: proportion of adult dose according to body weight.

Method of administration
For oral administration.

Duration of administration
No restriction; neither dependence nor withdrawal symptoms have been reported.

Contra-indications
None known.

E/S/C/O/P MONOGRAPHS

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Special warnings and special precautions for use
None required.

Interaction with other medicinal products and other forms of interaction
None reported.

Pregnancy and lactation
No data available.

The use of passionflower herb dry extracts, fluid extracts and mother tinctures is assumed to be safe in the first and second trimester as well as during lactation, but should not take place without medical advice. The use in the third trimester is not recommended [Herba Pro Matre 2014].

Effects on ability to drive and use machines
None known.

Undesirable effects
Hypersensitivity possible in very rare cases [Smith 1993, Echechipia 1996].

One case of nausea, bradycardia and ventricular arrythmia was reported [Fisher 2000].

Overdose
No case of overdose reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Effects on receptor binding
Two dry extracts (3.0% and 9.1% total flavonoids respectively) were studied for their binding affinity to three receptors. At concentrations from 10 to 1000 µg/mL they did not interact with the binding sites of benzodiazepine, dopaminergic and histaminergic subtypes. Isovitexin-2"-*O*-glucoside and isoorientin-2"-O-glucoside did not inhibit the benzodiazepine binding site in concentrations up to 30 µM [Burkard 1997].

An ethanolic extract (not further specified) did not inhibit various CNS receptors expressed in Chinese hamster ovary cells, such as opioid (µ, κ, δ, OFQ), serotonin (5-$HT_{1Dβ}$, 5-HT_6, 5-HT_7), α-oestrogen, histamine (H-1, H-2), neurokinin-1 and metabotropic glutamate receptors. Only binding of ^{3}H-GABA to the $GABA_A$-receptor was potently inhibited at 1 µg/mL [Simmen 1999].

In CA1 pyramidal cells in hippocampal slices from male Sprague–Dawley albino rats, a dry extract (5.6:1; 44% ethanol) at concentrations from 128 to 512 µg/mL evoked a direct current with a dose-dependent amplitude. The current was obviously mediated by $GABA_A$-receptors as it was completely but reversibly blocked by the $GABA_A$-receptor antagonist GABAzine. After removal of amino acids from the extract, including GABA, no effect was observed at a dose of 138 µg/mL [Elsas 2010].

[^{3}H]-GABA uptake into rat cortical synaptosomes was inhibited by a dry extract (5-7:1, 50% ethanol) with an IC_{50} value of 97.5 µg/mL, but no effects on GABA release and GABA transaminase activity were seen. The binding of the specific $GABA_A$-receptor antagonist [^{3}H]-SR95531 to rat $GABA_A$-receptors and of the $GABA_B$ receptor antagonist [^{3}H]-CGP 54626 to $GABA_B$-receptors were inhibited by the extract in a concentration-dependent manner (IC_{50} 101 µg/mL and 120 µg/mL respectively). The ethanol- and benzodiazepine-sites of the $GABA_A$-receptor were not affected [Appel 2011].

Other effects
Fifteen strains of *Helicobacter pylori* were inhibited by a 95% methanolic extract (MIC 50 µg/mL) [Mahady 2005].

In a study on circadian rhythm in NIH3T3 clone 5611 cells, an extract (50% ethanol, yield 20.5%) at 100 µg/mL significantly reduced the mRNA expression of the core clock genes brain-muscle ARNT-like protein 1 (Bmal1) and cryptochrome 1 (Cry1) at 4 h, circadian locomotor output cycles kaput at 4 and 8 h and period circadian protein 1 (Per1) at 8 h ($p<0.05$ and $p<0.01$). From 12 hours onwards until 24 hours it increased the mRNA expression of Bmal1, Per1, Per2 and Cry1 as well as of SOD1 and GPx. Isovitexin-2"-*O*-glucoside and homoorientin at 10 µg/mL and isoschaftoside and homoorientin at 30 µg/mL significantly ($p<0.05$ and $p<0.01$) enhanced the circadian expression of Per2 [Toda 2017].

In rat C6 glioma cells, an extract (60% ethanol; leaves and fruits 8:2) at 0.6 and 6 µg/mL significantly ($p<0.05$ and $p<0.005$ respectively) increased the ROS production as compared to vehicle [Kim 2020].

A methanolic extract significantly ($p<0.05$) increased the expression of the brain derived neurotrophic factor (BDNF) in human neuroblastoma SH-SY5Y cells by 74.1% at 30 µg/mL and by 56.6% at 10 µg/mL. In a metabolomic approach, primary metabolites belonging to the Krebs cycle were shown to be positively correlated with BDNF activity [Gonulalan 2020].

The organic anion transporting polypeptides 2B1 and 1A2 (OATP2B1 and OATP1A2) are involved in the transport of endogenous sulphated steroid hormones, which are assumed to play a role in neurosteroid homeostasis. The effect of apigenin, orientin and vitexin, as well as of two preparations (425 mg dry extract, 5-7:1, 50% ethanol or 300 mg powdered drug respectively), on the OATP2B1- and OATP1A2-mediated transport of dehydroepiandrosterone sulphate (DHEAS), estrone-3-sulphate (ES) and pregnenolone sulphate (PS) were tested. Apigenin decreased the accumulation of all three sulphates in OATP2B1 and OATP1A2 overexpressing cells. Orientin reduced the cellular amount of DHEAS and PS in OATP2B1 expressing cells and showed no influence in OATP1A2 expressing cells. For vitexin, a reduction of ES and PS in OATP2B1 and of PS in OATP1A2 expressing cells was observed. Orientin was identified as inhibitor and substrate for both transporters, vitexin only for OATP2B1 and apigenin only for OATP1A2. At a concentration of 50 µg/mL, the dry extract significantly ($p<0.05$) reduced the transport of all steroids with both transporters, whereas the effect of the powdered drug was distinctly less [Schäfer 2022].

In vivo experiments

Sedative and anxiolytic effects
After oral administration of a dry extract (2.6% flavonoids) at a dose of 800 mg/kg b.w. to mice, a significant ($p<0.01$) anxiolytic effect was observed with a prolongation of the hexobarbital-induced sleeping time whereas the locomotor activity remained unaffected [Della Loggia 1981].

After i.p. administration in rats, a hydroethanolic dry extract (not further specified) at a dose of 160 mg/kg b.w. significantly ($p<0.01$) prolonged sleeping time induced by pentobarbital, and at 50-400 mg/kg, reduced spontaneous locomotor activity in a dose-dependent manner. Administered orally or i.p. at a dose of 160 mg/kg, the extract significantly ($p<0.05$ to $p<0.01$) raised

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the threshold to nociceptive stimuli in the tail flick and hot plate tests [Speroni 1988].

Rats showed reduced activity in a one-arm radial maze after one week of daily oral administration with an alcohol-free hydroethanolic extract (10 mL/kg b.w., corresponding to 5 g/kg of the drug) [Sopranzi 1990].

The activity of a liquid hydroethanolic extract from cryoground fresh plant after removal of ethanol was determined in mice. Oral treatment of mice with doses of 25 and 50 mL/kg b.w. (corresponding to 1.25 and 2.5 g/kg of dried drug respectively [Chefaro-Ardeval]) reduced exploratory and spontaneous motor activities, prolonged sleeping time induced by pentobarbital and inhibited aggressiveness and restlessness caused by amphetamine tartrate. The sedation index was comparable with that of meprobamate (250 mg/kg b.w.) and higher than that of diazepam (10 mg/kg b.w.) and chlordiazepoxide (10 mg/kg b.w.) [Galliano 1994].

No sedative effects were observed in two different experimental models in mice (hexobarbital-induced sleeping time, exploratory activity) after oral administration of a dried 30% hydroethanolic extract (1.75 and 3.5 mL/kg b.w.) [Weischer 1994].

The sedative effect of a dry extract (4.5:1, ethanol 70%), was investigated in male Swiss mice. The extract (100 mg/kg b.w.) was administered i.p. 10 min before s.c. injection of sodium pentobarbital (40 mg/kg). A distinct prolongation of mean sleeping time by 40% was observed compared to control. When administered by gastric tube 1 h before s.c. injection of amphetamine sulphate (5 mg/kg b.w.), the extract (50 mg/kg) caused a significant reduction ($p<0.05$) in hypermotility in an activity cage. In comparison with the control group an average reduction in motility of 17% was observed 120 minutes after administration of amphetamine sulphate [Capasso 1995].

An aqueous extract (79:1) administered i.p. to male Swiss mice showed sedative activity at doses of 25, 50 and 100 mg/kg b.w.. A dose-dependent reduction of the locomotor activity in an activity cage and a significant ($p<0.05$ to $p<0.01$) decrease of motor coordination at 20, 40, 80 and 120 minutes after treatment were observed in a rotarod apparatus. The pentobarbital-induced sleeping time was prolonged dose-dependently, significantly ($p<0.01$) at doses of 50 and 100 mg/kg b.w. [Capasso 1996].

Swiss mice received a hydroethanolic dry extract (2% flavonoids) at i.p. doses of 60, 125 and 250 mg/kg b.w.. A dose-dependent reduction of the spontaneous locomotor activity was recorded, significant ($p<0.05$ and $p<0.01$ respectively) at the two higher doses. Intragastric administration at the same doses resulted in a less distinct decrease of activity. Oral treatment with 30, 60 and 125 mg/kg b.w. of a more hydrophilic extract (7.4% flavonoids) induced a dose-dependent reduction of the locomotor activity, significant ($p<0.01$) at doses of 60 and 125 mg/kg. The sodium pentobarbital-induced sleeping time was increased significantly ($p<0.01$) at these doses. Additionally, a significant ($p<0.01$) delay in the onset of convulsive episodes after administration of 50 mg/kg pentetrazol i.p. and an increase in the number of survivals were observed after pre-treatment of the animals with 60 and 125 mg/kg of the extract [Speroni 1996].

The sedative and anxiolytic properties of two lyophilized extracts (30% ethanol, 7.2:1; and aqueous, 7.6:1) from cryoground fresh aerial parts were assessed after i.p. administration to male Swiss albino mice. The aqueous extract reduced the activity in the staircase and free exploratory tests and prolonged the pentobarbital-induced sleeping time at doses of 400 and 800 mg/kg b.w. (expressed in terms of dry plant material). Pre-treatment with the benzodiazepine receptor antagonist flumazenil (10 mg/kg b.w.) did not influence the activity in the staircase test. The hydroethanolic extract significantly ($p<0.05$) enhanced the activity in the staircase and the light/dark avoidance tests at 400 mg/kg b.w. [Soulimani 1997].

The anxiolytic effect of various extracts was tested in the elevated plus-maze model in mice. After oral administration of 75 to 300 mg/kg b.w. neither the petroleum ether nor the chloroform and water extracts modified the behavioural parameters. The methanolic extract showed a significant ($p<0.001$) effect at doses of 75, 100, 125 and 200 mg/kg. At doses of 125 and 200 mg/kg, the effect was comparable to that of 2 mg/kg b.w. diazepam [Dhawan 2001b].

These findings were confirmed in another study using the same test, in which extracts of the different plant parts were compared. The methanolic extracts of all plant parts were the most effective ($p<0.05$ to $p<0.001$) [Dhawan 2001c].

Five dried tinctures (not further specified; 100, 200, 300 and 400 mg/kg b.w.) and a methanolic extract (125 mg/kg b.w.) were administered orally to Swiss Albino mice before testing in the elevated plus-maze. Four tinctures and the extract showed significant ($p<0.05$) anxiolytic effects as measured by the mean time spent in the open arms. Two of the tinctures were most active at a dose of 400 mg/kg, one at 300 mg/kg and one at 200 mg/kg, the fifth one was almost without effect [Dhawan 2002a].

No significant effects on sleep latency in sleep disturbed Wistar rats and on delta power during non-REM sleep were noted 6 hours after oral administration of an aqueous extract at a dose of 300 mg/kg or at the very high doses of 1000 and 3000 mg/kg b.w. [Shinomiya 2005].

An extract (70% ethanol, standardized to 4% flavonoids) administered orally to male Swiss mice at a dose of 250 mg/kg b.w. two hours before s.c. injection of 5 mg/kg amphetamine significantly reduced the amphetamine-induced hypermotility at 30, 60, 90 and 120 minutes ($p<0.05$ to $p<0.01$) as compared to vehicle control. The barbiturate-induced sleeping time was significantly ($p<0.05$) prolonged [Capasso 2005].

Male BL6/C57J mice were treated orally with 150, 375 or 600 mg/kg b.w. of an extract (not further specified) one hour before testing in the elevated plus maze model. Diazepam (1.5 mg/kg p.o.) served as a positive control. At 375 mg/kg the extract led to a significant ($p<0.01$) increase in the time spent in the open arms and in the number of open arm entries as compared to control. The effects were comparable to the positive control. Doses of 150 and 600 mg/kg had no effect [Grundmann 2009].

In a comparable study with an extract (5-7:1, 50% ethanol) at the dose of 375 mg/kg b.w., significant increments in the time spent in the open arms ($p<0.001$) and in the number of open arm entries ($p<0.05$) were observed as compared to control. The effects were abolished by pre-treatment with the $GABA_A$/benzodiazepine receptor antagonist flumazenil (3 mg/kg, i.p., 15 min before oral treatment with the extract), but not with the 5-HT_{1A}-receptor antagonist WAY-100635 (0.5 mg/kg, i.p., 15 min before oral treatment with the extract). Doses of 150 and 600 mg/kg of the extract did not show an effect [Grundmann 2008].

Five extracts (containing 2.8-46.4% total flavonoids) were prepared from fresh or dried material using 65% ethanol or water at different temperatures and extraction times. The extracts were given to CF-1 mice at 1 g/kg b.w./day for 1 week with the drinking water. Compared to control, one of the aqueous and one of the ethanolic extracts reduced pentetrazol-induced stage 4 seizures. No influence was seen on the sensomotor activity in the rotarod model for any of the extracts. In contrast to several

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other studies, all extracts increased the anxiety level as shown by the time spent in the open arms, the extending over edges of the open arms, the ratio of open arm entries and the distance moved in the open arms in the elevated plus maze model [Elsas 2010].

BALB/c mice were treated with a methanolic extract (200, 400 or 600 mg/kg b.w. p.o.) or diazepam (2 mg/kg i.p. in the staircase test or 4 mg/kg i.p. in the locomotor activity assay). In the staircase assay the extract at 200 mg/kg and diazepam significantly ($p<0.05$ and $p<0.001$ respectively) increased the number of steps climbed. At 400 mg/kg no change was observed as compared to control, at 600 mg/kg the number of steps was significantly ($p<0.05$) reduced. The number of rears significantly decreased after all treatments ($p<0.05$ to $p<0.01$). The latter effect was reversed by pre-treatment with pentetrazol (PTZ; 10 mg/kg i.p.). A significant reduction in locomotor activity was seen with the two higher extract doses ($p<0.01$ and $p<0.001$) as well as with diazepam ($p<0.001$). PTZ only reverted the effects of the extract but not of diazepam [Aman 2016].

Male Wistar albino rats received an extract (60% ethanol; 5-7:1; 4.43% flavonoids) at dosages of 30, 100 or 300 mg/kg b.w./day with their drinking water for 7 weeks before and 2 weeks during testing. In the water maze test, the extract at 100 and 300 mg/kg significantly (all $p<0.05$ compared to control) decreased the mean latency and the mean path to the platform; the mean percentage of time the animals searched actively for the platform was significantly increased. The mean velocity of swimming was significantly ($p<0.0001$) higher after 30 and 300 mg/kg. In the memory test, a significant increase in the number of crossings over the previous platform position ($p<0.05$) and a significantly lowered stress level ($p<0.0001$) were observed after all doses. The experimental groups showed changes in the concentrations of 5-HT in the prefrontal cortex, hippocampus, hypothalamus and striatum and in the 5-HT turnover in the prefrontal cortex, hippocampus and striatum as well as of the noradrenaline metabolite 3-methoxy-4-hydroxyphenylglycol in the hypothalamus. A significant ($p<0.05$) difference was found between experimental groups for the concentration of glutamic acid in the hippocampus [Jawna-Zboińska 2016].

An extract (50% ethanol, yield 20.5%) was administered orally to male ICR mice at a dose of 100 mg/kg/day for 15 days. Significant reduction of sleep latency and an increase in sleeping time were observed in the pentobarbital-induced sleep test (both $p<0.05$ as compared to control). In the serum, the extract induced high-amplitude rhythms in the corticosterone levels and enhanced the circadian expression of Bmal1, circadian locomotor output cycles kaput and Per1. In the liver, high-amplitude rhythms in Per1, Per2, and Cry1 gene expression were observed. In the cerebrum, dopamine levels and the mRNA expression of the monoamine oxidases A and B, of catechol-*O*-methyltransferase and of glutamic acid decarboxylase were enhanced [Toda 2017].

In 6 Wistar rats, the EEG, the ocular activity and the electromyogram were measured for 9 hours the day before treatment and for 9 hours after i.p. administration of 500 mg/kg b.w. of an extract (60% ethanol; removal of the ethanol and replacement to the original volume by water). A significant ($p<0.05$) decrease in the time of wakefulness and an increase ($p<0.05$) in the time of slow wave sleep (SWS) were observed. The latency of SWS dropped from 44.4 min to 25.9 min. The effects on REM sleep were inconsistent and not significant. A trend towards a reduction of the number of REM sleep phases was observed [Ayala Guerrero 2017].

ICR mice treated orally with an extract (60% ethanol, leaves and fruits 8:2) were administered either single doses of 250 and 500 mg/kg b.w. or 250 mg/kg b.w. daily for 5 days. Immunohistochemical staining showed the primary reaction area in the brain around the mammillary body. Compared to vehicle, the serum melatonin levels were significantly elevated after the single dose of 500 mg/kg ($p<0.01$) and after the repeated doses ($p<0.05$). The immobility time increased with the single dose of 500 mg/kg ($p<0.0001$) and with the repeated doses on days 1 to 5, reaching significance ($p<0.05$) only on day 5. In SD rats, an increase in the eye closing time was observed after a single oral dose of 500 mg/kg ($p<0.0001$) and after repeated oral doses of 250 mg/kg on days 3 ($p<0.05$) and 5 ($p<0.005$) [Kim 2020].

In C57BL/6 mice, receiving the same extract orally at 500 mg/kg b.w. daily for 5 days, significant decreases in energy expenditure and activity during the dark cycle ($p<0.0005$ and $p<0.005$ respectively) as well as over 24 hours ($p<0.005$ and $p<0.05$ respectively) were observed. The serum level of melatonin and the expression of calretinin in the hypothalamus and the hippocampus were increased significantly (all $p<0.0005$). The serum serotonin level was not changed [Kim 2019a].

Addition of the same extract at 2 mg/mL to the drinking water of Sprague Dawley rats for 12 days resulted in significant increases in the melatonin and serotonin concentrations in the serum ($p=0.0339$ and $p=0.0396$ respectively) as compared to control. No significant differences in body weight gain, food intake and water consumption were seen [Kim 2019b].

Weaned piglets (n=120) received either a control diet or this diet supplemented with an extract (70% ethanol) equivalent to 1 g passionflower herb /kg fodder, standardized to 3.5% total flavonoids, for 28 days. No differences between the groups were seen in growth performance. The presence of skin lesions and the IgA and cortisol concentrations in saliva were assessed on days 1, 10, 19 and 28. In the extract group fewer ear lesions ($p<0.05$), less aggressive behaviour ($p<0.001$), higher enrichment exploration ($p<0.001$) and lower cortisol levels ($p<0.01$) were observed. The effect on tail lesions ($p<0.001$) and behavioural observations ($p<0.001$) were time-dependent [Pastorelli 2022].

Three fractions from an extract (5-7:1, 50% ethanol, fractionated with petroleum ether, chloroform and butanol) were tested in mice in the elevated plus-maze model. The animals received oral doses of the fractions equivalent to 150, 300 and 750 mg/kg b.w. of the extract. At the two lower doses, the butanol fraction significantly ($p<0.05$) increased the number of open arm entries and the time spent in open arms. The highest activity for both parameters was observed with the chloroform fraction at the lowest dose ($p<0.001$). The petroleum ether fraction remained without effect [Sampath 2011].

Effects on withdrawal symptoms

Male Wistar rats were sensitized with 0.4 mg/kg b.w. nicotine for 4 days. On day 5 their spontaneous locomotor activity was determined in an activity chamber. After 25 min habituation time, the animals received an extract (water-glycerol 1:1; 800 mg/kg b.w. i.p.) or vehicle and after another 25 min. a challenge dose of nicotine. From 20 to 40 minutes after challenge a significant ($p<0.05$ to $p<0.01$) decrease in average velocity was observed in the passionflower herb group as compared to vehicle [Breivogel 2012].

Four groups of male Wistar rats received by oral gavage either i) water for 19 days, and after one day of no treatment water for another 8 days, or ii) water for 19 days, and after one day of no treatment an extract (standardized to 7% of flavonoids, 200 mg/kg b.w. for 8 days, or iii) ethanol for 19 days (4 g/kg b.w.), and after one day of no treatment water for another 8 days, or iv) ethanol for 19 days, and after one day of no treatment the extract for 8 days. In the ethanol-treated groups the nociceptive threshold in the hot plate test increased after ethanol withdrawal. The increase was reversed by the extract ($p<0.05$). No improvement of the increased nociceptive threshold was observed in the tail

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flick test. The extract did not influence the levels of BDNF and interleukin-10 in the prefrontal cortex, the brainstem and the hippocampus [Schunck 2017].

Antitussive effects
A methanolic extract from dried leaves (yield 4.1%) was administered orally to Swiss mice at doses of 100 and 200 mg/kg b.w. before exposure to SO_2 and coughing was recorded for 5 minutes after challenge. The extract led to an inhibition of 39.4% and 65.0% respectively ($p<0.01$ compared to vehicle control). With codeine as positive control, inhibitions of 34% at 10 mg/kg and 61% at 20 mg/kg were observed [Dhawan 2002b].

The same extract was tested in an asthma model in guinea pigs measuring bronchospasms induced by acetylcholine chloride aerosol. The time until the appearance of bronchospasms (preconvulsive time PCT) was measured. Then the animals received the extract (50, 100 or 200 mg/kg b.w. p.o) for seven days. Two hours after the last dose the acetylcholine chloride challenge was repeated. At 100 and 200 mg/kg the extract showed a significant ($p<0.05$) increase in the PCT with a protection of 53.3% and 28.9% respectively, as compared to control [Dhawan 2003a].

Anticonvulsive effects
Male Balb/c mice received a hydroalcoholic extract (4% flavonoids; 0.05, 0.1, 0.2 or 0.4 mg/kg b.w. i.p.) 30 min before i.p. injection of PTZ. At 0.2 and 0.4 mg/kg, the extract significantly ($p<0.001$) prolonged the onset time of seizures as compared to vehicle. The highest dose also decreased the duration of seizures ($p<0.001$). Pre-treatment with flumazenil or naloxone 5 min before the administration of the extract reversed the effects [Nassiri-Asl 2007].

The same extract was injected intracerebroventricularly in male Wistar rats 30 min before i.p. injection of PTZ. At a dose of 1.5 µg/kg b.w., the latency to first minimal clonic seizures was significantly ($p<0.01$) prolonged and at 0.55 and 1.5 µg/kg also the latency to the first generalized clonic seizures ($p<0.01$) as compared to vehicle control. The survival rate increased dose-dependently. Intracerebroventricular pre-treatment with flumazenil abolished the effects of the extract [Nassiri-Asl 2008].

Male Swiss albino mice were injected with PTZ (50 mg/kg b.w. i.p.) on days 1, 5, 10 and 15. From days 5 to 15 the animals received daily either a hydroethanolic extract (not further specified) at 150, 300 or 600 mg/kg b.w. i.p., diazepam (2 mg/kg i.p.) or vehicle. The extract significantly ($p<0.05$) decreased the seizure severity score at 300 and 600 mg/kg on day 5, and at all doses on days 10 and 15 as compared to vehicle control. At 300 and 600 mg/kg no seizures were observed on day 15 and on days 10 and 15 respectively. The extract at the highest dose significantly ($p<0.05$) reduced the immobility time in the forced swimming test on day 15 as compared to vehicle control. Brain noradrenaline and serotonin levels were significantly ($p<0.05$) decreased by PTZ as compared to naïve control. The extract groups showed significant ($p<0.05$) increases in the noradrenaline level at 300 and 600 mg/kg, and in the serotonin level at all doses compared to vehicle control [Singh 2012].

In an epilepsy model, chemical kindling was induced in Swiss albino mice by sequential i.p. injections of subconvulsive doses of PTZ (35 mg/kg b.w. every 48 hours) for up to 45 days, and saline was administered for the naïve control. Following established kindling, mice received single oral doses of either vehicle or an extract (70% ethanol; yield 15%; 1.57 µg/g harmine, 0.32 µg/g harmaline, 10.45 µg/g harmalol) at doses of 150, 300 or 600 mg/kg. On days 5, 10 and 15 after the treatment the animals received a PTZ-challenge. After this challenge, significant ($p<0.05$) reductions were observed in the seizure severity scale, in the transfer latency in the elevated plus maze model and in the number of mistakes in the passive shock avoidance model in all verum groups at all time points as compared to kindled vehicle control. In the latter model, an increase in the step-down latency ($p<0.05$ for 300 and 600 mg/kg at all time points and for 150 mg/kg after 10 and 15 days) was shown compared to kindled vehicle control. The immobility period in the tail suspension test was reduced by all doses on days 5, 10 and 15, and significantly ($p<0.05$) so in the forced swimming test as compared to kindled and naïve vehicle controls. In the cortex and the hippocampus, compared to kindled control, all doses of the extract significantly ($p<0.001$) decreased the PTZ-induced elevated AChE levels. The two higher doses increased the PTZ-lowered dopamine and serotonin levels ($p<0.05$ to 0.001) and all extract doses the noradrenaline levels ($p<0.001$); the latter also compared to naïve control. PTZ-elevated nitrite levels decreased in the cortex ($p<0.001$ for all doses) and for 150 and 300 mg/kg in the hippocampus [Goel 2015].

Neuroprotective effects
In a Parkinson's disease model, neurodegeneration was induced in male Swiss albino mice with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; 20 mg/kg b.w. i.p. daily) for 4 days. Mice were subsequently treated orally with a methanolic leaf extract (200 and 400 mg/kg b.w.) for 14 days. Behavioural abnormalities were assessed in several models such as the Rotarod test, the Pole test, the catalepsy test, the beam walk test, Gait analysis and the adhesive removal test. In all models treatment with the extract significantly ($p<0.01$) and dose-dependently improved the behavioural deficits induced by MPTP. Both doses of the extract also significantly ($p<0.01$) ameliorated the reduced levels of dopamine, SOD, glutathione peroxidase and reduced glutathione and the increased concentrations of MAO B and glutamate in the corpus striatum of MPTP control mice [Narayanan 2011].

In a mouse model for insomnia, DBA/2 mice received an extract (60% ethanol, leaves and fruits 8:2) at oral doses of 10 or 50 mg/kg b.w. for 3 days. An improvement of neurogenesis was demonstrated by a significant ($p<0.001$) increase in the number of doublecortin- and Ki67-positive cells in the hippocampal gyrus. Administration of 100 mg/kg/day to these animals resulted in improved memory in the water maze model. In Sprague Dawley rats receiving the extract at 2 mg/mL with the drinking water for 12 days, doublecortin, parvalbumin, the BDNF and the melatonin receptor 1 were significantly increased ($p<0.02$ to $p<0.0012$). In both rodent models the expression of Tau was significantly higher ($p<0.007$ and $p<0.0011$), while the phosphoTau expression and the phosphoTau/Tau ratio were significantly lower (all $p<0.0001$) as compared to control [Kim 2019b].

Other effects
In carrageenan-induced paw oedema, dextran pleurisy and cotton pellet granuloma tests in rats, a dry extract (mixture obtained from a two-step hydroethanolic and aqueous extraction) exerted a dose-dependent anti-inflammatory effect after oral administration. Significant inhibition of carrageenan oedema at doses of 125 (19.5%, $p<0.05$), 250 and 500 mg/kg b.w. (48.1% and 55.7%, $p<0.01$) and of cotton pellet granuloma at doses of 250 (15.7%, $p<0.05$) and 500 mg/kg b.w. (20.2%, $p<0.01$) were observed [Borelli 1996].

A methanolic extract from dried leaves (yield 4.1%) was administered orally to Swiss male mice at single doses of 75, 100 and 150 mg/kg b.w. The mounting behaviour was tested three times for 15 min during three hours after administration. Compared to control, during all observation periods significant increases in the number of mounts on non-oestrous females were observed ($p<0.01$ 15 min after administration and $p<0.001$ 1.5 and 2.5 hours after administration). The dose of 100 mg/kg was most effective [Dhawan 2003b].

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A methanolic leaf extract (yield 22.9%) was administered orally at daily doses of 100 and 200 mg/kg b.w. to streptozotocin (STZ)-induced diabetic Swiss albino mice for 14 days. At the end of the treatment the reduced body weight observed in diabetic controls was significantly ($p<0.05$) improved by the extract at 200 mg/kg, returning towards the normal level. At this dose of extract, on days 4, 7, 10 and 15 the elevated fasting blood glucose level in diabetic mice also decreased significantly ($p<0.01$ to $p<0.001$) and the diabetes-induced urine glucose level was reduced on days 7, 10 and 15, whereas the reduced liver glycogen level was significantly ($p<0.001$) increased. Significant ($p<0.01$ to $p<0.001$) improvements of the STZ-induced changes in the serum lipid profile (total glycerides, TC, VLDL-c, LDL-c, HDL as well as STC/HDL-c and LDL-c/HDL-c ratios) were observed. The extract at 200 mg/kg enhanced the regeneration of islets of Langerhans in the pancreas and the restoration to normal cellular size of islets with hyperplasia.

In an oral glucose tolerance test in normal fasting mice treated in the same way, the extract at 200 mg/kg significantly ($p<0.001$) reduced the serum glucose concentration at 0, 30, 60 and 120 min as compared to control. The effect was comparable to glibenclamide at 10 mg/kg p.o. [Gupta 2012].

Antinociceptive effects were shown for a methanolic extract (not further specified) in the abdominal restriction assay and the hot plate test in BALB/c mice. The animals received the extract at single doses of 150, 200 or 250 mg/kg b.w. p.o. or morphine (5 mg/kg i.p) or diclofenac (50 mg/kg i.p.). A significant attenuation of acetic acid-induced abdominal constriction was observed after the two higher doses of the extract ($p<0.01$ and $p<0.001$ as compared to saline control) as well as after diclofenac and morphine (both $p<0.001$). Naloxone (0.5 mg/kg s.c.) reversed the antinociceptive activity of the extract and of morphine but not of diclofenac.

The same extract was administered orally at 200 or 300 mg/kg to streptozotocin-induced diabetic Sprague Dawley rats in models of allodynia and vulvodynia. Gabapentin (75 mg/kg i.p.) served as positive control. In allodynia, 1 hour after administration a significant ($p<0.001$) increase in the paw withdrawal threshold was observed for both extract doses as well as for gabapentin, and in the paw withdrawal latency with the higher extract dose and with gabapentin. The extract did not show an effect in static vulvodynia. A significant ($p<0.001$) relief in dynamic vulvodynia was seen for both extract doses and gabapentin [Aman 2016].

In worm models (*Caenorhabditis elegans*), an extract (60% ethanol, leaves and fruits 8:2) at dilutions of 50 mg/L, 100 mg/L and 500 mg/L increased the survival rate under oxidative stress, significant at the highest dose ($p=0.0485$), and the mean and maximum lifespan of the worms were extended significantly ($p<0.025$ to $p<0.001$). The expression of the heat shock protein hsp-16.2, which is positively correlated with lifespan, was up-regulated in the long-lived worms treated with 250 mg/L of the extract ($p<0.0001$) [Kim 2019b].

Several studies using an unknown benzoflavone moiety from a methanolic leaf extract showed a reversal of tolerance and dependence of several addiction-prone psychotropic drugs in mice [Dhawan 2002c; 2002d; 2002e; 2002f; 2003c] as well as of declined sexuality in rats induced by the psychotropic drugs [Dhawan 2002g; 2003d; 2003e]. In male rats, the compound increased libido, sperm count and fertilization potential, which resulted in greater litter size after mating [Dhawan 2002h]. Nevertheless, the structure of this compound was never elucidated and re-isolation was not successful [Holbik 2010].

Pharmacological studies in humans

In a randomized, placebo-controlled, double-blind study performed in a cross-over design 12 healthy, female volunteers received either 1.2 g of an extract (5.9:1, solvent not specified), 10 mg of diazepam or placebo and 140 minutes later 100 mg of caffeine. The current state of alertness was rated by the subjects on a visual analogue scale (VAS) from "wide awake" to "almost asleep". VAS results 120 min after treatment with the extract reached about one third of the diazepam value compared to placebo. Quantitative EEG, for 5 minutes under vigilance-controlled reaction time conditions followed by 5 min under resting conditions, was measured before administration and 120 and 180 min after intake. The effects after administration of the extract were difficult to distinguish from placebo [Schulz 1998].

Healthy volunteers (27 female, 14 male; aged 18 to 35 years) consumed one cup of a tea (2 g passionflower herb/250 mL boiling water or 2 g parsley as placebo) 1 hour before bedtime for 7 days in a double-blind, placebo-controlled, repeated-measures design trial. After a one week wash-out period they changed to the other tea. On each treatment day, the participants completed a sleep diary preceding sleep and upon rising. On day 7 of each treatment the State-trait anxiety inventory (STAI) was completed. Ten participants also underwent overnight polysomnography on the last night of each treatment period. Of six parameters in the sleep diary, only subjective sleep quality showed a significantly ($p<0.002$) improved result for passionflower herb compared with placebo. In all other subjective and objective parameters, no differences between verum and placebo were observed [Ngan 2011].

In a randomized, double-blind, placebo-controlled study, stress was induced in healthy volunteers (30 female, 30 male; aged 24 to 31 years) by a simulated public speaking test. The participants received a dry extract (500 mg, standardized to 0.29% vitexin) or placebo 1.5 hours before the start of the stressful situation. During the stressful phase, the heart rate significantly ($p<0.05$) increased in the experimental group as compared to placebo, but significantly ($p<0.05$) declined throughout the speech compared to the placebo group. No differences between the groups were observed in the standardized anxiety measure STAI [Avelino da Silva 2017].

Healthy volunteers (n=44; ca. 22 of each gender) took 200 mg/day of a dry extract (50% ethanol; yield 20.5%; 3% flavonoid glycosides) one hour before going to sleep for 12 weeks in a randomized, placebo-controlled, double-blind trial. The Japanese version of the Medical Outcomes Study Short Form 36-Item Health Survey (SF-36) and the Oguri-Shirakawa-Azumi sleep score were used to assess emotional status and sleep quality at weeks 6 and 12. After 6 weeks significant improvements were seen in the SF-36 parameters role/social component summary ($p<0.01$), social functioning, role emotional and mental health (all $p<0.05$) as compared to placebo. After 12 weeks the mental component summary and vitality were significantly ($p<0.05$) better in the verum group. No significant differences between the groups were observed for any sleep parameters, blood pressure, pulse rate, haematological and biochemical components as well as urinary parameters at both time points of assessment [Takara 2019].

In a randomized, double-blind, placebo-controlled study, the influence of a single dose of a dry extract (50% ethanol, 850 mg corresponding to 4.25–5.95 g of passionflower herb) on EEG parameters in healthy volunteers (23 female, 17 male) was investigated. Quantitative EEG and eye tracking were recorded before and 45 min after intake of verum or placebo during observation of a video showing various audio-visual cognitive and emotional challenges in series. Before the intake, four cognitive and four emotional challenges induced increases in the beta-, delta- and theta spectral power mainly in fronto-temporal brain areas. These increases were attenuated 45 min after intake in the verum group as compared to placebo. The effects were

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attributed to an influence on glutamatergic and GABAergic neurotransmission [Dimpfel 2016].

Clinical studies

Systematic reviews evaluating 9 and 6 studies respectively concluded that there is a potential for passionflower herb to alleviate some symptoms of neuropsychiatric origin [Miroddi 2013, Janda 2020].

In a randomized, double-blind, controlled study involving 36 patients (20 female, 16 male) with generalised anxiety disorder according to the Diagnostic and Statistical Manual of Mental Disorders (DSM IV) and a score of 14 or more on the Hamilton anxiety rating scale (HAM-A), 18 were treated for 4 weeks with 45 drops/day of a hydroethanolic extract (not further specified) and a placebo tablet. The control group (n=18) received 45 drops/day of a placebo and 30 mg of oxazepam per day. The HAM-A score was assessed on days 0, 4, 7, 14, 21 and 28. There were no differences between the groups in HAM-A score on days 0, 21 and 28. At day 4 the score in the passiflora group was significantly higher ($p<0.008$) than in the oxazepam group. At day 7 and 14 the difference was not significant. At all time points the effect of both treatments was significant ($p<0.001$) when compared to baseline [Akhondzadeh 2001a].

A total of 65 male opiate addicts going through withdrawal were treated in a randomized, double-blind, controlled study with 60 drops of a hydroethanolic extract (not further specified) and 0.8 mg clonidine or placebo drops and clonidine. The severity of the withdrawal syndrome was measured on days 0, 1, 2, 3, 4, 7 and 14 using the "Short Opiate Withdrawal Score". Both protocols were equally effective with regard to the physical symptoms of withdrawal; the passiflora/clonidine treatment was superior to clonidine alone for the psychological symptoms [Akhondzadeh 2001b].

In a randomized, double-blind, parallel-group trial, children (aged 6 to 13 years) with attention deficit hyperactivity disorder (ADHD) received 0.04 mg/kg/day of a preparation (not further specified) (n=17) or 1 mg/kg/day methylphenidate (n=17) for 8 weeks. The effects were assessed by the Parent ADHD rating scale and the Teacher rating scale at 2, 4, 6 and 8 weeks. In both scales a significant decrease in the scores was seen at all times points for passionflower herb as compared to baseline ($p<0.05$ to 0.001). For methylphenidate the decrease was significant from week 4 ($p<0.05$ to 0.001) compared to baseline. No significant differences were observed between the passionflower herb and methylphenidate groups [Akhondzadeh 2005].

In a randomized, double-blind, placebo-controlled study, 30 female and 30 male patients undergoing ambulatory inguinal surgical hernia repair (herniorrhaphy) received either 500 mg of a preparation (not further specified) or placebo as premedication 90 min before surgery. Anxiety and sedation were assessed 10, 30, 60 and 90 min after premedication. The Trieger Dot Test and the Digit-Symbol Substitution Test were used for the assessment of the psychomotor function at arrival in the operating room as well as 30 and 90 min after tracheal extubation. The time between arrival in the anaesthetic recovery room and discharge was recorded. Compared with placebo, the oral pre-treatment with verum resulted in a significant ($p<0.001$) decrease in anxiety levels with the onset and peak effect at 10 and 30 min respectively. All other assessed parameters did not show any significant difference [Movafegh 2008].

Patients (8 female, 52 male) scheduled for inguinal herniorrhaphy under spinal anaesthesia were enrolled in a prospective, randomized, double-blind, placebo-controlled study. Thirty minutes before spinal anaesthesia, haemodynamic parameters, STAI scores, the sedation score and psychomotor function were assessed and the patients received either a preparation (700 mg passionflower herb / 5 mL aqueous extract; not further specified) or placebo. Just before spinal anaesthesia, the tests were repeated and haemodynamics, sedation score, sensory-motor block and side effects were controlled during the operation. Psychomotor function tests were repeated at the end and 60 min after the operation. A significant increase in the STAI anxiety scores ($p<0.01$ and 0.001 respectively) was observed in the placebo group just before spinal anesthesia as compared to baseline. In the verum group no change in anxiety scores occurred. No differences between the groups were seen for all other parameters [Aslanargun 2012].

Patients (39 female, 24 male) undergoing planned dental treatment received either 20 drops of a preparation (not further specified), or a placebo or no pre-treatment the night before and a further 20 drops the following morning at least 90 min before treatment. Anxiety was measured using a VAS. With verum a significant ($p<0.001$) decrease from 12.1 points to 8.5 points was observed as compared to placebo and no pre-treatment [Kaviani 2013].

In a multicenter, prospective, non-interventional trial the effects of a dry ethanolic extract (50% ethanol; DER 5-7:1; 425 mg; 3 times daily for 12 weeks) on stress resistance (resilience) and quality of life (QoL) in patients (115 female, 38 male) with nervous restlessness were studied. Standardised questionnaires were used to evaluate resilience (RS-13), QoL (EQ-5D including EQ-VAS) and addiction potential (BDEPQ). After 12 weeks, significant ($p<0.001$) improvements in resilience from 52.1 to 67.7 points and QoL from 47.9 to 75.0 points, as well as of all accompanying symptoms such as felt restlessness, sleep disturbance, exhaustion, fear, lack of concentration, transpiration, nausea, trembling and palpitation were observed and the mean BDEPQ score was significantly ($p<0.001$) reduced from 23.0 to 19.3 points [Gibbert 2017].

In a double-blind, randomized and parallel group study, patients undergoing planned inguinal herniorrhaphy received either 500 mg of a preparation (not further specified; 30 female, 38 male) or 10 mg of oxazepam (24 female, 36 male) as a premedication 90 min before surgery. Anxiety was assessed with a numerical rating scale (NRS) both before and 90 min after the premedication. Psychomotor function was evaluated with Trieger Dot Test and the Digit-Symbol Substitution Test at arrival in the operating room and 90 min after tracheal extubation. At 90 min a significantly ($p<0.001$) better result in the NRS anxiety score was seen in the passiflora group (4.4±1.2) as compared to oxazepam (6.1±1.3). No significant differences were observed in the psychomotor function scores. Recovery of these scores was comparable in both groups reaching baseline values 90 min after extubation [Azimaraghi 2017].

A double-blind, randomized, placebo-controlled study investigated the effects of an extract (60% ethanol, leaves and fruits 8:2) in patients with insomnia disorder according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). The two groups received either 60 mg of the extract (27 female, 18 male) or placebo (23 female, 16 male) every night for two weeks. The total sleep time significantly ($p<0.05$) increased in the verum group as compared to placebo. No differences between the groups were observed in sleep efficiency, sleep latency, total arousal number, wake after sleep onset, subjective sleep parameters and scores for depression, anxiety and stress. However, in the verum group significant improvements of sleep efficiency, wake after sleep onset as well as of scores for depression, anxiety and stress were seen as compared to baseline ($p<0.002$ to $p<0.025$) [Lee 2020].

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Pharmacokinetic properties
In subcellular systems with human liver microsomes and a human liver S9 fraction containing microsomal and cytosolic fractions including important phase I and II enzymes, the metabolism of orientin, isoorientin, schaftoside, isoschaftoside, vitexin, and isovitexin was investigated. Orientin and isoorientin were metabolized to several mono-glucuronidated and mono-sulphated compounds, vitexin and isovitexin only to mono-glucuronides. The major metabolite of isoorientin was isoorientin-3'-*O*-α-glucuronide. For schaftoside and isoschaftoside, no phase I or II metabolites were identified [Tremmel 2021a].

In a Caco-2 cell monolayer model these six flavonoids underwent various metabolism pathways. At 10 and 100 µM, vitexin, schaftoside and isoschaftoside showed very limited metabolism with only hydroxylated/methoxylated glycosylflavonoids as metabolites. In contrast, orientin, isoorientin and isovitexin resulted in more phase I and II metabolites at 10 µM including sulphates as conjugation products and at 100 µM the diversity of metabolites further increased with sulphates and glucuronides [Tremmel 2021b].

Preclinical safety data

Single dose toxicity
No acute toxicity was observed in mice after i.p. administration of extracts in doses up to 500 mg/kg b.w. [Aoyagi 1974] and 900 mg/kg b.w. [Speroni 1988] as well as after oral administration of a methanolic leaf extract at doses between 50 and 1600 mg/kg [Gupta 2012].

Repeated dose toxicity
No change compared to control animals was observed in weight, rectal temperature and motor coordination of male Sprague-Dawley rats following 21 days of subacute oral treatment with 10 mL/kg b.w. of a hydroethanolic extract, equivalent to 5 g/kg b.w. of the drug [Sopranzi 1990].

An extract (60% ethanol, leaves and fruits 8:2) was administered orally to C57BL/6 mice at 500 mg/kg b.w. daily for 5 days. No differences in body weight, food and water consumption or body composition were observed as compared to the vehicle control [Kim 2019a].

Mutagenicity
In the somatic segregation assay in the diploid strain *Aspergillus nidulans* D-30, no genotoxic effects (neither chromosomal damage caused by aneuploidy and clastogenic effects nor mitotic crossover) were detected after plate incorporation of 1.30 mg/mL of a fluid extract (16.2% dry matter, 0.32% ethanol) [Ramos Ruiz 1996].

Clinical safety data
In children with attention deficit hyperactivity disorder receiving 0.04 mg/kg b.w./day of a preparation (not further specified) (n=17) or 1 mg/kg b.w./day methylphenidate (n=17) for 8 weeks, a few adverse reactions such as palpitations, weight loss, dry mouth, constipation, headache, early awakening and difficulty in falling asleep were observed in both groups. The number of events was higher in the methylphenidate group with 35 events as compared to 11 events in the passionflower herb group. With regard to decreased appetite and anxiety/nervousness, passionflower herb was tolerated significantly better ($p<0.03$ and 0.01 respectively) [Akhonzadeh 2005].

In a multicenter, prospective, non-interventional trial with a dry ethanolic extract (50% ethanol; 5-7:1; 425 mg; 3 times daily for 12 weeks) tolerability was rated as very good or good by more than 99% of the patients (n=153). Three cases of tiredness were reported [Gibbert 2017].

In a double-blind, randomized, placebo-controlled study with an extract (60% ethanol, leaves and fruits 8:2) in patients with insomnia disorder no adverse events occurred in the verum group (n=45) and laboratory results were all in the normal ranges [Lee 2020].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Online Series, 2019
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Online Series, 2021
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Online Series, 2019
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Online Series, 2019
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHAMOMILLAE ROMANAE FLOS	Roman Chamomile Flower	Online Series, 2019
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Online Series, 2021
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Online Series, 2021
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Online Series, 2023
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil´s Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HELICHRYSI FLOS	Sandy Everlastin Flower	Online Series, 2019
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John´s Wort	Online Series, 2018

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JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Online Series, 2020
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Online Series, 2021
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passionflower Herb	Online Series, 2023
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Online Series, 2020
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Online Series, 2021
PRUNI AFRICANAE CORTEX	Pygeum Bark	Online Series, 2020
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher´s Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Online Series, 2021
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TILIAE FLOS	Lime Flower	Online Series, 2022
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Online Series, 2020
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Online Series, 2020
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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Online Series

The Scientific Foundation for Herbal Medicinal Products

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (well-established use) or on experience and historical use of that product (traditional use) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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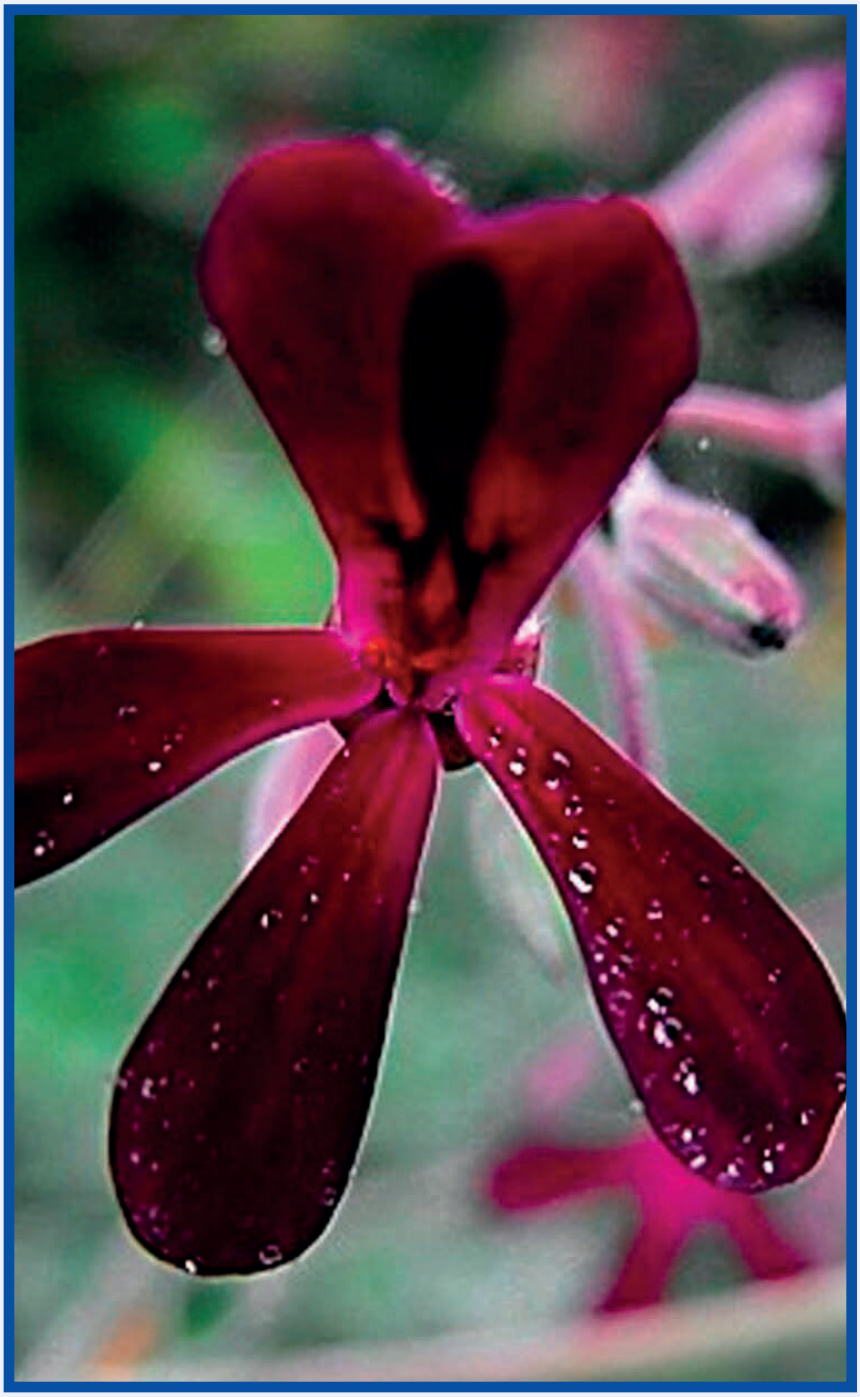
E/S/C/O/P Monographs

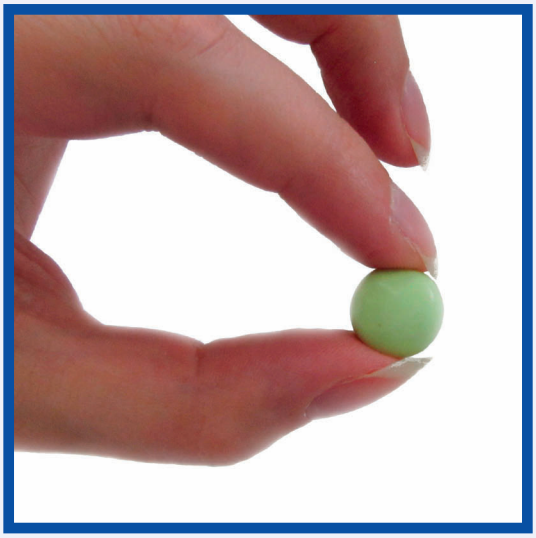
Online Series

The Scientific Foundation for Herbal Medicinal Products

Pelargonii radix
Pelargonium Root

2015





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MONOGRAPHS

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PELARGONII RADIX
Pelargonium Root

2015

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On Phytotherapy

ESCOP Monographs were first published in loose-leaf form progressively
from 1996 to 1999 as Fascicules 1-6, each of 10 monographs

Second Edition, completely revised and expanded

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Pelargonii radix - Pelargonium Root

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Plant illustrated on the cover: *Pelargonium sidoides*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

PELARGONII RADIX

2015

Pelargonium Root

DEFINITION

Dried, unpeeled, usually fragmented, underground organs of *Pelargonium sidoides* DC and/or *Pelargonium reniforme* CURT. It contains not less than 2.0 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; Mr 126.1) (dried drug).

The material complies with the monograph of the European Pharmacopoeia [Pelargonium root].

CONSTITUENTS

Oligomeric and polymeric proanthocyanidins (mainly with catechin and gallocatechin units, approx. 9%); flavan-3-ols (afzelechin, catechin and gallocatechin); phenolic acids (gallic acid and its methylester); hydroxycinnamic acids (caffeic acid, *p*-coumaric acid); flavonoids (*P. reniforme*); highly oxygenated coumarins (approx. 0.05% for *P. sidoides* and 0.03% for *P. reniforme*) such as 7-hydroxy-5,6-dimethoxycoumarin (umckalin), 5,6,7-trimethoxycoumarin (TMC), 5,6,7,8-tetramethoxycoumarin and 6,8-dihydroxy-5,7-dimethoxycoumarin (DHDMC) (all characteristic for *P. sidoides*), 6-hydroxy-5,7-dimethoxycoumarin (fraxinol), 8-hydroxy-6,7-dimethoxycoumarin (fraxidin) and 5,6-dihydroxy-7-methoxycoumarin (isofraxetin) (characteristic for *P. reniforme*), 8-hydroxy-5,6,7-trimethoxycoumarin and 6,7,8-trihydroxycoumarin (present in both species), *P. sidoides* contains also coumarins as glycosides and sulfates [Kolodziej 1995,1998,2000,2003b, 2007, Kayser 1995, Latté 2000, Schoetz 2008].

CLINICAL PARTICULARS

Therapeutic indications
Symptoms of upper respiratory tract infections including common cold, such as blocked or runny nose, sore throat and cough [Timmer 2013, Kamin 2010a-b, Brendler 2009, Bachert 2009, Agbabiaka 2008, Matthys 2010a/2007c/2003, Lizogub 2007, Chuchalin 2005, Bereznoy 2003].

Posology and method of administration

Dosage

Ethanolic extract (1:8-11; 12% V/V)
Adults and children over 12 years: 2.5-7.5 mL daily [Timmer 2013, Kamin 2010a, Brendler 2009, Bachert 2009, Agbabiaka 2008, Matthys 2007b-c/2003, Lizogub 2007, Chuchalin 2005, Heil 1994].
Children aged 6-12 years: 1.25–2.5 mL daily [Timmer 2013, Kamin 2010a, Brendler 2009, Agbabiaka 2008, Matthys 2007b, Bereznoy 2003, Haidvogl 1996, Heil 1994].
Children aged 2-6 years: 0.6-1.25 mL daily [Timmer 2013, Kamin 2010a, Brendler 2009, Agbabiaka 2008, Matthys 2007b, Haidvogl 1996, Heil 1994].

Method of administration
For oral administration.

Duration of administration
If symptoms persist or worsen, medical advice should be sought.

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

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Pregnancy and lactation
No data available.

In accordance with general medical practice, the product should not be used during pregnancy or lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
Gastro-intestinal complaints and allergic skin reactions [Agbabiaka 2008, Timmer 2013, Brendler 2009, Brown 2009]. Hepatotoxicity has been reported but causality for pelargonium could not be established [Teschke 2012a and 2012b].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Almost all pharmacodynamic and clinical studies were performed with an ethanolic extract of *P. sidoides* root (1:9-11 or 1:8-10; 11% ethanol (m/m)) as an 80:20 mixture with glycerol 85%; this will be subsequently cited as the liquid extract.

Pharmacodynamic properties

In vitro experiments

Antibacterial effects
Methanolic extracts of the roots showed antibacterial activity against 8 bacteria: *Staphylococcus aureus* (Sa), *Streptococcus pneumoniae*, β-haemolytic *Streptococcus, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa* and *Haemophilus influenzae* (MIC values: 5 – 7.5 mg/mL). The ethyl acetate, butanol and remaining water fractions of these extracts gave MIC values from 0.6 to 2.5 mg/mL. From the 7 isolated compounds umckalin and DHDMC were the most potent (MIC values: 0.2-0.5 mg/mL) [Kayser 1997].

The liquid extract demonstrated antibacterial activity against several multiresistant strains of Sa (MIC 3.3 mg/mL). An 80% acetone extract (4:1; 12.5 µg/mL) inhibited the growth of *Mycobacterium tuberculosis* by 96% in the radiospirometric bioassay. In the Alamar blue assay the same extract exhibited an MIC of 100 µg/mL (compared to rifampicine MIC 0.06 µg/mL) [Kolodziej 2003a].

The liquid extract reduced the growth of *Helicobacter pylori* by 43% of the control value at a concentration of 100 µg/mL. The extract (50 and 100 µg/mL) significantly reduced ($p<0.05$) the quantity of bacteria attached to gastric epithelial AGS cells by 77% and 91% respectively; while amoxicillin (up to 64 µg/mL) was inactive. At the same concentrations the adherence to these cells was significantly reduced ($p<0.05$) [Beil 2007].

The same extract significantly reduced adhesion of *Streptococcus pyogenes* (Sp) to human Hep-2 epithelial cells dose-dependently by up to 46% ($p<0.001$) while the adhesion to decaying buccal epithelial cells was increased by 7-fold ($p<0.001$) [Conrad 2007b].

In another experiment the extract also inhibited the adhesion of Sp to Hep-2 cells but the polyphenol-free extract was inactive. When highly purified proanthocyanidin fractions from *P.sidoides* were evaluated, only the prodelphinidins showed anti-adhesive properties [Janecki 2011].

Immunomodulatory properties
A methanolic extract, as well as petrol ether, ethyl acetate and butanol fractions thereof, reduced the intracellular survival of *Leishmania donovani* amastigotes within murine macrophages (EC_{50} 2.7, <0.1, <0.1 and 3.3 µg/mL respectively). A bioassay-guided isolation led to the characterization of gallic acid and its methyl ester with EC_{50} values of 4.4 and 12.5 µg/mL (compared to the reference sodium stibogluconate, EC_{50} 2.7 µg/mL). Isolated coumarins proved to be inactive at concentrations up to 25 µg/mL. This was possibly due to macrophage activation which was confirmed by detection of TNF-α and NO-inducing activity. The most potent NO inducers were gallic acid, umckalin and DHDMC (35-45% of the effect of LPS), whereas gallic acid and its methyl ester exhibited the strongest TNF-α-inducing potential (24 and 19% of LPS stimulus). Gallic acid also showed an interferon-like activity by reducing the cytopathogenic effect of encephalomyocarditis virus in fibroblasts [Kayser 2001].

The liquid extract (1 – 30 µg/mL) dose-dependently increased the release of NO, IL-1, IL-12 and TNF-α and changed the expression of the surface markers CD40 and CD119 in bone-marrow derived macrophages infected with *Listeria monocytogenes* [Thäle 2008].

The liquid extract (3 µg/mL) increased β-interferon secretion in MG-63 cells by 200% [Kolodziej 2003a].

Cytotoxic effects
In the brine shrimp lethality bioassay, neither pelargonium extracts nor phenolic constituents such as benzoic and cinnamic acid derivatives, hydrolysable tannins and C-glycosylflavones showed cytotoxic effects (LC_{50} > 1 mg/mL for the extracts; LC_{50} > 0.2 mg/mL for the pure compounds) [Kolodziej 2002].

The cytotoxicity of scopoletin, umckalin and DHDMC was evaluated in a human small cell lung carcinoma line and in a human colorectal cancer cell line, using the micro culture tetrazolium assay. Only DHDMC showed a moderate cytotoxicity with IC_{50} values of 22.1 and 9.5 µM respectively (compared to cisplatin: IC_{50} of 1.0 and 2.7 µM respectively) [Kolodziej 1997].

Other effects
The liquid extract showed a dose-dependent anti-influenza virus activity with an EC_{50} of 6.6 µg/mL and corresponding to a selectivity index of 84 (CC_{50}/ EC_{50}). The concentrations required for complete virus clearance (determined on 6 different strains) varied from 16 µg/mL up to 300 µg/mL. It was demonstrated that the extract had no virucidal activity but affected an early step in the virus life cycle (presumably viral entry into the host cell). A polyphenol-free extract had no activity while an oligo-/polymeric prodelphinidin fraction had a EC_{50} of 2.8 µg/mL [Theisen 2012].

The extract exhibited a free radical scavenging activity in the DPPH assay (IC_{50} 14.7 ± 0.85 µg/mL) (Rezaizadehnajafi 2014).

The liquid extract (30 µg/mL) enhanced phagocytosis by 56% at 2 min ($p=0.002$) and oxidative burst (maximum increase of 120% after 4 min, $p<0.001$). The extract also enhanced intracellular killing, demonstrated by a significant reduction of surviving *Candida albicans* cells (maximum reduction of 31% after 120 min, $p<0.001$) [Conrad 2007a].

The liquid extract significantly ($p<0.05$) increased the ciliary beat frequency in human nasal epithelium cell cultures to 123% at 30 µg/mL and to 133% at 100 µg/mL, compared to the equilibration phase (100%) [Neugebauer 2005].

In vivo experiments
The liquid extract administered by inhalation to influenza-infected mice for 10 days significantly ($p<0.003$) increased survival without obvious toxicity (no difference in weight of lungs, liver, spleen and kidneys) [Theisen 2012].

In nematodes (*Caenorhabditis elegans*) pre-treated with 40, 50 and 100 µg/mL of the extract for 48 h before the addition of 20 µM juglone, a significant reduction (32% to 58%; $p<0.001$) in hsp-16.2::GFP activity (induced by oxidative stress) was observed as compared to controls. The same doses of the extract significantly increased the survival rate (22 – 24%; $p<0.05$) in the nematodes after the addition of a lethal dose of 300 µM juglone for 24 h. Application of the extract (50 µg/mL) to TJ356 worms induced the migration of the transcription factor DAF-16 from cytosol to the nucleus (this is essential for the activation of the transcription of various genes mediating stress resistance) [Rezaizadehnajafi 2014].

Pre-treatment of mice with a single oral dose of liquid extract (400 µg/kg b.w.) significantly inhibited lipopolysaccharide-induced sickness behaviour ($p<0.05$) [Noldner 2007].

Studies in humans
All studies were performed with the liquid extract except a few where it was dried but no DER is given for the dry extract.

Pharmacological studies in humans
In a randomized, double-blind, placebo-controlled study 28 athletes received the liquid extract (4.5 mL daily) or placebo for 28 days. The relative salivary IgA level was significantly increased in the verum group ($p<0.001$) while nasal IL-15 ($p<0.05$), serum IL-15 ($p<0.02$) and serum IL-6 levels ($p<0.05$) were significantly decreased [Luna 2011].

Clinical Studies
In a systematic review and meta-analysis, 6 randomized clinical trials (Matthys 2003, Chuchalin 2005, Matthys 2007c, Blochin and 2 unpublished trials) met the inclusion criteria, of which 4 were suitable for statistical pooling. Only mono-preparations containing the liquid extract used for the treatment of patients with acute bronchitis were included. Meta-analysis of the 4 placebo-controlled trials indicated that pelargonium significantly decreased the Bronchitis Severity Score (BSS assessing cough, sputum, rales/rhonchi, chest pain during coughing and dyspnoea) within 7 days of treatment (weighted mean difference: 2.80 points, 95% CI interval 2.44-3.15) [Agbabiaka 2008].

A Cochrane review evaluated 10 randomized clinical trials dealing with acute bronchitis in adults and children, sinusitis and the common cold in adults, and sore throat in children. The quality of 8 of the 10 studies was considered to be adequate for inclusion in the analysis (Chuchalin 2005, Matthys 2007c, Matthys 2010a, Lizogub 2007, Bachert 2009, Kamin 2010a, Kamin 2010b and Kamin 2012). It was concluded that based on the limited evidence from the few clinical trials with acceptable methodology, pelargonium may offer symptom relief in acute bronchitis in children and adults, and in rhinosinusitis and the common cold in adults [Timmer 2013].

Other clinical reviews, which assessed open as well as controlled studies, involved a total of 5400 patients. They all concluded that pelargonium is efficient in the treatment of upper respiratory tract infections [Brown 2009, Brendler 2009, Kolodziej 2002].

Acute bronchitis
In two randomized, double-blind, placebo-controlled, multicentre trials, patients (1-18 years) with acute bronchitis received the liquid extract (1-6 years: 3x0.5 mL; 6-12 years: 3x1 mL; 12-18 years: 3x1.5 mL daily) or placebo for 7 days. From baseline to day 7, the decrease in the BSS total score was significantly higher for the verum group compared to placebo:
- *Study 1* (n=200): 3.4±1.8 points versus 1.2±1.8 points ($p<0.0001$) [Kamin 2010a].
- *Study 2* (n=220): 4.4±1.6 points versus 2.9±1.4 points ($p<0.0001$) [Kamin 2012].

Treatment outcome and satisfaction with treatment were also significantly better compared to placebo ($p<0.0001$).

In three randomized, double-blind, placebo-controlled, multicentre studies patients with acute bronchitis received the liquid extract (1.5 mL 3 times daily) or placebo for 7 days. In all studies the decrease of BSS from baseline to day 7 was significantly higher for the verum group compared to placebo:
- *Study 1* (n=217): 7.6±2.2 points versus 5.3±3.2 points; $p<0.0001$ [Matthys 2007c]
- *Study 2* (n=124): 7.2±3.1 points versus 4.9±2.7 points; $p<0.0001$ [Chuchalin 2005]
- *Study 3* (n=468): 5.9±2.9 points versus 3.2±4.1 points; $p<0.0001$ [Matthys 2003]

In addition, the duration of illness was shorter and satisfaction with treatment was better with verum compared to placebo.

In a prospective, open, multicentre study, 205 patients (42±16 years) suffering from acute bronchitis, or an acute exacerbation of chronic bronchitis, were treated with 1.5 mL of the liquid extract, three times daily for 7 days. The total BSS decreased by 3.3±3.8 points and 60.5% of the patients assessed their health condition at the end of the study as much improved or free from symptoms [Matthys 2007a].

In a prospective, open, multicentre study, 2099 patients (<3 years: n = 78; 3-18 years: n = 420; >18 years: n = 1601) with acute bronchitis were treated with the liquid extract at an age-dependant dosage three times daily for a maximum of 14 days (<6 years: 0.5 mL; 6-12 years: 1 mL; >12 years: 1.5 mL). The mean BSS of all patients decreased from 7.1±2.9 points at baseline to 1.0±1.9 points at patient's last visit. Subgroup analysis for children (<18 years, n = 498) and infants (<3 years) showed a decrease in mean BSS from 6.3±2.8 to 0.9±1.8 points and from 5.2±2.5 to 1.2±2.1 points, respectively [Matthys 2007b].

A prospective, open, multicentre study evaluated the treatment of 742 patients (<12 years) with acute bronchitis or acute exacerbations of chronic bronchitis. The children were treated with the liquid extract at a dosage according to their age for a maximum of 2 weeks: <2 years 0.75 mL daily, 2-6 years 1.5mL daily and 6-12 years 3 mL daily. The overall BSS decreased significantly from 6.0±3.0 points to 1.4±2.1 points ($p<0.001$). The assessment of the individual symptoms (coughing, expectoration, difficulty in breathing, wheezing and chest pain) gave a response rate (remission and improvement) of more than 80% [Haidvogl 1996]. In a similar study with 259 children and the same treatment, all the individual symptoms showed remission or improvement rates of more than 80% [Dome 1996].

In a randomized, double-blind, placebo-controlled, dose-finding study, 400 patients (6-18 years) with acute bronchitis received either 30 mg, 60 mg or 90 mg of the dry extract or placebo daily for 7 days. The decrease of total BSS from baseline to day 7 was significantly higher for the verum groups (60 mg, $p=0.0004$; 90 mg, $p<0.0001$) compared to placebo (4.4±2.4 and 5.0±1.9 points respectively versus 3.3±2.6 points) without relevant differences between these 2 verum dosages [Kamin 2010b].

In a randomized, double-blind, placebo-controlled, multicentre, dose-finding trial, 406 patients (>18 years) with acute bronchitis

received either 30 mg, 60 mg or 90 mg of the dry extract or placebo daily for 7 days. The decrease of total BSS from baseline to day 7 was significantly higher for the verum groups ($p<0.0001$) compared to placebo (4.3±1.9, 6.1±2.1 and 6.3±2.0 points respectively, versus 2.7±2.3 points) without relevant differences between the 2 highest dosages [Matthys 2010a]. The HRQL (health-related quality of life) and PRO (patient-reported outcome) questionnaires, assessing the secondary outcome measures, demonstrated significantly ($p<0.05$ or $p<0.0001$) greater improvement in all three of the verum groups compared to placebo (physical score, impact of patient's sickness, duration of activity limitation, patient-reported treatment outcome, satisfaction with treatment) [Matthys 2010b].

According to two reviews, other studies also showed an improvement of the BSS with the liquid extract given at an age-dependant dosage three times daily (<6 years: 0.5 mL; 6-12 years: 1 mL; >12 years: 1.5 mL) [Kolodziej 2003b; Brendler 2009].

TABLE 1. Clinical and surveillance studies in patients with acute bronchitis (Kolodziej 2003b)

Number of patients	Duration (days)	Control	BSS decrease after treatment	
			Verum	Control
205 adults	7	placebo	7.7 points	5.3 points
220 adults & children	7	placebo	4.4 points	2.9 points
60 children (6-12 y.)	7	AcC	7[1] points	6[1] points
213 children (6-12 y.)	7	AcC	6.7 points	6.6 points
205 adults	7	-	3.3 points	-
1042 children (up to 12 y.)	14	-	5.4 points	-

AcC = acetylcystein

[1] result given as such in Kolodziej 2003b

Chronic bronchitis
In a randomized, double-blind, placebo-controlled trial, 200 patients (> 18 years) with a history of chronic bronchitis were allocated to a 24-week add-on therapy with 1.5 mL of the liquid extract 3 times daily or placebo, alongside a standardised baseline-treatment. Median time to exacerbation was significantly ($p=0.005$) prolonged with verum compared to placebo (57 versus 43 days). Analysis of the secondary endpoints showed fewer exacerbations, less patients with antibiotic use, improved quality of life and less days of inability to work [Matthys 2013].

Tonsillopharyngitis
In a randomized, double-blind, placebo-controlled trial, 143 patients (6-10 years) with acute non-group A β-haemolytic *Streptococcus* tonsillopharyngitis received the liquid extract (3 mL daily) or placebo for 6 days. The decrease of the Tonsillopharyngitis Severity Score (TSS: assessing sore throat, difficulty in swallowing, pharyngeal erythema and fever) from baseline to day 4 was significantly higher for the verum group compared to placebo (7.1±2.1 points versus 2.5±3.6 points; $p<0.0001$). Also, the severity of the symptoms was reduced and the duration of illness was shortened by at least 2 days [Bereznoy 2003].

According to two reviews, other studies also showed an improvement of the TSS with the liquid extract given in a dosage of 1 mL three times daily [Kolodziej 2003b; Brendler 2009].

TABLE 2. Clinical and surveillance studies in patients with acute tonsillopharyngitis (Kolodziej 2003b)

Number of patients	Duration (days)	Control	TSS decrease after treatment	
			Verum	Control
124 children (6-10 y.)	6	placebo	6.8 points	3.4 points
78 children (6-10 y.)	6	placebo	6.7 points	3.6 points
60 children (6-10 y.)	10	gargle	5[1] points	3[1] points
1000 (2-35 years)	7	-	11[1] points	-

[1] result given as such in Kolodziej 2003b

Rhinosinusitis
In a randomized, double-blind, placebo-controlled, multicentre trial, 103 patients (18 – 60 years) with acute rhinosinusitis received the liquid extract (3 mL three times daily) or placebo for a maximum of 22 days. From baseline to day 7, the mean decrease in Sinusitis Severity Score (SSS; headache, maxillary pain, nasal obstruction and purulent nasal secretion) was 5.5 points in the verum group compared to 2.5 points in the placebo group ($p<0.00001$). Analysis of the secondary outcome measures showed a remission or major improvement in 30% of the patients in the verum group, compared to 5.8% in the placebo group ($p<0.0001$) [Bachert 2009].

In a randomized, double-blind, placebo-controlled trial, 272

patients with acute sinusitis were treated with 3 mL of the liquid extract three times daily or placebo for a maximum of 3 weeks. In the verum group, the mean SSS decreased from 14.4±1.8 points at baseline to 7.4±3.2 points at the patient's last visit, while the baseline value of 13.9±1.7 points in the placebo group remained unchanged ($p<0.0001$) [Bachert 2005 in Brendler 2009].

In a prospective, open, multicentre study, 361 patients (1-94 years) with acute sinusitis or acute exacerbation of chronic sinusitis were treated with the liquid extract. Adults/children (< 12 years) received 1.5/1 mL every hour up to 12 times daily on the first 2 days and thereafter 1.5/1 mL three times daily for 28 days. Patients with chronic sinusitis received prophylactic treatment for a further 8 weeks at 1.5/1 mL two times daily. The mean SSS of all patients decreased from 15.2±4.6 points at baseline to 2.4±3.2 points at day 28. Within 4 weeks 82.3% of the patients showed a complete remission or a clear improvement in symptoms [Schapowal 2007].

Common cold
In a randomized, double-blind, placebo-controlled, multicentre trial, 103 patients (18 – 55 years) with common cold (at least 2 major and 1 minor, or 1 major and 3 minor cold symptoms) received the liquid extract (1.5 mL three times daily) or placebo for 10 days. The decrease of the mean SSID (sum of symptom intensity differences) of the cold intensity score (CIS) from baseline to day 5 was significantly improved for the verum group ($p<0.0001$) compared to placebo (14.6±5.3 versus 7.6±7.5). The mean CIS decreased by 10.4±3.0 points in the verum group versus 5.6±4.3 points in the placebo group. After 10 days 78.8% of the patients in the verum group were free of symptoms (CIS = 0) versus 31.4% in the placebo group ($p<0.0001$) [Lizogub 2007].

Upper respiratory tract infections
A multicentre, post marketing surveillance study was carried out in 166 patients (1-19 years) with acute and chronic ear, nose, throat and respiratory tract infections. They were treated for up to 7 days (88 patients), 8 to 14 days (60 patients) and more than 14 days (16 patients) with the liquid extract at a dosage according to their age: 1-6 years 0.75–1.5 mL daily, 6-12 years 1.5–3 mL daily and >12 years 3–4.5 mL daily. The assessment of the subjective symptoms was performed by both physicians and patients/parents on a 4-point rating scale. The response rate (remission and improvement) was between 70% and 90% for the different symptoms such as coughing, fever, expectoration and pain [Heil 1994].

In a study investigating the prevention of asthma attacks during upper respiratory tract viral infections, 61 children received the liquid extract at a daily dosage according to their age (1-5 years: 3x0.5 mL, 6-12 years: 3x1 mL and >12 years: 3x1.5 mL) or placebo for 5 days. After assessment of the symptoms, significant improvement ($p<0.05$) of cough frequency and nasal congestion was observed in the verum group; for fever and muscles aches there was no significant difference. The frequency of asthma attack was also significantly ($p<0.05$) reduced in the verum group [Tahan 2013].

In a prospective, open, multicentre study, 641 patients (10-60 years) were treated with the liquid extract at an age-dependant dosage for a maximum of 14 days. Improvement of symptoms was observed after 7 days (n=240) and 14 days (n=305). In 88.9% efficacy was assessed as "very good" or "good" [König 1995 in Brendler 2009].

Pharmacokinetic properties
No data available.

Preclinical safety data
No effect on thromboplastin time (TPT), partial TPT or thrombin time (TT) was observed in rats after oral administration of the liquid extract (up to 500 mg/kg b.w, for 2 weeks), while treatment with warfarin (0.05 mg/kg b.w.) resulted in significant changes in TPT and partial TPT. The anticoagulant activity of warfarin was not influenced when warfarin (0.05 mg/kg b.w.) and the extract (500 mg/kg b.w.) were given concomitantly [Koch 2007].

The coumarins found in pelargonium root do not possess the structure required for anticoagulant activity [Arora 1963, Williamson 2009].

Toxicological studies in rats and dogs revealed a no observed effect level of more than 750 mg liquid extract/kg b.w. At a dose of 3 g/kg no signs of hepatotoxicity were found after morphological and histopathological examination. Incubation of human hepatocytes and hepatoma cells with 50 µg/mL extract confirmed the non-hepatoxicity. Based on theoretical considerations the metabolism of 7-hydroxycoumarins (present in pelargonium root) by 3,4-epoxidation leading to formation of hepatotoxic metabolites is very unlikely [Loew 2008].

The effects of oral administration to rats of an aqueous extract (approx.16:1), at 100, 200 and 400 mg/kg b.w. for 21 days, on haematological and biochemical parameters, and on the organ body-weight ratio were investigated. Red blood cell count, haemoglobin, platelets, lymphocytes, total proteins, globulin and sodium levels were significantly increased, while the levels of alkaline phosphatase, chloride and uric acid were significantly reduced ($p<0.05$). No deaths or clinical signs were observed [Adewusi 2009].

Clinical safety data
The available data from clinical trials do not show an elevated risk of serious adverse events. However, gastrointestinal complaints such as nausea, vomiting, diarrhoea and heartburn, and allergic skin reactions with pruritus and urticaria, have been reported [Agbabiaka 2008, Timmer 2013, Brendler 2009, Brown 2009]. The following are adverse reports from clinical papers cited above.

Fifty one patients from the verum group (n=99) experienced 79 adverse events, compared to 40 patients with 46 adverse events in the placebo group (n=101). Most were gastrointestinal complaints and none were classified as serious. The incidence of suspected adverse reactions (events/day of exposure) was 0.001 [Matthys 2013].

Only 3 adverse events were observed in 2 of 111 patients in the verum group and all were classified as non-serious with no causal relationship with the medication [Kamin 2012].

A total of 59 adverse events were observed in 55 of 200 patients, for 8 events a causal relationship with the medication could not be excluded. None of the adverse events was classified as serious and most of the events were gastrointestinal complaints (17 patients in the verum group and 7 in the placebo group). The mean values of clinical laboratory parameters, such as different transferase enzymes, showed no group differences [Kamin 2010a].

At least one adverse event occurred in 47 of 217 patients (23 verum, 24 placebo), in 25 of 124 patients (15 verum, 10 placebo) and in 36 of 468 patients (20 verum, 16 placebo). All events were assessed as non-serious [Matthys 2007c, Chuchalin 2005, Matthys 2003].

Eighteen adverse events were observed in 16 of 205 patients,

all were assessed as non-serious [Matthys 2007a].

A total of 28 adverse events occurred in 26 of 2099 patients, of which 14 were in children (13/420 patients) and 4 in infants (3/78 patients). Most of them were coded as gastrointestinal disorders. For 9 adverse events a causal relationship to the medication could not be excluded, but was assessed as unlikely in 8 cases. In one child there was a hypersensitivity reaction possibly related to the medication [Matthys 2007b].

Only 8 of 742 patients (<12 years) experienced adverse events which were probably related to the medication. In 2 patients exanthema occurred and in one diarrhoea; in 2 patients psychomotor restlessness was reported [Haidvogl 1996].

In 152 of 806 patients 155 adverse events occurred, most of them classified as gastrointestinal disorders. No serious events were reported [Kamin 2010b, Matthys 2010a].

Adverse events occurred in 15 of 143 patients (1 verum; 14 placebo) and were not related to the medication [Bereznoy 2003].

At least one adverse event occurred in 8 of 103 patients. All events were assessed as non-serious. In 4 cases a causal relationship with the drug could not be excluded (3 gastrointestinal complaints and 1 allergic skin reaction) [Bachert 2009].

For 21 of 67 adverse events, in 17 of 361 patients, a causal relationship with the medication could not be excluded. Most of the events were gastrointestinal complaints [Schapowal 2007].

Adverse events occurred in 3 of 103 patients (2 verum; 1 placebo) and all were assessed as non-serious [Lizogub 2007].

In a study with 166 patients neither interactions nor undesirable effects were observed [Heil 1994].

In the period from 2002 to 2006, the Uppsala Monitoring Centre received 34 case reports of hypersensitivity reactions suspected to be associated with the use of pelargonium. In 14 of these reports, the description and timing of the event was indicative of an acute Coombs and Gell type I hypersensitivity reaction. Two of the patients needed treatment for circulatory failure or anaphylactic shock [de Boer 2007].

In two studies, a total of 28 spontaneous reports of primarily assumed hepatotoxicity associated with the use of pelargonium were assessed using the liver specific scale of the Council for International Organisations of Medical Sciences. None of the cases of liver disease generated a positive signal of safety concern since causality for pelargonium could not be established [Teschke 2012a and 2012b].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Supplement 2009
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Second Edition, 2003
FUMARIAE HERBA	Fumitory	Supplement 2009
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Supplement 2009
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Second Edition, 2003
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Second Edition, 2003

LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Supplement 2009
MARRUBII HERBA	White horehound	Online Series, 2013
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MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
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MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Second Edition, 2003
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Second Edition, 2003
RATANHIAE RADIX	Rhatany Root	Supplement 2009
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Second Edition, 2003
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Second Edition, 2003
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Second Edition, 2003
SALICIS CORTEX	Willow Bark	Second Edition, 2003
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
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SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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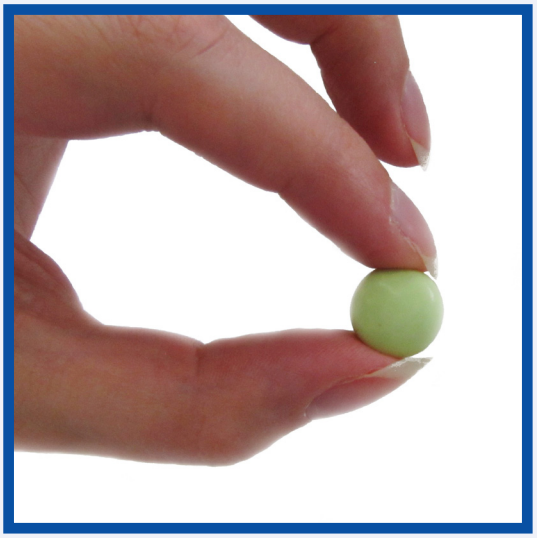
Plantaginis lanceolatae folium/herba

Ribwort Plantain Leaf/Herb

2013



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***The* Scientific Foundation for**
Herbal Medicinal Products

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
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Notes for the Reader
Abbreviations
The monograph text
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Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

PLANTAGINIS LANCEOLATAE FOLIUM/HERBA 2013

Ribwort Plantain Leaf/Herb

DEFINITION

Ribwort plantain leaf consists of whole or fragmented dried leaf and scape of *Plantago lanceolata* L.*s.l.* It contains not less than 1.5 per cent of total *ortho*-dihydroxycinnamic acid derivatives, expressed as acteoside ($C_{29}H_{36}O_{15}$; M_r 624.6) (dried drug). Ribwort plantain leaf complies with the monograph of the European Pharmacopoeia [Ribwort Plantain].

Ribwort plantain herb consists of the dried flowering aerial parts of *Plantago lanceolata* L. The material complies with the Deutsches Arzneibuch [Spitzwegerichkraut 1996].

Fresh material may also be used, provided that when dried it complies with the monograph of the respective pharmacopoeia.

CONSTITUENTS

The characteristic constituents are complex mucilage polysaccharides (6.5%): consisting of galactose (44%), arabinose (32%), glucose (9%), mannose (4%), rhamnose (7%), galacturonic acid, glucuronic acid, fucose and xylose [Bräutigam 1985a], iridoid glycosides principally aucubin (1-3%) and catalpol (1%), dihydroxycinnamic acid derivatives such as acteoside (3.5%), plantamajoside (1%), isoacteoside and lavandulifolioside and further caffeoyl derivatives. Further constituents are flavonoids (glycosides of apigenin and luteolin), saponins and tannins [Blaschek 2007; Bräutigam 1985a; Bräutigam 1985b; Darrow 1997; Handjieva 1991; Háznagy 1976; Kardosova 1992; Marchesan 1998a; Murai 1995; Paper 1999].

CLINICAL PARTICULARS

Therapeutic indications

Catarrh of the respiratory tract [Blaschek 2007; Kraft 1996; Kraft 1997; Kraft 1998; Schilcher 2007; Willuhn 2002].

Temporary, mild inflammatory conditions of the oral and pharyngeal mucosa [Blaschek 2007; Schilcher 2007; Willuhn 2002]. Insects bites and wound healing [Marchesan 1998a,b; Schilcher 2007].

Efficacy in these latter indications is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Adults: average daily dose, 3-6 g of the drug or equivalent preparations [Kraft 1996; Kraft 1997; Willuhn 2002].
Elderly: dose as for adults.
Children, average daily dose: *>1-4 years of age*, 1-2 g; *4-10 years*, 2-4 g; *10-16 years*, 3-6 g [Kraft 1998; Dorsch 2002].

Fresh crushed leaves to be applied on insect bites [Schilcher 2007].

Method of administration
For oral and topical administration.

Duration of administration
If symptoms persist or worsen, medical advice should be sought.

Contraindications
None known.

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Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None known.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Anti-inflammatory activity
Four different freeze-dried extracts (ethanol 28%) from ribwort plantain herb were evaluated for anti-inflammatory activity in a modified hen's egg chorioallantoic membrane (HET-CAM) test using sodium dodecyl sulphate as the membrane irritant. At concentrations of 500 µg/pellet all the extracts inhibited the formation of blood vessels around the granuloma and the total blood vessel net appeared normal. Two extracts inhibited membrane irritation by 100%, the other two by 67 and 93% respectively. The activity of the extracts was comparable to that of hydrocortisone, phenylbutazone and diclofenac, each at 50 µg/pellet [Marchesan 1998a; Marchesan 1998b].

Acteoside and plantamajoside inhibited 5-lipoxygenase with IC_{50} values of 13.6 and 3.75×10^{-7} M respectively [Ravn 1990].

In human polymorphonuclear leukocytes acteoside inhibited the production of 5-HETE with an IC_{50} of 4.85 µM and leukotriene B_4 with an IC_{50} of 2.93 µM. In peritoneal leukocytes from mice, 5-HETE production was inhibited with an IC_{50} of 5.27 µM [Kimura 1987].

An ethanolic extract of the whole plant inhibited nitric oxide (NO) production dose-dependently in the murine macrophage cell line J774a.1. Moreover, the extract was shown to have NO-scavenging activity and an inhibitory effect on nitric oxide synthase gene expression (Vigo 2005).

Antibacterial and antiviral activity
Expressed juice and aqueous, methanolic and ethanolic extracts of ribwort plantain leaf have shown inhibitory activity against various microorganisms such as *Bacillus subtilis*, *B. cereus*, *Klebsiella pneumoniae*, *Micrococcus flavus*, *Mycobacterium phlei*, *Pseudomonas aeruginosa*, *Proteus vulgare*, *Staphylococcus aureus*, *Streptococcus aureus*, *S.* β-*haemolyticus* and *S. pyocyaneus*, and against several strains of *Salmonella* and *Shigella* in the plate diffusion test [Blaschek 2007; Bräutigam 1985c; Elich 1966; Felklová 1958; Hänsel 1966; Tarle 1981].

Aucubigenin, the aglycone of aucubin, has been shown to be mainly responsible for antibacterial activity of the drug and extracts [Háznagy 1976; Elich 1966; Hänsel 1966; Elich 1962; Háznagy 1970]. Extracts without aucubin exerted no antibacterial effects [Elich 1962].

Aucubin preincubated with β-glucosidase suppressed hepatitis B virus DNA replication in HepG2 cell cultures in a dose-dependent manner [Chang 1997].

Spasmolytic activity
A fluid extract (1:1) from ribwort plantain herb inhibited contractions of isolated guinea pig ileum induced by acetylcholine, histamine, K^+ and Ba^{2+} by 50% at 10 mg/ml and 100% at higher concentrations. The effects were comparable to those of atropine, diphenhydramine and papaverine. With preparations of isolated guinea pig trachea, only Ba^{2+}-induced contractions were inhibited, by 30% at 10 mg/ml [Fleer 1997].

Acteoside inhibited histamine- and bradykinin-induced contractions of isolated guinea-pig ileum with pA values of 6.31 and 6.51 respectively [Schapoval 1998].

An extract (20% ethanol) from ribwort plantain (not specified) and isolated constituents were investigated for antispasmodic activity in the isolated guinea-pig ileum and trachea models. Ribwort plantain extract showed antispasmodic activity in a dose-dependent manner in both the ileum and the trachea. Luteolin, acteoside and plantamajoside, but not catalpol, isoacteoside, lavandulifolioside nor aucubin, inhibited agonist-induced contractions in the ileum. In addition, luteolin and acteoside also inhibited agonist-induced contractions in the trachea (Fleer 2007).

Immunostimulant activity
At a concentration of 0.0002% the polysaccharides of ribwort plantain leaf increased phagocytosis of granulocytes by 20.5%. Chemiluminescence was increased by 36% at a concentration of 0.001% [Bräutigam 1985c].

Polysaccharides (consisting of galactose, arabinose and rhamnose) isolated from the leaves showed immunomodulatory activity in the mitogenic and comitogenic rat thymocyte tests. The activity was dose-dependent and reached a maximum stimulatory response at a concentration of 1.0 mg/ml (Ebringerová 2003).

Antioxidant activity
A methanolic extract from ribwort plantain (not specified) was shown to have antioxidant activity in the DPPH radical scavenging test and the inhibition of Fe^{2+}/ascorbate-induced lipid peroxidation on bovine brain liposomes (Galvez 2005).

Other effects
The mucociliary transport velocity in isolated ciliated epithelium from the frog oesophagus was not influenced by application of 200 µl of an infusion from ribwort plantain herb (4.6 g per 100 ml of water) [Müller-Limmroth 1980].

Polysaccharides from ribwort plantain were investigated for bioadhesion to epithelial tissues in a novel ex vivo model using porcine buccal membranes. Adhesion to epithelial tissues was measured as a decrease in polysaccharide concentration. The decrease was approximately 5 and 20 % after 1 and 3 hours of treatment respectively (Schmidgall 2000).

In vivo experiments

Anti-inflammatory effects
Oral pre-treatment of rats with a dry 80%-ethanolic extract

from ribwort plantain leaf at 100 mg/kg body weight inhibited carrageenan-induced paw oedema by 11% (not significant) compared to 45% inhibition by indomethacin at 5 mg/kg [Mascolo 1987].

A freeze-dried extract from ribwort plantain leaf, administered intraperitoneally to rats, reduced the inflammatory effect and leukocyte infiltration induced by simultaneous subplantar injection of carrageenan and prostaglandin E1 [Shipochliev 1981].

Aucubin administered orally at 100 mg/kg body weight inhibited carrageenan-induced mouse paw oedema by 33.0% after 3 hours ($p<0.01$), compared to 44% inhibition by oral indomethacin at 7 mg/kg ($p<0.01$). In the 12-*O*-tetradecanoylphorbol acetate (TPA)-induced mouse ear oedema test, aucubin applied topically at 1 mg/ear inhibited oedema by 80% ($p<0.01$), compared to 87% inhibition by indomethacin at 0.5 mg/ear ($p<0.01$) [Recio 1994].

Oral pretreatment of rats with acteoside at 150 mg/kg body weight significantly inhibited carrageenan-induced rat paw oedema by 96 and 94% after 2 and 4 hours respectively compared to vehicle controls; oral indomethacin at 10 mg/kg caused 25 and 40% inhibition after 2 and 4 hours respectively [Schapoval 1998].

Topical pretreatment with acteoside or plantamajoside at 3 mg/ear inhibited arachidonic acid-induced mouse ear oedema by 14% ($p<0.05$) and 25% ($p<0.01$) respectively, compared to 38% inhibition by phenidone at 0.1 mg/ear ($p<0.01$) [Murai 1995].

Following intraperitoneal administration of acteoside in the mouse dextran sulphate sodium-induced colitis model, significant improvements in histological scores of colonic tissues compared to controls were observed ($p < 0.02$). A decrease in production of immunoregulatory and proinflammatory cytokines was also observed (Hausmann 2007).

Antiulcerogenic effects
Administration of an aqueous extract of ribwort plantain leaf to mice (200 or 400 mg/kg p.o.) and rats (140 or 280 mg/kg intraduodenally) demonstrated activity in a variety of ulcer models, with the higher doses providing better protection than lower doses. The higher dose significantly ($p<0.001$) reduced the ulcer index compared with control in a mouse model of acetic acid-induced ulcer, and had slightly greater activity than ranitidine (70 mg/kg) and pure mucilage (172 mg/kg). In a mouse model of indomethacin-induced ulcer, the higher dose significantly ($p<0.001$) reduced the ulcer score compared with control and had a slightly greater effect than misoprostol (280 µg/kg). In a mouse model of cysteamine HCl-induced ulcer, the higher dose significantly ($p<0.001$) reduced the ulcerated area compared with control, but to a slightly lesser degree than ranitidine (70 mg/kg). In a rat model of pylorus ligation-induced ulcer, the higher dose significantly ($p<0.01$) reduced ulcer score compared with control and conferred better protection than ranitidine (50 mg/kg; $p<0.05$). In this model both doses of the leaf extract also significantly ($p<0.001$) increased mucin levels compared with control (Melese 2011).

Immunostimulant effects
Interferon production in mice increased by 15-fold and 3-fold respectively 24 and 48 hours after intravenous administration of 0.2 ml of a decoction from ribwort plantain leaf (2 g in 100 ml of water) [Plachcinska 1984].

An aqueous extract from ribwort plantain leaf increased SRBC-antibody production and the liberation of angiogenesis factor in mice [Strzelecka 1995].

Analgesic activity
Three phenylethanoid-based fractions of a methanolic extract from ribwort plantain: phenylethanoid-flavonoidic fraction (49.5% acteoside, 11.6% luteolin-7-glucoside, free of iridoids); phenylethanoid fraction (46.6% acteoside); phenylethanoid-iridoidic fraction (48.8% acteoside, 4.9% catalpol, 7.8% aucubin, free of flavonoids) were investigated in the hot-plate test and the acetic acid-induced writhing test in mice (2 testing groups and one blank control group, 10 mice for every experiment). Following intraperitoneal administration all 3 fractions showed analgesic activity. The phenylethanoid fraction had a more rapid and prolonged effect compared to the phenylethanoid-flavonoidic and phenylethanoid-iridoidic fractions (Armatu 2007).

Anthelmintic activity
Dry extracts (90% ethanolic 9:1 and aqueous 14:1) from the leaves were investigated for anthelmintic activity against pinworm parasites in male swiss albino mice (groups of 6 mice). The extracts were administered orally at a dose of 100 mg/kg body weight. The ethanolic extract demonstrated significant activity against the pinworm parasites: 41.6 % efficacy in eliminating *Syphacia obvelata* ($p<0.05$) and 44.5 % efficacy in eliminating *Aspiculuris tetraptera* ($p<0.05$). The aqueous extract also showed activity but this was not significant: 30.7 % efficacy in eliminating *Syphacia obvelata* and 35.9 % efficacy in eliminating *Aspiculuris tetraptera* (Kozan 2006).

Other effects
In a model of diet-induced obesity in C57BL/6J mice, addition of 10 % ribwort plantain leaf powder to a high fat diet for 28 days led to a significant reduction in body weight ($p<0.05$) and visceral fat ($p<0.01$) despite not affecting food intake. Mice consuming the ribwort plantain also exhibited significantly lower ($p<0.05$) serum free-fatty acids and glucose levels than control mice (Yoshida 2012).

Pharmacological studies in humans
No data available.

Clinical studies
In an open study 593 patients with acute respiratory complaints such as acute bronchitis and post-infectious dry cough were treated, for 10 days on average, with a mean daily dose of 31.3 ml of a syrup, equivalent to about 6.3 g of ribwort plantain herb (100 ml of the syrup contained 20 g of fluid extract 1:1 of the herb). Symptoms declined significantly ($p<0.001$), the overall symptom score declining by about 65%. Compared to initial values, the subjective score reductions were: cough frequency, 65.5%; cough intensity, 66.9%; cough-related chest pain, 79.9%; dry cough, 68.5%; dyspnoea, 69.6%; rales, 69.9%; and obstructive "whistling", 74.6%. Expectoration improved by 73.3% and purulent sputum decreased by 66.4%. Global efficacy evaluated by the physicians was excellent in 25.9% of patients, good in 61.8%, moderate in 7.8% and minimal in 2% [Kraft 1996; Kraft 1997].

Similar results were reported in a subgroup of the 91 patients under 18 years of age (58 of them under 13 years) in the above study. In this subgroup the mean daily dose was 22.4 ml of the syrup, equivalent to about 4.5 g of ribwort plantain herb, and the mean duration of treatment was 8.8 days. The overall symptom score declined by about 58.3%. The physicians evaluated global efficacy as excellent in 22%, good in 63.7%, moderate in 9.9% and minimal in 1.1% of patients [Kraft 1998].

Pharmacokinetic properties
No data available.

Preclinical safety data
An aqueous extract of *Plantago lanceolata* leaf did not produce acute toxicity in rodents (species undefined) at a dose of 2000 mg/kg; the authors state that the LD_{50} of the extract was above 2000 mg/kg (Melese 2011).

No repeated-dose or reproductive toxicity data are available.

A tincture of ribwort plantain herb (1:5, 70 % ethanol) showed no mutagenicity in the Ames Test using *Salmonella typhimurium* strains TA98 and TA100, with or without metabolic activation [Schimmer 1994].

In a somatic segregation assay in the diploid strain *Aspergillus nidulans* D-30 no genotoxic effects (neither chromosomal damage caused by aneuploidy and clastogenic effects nor mitotic crossover) were detected after plate incorporation of 4.76 mg/ml of a fluid extract of ribwort plantain leaf [Ramos Ruiz 1996].

Clinical safety data
In the open study involving 593 patients there were only 7 minor adverse events, mainly diarrhoea (n = 5) [Kraft 1996; Kraft 1997; Kraft 1998].

Two cases of phytophotodermatitis have been reported following the consumption of ribwort plantain herb and exposure to sunlight. A 42-year-old woman and her 2-year-old daughter presented to an outpatient clinic with a blistered rash on sun exposed areas that had appeared 4 to 5 hours after a 2 hour exposure to sunlight, 24 hours after consumption of an undefined amount of the plant (identity confirmed by the Department of Medical Biology, Yüzüncü Yil University, Turkey)[Ozkol 2012].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Supplement 2009
ALOE CAPENSIS	Cape Aloes	Second Edition, 2003
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Second Edition, 2003
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
BALLOTAE NIGRAE HERBA	Black Horehound	Supplement 2009
BETULAE FOLIUM	Birch Leaf	Second Edition, 2003
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Second Edition, 2003
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Supplement 2009
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Second Edition, 2003
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Second Edition, 2003
FUMARIAE HERBA	Fumitory	Supplement 2009
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Supplement 2009
GRINDELIAE HERBA	Grindelia	Supplement 2009
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Second Edition, 2003
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Second Edition, 2003
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003

LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Supplement 2009
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Second Edition, 2003
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Second Edition, 2003
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Second Edition, 2003
RATANHIAE RADIX	Rhatany Root	Supplement 2009
RHAMNI PURSHIANI CORTEX	Cascara	Second Edition, 2003
RHEI RADIX	Rhubarb	Second Edition, 2003
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Second Edition, 2003
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Second Edition, 2003
SALICIS CORTEX	Willow Bark	Second Edition, 2003
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Second Edition, 2003
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VIOLAE HERBA CUM FLORE	Wild Pansy	Supplement 2009
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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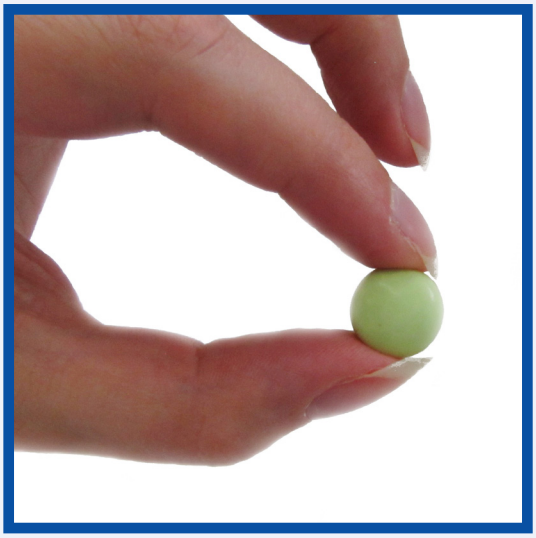
The Scientific Foundation for Herbal Medicinal Products

Plantaginis ovatae semen

Ispaghula Seed

2020



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Herbal Medicinal Products

PLANTAGINIS OVATAE SEMEN
Ispaghula Seed

2020

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

Edited by Roberta Hutchins and Simon Mills
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Plant illustrated on the cover: *Plantago ovata*

FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

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Members of ESCOP
Board of Supervising Editors
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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

PLANTAGINIS OVATAE SEMEN 2020

Ispaghula Seed

DEFINITION

Ispaghula seed consists of the dried ripe seeds of *Plantago ovata* Forssk. (*Plantago ispaghula* Roxb.).

The material complies with the monograph of the European Pharmacopoeia [Plantaginis ovatae semen].

CONSTITUENTS

About 80% [Leng-Peschlow 1991] of total dietary fibre when determined by the AOAC method [Prosky 1985], consisting predominantly of insoluble fibre (ratio of soluble to insoluble fibre, 47:53) [Leng-Peschlow 1991]. Soluble fibre occurs in the epidermis as a mucilage polysaccharide consisting mainly of a highly-branched arabinoxylan with a xylan backbone and branches of arabinose, xylose and 2-*O*-(galactosyluronic acid)-rhamnose residues [Kennedy 1979; Sandhu 1981].

The seeds also contain proteins, fixed oil, sterols and the trisaccharide planteose [Heckers 1984; Sharma 1986; Wichtl 1999].

CLINICAL PARTICULARS

Therapeutic indications

Treatment of occasional constipation [Kay 1978; Sölter 1983; Marlett 1987; Brunton 1990; Brayfield 2014].
Conditions in which easy defecation with soft stools is desirable, e.g. in cases of anal fissures or haemorrhoids [Webster 1978], after rectal or anal surgery, and during pregnancy.
Conditions which need an increased daily intake of fibre, e.g. irritable bowel syndrome [Sölter 1983; Ligny 1988].
Adjuvant symptomatic therapy in cases of diarrhoea from various causes [Bradshaw 1983; Kovar 1983; Sölter 1983; Koch 1984; Hamouz 1984; Qvitzau 1988].

Posology and method of administration

Dosage

Adults and children over 12 years of age: as a laxative, 7-30 g of the seeds [Sölter 1983; Marlett 1987; Ligny 1988, Brayfield 2014] or equivalent preparations daily; in cases of diarrhoea, 20 g up to 40 g daily, divided into 2-3 doses [Sölter 1983].
Children 6-12 years: half the adult dose.

Method of administration
For oral administration.

It is very important to take the seeds with a large amount of liquid, e.g. mix approx. 5 g with 150 ml of cool water, stir briskly and swallow as quickly as possible, then maintain adequate fluid intake.

Ispaghula seed should preferably be taken at mealtimes; it should not be taken immediately prior to going to bed. Any other medications should be taken at least 30-60 minutes before ispaghula seed in order to avoid delayed absorption.

Duration of use
In cases of diarrhoea, medical advice should be sought if the symptoms persist for more than 3 days in order to ensure definitive diagnosis of the cause.

Contraindications
Children under 6 years of age.
Known hypersensitivity to ispaghula seed [Suhonen 1983; Zaloga 1984; Lantner 1990; Brayfield 2014].

Ispaghula seed should not be used by patients with the following conditions unless advised by a physician: faecal impaction; undiagnosed abdominal symptoms; a sudden change in bowel habit that persists for more than 2 weeks; rectal bleeding; failure to defecate following the use of a laxative; abnormal constrictions in the gastrointestinal tract; potential or existing intestinal obstruction (ileus); diseases of the oesophagus and cardia; megacolon [Brunton 1990; Brayfield 2014]; diabetes mellitus which is difficult to regulate [Kay 1978; Cummings 1978; Brunton 1990].

Special warnings and precautions for use
A sufficient amount of liquid should always be taken: at least 150 ml of water per 5 g of seed [Brayfield 2014].

Taking ispaghula seed without adequate fluid may cause it to swell and block the throat or oesophagus and may cause choking. Intestinal obstruction may occur should adequate fluid intake not be maintained. Do not take ispaghula seed if you have ever had difficulty in swallowing or have any throat problems. If you experience chest pain, vomiting, or difficulty in swallowing or breathing after taking it, seek immediate medical attention. Treatment of the elderly and debilitated patients requires medical supervision. Administration to the elderly should be supervised.

In cases of diarrhoea sufficient intake of water and electrolytes is important.

Interactions with other medicinal products and other forms of interaction
Enteral absorption of concomitantly administered minerals (e.g. calcium, iron, lithium, zinc), vitamins (B12), cardiac glycosides and coumarin derivatives may be delayed [Kay 1978; Cummings 1978; Brunton 1990]. For this reason, other medications should be taken at least 30-60 minutes before ispaghula seed. In the case of insulin-dependent diabetes it may be necessary to reduce the insulin dose [Kay 1978; Cummings 1978; Capani 1980; Frati-Munari 1989].

Pregnancy and lactation
No restriction [Lewis 1985].
A risk is not to be expected since the constituents of ispaghula seed are not absorbed and have no systemic effects.

Effects on ability to drive and use machines
None known.

Undesirable effects
Flatulence may occur, but generally disappears during the course of treatment. Abdominal distension and risk of oesophageal or intestinal obstruction and faecal impaction may occur, particularly if ispaghula seed is ingested without sufficient fluid.

There is a risk of allergic reaction from inhalation of the powder during occupational exposure [Busse 1975]. There have been reports of single cases of hypersensitivity with the possible risk of anaphylaxis [Suhonen 1983; Zaloga 1984; Lantner 1990; Brayfield 2014].

Overdose
Overdosage may cause abdominal discomfort and flatulence, or even intestinal obstruction. Adequate fluid intake should be maintained and management should be symptomatic.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties
Note: Due to the fact that the ripe seeds of ispaghula seed contain the epidermis ('husk') with mainly soluble fiber (see Constituents), some pharmacological studies with ispaghula husk are referenced in the following as well.

Ispaghula seed has its effects by absorbing fluid, increasing bulk and so physically stimulating the gut wall. The intraluminal pressure is decreased and colon transit is accelerated, increasing the number of bowel evacuations and softening the stools. Ispaghula seed increases stool weight and water content due to the fibre residue, the water bound to that residue, and the increased faecal bacterial mass [Read 1986, Sharma 1986].

In vivo experiments

Metabolic effects
In rats adapted to a fibre-free, cholesterol-rich diet, dietary starch was replaced for 4 weeks by ispaghula seed or ispaghula husk. Within 2 weeks, ispaghula seed normalized plasma total cholesterol levels which were elevated 5-fold compared to a cholesterol-deficient diet. HDL significantly increased in fibre-free controls from 5% to 47% (in relation to total cholesterol). LDL was greatly reduced (1.14 versus 12.6 mmol/L) and liver cholesterol content significantly lowered (15.5 versus 22.1 µmol/L). Ispaghula seed was as effective as the husks [Leng-Peschlow 1989].

Rats were fed with a diet containing 0.5% cholesterol and 10% fibre (cellulose, pectin, ispagula seed or husk). Two control groups were fed a fibre-free diet, one of which also received 0.5% cholesterol in the diet. Ispaghula seed exerted an effect on total cholesterol equal to that of pectin but resulted in higher HDL levels. The effect on liver lipids was more pronounced than that of pectin. Ispaghula husk normalized serum triglyceride levels and produced lower serum total cholesterol and higher HDL levels compared to normal controls [Kritchevsky 1995].

Rats fed for 4 weeks with a diet supplemented with 10% ispaghula husk had significantly ($p<0.05$) higher levels of faecal bile acids than rats fed a fibre-free diet [Vahouny 1987].

After feeding 3 groups of hamsters of both sexes for 30 days with either a fibre-free diet (n=15), a high fibre diet with cellulose (n=8) or a high fibre diet with ispaghula husk (n=8), proximal, middle and distal regions of the small intestine were analyzed for their digestive-absorptive function of sucrose. Ispaghula husk significantly ($p<0.05$) reduced the level of monosaccharides crossing the intestinal tissue compared with the fibre-free diet, as did cellulose. Uniquely for isphagula however this effect was consistent through all segments of the bowel [Mahapatra 1988].

Male guinea pigs (n = 30; 10 per group) were fed either with a control diet or diets containing 7.5 g or 10 g ispaghula seed per 100g of diet for 4 weeks. Although a dose response was not observed, plasma triglycerides and LDL cholesterol were 34% and 23% lower compared to control ($p<0.01$). Lecithin cholesterol acyltransferase (LCAT) and cholesterol ester transfer protein (CETP) activities were significantly affected in the ispaghula seed treatment groups. The control group showed 100% and 36% higher LCAT and CETP ($p< 0.01$) activities respectively when compared to the ispaghula seed groups. Hepatic total and free cholesterol concentrations were not affected but cholesterol ester concentrations were 50% ($p< 0.01$) lower in the ispaghula seed groups. The activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (rate-limiting enzyme of cholesterol synthesis) was up-regulated in the treatment groups by 37%. Similarly, the activity of cholesterol 7α-hydroxylase (enzyme of cholesterol catabolism to bile acids) was 33% higher in the treatment groups ($p<0.02$). Faecal bile acids

were 3 times higher in the ispaghula seed groups compared to control [Romero 2008].

Mucosa-protective effects
Rats (n=70; 10 per group) were fed for 4 weeks with a diet supplemented with either 100 g or 200 g of ispaghula seed, ispaghula husk or wheat bran per kg of standard diet, and compared to controls given the fibre-free standard diet only. β-glucuronidase activity of colonic bacteria (potentially producing toxic metabolities from bile), decreased in a time-dependent manner in both ispaghula seed groups, at day 8 reaching 25% of the activity of the control group for the seed and 10% of the activity of the control group for the husk (both $p<0.001$), both having greater impact than wheat bran. Acetate is a short-chain fatty acid released from the digestible part of the fibre by bacterial fermentation, and has a trophic and differentiating effect on the mucosal cell; it was increased significantly ($p<0.001$) in caecal contents of the ispaghula groups compared to the fibre-free control (wheat bran having no significant effect) [Leng-Peschlow 1991].

Similar results have been shown in monkeys in which β-glucuronidase activity was significantly ($p<0.05$) decreased in those fed a diet supplemented with ispaghula husk compared to cellulose over a 3.5 year period [Costa 1989].

Although human clinical evidence is lacking [Yao 2017], elevated β-glucuronidase activity in rats is correlated with increased induction or promotion of colonic carcinogenesis [Bauer 1979; Takada 1982; Sun 1988].

Experiments in rats demonstrated that the microbial production of short-chain fatty acids is required to maintain normal epithelial cell proliferation not only in the hindgut but also in the small intestine [Sakata 1988], where butyrate is needed for normal differentiation of the cells [Muller 1986].

HLA-B27 transgenic rats (as an experimental model for ulcerative colitis) were fed with a fibre supplemented diet (5% ispaghula seed) for 13 weeks before evaluation of the colonic inflammatory status, both histologically and biochemically, and the luminal colonic production of short-chain fatty acids (SCFA, i.e. propionate, butyrate) was quantified. Dietary fibre supplementation ameliorated the development of colonic inflammation by a decrease of pro-inflammatory mediators, e.g. nitric oxide, leukotriene B4, TNFα. The intestinal contents of treated rats compared to non-treated animals showed a significantly higher production of SCFA which was associated with inhibition of production of pro-inflammatory mediators [Rodriguez-Cabezas 2003].

Pharmacological studies in humans

Laxative effects
Gastrointestinal transit time, frequency of defecation, stool weight and stool consistency were studied in 12 women who were each given fibre supplements (40g daily) wheat bran, psyllium gum (ispaghula), a combination of both, or a low fibre control (21g daily) for 2 weeks. Fibre supplementation decreased transit time and increased the daily number of defecations and both the wet and dry weights of stools. Bran had a greater effect on transit time than psyllium; whereas psyllium was superior to bran regarding the amount of water in the stools and the total stool weight. In subjects receiving the control, 50% of the stool ratings were scored as "hard" compared to 10% in those receiving the high-fibre supplements [Stevens 1988].

Metabolic effects
Ispaghula husk (9.6 g daily) was administered for 6 weeks to 2 normally active young adult males after a 4-week pre-experimental control period. Total faecal bile acids per 24 hours averaged 3 times (302%) the excretion level of the control period. Serum cholesterol levels averaged 17% lower than during the control period ($p<0.005$) [Forman 1968].

Clinical studies

Constipation
In a short-term study, 42 adults (aged 18 to 35) with a history of chronic constipation and having ≤3 bowel movements during one week of a single blind placebo treatment were randomized to receive either 7.2 g of ispaghula husk per day or a combination of ispaghula seed plus senna (6.5 g + 1.5 g per day) for 14 days. Both laxatives increased defecation frequency although the added effect of senna was clearly evident. Constipation was assessed by stool frequency and weight, with laxation attained by 63% of the ispaghula/senna combination group and 48% of the ispaghula husk group alone [Marlett 1987].

In 15 short-term open controlled clinical trials 669 patients in total (266 males and 403 females; aged 13 to 90 years) were treated with ispaghula seed (product containing 65 g ispaghula seed with 2.2 g ispaghula husk and excipients up to 100 g of finished product) for 7 days. The standard daily dose of the product administered with a glass of cold water was 10 g before the evening meal and if necessary an extra 5 g before breakfast. Number of daily bowel evacutions, stool consistency and associated symptoms were noted on days 1, 3, 4 and 7. 309 patients with constipation (in some cases post surgery), 74 pregnant women, 116 patients with haemorrhoids (including post surgery), anal fissures and abscesses as well as 142 patients with constipation from various reasons (in total 641 patients) were suitable for assessment [Sölter 1983].

In the short-term trial with the largest number of patients (200 of which 197 considered for evaluation) treatment of constipation in bedridden patients (n = 89) was studied. After 1 day of treatment 74% of the patients had at least one bowel movement, after 3 days bowel movement was achieved in 92% and after 7 days in 99% of the cases. In 27 other patients constipation post surgery was treated and success rates of 48%, 100%, 100% and 100% were noted on days 1, 3, 4 and 7 respectively [Sölter 1983].

Of 28 patients treated with the ispaghula seed combination because of haemorrhoids, anal fissures and abscesses at least one bowel movement started already on the first day of treatment in 82% and by the third day in 96% of cases [Sölter 1983].
In 17 patients with recent hemorrhoidectomy or other anal operations, defecation began in 35%, 94%, 100% and 100% of the patients on days 1, 3, 4 and 7 respectively [Sölter 1983].
To summarize the results in the remaining short-term trials, a response to treatment in the form of at least one daily bowel evacuation was achieved in 53% on the first day, in 83% on the third day and in 90% of the patients on the fourth day of treatment [Sölter 1983].

Regarding the parameter "changes in stool consistency (measured in a 5 point scale from liquid to no evacution)" the result of all 15 short-term trials was a significant effect ($p<0.01$; at least 0.69 scale units) in 7 of 13 trials.

Regarding stool consistency a significant improvement was noted in constipation in pregnancy, in 3 of the 4 studies ($p<0.01$) [Sölter 1983].

In a 3-center long-term open clinical trial 139 patients (59 males and 80 females; 136 suitable for assessment; aged 9 to 80 years) were treated with the same daily dose as in the

short-term trials over periods of up to 12 weeks (110 patients were treated for the full 12 weeks, the others dropped out because they no longer required medication but were however included for evaluation). Number of daily bowel evacuations and stool consistency improvement were noted on day 8 and at 4, 8 and 12 weeks. The total number of patients successfully treated regarding number of daily bowel evacuations and stool consistency was 123 (88.4%) [Sölter 1983].

Irritable bowel syndrome
In a randomized, double blind, placebo controlled clinical study, 60 patients with irritable bowel syndrome were treated 4 times daily for 30 days with a combination of ispaghula seed (3.25 g per dose) and husk (0.11 g per dose) or placebo, after meals and before going to bed (total daily dose:13.0 g of seed and 0.44 g of husk). Patients were allowed to take a concomitant antispasmodic at doses of up to 5 tablets daily. The number of tablets taken and severity of condition were assessed. After 15 days of treatment there was a significant ($p<0.05$) improvement in both groups. After 30 days of treatment, 27 out of 30 patients in the ispaghula group claimed a symptomatic improvement, their antispasmodic requirements dropped by more than 50%. In the placebo group, 19 out of 30 patients showed an improvement in symptoms but their antispasmodic intake remained as high as before the trial [Ligny 1988].

Diarrhoea
In an open study, 36 patients (28 females, 8 males) with symptoms of diarrhoea for 2 days (on average 7 evacuations before starting therapy) were treated with a combination of ispaghula seed (6.5 g) and husk (0.22 g) 3 times daily (total daily dose 19.5 g of seed and 0.66 g of husk). Frequency of bowel evacuations was reduced on the first day of treatment to an average of 3 and to <2 on the second day of treatment. On day 3 only one patient had more than 1 evacuation [Kovar 1983].

In an open, controlled study, 50 inpatients with acute (n=22) and chronic (n=28) diarrhoea were treated for 3 days with a combination of ispaghula seed (6.5 g per dose) and husk (0.22 g per dose) 3 times daily (total daily dose 19.5 g of seed and 0.66 g of husk) and individually dosed for a further 4 days. Firming of stools and a reduction of evacuation frequency were established on day 3 in all patients. On day 7 diarrhoea symptoms were no longer present, with no recurrence one week after discontinuation of treatment [Hamouz 1984].

In an open, randomized, crossover study, 25 patients (18 females and 7 males) with chronic diarrhoea were treated either with loperamide (4 mg daily initially and 2 mg after each loose stool with a maximum of 16 mg per day) or ispaghula husk and calcium (husk/calcium carbonate/calcium hydrogen phosphate in a weight ratio of 4:1:1; 5 g twice daily). The median duration of diarrhoea was 12 months (range: 0.5 – 360 months). After 7 days of treatment with either loperamide or ispaghula husk-calcium, treatment was stopped. When diarrhoea recurred, the alternative treatment was started for a further 7 day period. Treatment sequence was decided randomly. Before treatment, the median number of daily stools was 7 (range: 4-13). Both treatments reduced stool frequency by 50%, but the ispaghula husk/calcium was significantly ($p<0.05$) more effective with regard to urgency and stool consistency [Qvitzau 1988].

In an open, controlled study, 84 hospital inpatients with diarrhoea (48 psychiatric patients and 36 nursing home patients) were treated for up to 3 days with a combination of ispaghula seed (6.5 g) and husk (0.22 g) 3 times daily (total daily dose 19.5 g of seed and 0.66 g of husk). The dose was then reduced to 3.25 g of seed and 0.11 g of husk 3 times daily (total daily dose 9.75 g of seed and 0.33 g of husk), if necessary.

In the study group of psychiatric patients (28 females and 20 males), 20 had acute and 28 had chronic diarrhoea. During treatment of the subgroup with chronic diarrhoea, the ispaghula combination showed a good response in 16 cases and an adequate response in 8 others. After one week of treatment, the average daily frequency of bowel movements was reduced from 3.4 (before treatment) to 1.5.

In the 20 psychiatric patients with acute diarrhea, stool frequency decreased from a daily average of 4.7 before treatment to 2.3 on the third day of treatment.

The nursing home patients (28 females and 8 males, age 40 to 90 years) were all suffering from acute diarrhoea. Before treatment, average daily stool frequency was 6.94 which dropped to 3.28 on the first day of treatment and to 2 on the third day. All stools were soft but formed or solid, and there were no accompanying symptoms [Sölter 1983].

In a randomized, controlled, crossover study, 30 patients with Crohn's disease were either treated after their hospital stay with a combination of ispaghula seed (3.25 g per dose) and husk (0.11 g per dose) 2 times daily (in total 6.5 g of seed and 0.22 g of husk) or remained untreated. Treatment with the ispaghula combination was discontinued in the first group after 3 months, and subsequently given for a further 3 months to the previously untreated group. Both arms of the study were completed by 20 patients. Disregarding the periods of active inflammatory exacerbation, the average number of daily bowel evacuations significantly ($p<0.01$) decreased with the ispaghula seed combination from 2.71 to 2.0 per day. Including the periods of active inflammation, the average number of evacuations dropped from 3.79 to 2.19 per day ($p<0.01$) [Sölter 1983, Koch 1984].

Lipid lowering activity
Chronic inpatients (6 of each sex, aged between 57 and 76), suffering all but one from constipation and being confined to bed, were given up to 15-18 g of ispaghula husk shortly before breakfast for 16 weeks. Stool consistency and ease of passage significantly improved in all patients. In all but 2 patients a decrease in serum cholesterol also occurred. The mean cholesterol level was significantly ($p<0.005$) reduced from 6.1 to 5.1 mmol/L [Burton 1982].

In a double-blind, placebo-controlled, parallel study, 28 men with mild to moderate hypercholesterolemia (range: 4.86 to 8.12 mmol/L) were treated for 8 weeks with 3.4 g of ispaghula husk, or cellulose as placebo, three times daily at mealtimes. Ispaghula husk significantly reduced total cholesterol by 14.8%, from 6.39 to 5.45 mmol/L ($p<0.05$), and LDL by 20.2%, from 4.18 to 3.34 mmol/L ($p<0.05$) [Anderson 1988].

Mildly hypercholesterolemic male and female adults (n=28) were administered ispaghula seed (4.06 g twice daily p.o.) for 3 months. Compared to pre-treatment values total cholesterol was reduced by 7.2% ($p<0.007$) and LDL by 13% ($p<0.0003$), while the other parameters including HDL, triglycerides and urea nitrogen remained unaffected [Segawa 1998].

Hypoglycemic effects
In an open, randomized, crossover study, 20 postgastrectomy patients received a glucose solution with or without ispaghula husk to determine its effects on blood glucose and breath hydrogen. In the high responder group (n=15; 20 ppm hydrogen/2h) the mean hydrogen response was 78 ppm/2h with the glucose meal and reached a blood glucose level of 195 mg/dl. The mean hydrogen response was reduced 20 ppm/2h following a glucose meal with ispaghula husk and blood glucose level decreased to 153 mg/dl. The differences were significant ($p<0.05$) [Welsh 1982].

In an open, controlled trial, 19 male and 21 female outpatients (aged 39 to 77 years) suffering from constipation and non-insulin dependent diabetes were allocated into 2 treatment groups, receiving either 3.6 g or 7.2 g ispaghula husk daily for 2 months. After this period all patients received 10.8 g of ispaghula husk daily for a further 2 months. Fasting glucose after 3.6 g of ispaghula husk decreased from an average of 10.9 mmol/l to 8.8 ($p<0.01$) and after 7.2 g of ispaghula husk from 10.7 to 8.8 ($p<0.01$). After 4 months of treatment the average was significantly ($p<0.001$) reduced to 8.8 mmol/l glucose from a starting average of 10.8 mmol/l [Fagerberg 1982].

Pharmacokinetic properties
The vegetable fibre of ispaghula seed is resistant to digestion in the upper intestinal tract; thus it is not absorbed. Part of the fibre is degraded by colonic bacteria in the lower intestinal tract to short-chain fatty acids, methane or carbon dioxide [Kay 1978].

Preclinical safety data
No data available.

Clinical safety data

Constipation
Among the symptoms of constipation are gaseous distension, abdominal pain, nausea and vomiting. The same symptoms may arise from medication with a bowel-regulating agent like ispaghula seed. Therefore it is often difficult to distinguish between a symptom due to constipation and a side effect due to a bulk laxative. In short-term clinical trials with 669 patients with constipation of various aetiologies, the most commonly reported symptoms were gaseous distension, and occasionally abdominal pain and slight nausea which disappeared with continuation of treatment [Sölter 1983].

Adverse effects also occurred in a long-term study over 12 weeks in 92 out of 139 patients, mainly in the first 2 weeks of treatment in the form of gaseous distension and abdominal pain, but tended to subside as treatment was continued. Proctoscopy, carried out in a random sample of patients (n = 9) during the 12 weeks of treatment, showed no macroscopic or histological evidence of any changes in the rectal mucosa. Checks of electrolyte levels, also in random samples, showed no changes in potassium, sodium or calcium levels [Sölter 1983].

Diarrhoea
In open clinical studies with a total of 170 patients with acute and chronic diarrhoea of various origin, an ispaghula seed and husk combination was well tolerated. Gaseous distension occurred in most cases on the first day of treatment but disappeared when treatment was continued [Kovar 1983; Sölter 1983; Hamouz 1984].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Online Series, 2019
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Online Series, 2019
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Online Series, 2019
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHAMOMILLAE ROMANAE FLOS	Roman Chamomile Flower	Online Series, 2019
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HELICHRYSI FLOS	Sandy Everlastin Flower	Online Series, 2019
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013

HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Online Series, 2020
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Online Series, 2020
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Online Series, 2020
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Online Series, 2020
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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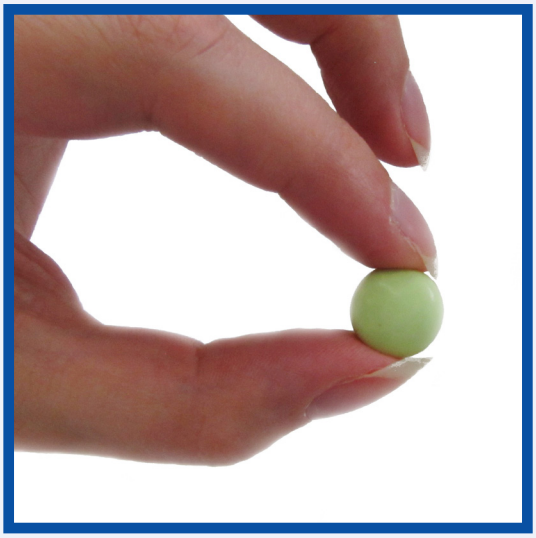
The Scientific Foundation for Herbal Medicinal Products

Plantaginis ovatae testa

Ispaghula Husk, Blond Psyllium Husk

2016



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***The* Scientific Foundation for**
Herbal Medicinal Products

PLANTAGINIS OVATAE TESTA
Ispaghula Husk, Blond Psyllium Husk

2016

E/S/C/O/P
European Scientific Cooperative
On Phytotherapy

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Second Edition, completely revised and expanded

Second Edition, Supplement 2009

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Edited by Simon Mills and Roberta Hutchins
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Stan Shebs [CC BY-SA 3.0 (http://creativecommons.org/licenses/by-sa/3.0)] (*Plantago ovata*)
Cover and text design by Martin Willoughby
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Plant illustrated on the cover: *Plantago ovata*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

PLANTAGINIS OVATAE TESTA 2016

Ispaghula Husk, Blond Psyllium Husk

DEFINITION

Ispaghula husk consists of the episperm and collapsed adjacent layers removed from the seeds of *Plantago ovata* Forssk. (*P. ispaghula* Roxb.).

The material complies with the monograph of the European Pharmacopoeia [Ispaghula Husk].

CONSTITUENTS

Dietary fibre, principally as mucilage polysaccharide consisting of a highly-branched arabinoxylan with a xylan backbone and branches of arabinose, xylose and 2-*O*-(galactosyluronic acid)-rhamnose residues [Kennedy 1979; Sandhu 1981; Hänsel 1999; Marlett 2003; Fischer 2004].

CLINICAL PARTICULARS

Therapeutic Indications
Treatment of occasional constipation [Sweetman 2002; Brunton 1990; Kay 1978; Borgia 1983; Stephen 1980; Marlett 1987; Thorburn 1992; McRorie 1998; Wang 2004].
Conditions in which easy defaecation with soft stools is desirable, e.g. in cases of anal fissures or haemorrhoids [Webster 1978; Kecmanovic 2006], after rectal or anal surgery [Eogan 2007], and during pregnancy.
Conditions which need an increased daily intake of fibre, e.g. irritable bowel syndrome [Prior 1987; Golecha 1982; Kumar 1987; Bijkerk 2009; Ford 2008].
Adjuvant symptomatic therapy in cases of diarrhoea from various causes [Bradshaw 1983; Qvitzau 1988; Hamouz 1984; Bobrove 1990; Smalley 1982; Frank 1979; Eherer 1993].
As an adjunct to a low fat diet in the treatment of mild to moderate hypercholesterolaemia [Bell 1989; Levin 1990; Anderson 1991; Sprecher 1993; Weingand 1997; Anderson 2000a; MacMahon 1998; Anderson 2000b; Ciba 1992a/b/c; Ciba 1993a/b/c; Neal 1990; Bell 1990; Anderson 1992; Roberts 1994; Stoy 1993; Wolever 1994a; Summerbell 1994; Jenkins 1997; Olson 1997; Davidson 1998; Davidson 1996; Spence 1995; Anderson 1988; Everson 1992; Rodriguez-Morán 1998; Gupta 1994; FDA 1998; Ganji 2008; Uehleke 2008; Jayaram 2007; Jenkins 2002; Solà 2010].

Posology and method of administration

Dosage
Adults and children over 12 years of age: 4-20 g daily, divided into 2-3 doses.
Children 6-12 years: half the adult dose.

Special Dosage Instructions
For the treatment of hypercholesterolaemia approx. 10 g of ispaghula husk should be given daily in 2 or 3 doses [Bell 1989; Levin 1990; Anderson 1991; Sprecher 1993; Weingand 1997; Anderson 2000a; MacMahon 1998; Anderson 2000b; Ciba 1992a/b/c; Ciba 1993a/b/c; Bell 1990; Anderson 1992; Roberts 1994; Stoy 1993; Summerbell 1994; Jenkins 1997; Olson 1997; Davidson 1998; Anderson 1988; FDA 1998].

Method of administration
For oral administration.

It is very important to take the material with a large amount of liquid, e.g. mix approx. 5 g with 150 ml of cool water, stir briskly and swallow as quickly as possible, then maintain adequate fluid intake.

Ispaghula husk should preferably be taken at mealtimes; it should not be taken immediately prior to going to bed. Any other medications should be taken at least 30-60 minutes before ispaghula husk in order to avoid delayed absorption.

Duration of administration
In cases of diarrhoea, medical advice should be sought if the symptoms persist for more than 3 days in order to ensure definitive diagnosis of the cause.

Contraindications
Children under 6 years of age.

Known hypersensitivity to ispaghula husk [Sweetman 2002; Brunton 1990; Zaloga 1984; Suhonen 1983; Lantner 1990].

Ispaghula husk should not be used by patients with the following conditions unless advised by a physician: faecal impaction; undiagnosed abdominal symptoms; a sudden change in bowel habit that persists for more than 2 weeks; rectal bleeding; failure to defaecate following the use of a laxative; abnormal constrictions in the gastrointestinal tract; potential or existing intestinal obstruction (ileus); diseases of the oesophagus and cardia; megacolon [Sweetman 2002; Brunton 1990]; diabetes mellitus which is difficult to regulate [Brunton 1990; Kay 1978; Cummings 1978].

Special warnings and special precautions for use
A sufficient amount of liquid should always be taken: at least 150 ml of water per 5 g of material [Thorburn 1992].

If you experience chest pain, vomiting, or difficulty in swallowing or breathing after taking ispaghula husk, seek immediate medical attention. Administration to the elderly should be supervised. In cases of diarrhoea sufficient intake of water and electrolytes is important.

Interactions with other medicaments and other forms of interaction
Enteral absorption of concomitantly administered minerals (e.g. calcium, iron, lithium, zinc), vitamins (B12), cardiac glycosides and coumarin derivatives may be delayed [Brunton 1990; Kay 1978; Cummings 1978; Perlmann 1990]. For this reason, other medications should be taken at least 30-60 minutes before ispaghula husk. In the case of insulin-dependent diabetics it may be necessary to reduce the insulin dose [Kay 1978; FDA 1998; Fagerberg 1982; Capani 1980; Frati-Munari 1989; Abraham 1988].

Fertility, pregnancy and lactation
No restriction.

A risk is not to be expected since the components of ispaghula husk are not absorbed and have no systemic effects [Lewis 1985].

Effects on ability to drive and use machines
None known.

Undesirable effects
Flatulence may occur, but generally disappears during the course of the treatment. Abdominal distension and risk of oesophageal or intestinal obstruction and faecal impaction may occur, particularly if ispaghula husk is ingested without sufficient fluid.

There is a risk of allergic reaction from inhalation of the powder during occupational exposure. Due to the allergic potential of ispaghula husk, patients must be aware of reactions of hypersensitivity including anaphylactic reactions in single cases [Zaloga 1984; Suhonen 1983; Lantner 1990].

Overdose
Overdose may cause abdominal discomfort and flatulence, or even intestinal obstruction. Adequate fluid intake should be maintained and management should be symptomatic.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

Dietary fibre such as ispaghula husk binds water and swells to form a demulcent gel or viscous solution in the intestine. By virtue of binding water in the colon, the faeces are softened and their bulk increased, thus promoting peristalsis and reducing transit time [Stephen 1980; Marlett 1987; Brunton 1990; Sweetman 2002]. Degradation products of dietary fibre resulting from bacterial action in the colon may also contribute to the laxative effect [Kay 1978; Brunton 1990].

The ability to absorb abundant water and to convert fluid in the intestine into a more viscous mass also makes ispaghula husk useful for the symptomatic relief of acute diarrhoea, enabling the patient to pass a formed stool [Smalley 1982; Bradshaw 1983; Brunton 1990].

In vitro experiments
After anaerobic fermentation of ispaghula husk with colonic bacteria, induction of apoptosis was induced in all primary tumour and metastatic cell lines of colorectal cancer cells, independent of p53, adenomatous polyposis coli, β-catenin or cyclo-oxygenase-2 status [Sohn 2012].

In vivo experiments
Ispaghula husk reduces experimentally-induced hyperlipidaemia and atherosclerosis in animals [Turley 1994; Turley 1991; McCall 1992a; Gallaher 1993; Matheson 1995; Horton 1994; Fernandez 1995; Arjmandi 1992; Matheson 1994; McCall 1992b]. Beneficial changes in serum lipid and lipoprotein profiles were greater in rats fed a mixture of ispaghula husk and margarine (rich in *trans*, as well as *cis*, fatty acids) than in rats fed a mixture of ispaghula husk and corn oil/olive oil (rich in *cis*, but free from *trans*, fatty acids) [Fang 2000].

In 2 groups of rabbits fed for 4 weeks with either standard feed (group 1; n=18) or with feed supplemented with ispaghula husk (3.5 mg/kg/d) (group 2; n=18), biochemical parameters were determined on day 14 after a glucose load of 3g. Alloxan was then used to induce experimental diabetes and animals were analysed after a further glucose load on day 28. In healthy animals, at day 14, the addition of ispaghula husk to feed had not modified glucose concentrations. On day 28, the diabetic animals (subdivided into mild and severe diabetes) fed with ispaghula husk had decreased glucose concentrations which were more pronounced in rabbits with mild diabetes (21.7%; $p<0.05$) than in rabbits with severe diabetes (9.6%; not significant). All other biochemical parameters were lower in group 2 than in group 1 (standard diet) although differences were significant only for total cholesterol, LDL and Hb1Ac ($p<0.05$) [Díez 2013].

Similar experiments in obese Zucker rats (model for metabolic syndrome) showed that ispaghula husk is more beneficial than insoluble fiber such as cellulose or methylcellulose [Galisteo 2010].

Ispaghula husk had little or no effect on the faecal excretion of neutral steroids or on cholesterol absorption [Turley 1994; Turley 1991; McCall 1992a] but apparently enhanced bile acid loss, due to its gel-forming ability and its viscosity [Gallaher 1993; Matheson 1995].

In C57BL/6J mice, the transcriptional response regarding genes encoding enzymes regulating key steps of lipid metabolism and their protein expression were tested 3 and 10 weeks after a control diet or a high fibre diet containing 10% ispaghula husk. In the verum group, plasma total cholesterol ($p<0.05$)

and triacylglycerol ($p<0.05$) levels were reduced which was most likely mediated by increased bile acid synthesis. After 3 weeks, genes involved in lipogenesis were downregulated, whereas those involved in cholesterol and bile acid synthesis were upregulated. With prolonged ispaghula husk feeding, genes involved in lipogenesis and the respective protein expression levels were significantly upregulated which could likely suggest a regulatory mechanism to restore the lowered plasma cholesterol and triacylglycerol levels [Chan 2008].

Male hamsters were fed for 4 weeks with a high n-3 PUFA (polyunsaturated fatty acid) diet including either ispaghula husk or methyl cellulose at 80 mg/kg of feed, and enriched with variable levels of cholesterol (0, 0.05, 0.1%, w/w). Plasma total cholesterol and LDL were significantly ($p<0.05$) lower for ispaghula husk compared to methyl cellulose in only the 0.1% cholesterol group. In a further experiment, hamsters were divided into four groups, two given a diet high in n-3 PUFA and 2 high in n-6 PUFA, all diets contained 0.2 % cholesterol and either psyllium husk or methyl cellulose at 80 mg/kg of feed. Psyllium husk led to significantly ($p<0.05$) lower plasma total cholesterol and VLDL-cholesterol levels when compared to methyl cellulose in only the hamsters fed the n-3 PUFA diet, whereas hepatic total cholesterol was significantly ($p<0.05$) lower for ispaghula husk compared to methyl cellulose in the n-3 and n-6 PUFA diet groups [Liu 2004].

Ispaghula husk was shown to increase the activity of hepatic 7α-hydroxylase, the initial and rate-limiting enzyme in the conversion of cholesterol to bile acids [Matheson 1995; Horton 1994; Fernandez 1995]. The conversion of hepatic cholesterol into bile acids has been established as a mechanism for reducing serum cholesterol [Matheson 1995; Arjmandi 1992; Matheson 1994] with a net negative sterol balance across the liver [Turley 1994].

A study testing various diets in guinea pigs investigated the mechanisms by which ispaghula husk influences plasma low-density lipoprotein (LDL) concentrations. Ispaghula husk altered hepatic cholesterol homeostasis by increasing the activity of HMG-CoA reductase ($p<0.001$) and reducing hepatic acyl-CoA:cholesterol acyltransferase activity ($p<0.001$). It increased hepatic membrane apoB/E receptor number ($p<0.005$), reduced plasma LDL concentrations ($p<0.001$) and modified LDL composition and size compared to LDL from control animals. The LDL from treated animals had a lower proportion of cholesteryl ester, a higher proportion of triacylglycerol, a lower molecular weight, a smaller diameter and a higher peak density ($p<0.001$) [Fernandez 1995].

Anti-carcinogenic effects
ICR:CD1 female mice exposed to carcinogenesis with azoxymethane (10mg/kg b.w. for 6 weeks) were fed with a diet containing 0.7% ispaghula husk. Two weeks after the last azoxymethane (AOM) injection, the caecum, colon and rectum were inspected for early precursor lesions of colorectal cancer such as Aberrant Crypt Foci (ACF), Mucin Depleted Foci (MDF) and Beta-Catenin-Accumulated Crypts (BCAC). ACF, BCAC and low-grade dysplasia were observed in the intestinal mucous membrane from AOM-treated animals, both with and without ispaghula supplementation, while MDF and high grade dysplasia were seen only in AOM-treated control animals fed with standard diet. Differences were not significant between the groups but the ispaghula supplement group had a trend towards reduced lesions [Díez-Liébana 2009].

Pharmacological studies in humans
In 10 healthy subjects, 20 g of ispaghula husk per day significantly decreased fat digestibility ($p<0.05$) and increased faecal fat excretion ($p<0.05$) [Ganji 1994]. However it did not affect faecal steroid excretion (neutral steroids and bile acids) [Abraham 1988; Gelissen 1994]. In16 healthy volunteers consuming 7 g of ispaghula husk per day for 8 weeks, faecal lithocholic and isolithocholic acids and the weighted ratio of lithocholic to deoxycholic acid were significantly lower after treatment. These changes in faecal bile acid profiles indicate a reduction in the hydrophobicity of bile acids in the enterohepatic circulation [Chaplin 2000].

In a placebo-controlled, crossover design study with a 21 day washout period, 68 healthy volunteers (24 male, 23 premenopausal female, 21 postmenopausal female) received 15g of ispaghula husk or placebo daily for 30 days. Mononuclear cell LDL receptor mRNA was not significantly modified by the ispaghula husk in any group compared to control. In contrast, HMG-CoA reductase mRNA was significantly ($p<0.05$) increased in the ispaghula husk groups by 8, 27 and 25% respectively in the men, pre- and postmenopausal women. Although ispaghula husk increased mononuclear cell lipoprotein lipase mRNA in men by 24% ($p<0.05$), postmenopausal women experienced a 23% decrease ($p<0.05$) whereas premenopausal women were not affected [Vega-López 2003].

Using radiotelemetry in patients with left sided diverticular disease, it was shown that ispaghula husk stimulates colonic motility and thus reduces mouth to rectum transit time, predominantly by decreasing the duration of right colonic transit [Thorburn 1992].

Clinical studies

Laxative effect
In a 2-week randomized study, the effects of ispaghula husk (2 × 5.1 g per day) were compared with those of docusate sodium (200 mg per day) in 170 patients suffering from chronic idiopathic constipation. Ispaghula husk was significantly superior to docusate sodium for softening stools by increasing stool water content and had greater overall laxative efficacy [McRorie1998].

In a randomised, controlled, open study with parallel group design male and female patients with chronic functional constipation were treated for 2 weeks with either low-dose polyethylene glycol plus electrolytes (PEG+E; 13.8g dissolved in water twice daily; n=63) or ispaghula husk (3.5g dissolved in water twice daily; n=63). The weekly defecation rates after treatment for 1 or 2 weeks were all statistically significant ($p<0.001$) compared to baseline for both groups and timepoints. In the PEG+E group the mean weekly defecation rate increased from 1.18 at baseline to 7.95 after 1 week and 8.48 after 2 weeks; in the ispaghula husk group it increased from 1.33 at baseline to 5.33 after 1 week and 5.71 after 2 weeks. After 2 weeks of treatment with PEG+E and ispaghula husk the stool was normalized in 55 out of 63 (87.3%) and 42 out of 63 (66.7%) patients respectively. The absolute mean values and increases of defecation rate achieved after both 1 and 2 weeks were all significantly greater in the PEG+E group than those in the ispaghula husk group ($p<0.001$) [Wang 2004].

For reduction of constipation in people prescribed opioids, a review up to the year 2009 found no clinically relevant results from randomised controlled studies with ispaghula husk [Ahmedzai 2010].

Conditions in which easy defecation is desired
Consistency of the faeces, ease of defecation and general bowel habit were significantly improved ($p<0.01$) in 53 patients with

haemorrhoids after a 6-week course of 2 × 3.5 g of ispaghula husk daily when compared to results from the 6-week placebo phase in a double-blind, crossover study [Webster 1978].

In a randomised, clinical trial involving 98 patients with grade III and IV haemorrhoids undergoing open haemorrhoidectomy, subjects were divided into two groups of 49 patients and treated postoperatively for 20 days with either 2 sachets daily of 3.26 g of ispaghula husk or glycerin oil as control. The individual pain score was significantly lower in the ispaghula husk group compared to control at the first bowel movement after surgery ($p<0.001$) and after 10 days ($p<0.01$); there was no significant difference in pain level after 20 days ($p>0.05$). Patients in the verum group had a significantly shorter mean hospital stay of 2.6 days ($p<0.001$) compared to the control group with 3.9 days [Kecmanovic 2006].

In a randomised clinical study involving 147 post partum women who had sustained anal sphincter injury at vaginal birth, one group (n=77) received lactulose alone three times daily for the first 3 postpartum days followed by sufficient lactulose over the following 10 days compared to a group (n=70) with the same lactulose regimen and an additional sachet of ispaghula husk (3.5 g) per day. Pain scores were similar in both groups and the additional treatment did not show any additional benefit [Eogan 2007].

Conditions requiring increased fibre intake, e.g. irritable bowel syndrome (IBS)
In another controlled crossover study a significant improvement ($p<0.05$) was observed from overall assessment after patients with irritable bowel syndrome (IBS) had taken ispaghula husk for 3 weeks, the best results being obtained in spastic colitis ($p<0.01$) and relief in severity of abdominal pain ($p<0.05$) [Golecha 1982]. Ispaghula husk was also shown to alleviate the symptoms of left-sided diverticular disease [Thorburn 1992].

In a randomised, placebo-controlled clinical trial 275 patients with IBS received either 10 g of ispaghula husk (n=85), 10 g of bran (insoluble fiber; n=97) or placebo (rice flour; n=93), taken with meals in two daily dosages for 12 weeks. The primary endpoint was adequate symptom relief analysed after 1, 2 and 3 months of treatment and secondary endpoints included IBS symptom severity score (validated visual analogue scale relating five aspects of bowel dysfunction to actual intensity of IBS symptoms), severity of abdominal pain (measured by means of first question of IBS symptom severity score), and IBS quality of life scale (validated in various populations). The 3 month treatment period was completed by 54 (64%) of the patients in the ispaghula husk group, 54 (56%) in the bran group and 56 (60%) in the placebo group. The primary outcome, as measured by the proportion of responders (with more than 2 weeks of adequate relief in previous 4 weeks) was significantly greater in the ispaghula husk group compared to placebo after 1 month (57% versus 35%) and after 2 months (59% versus 41%). After 3 months the difference compared to placebo was significant for the bran group (57% versus 32%) but not the ispaghula husk group. After 3 months of treatment symptom severity was significantly ($p = 0.03$) reduced by 90 points in the ispaghula husk group compared to 49 points in the placebo group, and non-signficantly decreased by 58 points ($p = 0.61$) in the bran group. No significant differences were found between the three groups with respect to changes in severity of abdominal pain related to IBS or in quality of life [Bijkerk 2009].

A review up to 2008 analysed 6 randomised clinical trials in order to determine the effect of ispaghula husk in the treatment of IBS. Persistent symptoms remained after treatment in 83 patients (52%) out of 161 receiving ispaghula husk compared to 103 out of 160 (64%) receiving placebo, showing some efficacy for ispaghula husk in treating IBS. However, only 5 of the studies had a Jadad score of 4 or more leaving the effect of ispaghula husk as no longer statistically significant [Ford 2008].

Antidiarrhoeal effect
In a crossover study 9 volunteers with diarrhoea induced by phenolphthalein were consecutively treated in a random sequence with 18 g of ispaghula husk, 6 g of calcium polycarbophil, 42 g of unprocessed wheat bran or placebo daily for 4 days. Only ispaghula husk treatment made stools firmer ($p<0.01$) and increased faecal viscosity ($p<0.001$). In a dose-response study in 6 subjects, doses of 9, 18 and 30 g of ispaghula husk per day caused a near linear increase in faecal viscosity [Eherer 1993].

Cholesterol-lowering effects
A meta-analysis has been conducted on 5 published studies [Bell 1989; Levin 1990; Anderson 1991; Sprecher 1993; Weingand 1997] and 3 unpublished studies involving a total of 656 subjects with mild to moderate hypercholesterolaemia (384 in ispaghula husk groups and 272 in placebo groups); 7 studies used a randomized, double-blind, placebo-controlled design, while one study [Weingand 1997] used a crossover design. All the studies met the following criteria: ispaghula husk was used as an adjunct to an American Heart Association (AHA) Step 1 diet (i.e. a diet with no more than 30% of calories from total fat, less than 10% calories from saturated fat, and less than 300 mg of cholesterol daily) with a pretreatment dietary lead-in period of 8-12 weeks; the subjects consumed 10.2 g of ispaghula husk daily in 2 or 3 divided doses for 8 weeks (4 studies), or 12-26 weeks (4 studies). The conclusions were that ispaghula husk significantly lowered serum total cholesterol concentrations by an additional 3.9% ($p<0.0001$) and serum LDL-cholesterol concentrations by an additional 6.7% ($p<0.0001$) relative to placebo in subjects already consuming a low fat diet. Ispaghula husk also significantly lowered serum ratios of apolipoprotein B to apolipoprotein A-I by an additional 6% ($p<0.05$) relative to placebo, but had little effect on serum HDL-cholesterol or triglyceride concentrations [Anderson 2000a].

340 patients with mild to moderate hypercholesterolaemia entered an initial 8-week diet-only period followed by a 12-week randomized, double-blind comparison of ispaghula husk and placebo groups. Ispaghula husk at daily doses of 7.0 g ($p = 0.009$) and 10.5 g ($p<0.001$) produced significantly greater reductions in serum LDL-cholesterol levels (8.7% and 9.7% respectively) than did placebo (4.3%). At both dose levels ispaghula husk also produced significantly greater reductions in total cholesterol levels than did placebo (7.0 g/day versus placebo, $p = 0.040$; 10.5 g/day versus placebo, $p = 0.010$) [MacMahon 1998].

The long-term cholesterol-lowering effect of ispaghula husk as an adjunct to diet therapy (AHA Step 1) was evaluated in a randomized, double-blind, placebo-controlled study involving 248 subjects with primary hypercholesterolaemia. After 24-26 weeks, daily treatment with 10.2 g of ispaghula husk had significantly reduced ($p<0.001$) serum total cholesterol (4.7%) and LDL-cholesterol (6.7%) compared to placebo [Anderson 2000b].

In a randomized, double-blind study in patients with mild to moderate hypercholesterolaemia, 2 × 5.1 g of ispaghula husk daily for 16 weeks as an adjunct to a low fat diet (AHA Step 1) significantly reduced total cholesterol ($p<0.02$), LDL-cholesterol ($p<0.01$) and apolipoprotein B ($p<0.04$) in comparison with placebo or diet alone [Ciba 1992a/b/c]. An open, 28-week extension of this study confirmed the reductions in total

cholesterol and LDL-cholesterol after taking 2 × 5.1 g of ispaghula husk daily in conjunction with a low fat diet [Ciba 1993a/b/c].

These results [Bell 1989; Levin 1990; Anderson 1991; Ciba 1992a/b/c; Ciba 1993a/b/c; Sprecher 1993; Weingand 1997; MacMahon 1998; Anderson 2000b] and another study [Neal 1990] showed that, where a good reduction of blood lipids has already been achieved by use of a low fat diet, a further reduction is possible by taking ispaghula husk as an adjunct to the diet.

Another meta-analysis was performed to determine the effects of ispaghula husk-enriched cereal products on blood total cholesterol, and LDL- and HDL-cholesterol levels, in mild to moderate hypercholesterolaemic adults who consumed a low fat diet. The 8 published [Bell 1990; Anderson 1992; Roberts 1994; Stoy 1993; Wolever 1994a; Summerbell 1994; Jenkins 1997] and 4 unpublished studies included, involving a total of 404 patients, met the criteria of randomized, controlled studies in adults using either a crossover design (7 studies) or a design with parallel arms (5 studies) to compare treatment and control conditions for determining the effects of ispaghula husk-enriched cereals on blood lipids, with control groups that ate cereals providing ≤3 g of soluble fibre per day. Three studies [Roberts 1994; Summerbell 1994 and one of the unpublished studies] had no dietary lead-in period whereas the others had a 3- to 6-week dietary lead-in period, during which time the subjects were instructed to adhere to a low fat diet. The daily dose of ispaghula husk was 9.4-12 g in 9 studies, but lower (3.0, 6.7 and 7.6 g) in the other 3 studies. The conclusions from this meta-analysis were that treatment with ispaghula husk-enriched cereals significantly lowered total cholesterol (by 5%; $p<0.0002$) and LDL-cholesterol (by 9%; $p<0.0001$) but had no effect on HDL-cholesterol. The results confirmed that consuming an ispaghula husk-enriched cereal as part of a low fat diet improves the blood lipid profile of hypercholesterolaemic adults beyond that which can be achieved with a low fat diet alone [Olson 1997].

The results of a randomized, double-blind, controlled study in patients with mild to moderate hypercholesterolaemia suggested that consumption of foods containing 10.2 g of ispaghula husk daily in conjunction with a low fat diet (AHA Step 1) results in the maintenance of reduced LDL-cholesterol concentrations without affecting HDL-cholesterol or triacylglycerol concentrations. At the conclusion of the 24-week treatment period, the mean LDL-cholesterol concentration was 5.3% lower in the verum group than in the control group ($p<0.05$) [Davidson 1998].

After 8 weeks of diet stabilisation, the effects of an ispaghula husk-enriched cereal (providing 6.4 g of ispaghula husk per day) or a matched control cereal, administered during 6-week phases (separated by a 6-week washout period) of a randomized, double-blind, crossover study, were compared in 25 hypercholesterolaemic children aged 6-18 years. Reductions in serum total cholesterol and LDL-cholesterol concentrations were highly significant in favour of the ispaghula husk-enriched cereal ($p = 0.03$ and 0.01 respectively); the reduction in LDL-cholesterol was 7% in the ispaghula husk group compared to nil in the control group [Davidson 1996]. In contrast, in a study involving 20 children aged 5-17 years with elevated serum LDL-cholesterol, who had already been on a low total fat, low saturated fat, low cholesterol diet for at least 3 months, treatment with an ispaghula husk-enriched cereal (providing 6 g of ispaghula husk per day) for 4-5 weeks had no additional lowering effect on total cholesterol or LDL-cholesterol levels [Dennison 1993].

In a randomized, double-blind, placebo-controlled study, after a low fat diet for 1 year (AHA Step 2, i.e. a diet with no more than 30% of calories from total fat, less than 7% of calories from saturated fat and less than 200 mg cholesterol daily), 105 moderately hypercholesterolaemic patients received either 5 g of colestipol (a bile acid sequestrant resin), 2.5 g of colestipol + 2.5 g of ispaghula husk, 5 g of ispaghula husk or 5 g of cellulose (as placebo) three times daily before meals for 10 weeks. The combination of colestipol + ispaghula husk reduced the ratio of total cholesterol to HDL-cholesterol significantly more than did colestipol alone or ispaghula husk alone ($p<0.05$). These findings suggested that adding ispaghula husk to half the usual dose of bile acid sequestrant resin maintains the efficacy and improves the tolerability of the resin [Spence 1995]. In another controlled study, ispaghula husk treatment significantly reduced the frequency and severity of constipation ($p<0.05$), abdominal discomfort ($p<0.01$) and heart-burn ($p<0.05$) induced by cholestyramine therapy [Maciejko 1994].

In a randomized, placebo-controlled, double-blind study, significant reductions ($p<0.01$) in serum total cholesterol (by 14.8%), LDL-cholesterol (by 20.2%) and the ratio of LDL- to HDL-cholesterol (by 14.8%) relative to baseline values were shown after 8 weeks of daily treatment with 10.2 g of ispaghula husk in 12 men with mild to moderate hypercholesterolaemia who maintained their usual diets. No significant reductions from baseline in these parameters were observed in 12 other volunteers after placebo treatment [Anderson 1988].

In a randomized, double-blind, placebo-controlled, crossover study involving 20 men with moderate hypercholesterolaemia who took 15 g of ispaghula husk daily for 40 days, a significant increase in bile acid synthesis was observed in those subjects whose LDL-cholesterol was lowered by more than 10% ($p<0.0002$), suggesting that ispaghula husk acts by stimulating bile acid synthesis [Everson 1992]. These findings are consistent with those of another randomized, double-blind, placebo-controlled, crossover study in which, besides significantly decreased serum LDL-cholesterol levels, significantly increased levels of the serum cholesterol precursors lathosterol and Δ8-cholestanol were observed after daily treatment with 10.2 g of ispaghula husk for 8 weeks ($p = 0.02$ vs. placebo), indicating increased endogenous cholesterol synthesis caused by elimination of bile acids. A trend towards decreased LDL production in response to ispaghula husk treatment was observed, suggesting that the reduction in serum LDL-cholesterol may be due to decreased LDL production [Weingand 1997].

In a randomised, double blind, placebo-controlled, multi-center study mild-moderate hypercholesterolaemic patients received 14 g/d of ispaghula husk (n=126) or placebo (microcrystalline-cellulose 14 g/d; n=128) in a low saturated fat diet for 8 weeks. Relative to placebo, ispaghula husk reduced plasma LDL by 6% ($p = 0.0002$), total cholesterol by 6.9% ($p<0.0001$), triglycerides by 21.6% ($p = 0.0004$), apolipoprotein B by 6.7% ($p<0.0001$), oxidised LDL by a mean of 6.82 U/L ($p = 0.0003$), insulin by 4.68 pmol/L ($p = 0.0218$) and systolic blood pressure by 4.0 mmHg ($p<0.0045$) after 8 weeks [Solà 2010].

The requirement that ispaghula husk should be consumed together with food for the treatment of hypercholesterolaemia has been shown in a study in which serum total cholesterol and LDL-cholesterol levels remained unchanged when ispaghula husk was taken between meals, whereas they decreased significantly when ispaghula husk was incorporated into a breakfast cereal. This suggests that the effect could be dependent on an interaction of ispaghula husk with other components of the meal [Wolever 1994b].

In a 6-week randomized, double-blind, placebo-controlled study, significant reductions in serum total cholesterol ($p = 0.03$),

LDL-cholesterol ($p = 0.01$) and triglycerides ($p = 0.005$), and a significant increase ($p<0.0001$) in HDL-cholesterol, were observed in 125 patients with type II diabetes after 6 weeks of daily treatment with 3 × 5 g of ispaghula husk [Rodriguez-Morán 1998]. The hypolipidaemic effect of ispaghula husk in patients with type II diabetes has also been shown in other studies [Frati-Munari 1989; Gupta 1994].

In a randomised, controlled, crossover study 68 hyperlipidaemic subjects (37 men, 31 postmenopausal women) consumed a test diet (high fibre diet with 2 g ispaghula husk 4 times daily) and a control low fat (25% of energy), low-cholesterol (<150 mg/d) diet. There were no differences in pretreatment values between the groups. Significant reductions in blood lipid values were seen after both diets. Mean values were significantly lower for the high fibre test diet compared to the low fat control diet for total cholesterol (2.1%; $p = 0.003$), triacylglycerols (5.2%; $p = 0.037$), apolipoprotein B (2.9%; $p<0.001$), and ratio of total cholesterol to HDL cholesterol (2.4%; $p = 0.015$) [Jenkins 2002].

In a randomised, multi-centre study 100 subjects (both sexes; 97 completed the study) with LDL >130 mg/dL and total cholesterol >220 mg/dL were treated for 12 weeks with either a combination of ispaghula husk (5.6 g twice daily) and atorvastation 10 mg once daily or atorvastation alone. After 8 weeks both groups had a significant reduction in mean LDL (20.5% for the combination group and 16% for atorvastation alone) as compared to baseline, however the difference between the groups was not significant. After 12 weeks the difference between the groups was significant ($p<0.05$), with an LDL reduction of 31.4% (compared to baseline) in the combination group compared to 22.8% in the atorvastation alone group. Serum cholesterol ($p<0.05$) and triglycerides ($p<0.05$) were significantly reduced, whereas HDL ($p<0.05$) was increased, in both groups at weeks 8 and 12; with no significant difference between the groups [Jayaram 2007].

In a prospective, open, observational study, 54 of 62 enrolled adult patients with mild to moderate hypercholesterolaemia and indications for laxative treatment completed a study protocol involving treatment with 3.5 g of ispaghula husk 3 times daily for 3 weeks. Total cholesterol significantly decreased from 252 mg/dL before treatment to 239 mg/dL after 3 weeks of treatment and LDL significantly decreased from 174 to 162 mg/dL respectively. Levels of triglycerides and HDL did not change significantly. Most of the patients reported gastrointestinal symptoms and adverse reactions in week 1, with the intensity and frequency decreasing week on week from weeks 1 to 3 [Uehleke 2008].

In a pilot clinical study 11 post-menopausal and 8 pre-menopausal women with total cholesterol >200 mg/dL were treated with 5g of ispaghula husk (baked in a cookie) 3 times daily for 6 weeks. Total cholesterol was significantly decreased in post-menopausal women (by 5.2 percent; $p< 0.05$) but not in pre-menopausal women. HDL also decreased in only the post-menopausal women (ca. 10.2%; $p<0.05$). There were no significant changes in LDL and triglycerides in either group [Ganji 2008]

Hypoglycaemic effects

A randomized, double-blind, crossover study was conducted in 18 non-insulin-dependent, type II diabetes patients, to whom 6.8 g of ispaghula husk or placebo was administered twice, immediately before breakfast and dinner (but none before lunch), during each 15-hour phase. Relative to placebo, for meals eaten immediately after ispaghula husk ingestion, postprandial maximum serum glucose elevations were reduced by 14% after breakfast and 20% after dinner; second meal effects after lunch showed a 31% reduction. Postprandial insulin concentrations measured after breakfast were reduced by 12% [Pastors 1991].

In a randomized, double-blind, placebo-controlled clinical study involving 49 patients with diabetes controlled by diet alone or diet plus glibenclamide or metformin, subjects were treated for 8 weeks with either 5.1 g ispaghula husk (n=27) or placebo (n=22) twice daily (20-30 min before breakfast and dinner) in combination with their existing drug regimen. Fasting plasma glucose (FBS) was measured every 2 weeks, plasma cholesterol, LDL, HDL, triglycerides and insulin levels every 4 weeks, HbA1c at the beginning and at the end of the study. At week 8, when compared with placebo, ispaghula husk demonstrated significantly lower levels of FBS and HbA1c ($p<0.05$) and significantly greater HDL ($p<0.05$). There were no significant changes in the levels of insulin, plasma cholesterol, LDL and triglycerides. Of the side effects recorded, flatulence was significantly higher in the placebo group ($p<0.0001$); the ispaghula husk group tolerated metformin better than placebo with significantly reduced hot flushing ($p<0.002$) [Ziai 2005].

In the second phase of an open study, 20 type II diabetic patients (12 male, 8 female) were treated for 6 weeks with 14 g of ispaghula husk per day (3.5 g before breakfast, lunch, afternoon snack and dinner) in addition to conventional dietary restrictions and standard treatment with glibenclamide. Mean serum glucose concentrations after a standard test meal were significantly lower in the ispaghula husk administration phase compared to phases 1 and 3 of the trial without ispaghula husk and only conventional dietary restrictions and standard treatment with glibenclamide. The same applied to serum total cholesterol and LDL; HDL values were unaffected by ispaghula husk supplementation [Sierra 2002].

A number of studies have shown that ispaghula husk lowers peak blood glucose levels due to delayed intestinal absorption [Kay 1978; Cummings 1978; Capani 1980; Fagerberg 1982; Florholmen 1982; Frati-Munari 1989; Abraham 1988; Wolever 1994b].

Other effects

In a 4-month randomized, double-blind, placebo-controlled, crossover study, ispaghula husk (2 × 3.5 g daily) was found to be superior to placebo ($p<0.001$) in relieving gastrointestinal symptoms, with a lower score on all of the eight scales (e.g. abdominal pain, diarrhoea, loose stools, bloating) in 36 colitis patients in remission. Less symptoms were reported in 69% of the patients while taking ispaghula husk, compared to 7% who felt better with placebo ($p<0.001$) [Hallert 1991].

A randomized, double-blind, placebo-controlled study assessed changes to the tolerability and efficacy of a 4-litre polyethylene based glycol lavage self-administered over 4 hours on the day prior to colonoscopy, following treatment for 4 days with ispaghula husk (approx 20 g per day; n=20) or placebo (n=20). Quality of preparation of the bowel was graded during colonoscopy and found to be significantly worse in the ispaghula husk group compared to placebo ($p<0.05$); tolerability was equivalent [Salwen 2004].

An open, cross-over clinical study involving 10 healthy volunteers compared the effects on gas expulsion following gas perfusion into the jejunum, after a standard diet alone and standard diet with ispaghula husk (30g/day) for seven days. Compared to standard diet alone, ispaghula husk significantly ($p<0.05$) prolonged the lag time before first gas expulsion from 1129 to 2265 seconds and significantly ($p<0.05$) reduced

the total gas evacuation volume from 1429 ml to 1022 ml, demonstrating promotion of gas retention after treatment with ispaghula husk [Gonlachanvit 2004].

In a 6-months, randomised, open clinical trial 141 hypertensive, overweight (BMI: 25-30) patients were treated with 3.5 g of ispaghula husk or guar gum 2 times daily (to be taken 20 minutes before the main 2 meals), or with standard diet alone additionally to standard hypertensive treatments which were equally distributed between the groups. After 6 months of treatment a significant improvement of both glycaemic and plasma lipid parameters in both fibre groups could be seen. Compared to baseline fasting plasma glucose decreased by 24 mg/dL ($p<0.001$), fasting plasma insulin by 5.1 µU/L ($p<0.001$), HbA1c by 0.7% ($p<0.001$), LDL by 9 mg/dL ($p<0.001$). Plasma HDL was not changed significantly in both fiber groups. Systolic blood pressure decreased by 5.2 mmHg ($p<0.001$) in the ispaghula husk group and by 5.3 mmHg in the guar group [Cicero 2007].

Pharmacokinetic properties
The vegetable fibre of ispaghula husk is resistant to digestion in the upper intestinal tract and thus is not absorbed. Subsequent degradation of the fibre by colonic bacteria results in the production of gas and short chain fatty acids. Studies in animals have demonstrated at least partial absorption of short chain fatty acids [Kay 1978; Cummings 1981; Brunton 1990].

Preclinical safety data
Not relevant.

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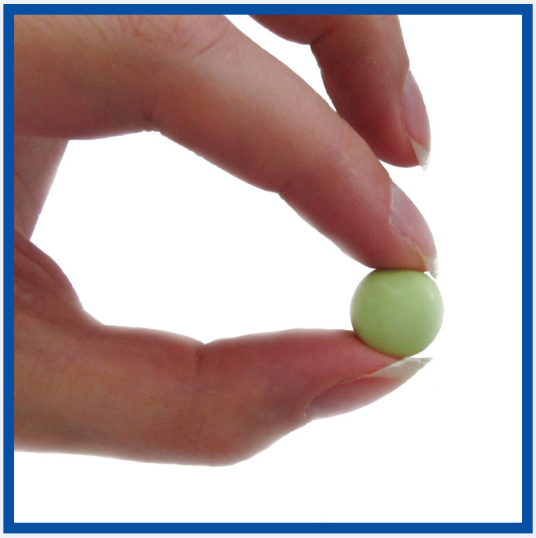
Primulae radix

Primula root

2021



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European Scientific Cooperative On Phytotherapy

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***The* Scientific Foundation for**
Herbal Medicinal Products

PRIMULAE RADIX
Primula root

2021

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European Scientific Cooperative
On Phytotherapy

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Plant illustrated on the cover: *Primula veris*

FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

PRIMULAE RADIX

2021

Primula root

DEFINITION

Primula root consists of the whole or cut, dried rhizome and root of *Primula veris* L. or *P. elatior* Hill.

The material complies with the monograph of the European Pharmacopoeia [European Pharmacopoeia].

CONSTITUENTS

Triterpene saponins of the oleanane-type usually in the range of 3-10 %. Saponins from *Primula elatior* (such as primulasaponin) are derived from the aglycone protoprimulagenin A, which is converted to primulagenin A. The main saponins of *Primula veris* are primacrosaponin and priverosaponin B, derived from anagalligenin, priverogenin B and priverogenin B-22-acetate respectively [Tschesche 1968,1975,1977,1983; Jentzsch 1973; Grecu 1975; Glasl 1984; Wagner 1986; Çalis 1992; Siems 1998; Hahn-Deinstrop 2000; Müller 2006; Schilcher 2010; Volk 2016; Apel 2017; Schöpke 2019; Włodarczyk 2020].

The phenolic glycosides primverin and primulaverin occur in both species, in amounts up to 2.3 %; they are the 2-primeverosides of 4-methoxy- and 5-methoxysalicylic acid methyl esters respectively [Wagner 1986; Thieme 1971; Müller 2006; Volk 2016; Bądczek 2017; Schöpke 2019]. These substances change during the drying process into the respective aglycones which are responsible for the characterictic odour of the roots [Schilcher 2010; Schöpke 2019].

CLINICAL PARTICULARS

Therapeutic Indications

Cough associated with common cold [Antonone 1988; Volk 2016; Schöpke 2019].

In this indication, the efficacy is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Adult daily dose: 0.5-1.5 g of the drug as a decoction or equivalent preparation, taken in small amounts 3 times daily [Antonone 1988; Schilcher 2010; Volk 2016].
Elderly: dose as for adults.
Children: 4-10 years, 0.5-1.0 g daily; 10-16 years, 0.5-1.5 g daily [Dorsch 1993].

Method of administration
For oral administration.

Duration of administration
No restriction.

Contra-indications
Known hypersensitivity to primula or its constituents [Schilcher 2010; Volk 2016].

Special warnings and special precautions for use
Caution is recommended in patients with gastritis or gastric ulcer.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy or lactation without medical advice.

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Effects on ability to drive and use machines
None known.

Undesirable effects
In rare cases gastrointestinal disturbance may occur [Schilcher 2010; Schöpke 2019]

Overdose
Overdosage may lead to stomach upset, vomiting or diarrhoea [Schöpke 2019].

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties
Primula root has an expectorant action arising primarily from local irritation of the gastric mucosa by saponins; this provokes a reflex increase in bronchial secretions, which dilutes the mucus and reduces its viscosity [Boyd 1954; Schilcher 2010; Schöpke 2019]. Irritation of mucous membranes in the throat and respiratory tract by saponins may also cause an increase in bronchial secretions and the surface tension-lowering action of saponins might help to reduce the viscosity of sputum, making it easier to eject [Hostettmann 1995].

In vitro experiments

Antimicrobial activity
Primula root saponins inhibit the growth of a variety of bacteria and fungi [Tschesche 1965; Wolters 1966]. An unspecified saponin mixture from *Primula veris* exhibited activity against influenza (A_2/Japan 305) virus, producing 89% inhibition at a concentration of 6.2 µg/mL [Rao 1974; Büechi 1996].

Other effects
A hexane extract (50 µg/mL) of *Primula veris* root inhibited COX-1 and COX-2 by 54% and 66% respectively [Lohmann 2000].

A tincture (1:5; ethanol 50% V/V) at concentrations between 0.1% and 1% dose-dependently inhibited the LPS-induced release of IL-8 in primary human monocytes [Nauert 2005b].

In vivo experiments
An undefined mixture of saponins from primula root at a concentration of 1:10,000 increased the ciliary activity of throat epithelium of curarized frog. This effect was assumed to be due to a decrease in surface tension of the mucus. The ciliary activity was less at a concentration of 1:6,000 and ceased at 1:3,000 due to toxic effects [Vogel 1963].

Saponins from *P. officinalis* (syn. *veris*) inhibited pain provoked by compression of inflamed paws of rats (n=16) at doses of 2.5 mg/kg p.o. (29.2 % inhibition; $p<0.02$) and 1.25 mg/kg i.v. (27.7 % inhibition; $p<0.001$) [Cebo 1976].

Pharmacological studies in humans
None.

Clinical studies
In a non-controlled study in patients (adults and children) with antibiotic-resistant stomatitis caused by *Candida albicans*, the topical application of an aqueous solution of saponins from *P. vulgaris* led to the disappearance of local symptoms within 2-3 days in more than 100 cases [Margineanu 1976].

Pharmacokinetic properties
No specific data are available on the pharmacokinetics of primula root saponins. In general, saponins are poorly absorbed by the body [Hostettmann 1995].

Preclinical safety data
The oral toxicity of saponins in mammals is relatively low due to their poor absorption from the gastrointestinal tract [Hostettmann 1995]. The LD_{50} for the saponin fraction from *P. veris* in mice was determined as 10.5 – 56 mg/kg b.w. after i.p. application and for primulic acid (mixture of saponins) in rats as 1.2 mg/kg b.w. after i.v. application [Vogel 1963; Grecu 1975; Cebo 1976; Schöpke 2019].

No signs of toxicity were found after oral administration of saponins to rats. Local irritating effects have been observed on the rabbit cornea [Vogel 1963; Schöpke 2019].

Clinical safety data
In various observational studies, a combination containing 5% thyme liquid extract and 2.5% primula root liquid extract (1:2-2.5; ethanol 70% m/m) was administered to children. In three studies involving 636, 330 and 839 children from 1-12 years of age, six adverse reactions (pruritus, itching, exanthema) occurred, while in another study involving 200 children between 6 and 12 months of age, one case of perioral oedema and one case of vomiting occurred [Nauert 2003, 2005a, 2006; Schmidt 2008].

In a randomized, double-blind, placebo-controlled, multicenter, prospective study, a combination containing 40% thyme liquid extract and 20% primula root tincture (1:5; ethanol 50% V/V) was administered to 150 patients (97 women, 53 men) suffering from acute bronchitis at a daily dose of 5 x 30 drops. The tolerability was good in both verum and placebo groups; neither serious adverse events nor clinically relevant findings in the safety parameters were observed. A total of 7 adverse events occurred, 2 in the verum group (slight gastrointestinal complaints) and 5 in the placebo group. One of the two adverse events in the verum group was considered to be possibly related to the intake of the study medication. In the global safety assessment, the tolerability of both medications was rated as “good” or “very good” by more than 90% of the patients and physicians [Gruenwald 2005].

In a randomized, single-blind, bi-centric, prospective study, a combination containing 5% thyme liquid extract and 2.5% primula root liquid extract (1:2-2.5; ethanol 70% m/m), as well as a combination containing 40% thyme liquid extract and 20% primula root tincture (1:5; ethanol 50% V/V), were given to 189 patients (121 women, 68 men) suffering from acute bronchitis over a time period of 7-9 days at a daily dose of either 6 x 5 mL of the first or 5 x 1 mL of the second test medication. The tolerability was very good in both groups; neither serious adverse events nor clinically relevant findings in the safety parameters were observed. A total of 10 adverse events (mild gastrointestinal complaints, headache) occurred, of which 5 were considered to be possibly or probably related to the intake of the study medication. In the global safety assessment, the tolerability of both medications was rated by about 90% of the patients and physicians as "good" or "very good" [Gruenwald 2006].

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CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHAMOMILLAE ROMANAE FLOS	Roman Chamomile Flower	Online Series, 2019
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Online Series, 2021
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Online Series, 2021
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HELICHRYSI FLOS	Sandy Everlastin Flower	Online Series, 2019
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013

HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Online Series, 2020
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Online Series, 2021
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Online Series, 2020
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Online Series, 2021
PRUNI AFRICANAE CORTEX	Pygeum Bark	Online Series, 2020
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Online Series, 2021
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Online Series, 2020
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Online Series, 2020
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

E/S/C/O/P Monographs

Online Series

The Scientific Foundation for Herbal Medicinal Products

The second edition of ESCOP Monographs, published as a hardback book in 2003 with a Supplement in 2009, has been widely acclaimed for its authoritative information on the therapeutic uses of herbal medicines. Monographs covering a total of 107 herbal substances include extensive summaries of pharmacological, clinical and toxicological data, and copious references to scientific literature form an important part of each text.

Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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The Scientific Foundation for Herbal Medicinal Products

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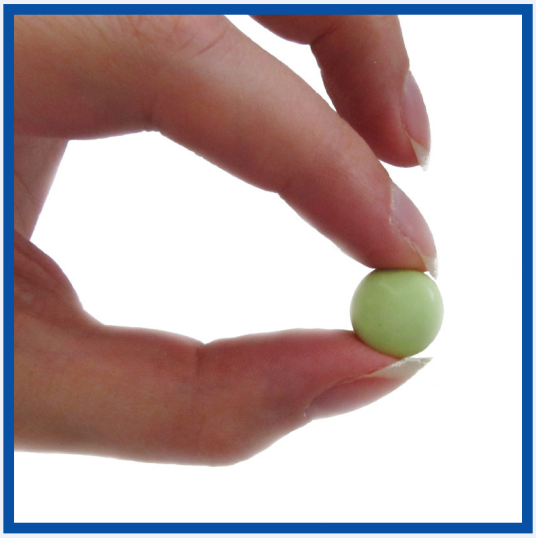
2020



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MONOGRAPHS

***The* Scientific Foundation for**
Herbal Medicinal Products

PRUNI AFRICANAE CORTEX
Pygeum africanum bark

2020

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On Phytotherapy

ESCOP Monographs were first published in loose-leaf form progressively
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Second Edition, completely revised and expanded

Second Edition, Supplement 2009

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FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

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Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in the production of ESCOP monographs and other activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

PRUNI AFRICANAE CORTEX 2020

Pygeum africanum bark

DEFINITION

Pygeum africanum bark consists of the whole or fragmented, dried bark of the stems and branches of *Prunus africana* (Hook f.) Kalkman (synonym: *Pygeum africanum* Hook.f.).

The material complies with the European Pharmacopoeia [Pygeum africanum bark].

CONSTITUENTS

The main characteristic consistuents are phytosterols (approx. 0.05 %) e.g. ß-sitosterol and its glucoside, ß-sitostenone, free fatty acids (C_{12} - C_{24}), pentacyclic triterpenic acids (ursolic and oleanolic acid derivatives), long chain aliphatic alcohols (*n*-docosanol and *n*-tetracosanol and their ferulic acid esters), atranorin and atraric acid (methyl 2,4-dihydroxy-3,6-dimethylbenzoate) [Longo 1981, 1983; Pierini 1982; Martinelli 1986; Bruneton 2009; Fourneau 1996; Bombardelli 1997; Liersch 2002; Schleich 2006a, 2006b; Nyamai 2015; Thompson 2019].

CLINICAL PARTICULARS

Therapeutic indications

Symptomatic treatment of micturition disorders (dysuria, pollakiuria, nocturia, urine retention) in benign prostatic hyperplasia (BPH) [Andro 1995; Bombardelli 1997; Wilt 1998; Ishani 2000; McQueen 2001] at stages I and II as defined by Alken [Alken 1973] or stages II and III as defined by Vahlensieck [Vahlensieck 1996].

Stages of Benign Prostatic Hyperplasia as defined by Alken [Alken 1973]	Stages of Benign Prostatic Hyperplasia as defined by Vahlensieck [V.1996]
Stage I Dysuria, pollakiuria, possibly nocturia, reduction in projection of the urine flow, no residual urine (stage of compensation of the bladder musculature).	**Stage I** No micturition problems More or less marked BPH Urine flow: greater than 15 mL/s maximal flow No residual urine No bladder trabeculation
Stage II Same symptoms as at stage I, but additionally residual urine (incipient decompensation of the bladder musculature).	**Stage II** Intermittent micturition problems (frequency, calibre of urine flow) More or less marked BPH Urine flow: between 10 and 15 mL/s maximal flow No or low (≤50 mL) residual urine No or incipient bladder trabeculation
Stage III Complete stoppage or overflow of the bladder (decompensation of the bladder musculature).	**Stage III** Permanent micturition problems (frequency, calibre of urine flow) More or less marked BPH Urine flow: less than 10 mL/s maximal flow Residual urine more than 50 mL Trabeculated bladder
	Stage IV Permanent micturition problems (frequency, calibre of urine flow) More or less marked BPH Urine flow: less than 10 mL/s maximal flow Residual urine more than 100 mL Overflowing bladder Stoppage of the upper urinary tract

E/S/C/O/P MONOGRAPHS

Posology and method of administration

Dosage

Daily dose: 100-200 mg of lipophilic extract [Andro 1995; Bombardelli 1997; Ishani 2000; McQueen 2001; Descazeaud 2015].

Method of administration
For oral administration.

Duration of administration
No restriction. Long-term administration may be advisable. If symptoms persist or worsen, medical advice should be sought.

Contra-indications
None known.

Special warnings and special precautions for use
All cases of difficulty in micturition require clarification by a physician and regular medical checks in order to rule out the need for other treatment, e.g. surgical intervention. Consultation with a physician is particularly necessary in cases of blood in the urine or acute urine retention.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
Not applicable.

Effects on ability to drive and use machines
None known.

Undesirable effects
In rare cases, mild gastrointestinal disturbances may occur [Ishani 2000; Andro 1995; Bombardelli 1997; McQueen 2001].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Most of the pharmacological and clinical studies have been performed with a chloroform extract containing 14% sterols.

Pharmacodynamic properties

The aetiology of BPH involves initially the microscopic growth of the prostate and its progression to a macroscopic enlargement of the gland. In the second phase, progression to clinical BPH, the patient develops symptomatic dysuria. About half of men with macroscopic BPH suffer from micturition complaints.

Although macroscopic growth of the prostate is a condition for the development of complaints, enlargement is not, by itself, sufficient for the progression to clinical BPH. Particularly in the case of irritative symptoms, additional factors such as prostatitis, vascular infarct or tensile strength of the glandular capsule are involved [Isaacs 1989; Sökeland 2000].

So far, the aetiology of this age-dependent disease is not completely understood. Various mechanisms have been postulated to be causally connected with prostatic growth:

- A shift in prostatic androgen metabolism due to an increase in 5α-reductase activity, which leads to an abnormal accumulation of dihydrotestosterone (DHT) [Lichius 2000; Carson 2003; Nahrstedt 1993; Miersch 1993].
- An increase in the oestrogen/androgen ratio and increased synthesis of sex hormone binding globulin (SHBG) [Farnsworth 1999; Miersch 1993].
- Growth factors, changes in prostatic stromal-epithelial interaction (embryonic reawakening) and/or an increase in prostatic stem cells [Lichius 2000; Isaacs 1989; Lawson 1990; Sökelandt 2000].
- An imbalance between cell proliferation and cell death (reduced apoptosis) [Carson 2003; Sökelandt 2000].
- Increased production of prolactin and elevated levels of prostaglandins and leukotrienes [Farnsworth 1999; Nahrstedt 1993; Miersch 1993].

A review of *in vitro* and *in vivo* pharmacological studies identifies various mechanisms of action of pygeum bark including [Edgar 2007]:

- Prostate hyperplasia via b-FGF (basic fibroblast growth factor), EGF (epidermal growth factor) and androgen-mediated cell proliferation.
- Bladder function with improvement in contractile dysfunction mediated via myosin isoform expression, lessened synaptic denervation and improved mitochondrial function.

In vitro experiments

Hormonal activity
A lipophilic extract (200:1, methylene chloride; standardized to 13% sterols) concentration-dependently inhibited the activity of 5α-reductase from rat prostate cells (IC_{50} 0.78 mg/mL) and of aromatase from human placenta cells (IC_{50} 0.98 mg/mL) [Hartmann 1996].

However, an earlier study demonstrated that a lipophilic extract had only a slight inhibitory activity (IC_{50} 63 µg/mL) against 5α-reductase from the human prostate in comparison with the synthetic 5α-reductase inhibitor finasteride IC_{50} 1ng/mL) [Rhodes 1993].

A dichloromethane extract showed an antiandrogenic activity in the luciferase assay (inhibition of hormone-activated human androgen receptor) at a concentration of 600 µg/mL. A bio-guided fractionation resulted in the isolation of atraric acid [Schleich 2006a, 2006b].

Atraric acid (10µM) inhibited both the hormone-activated human androgen receptor (AR) and the growth of AR expressing human prostate cancer cell lines LNCaP and C4-2 [Papaionnou 2008].

Anti-inflammatory activity
A lipophilic extract significantly inhibited the synthesis of 5-lipoxygenase metabolites in human polymorphonuclear cells stimulated with the calcium ionophore A23187: 5-HETE ($p<0.001$ at 1 µg/mL), LTB_4 ($p<0.01$ at 3 µg/mL), 20-hydroxy-LTB_4 ($p<0.001$ at 3 µg/mL) and 20-carboxy-LTB_4 ($p<0.01$ at 3 µg/mL) [Paubert-Braquet 1994a].

Antiproliferative effects
Growth factors seem to play a role in the pathogenesis of BPH, in particular b-FGF, which is present at an elevated level in BPH tissue. It has been shown that b-FGF, EGF and DHT stimulate the growth of fibroblasts from the adult prostate [Bombardelli 1997].

A lipophilic extract at 1µg/mL significantly ($p<0.05$) inhibited proliferation of mouse 3T3 fibroblasts stimulated by b-FGF and EGF [Paubert-Braquet 1994b].

The activity of a lipophilic extract on DNA synthesis was investigated by measuring the [^{3}H] thymidine incorporation into rat prostatic stromal cells. The extract inhibited the proliferation of both non-stimulated cells (IC_{50}: 14.4 µg/mL) and cells stimulated by the mitogenic growth factors EGF, insulin-like growth factor-I and b-FGF (IC_{50} values of 4.6, 7.7 and 12.6 µg/mL respectively) at similar concentrations to genistein, a known growth inhibitor in mitogenic studies. The proliferation induced by TPA (12-O-tetradecanoylphorbol-13-actate) and PDBu (phorbol-12,13-dibutyrate), direct activators of protein kinase C (PKC), was also concentration-dependently inhibited with IC_{50} values of 12.4 and 8.1 µg/mL respectively, compared to an IC_{50} of approx. 1nM for the PKC inhibitor staurosporine [Yablonsky 1997].

A lipophilic extract (5 – 100 µg/mL) dose-dependently inhibited proliferation of human prostatic fibroblasts and myofibroblasts stimulated by b-FGF and EGF [Boulbes 2006].

A 30% ethanolic extract inhibited the growth of PC-3 and LNCaP cells (IC_{50} 2.5 µl/mL for both cell lines). The extract significantly ($p<0.05$) induced apoptosis at 2.5 µl/mL in both cell lines and displaced bound estradiol (>70% at 5 µl/mL) and bound DHT (>60% at 5 µl/mL) in a competitive ligand-binding assay [Shenouda 2007].

Primary prostate stromal cells taken from patients both with and without BPH/LUTS (lower urinary tract symptoms) were treated with a lipophilic extract. Antiproliferative potency and extract-induced apoptosis were increased in the BPH versus the non-BPH stromal cells [Quiles 2010].

An ethanolic extract blocked the expression of IL-7 mRNA at 50 µg/mL and showed low cytotoxicity in the IEC-6 proliferation test (IC_{50} > 200 µg/mL) [Mwitari 2013].

Antioxidant effects
A chloroform extract was fractionated by partitioning between solvents of varying polarity and by column chromatography. The inhibitory activity of the extract and fractions from it on ferrous ion-induced stimulation of lipid peroxidation in microsomal preparations from rabbit livers was evaluated. The extract and fractions containing high levels of myristic acid and markedly inhibited lipid peroxidation with a potency comparable to that of α-tocopherol [Hass 1999].

Antibacterial activity
Methanolic extracts showed antibacterial activity in the diffusion method against *Salmonella typhi*, *Proteus vulgaris*, *Serratia marcescens*, *Escherichia coli*, *Bacillus cereus* [Ngule 2014], *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* [Mwitari 2013].

Other effects
A dichloromethane-methanol extract inhibited the proliferation of various cancer cell lines in the resazurin assay with IC_{50} values from 30 to 35 µg/mL [Ochwang'I 2018].

In vivo experiments

Effects on the prostate
Intragastric administration of a lipophilic extract to rats at 2 mg/kg b.w. daily for 20-50 days stimulated the secretory activity of the prostate in normal rats and prevented the development of prostatic hyperplasia induced by injection of human prostate adenoma tissue [Thieblot 1971]. Intraperitoneal administration of the extract at 1 and 10 mg/kg b.w. daily for 20 days enhanced secretory activity of the prostate and seminal vesicles in castrated rats. However, the activity of testosterone on these glands was antagonized, as shown by a significant reduction in weight gain of these organs ($p<0.05$). On the other hand, in castrated and adrenalectomised rats the extract potentiated the activity of testosterone on the target organs and increased the content of pituitary gonadotrophins [Thieblot 1977].

Intragastric administration of the extract to rats at 100 mg/kg b.w. daily for 3 days also increased prostate secretions [Clavert 1986].

A 30% ethanolic extract was administered to TRAMP mice (transgenic adenocarcinoma of the mouse prostate model) for 5 months (as 0.128 g/kg of the diet). The treated group showed a significant reduction ($p=0.034$) in well-differentiated prostate carcinoma (35%) compared to control (62.5%). There was no significant effect on total body weight, reproductive tract weight, testes or prostate weight [Shenouda 2007].

Effects on bladder function
Administration of a lipophilic extract to rats reduced the response of the bladder to electrical stimulation, phenylephrine, adenosine triphosphate and carbachol. The extract also reduced the bladder hyper-reactivity to carbachol in guinea pigs [Andro 1995].

After intragastric pre-treatment of rabbits with a lipophilic extract at 1, 10 and 100 mg/kg b.w. daily for 3 weeks, and further treatment for 2 weeks combined with partial bladder outlet obstruction, the bladders were excised, weighed and *in vitro* contraction studies were performed. The extract had no effect on bladder mass, but there was significant, dose-dependent preservation of the contractile responses of bladder strips ($p<0.05$).

In a further experiment (intragastric pre-treatment of rabbits at 100 mg/kg b.w. daily for 3 weeks; partial urethral obstruction for 1-14 days) the extract had no effect on bladder weight but reduced on day 1 and prevented from day 3 the severity of contractile dysfunctions associated with partial outlet obstruction. Pre-treatment restored the activities of citrate synthase and calcium-ATPase to nearly normal from day 7 onwards after an initial significant ($p<0.05$) reduction of their activities.

In another study, after 2 weeks of mild or severe partial outlet obstruction, the extract was intragastrically administered to rabbits at 100 mg/kg/day for 3 weeks. In contrast to the previous experiments, the rabbit bladder weight of the verum group with severe outlet obstruction was lower than in the placebo group but still significantly ($p<0.01$) higher than in the control group. Contractile dysfunction and reduction in compliance were reversed in the mild outlet obstruction group, while in the severe outlet obstruction group contractile dysfunction was improved [Levin 1996a; Levin 1996b; Levin 1997; Levin 2000].

In a subsequent study, rabbits received the extract intragastrically (100 mg/kg/day for 3 weeks) after 2 weeks of partial outlet obstruction. Partial obstruction resulted in a significant increase in bladder weight compared to that of unobstructed animals (8.8 g versus 2.3 g; $p<0.05$). Obstructed rabbits in the verum group had significantly lower bladder weights than placebo (4.2 g versus 8.8 g; $p<0.05$). The diminished contractile response to field stimulation and carbachol was restored to normal. Relative ratios for myosin heavy chain isoforms, altered at mRNA and protein levels by the obstruction, returned nearly to normal in the group treated with the extract ($p>0.01$) [Gomes 2000].

In a related study, intragastric administration of the extract to rabbits (30 mg/kg/day for 3 weeks) after 2 weeks of partial outlet obstruction resulted in reduced bladder hypertrophy, improved contractile responses and reversal of the induced structural

damage to cellular and subcellular membranes [Levin 2002]. Intragastric pre-treatment of rabbits at 3 mg/kg/day for 3 weeks protected the bladder from contractile dysfunctions induced by bilateral ischemia followed by reperfusion [Levin 2005].

In a 7-week experiment, partial urethral obstruction was created in rats by stimulating prostate growth with DHT, administered subcutaneously at 1.25 mg/kg b.w. during weeks 3 and 4. Intragastric administration of a lipophilic extract (100 mg/kg/day for 2 weeks before, 2 weeks during and 3 weeks after DHT treatment) significantly ($p<0.05$) reduced micturition frequency and normalized urethral opening pressure, voiding volume, as well as the weight of the total prostate and of the ventral lobe, compared to controls [Choo 2000]. A subsequent 6-week study in rats, during which DHT was administered in weeks 1 and 2, showed that co-treatment or post-treatment with the extract at 100 mg/kg/day suppressed the effects on micturition frequency and volume, but that only co-treatment could correct a developing increase in prostatic weight ($p<0.05$) [Yoshimura 2003].

A chloroform extract (100 mg/kg/day p.o. for 8 weeks) significantly ($p<0.05$) reduced the enhanced levels of hydroxyproline, transforming growth factor β1 and basic fibroblast growth factor in the bladder of streptozotocin-induced diabetic rats [Yongzhi 2008].

In an 8-week experiment streptozotocin-induced diabetic rats were fed with a chloroform extract (100 mg/kg/day). Catalase and SOD activities significantly ($p<0.05$) increased and MDA levels significantly ($p<0.05$) decreased in the treated group, compared to control. The number of iNOS-positive cells and the maximal bladder volume significantly ($p<0.05$) decreased, while bladder pressure and maximal bladder pressure significantly ($p<0.05$) increased in the treated group [Wang 2010].

Anti-inflammatory effects
A lipophilic extract, administered intragastrically at 400 mg/kg b.w., markedly reduced the carrageenan-induced paw oedema in rats. When administered intraperitoneally at 10 and 100 mg/kg the extract inhibited the increased vascular permeability caused by histamine [Marcoli 1986].

Pharmacological studies in humans
A histological study of prostate biopsy samples taken from 20 patients with BHP, following oral administration of a lipophilic extract at 100 mg/day or placebo for 20 days, revealed an improvement in capillary perfusion and fewer signs of inflammation in the verum group [Bongi 1972].

Another histological study of prostate biopsies ($n = 6$) taken before and after treatment with a lipophilic extract (75 mg/day for 1-3 months) indicated an improvement in secretory activity of the prostate without modifying its size [Dorémieux 1973].

In 22 men with insufficient prostatic secretion (reduced acid phosphatase activity) a lipophilic extract (150 mg/day for 3 months) significantly ($p<0.05$) increased prostatic secretion (increase in acid phosphatase and total protein concentration) [Clavert 1986].

In a study involving 18 men suffering from pollakiuria, dysuria and nocturia, a lipophilic extract (150 mg/day for 15-20 days) significantly reduced the volume of hypertrophic prostate ($p<0.01$) [Mathé 1995].

Serum collected from a 46 year old man without clinical BPH, after oral intake of a chloroform extract at 100 mg daily for 5 days, induced significant decreases in the proliferation of primary fibromuscular prostatic cells ($p<0.001$), organotypic fibromuscular prostatic cells ($p<0.001$) and WPMY cells ($p<0.05$). The transcriptome analysis revealed an upregulation of genes involved in multiple tumour suppression pathways and a downregulation of genes involved in inflammation and oxidative-stress pathways [Larré 2012].

Clinical studies
A systematic review of randomized controlled studies with lipophilic extract assessed 18 studies involving 1562 men with symptomatic BPH; 17 studies were double-blinded but only 1 reported a method of treatment allocation concealment. The mean study duration was 64 days and the daily dose of extract ranged from 75 to 200 mg. Data from 6 placebo-controlled studies involving 474 participants were suitable for pooling to provide a weighted estimate of effectiveness and meta-analysis indicated moderate improvement in the combined outcome of urologic symptoms and flow measures; nocturia decreased by 19% and residual urine volume by 24%; peak urine flow increased by 23%. Although the duration of the studies was short and the study design and types of reported outcome varied greatly, it was concluded that pygeum bark modestly, but significantly ($p<0.001$ to $p<0.05$), improves urologic symptoms and flow measures [Ishani 2000].

Other clinical reviews, which assessed open as well as controlled studies, involved a total of 1310 patients, daily doses of 75-200 mg of lipophilic extracts and treatment periods ranging from 15 to 120 days. They all concluded that pygeum bark improved the symptoms and objective measures of BPH and was well tolerated [Andro 1995; Bombardelli 1997; McQueen 2001].

The following results were obtained in randomized, double-blind, placebo-controlled studies with lipophilic extracts:

- 50 BPH patients treated with 75 mg of extract or placebo daily for 60 days: significant improvements in nocturnal micturition frequency ($p<0.01$), dysuria ($p<0.05$) and volume of residual urine ($p<0.01$) in the verum group [Bongi 1972].

- 120 BPH patients treated with 100 mg of extract or placebo daily for 6 weeks: significant ($p<0.01$) improvement in nocturnal micturition frequency, difficulty in starting micturition and sensation of incomplete voiding of the bladder in the verum group [Dufour 1984].

- 40 BPH patients treated with 200 mg of extract or placebo daily for 60 days: significant improvements in micturition frequency ($p<0.05$), nocturia ($p<0.01$), dysuria ($p<0.01$) and maximum and mean urine flow ($p<0.05$) in the verum group [Rizzo 1985].

- 263 BPH patients at Alken stage I treated with 100 mg of extract or placebo daily for 60 days: significant improvements in nocturia ($p = 0.007$), peak urine flow ($p = 0.002$), residual volume ($p = 0.02$) and overall symptoms ($p = 0.001$) in the verum group [Barlet 1990].

- 40 BPH patients treated with 100 mg of extract or placebo daily for 60 days: significant improvements in micturition frequency ($p<0.001$), urgency ($p<0.02$), dysuria ($p<0.02$) and peak urine flow ($p<0.05$) in the verum group [Bassi 1987].

Two different daily dosage regimens for a lipophilic extract were compared in a randomized study. Out of 235 BPH patients on commencement, 209 completed a 2-month double-blind phase, receiving either 50 mg of the extract twice daily (group A, 101

patients) or 100 mg once daily in the evening (group B, 108 patients). The primary efficacy parameter was the International Prostate Symptom Score (IPSS), a 40% or greater reduction from baseline being considered a significant improvement. Both treatments had similar efficacy after 2 months: in group A the IPSS decreased by 38%, the quality of life score improved by 28% and the maximum urinary flow rate increased by 16%; in group B the figures were 35%, 28% and 19%. In a subsequent 10-month open phase 174 patients took 100 mg of the extract once daily. After 12 months the overall IPSS had decreased by 46% and the maximum urinary flow rate had increased by 15% [Chatelain 1999].

In a comparison with a pollen extract, 38 BPH patients at Alken stages I and II received 100 mg of a lipophilic extract daily for 4 months. From evaluation of urodynamic and ultrasonographic parameters and subjective assessment, improvements compared to baseline were evident in peak flow rate (+11%), residual urine volume (-22%), obstructive symptom score (-46%) and irritative symptom score (-40%) [Dutkiewicz 1996].

A TRIUMPH (Trans European Research Into the Use of Management Policies for LUTS suggestive of BPH in Primary Healthcare) study recorded the treatment and outcomes of 2351 LUTS/BPH patients in 6 European countries over a 1-year follow-up period. Pygeum bark, administered to 90 of the patients, gave a mean reduction of 3.4 IPSS points. Clinically significant improvements (IPSS reduction >4) were seen in 43% of the patients [Hutchison 2007].

Pharmacokinetic properties
No data available.

Preclinical safety data

Acute toxicity
A single dose of the lipophilic extract up to 8 g/kg b.w. administered intragastrically to mice and rats was well tolerated [Andro 1995].

Neither mortality nor signs of adverse effects were observed after oral administration of single doses of the lipophilic extract to mice at 1-6 g/kg b.w. and to rats at 1-8 g/kg b.w. [Bombardelli 1997].

Repeated dose and chronic toxicity
Short-term (1 month) and long-term (6 months) intragastric administration of the extract to dogs at 375 mg/kg/day and to rats at 750 mg/kg/day caused no adverse effects to haematologic, biochemical or anatomical/pathological parameters [Andro 1995].

No adverse reactions were observed after daily intragastric administration of the extract to mice at 60 mg/kg or rats at 600 mg/kg for 11 months [Bombardelli 1997].

Oral administration of the extract to rats at up to 1 g/kg b.w. daily for 8 weeks did not cause clinical or pathological signs of toxicity, but moderate rises were observed in serum ALAT and blood urea nitrogen levels. At 3.3 g/kg daily for 6 days the extract caused marked clinical signs of toxicity, organ damage and a 50% mortality rate; the main lesions were hepatocellular degeneration and necrosis, diffuse nephrosis and myocardial degeneration. These findings confirmed the safety of the extract at therapeutic dosages, since signs of toxicity were observed only at very high doses [Gathumbi 2000&2002].

Reproductive toxicity
At up to 80 mg/kg/day the extract had no effect on fertility in male rats or rabbits [Andro 1995].

Carcinogenicity
In vivo and in vitro mutagenicity studies on the extract indicated a complete absence of mutagenic or clastogenic potential [Andro 1995].

Clinical safety data
The use of Pygeum africanum bark is considered to be well tolerated [Descazeaud 2015]. A number of controlled and uncontrolled clinical studies involving 2872 patients provided information on specific adverse events. Adverse events due to Pygeum africanum bark were generally mild in nature and similar in frequency to placebo. The most common complaint was mild gastrointestinal upset [Ishani 2000; Andro 1995; Bombardelli 1997; McQueen 2001].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Online Series, 2019
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Online Series, 2019
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Online Series, 2019
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHAMOMILLAE ROMANAE FLOS	Roman Chamomile Flower	Online Series, 2019
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HELICHRYSI FLOS	Sandy Everlastin Flower	Online Series, 2019
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013

HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Online Series, 2020
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Online Series, 2020
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Online Series, 2020
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Online Series, 2020
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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The second edition of ESCOP Monographs, published as a hardback book in 2003 with a Supplement in 2009, has been widely acclaimed for its authoritative information on the therapeutic uses of herbal medicines. Monographs covering a total of 107 herbal substances include extensive summaries of pharmacological, clinical and toxicological data, and copious references to scientific literature form an important part of each text.

Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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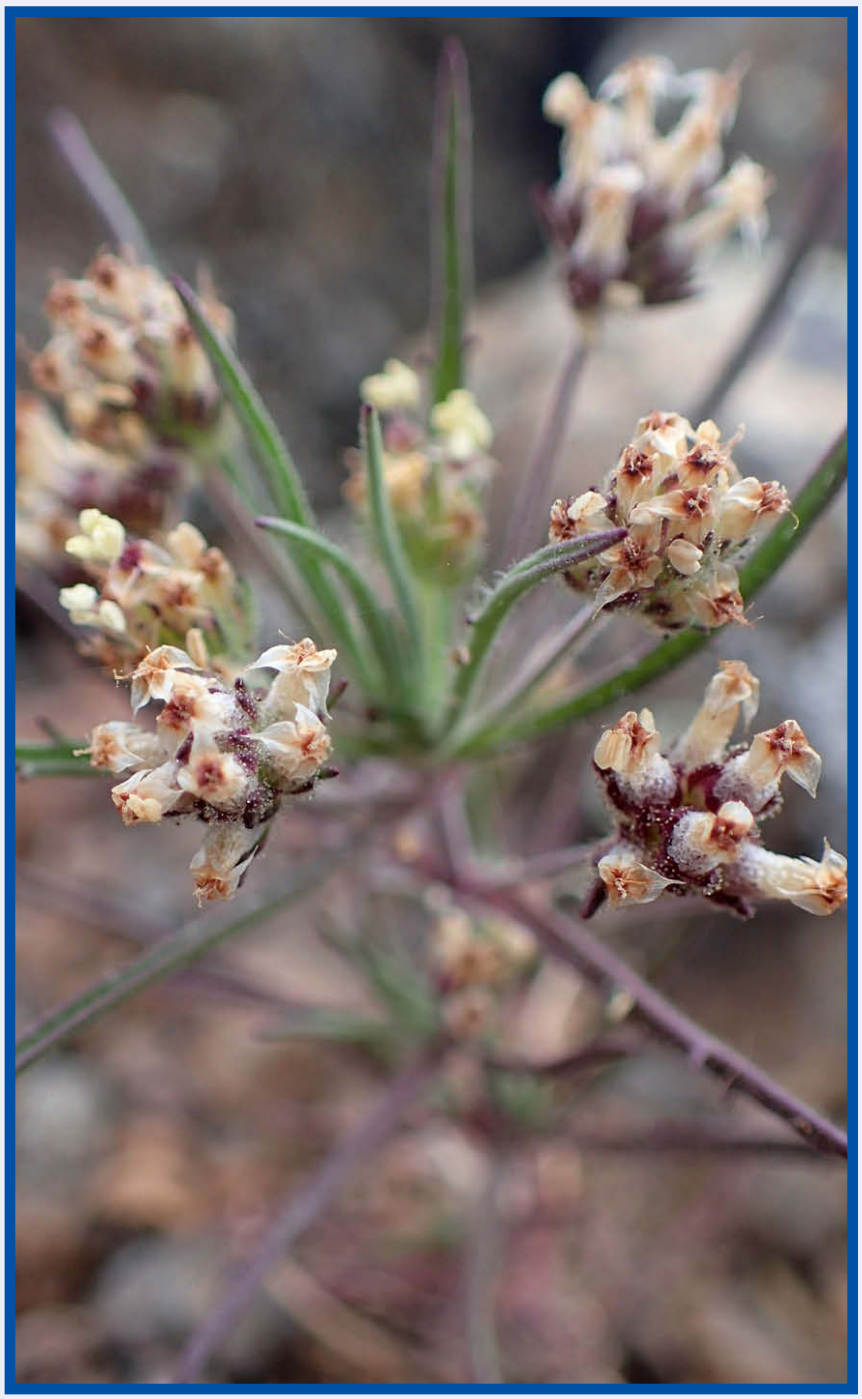
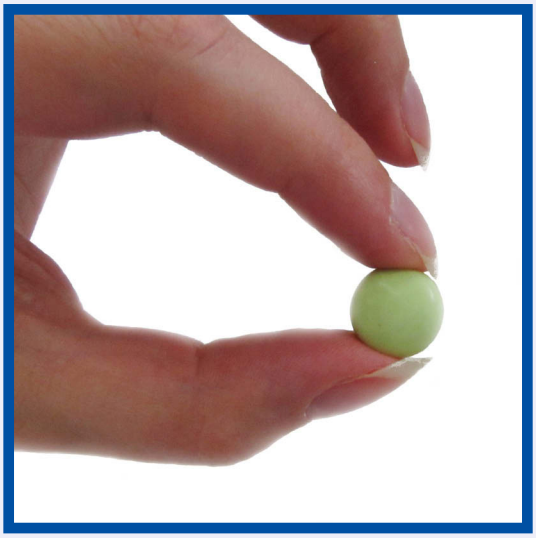
The Scientific Foundation for Herbal Medicinal Products

Psylli semen
Psyllium Seed

2017





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MONOGRAPHS

***The* Scientific Foundation for**
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PSYLLI SEMEN
Psyllium Seed

2017

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
CCl_4	carbon tetrachloride
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase

INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

PSYLLII SEMEN

Psyllium Seed

2017

DEFINITION

Psyllium seed consists of the ripe, whole, dry seeds of *Plantago afra* L. (*Plantago psyllium* L.) or *Plantago indica* L. (*Plantago arenaria* Waldstein and Kitaibel).

The material complies with the monograph of the European Pharmacopoeia [Psyllium seed].

CONSTITUENTS

Mucilage polysaccharide (10-15%) [Karawya 1971a; Bräutigam 2007] in the epidermis, consisting of xylose, galacturonic acid, arabinose and rhamnose residues [Karawya 1971a]. The seeds also contain protein (15-20%), fixed oil (5-13%) [Sticher 2015], the trisaccharide planteose [Sticher 2015; Balbaa 1971] and small amounts of phytosterols, triterpenes [Sticher 2015; Balbaa 1971], the iridoid glucoside aucubin and alkaloids (plantagonine, indicaine and indicamine) [Sticher 2015; Balbaa 1971; Karawya 1971b], but no starch [Karawya 1971a; Bräutigam 2007].

CLINICAL PARTICULARS

Therapeutic indications

Treatment of occasional constipation [Brunton 1996; Kay 1978].
Conditions in which easy defecation with soft stools is desirable, e.g. in cases of anal fissures or haemorrhoids, after rectal or anal surgery, and during pregnancy [Bräutigam 2007; Wichtl 2009; USP Dispensing Information 1994].
Adjuvant symptomatic therapy in cases of diarrhoea from various causes [Brunton 1996].

Posology and method of administration

Dosage
Adults and children over 12 years of age: as a laxative, 10-30 g daily of the seeds [Bräutigam 2007; Wichtl 2009] or equivalent preparations; in cases of diarrhoea, up to 40 g daily [Bräutigam 2007] divided into 2-3 doses.
Children 6-12 years: as a laxative, half the adult dose [Dorsch 2002].

Method of administration
For oral administration.

It is very important to take the seeds with a large amount of liquid, e.g. mix approx. 5 g with 150 ml of cool water, stir briskly and swallow as quickly as possible, then maintain adequate fluid intake.

Psyllium seed should preferably be taken at mealtimes; it should not be taken immediately prior to going to bed. Any other medications should be taken at least 30-60 minutes before psyllium seed in order to avoid delayed absorption.

Duration of administration
In cases of diarrhoea, medical advice should be sought if the symptoms persist for more than 3 days in order to ensure definitive diagnosis of the cause.

Contra-indications

Children under 6 years of age.
Known hypersensitivity to psyllium seed [Brayfield 2014].

Psyllium seed should not be used by patients with the following conditions unless advised by a physician: faecal impaction; undiagnosed abdominal symptoms; a sudden change in bowel habit that persists for more than 2 weeks; rectal bleeding; failure to defecate following the use of a laxative; abnormal constrictions in the gastrointestinal tract; potential or existing intestinal obstruction (ileus); diseases of the oesophagus and cardia; megacolon [Bräutigam 2007, Brunton 1996];

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MONOGRAPHS

diabetes mellitus which is difficult to regulate [Bräutigam 2007, Brunton 1996, Kay 1978].

Special warnings and special precautions for use
A sufficient amount of liquid should always be taken: at least 150 ml of water per 5 g of seed [Brayfield 2014].

Taking psyllium seed without adequate fluid may cause it to swell and block the throat or oesophagus and may cause choking. Intestinal obstruction may occur should adequate fluid intake not be maintained. Do not take psyllium seed if you have ever had difficulty in swallowing or have any throat problems. If you experience chest pain, vomiting, or difficulty in swallowing or breathing after taking it, seek immediate medical attention. Treatment of the elderly and debilitated patients requires medical supervision. Administration to the elderly should be supervised.

In cases of diarrhoea sufficient intake of water and electrolytes is important.

Interaction with other medicaments and other forms of interaction
Enteral absorption of concomitantly administered minerals (e.g. calcium, iron, lithium, zinc), vitamins (B12), cardiac glycosides and coumarin derivatives may be delayed [Bräutigam 2007; USP Dispensing Information 1994; Brunton 1996; Drews 1979; Cummings 1978]. For this reason, other medications should be taken at least 30-60 minutes before psyllium seed. In the case of insulin-dependent diabetics it may be necessary to reduce the insulin dose [Cummings 1978; Kay 1978].

Pregnancy and lactation
The product can be used during pregnancy and lactation [Lewis 1985].
A risk is not to be expected since the constituents of psyllium seed are not absorbed and have no systemic effects.

Effects on ability to drive and use machines
None known.

Undesirable effects
Flatulence may occur, but generally disappears during the course of treatment.
Abdominal distension and risk of oesophageal or intestinal obstruction and faecal impaction may occur, particularly if psyllium seed is ingested with insufficient fluid.

There is a risk of allergic reaction from inhalation of the powder during occupational exposure. Due to the allergic potential of psyllium seed, patients must be aware of reactions of hypersensitivity including anaphylactic reactions in single cases [Brayfield 2014].

Overdose
Overdosage may cause abdominal discomfort and flatulence, or even intestinal obstruction. Adequate fluid intake should be maintained and management should be symptomatic.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

Laxative effects
Psyllium seed increases the volume of intestinal contents by the binding of fluid, resulting in increased faecal weights. This leads to physical stimulation of the gut [Bräutigam 2007; Brunton 1996].

Psyllium seed increases stool weight and water content due to the fibre residue, the water bound to that residue [Bräutigam 2007; Brunton 1996] and the increased faecal bacterial mass [Brunton 1996].

Antidiarrhoeal effects
In cases of diarrhoea, psyllium seed binds fluids and thus increases the viscosity of intestinal contents [Brunton 1996], thereby normalizing transit time and frequency of defecation [Bräutigam 2007].

Pharmacokinetic properties
The vegetable fibre of psyllium seed is resistant to digestion in the upper intestinal tract; thus it is not absorbed. Part of the fibre is degraded by colonic bacteria [Bräutigam 2007; Kay 1978].

Preclinical safety data
After 125 days on a diet containing 25% of psyllium seed, albino rats showed a dark pigmentation of the suprarenal gland, the kidney marrow and the liver. Dogs showed a grey colour of the kidneys after being fed a diet containing 25% of psyllium seed for 30 days. Similar effects have not been observed in humans. The pigment probably originates from the black pericarp of *Plantago afra*. When the seeds were extracted with hot water prior to feeding and then fed to the animals as whole seeds no pigmentation was observed [Bräutigam 2007].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Supplement 2009
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Supplement 2009
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Second Edition, 2003
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003

LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Second Edition, 2003
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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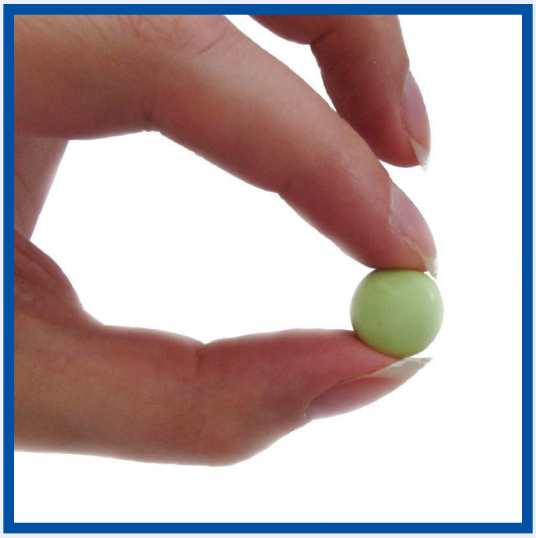
Ratanhiae radix
Rhatany Root

2017



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Plant illustrated on the cover: *Krameria triandra*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
CCl_4	carbon tetrachloride
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase

INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

RATANHIAE RADIX

2017

Rhatany Root

DEFINITION

Rhatany root consists of the dried, usually fragmented, underground organs of *Krameria triandra**) Ruiz et Pav., known as Peruvian rhatany. It contains minimum 5.0 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) and calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Rhatany root].

* According to latest taxonomy *Krameria triandra* Ruiz & Pav. is considered to be a synonym of *Krameria lappacea* (Dombey) Burdet & B.B. Simpson [Medicinal Plant Names Services].

CONSTITUENTS

The main characteristic constituents are complex polyphenols (proanthocyanidins) consisting of 2-14 flavan-3-ol units with mainly 2,3-*cis* configuration, a predominant 4-8 interflavan linkage and a propelargonidin : procyanidin ratio of approximately 7:3 [Scholz 1989; Schilcher 2010; Blaschek 2016].

Lignans, neolignans and nor-neolignans, generally with an arylbenzofuran moiety, are also present [Stahl 1981; Arnone 1988, 1990; De Bellis 1994; Baumgartner 2011 a,b; Blaschek 2016], the most abundant ones being conocarpan and eupomatenoid 6 [Arnone 1988, 1990].

CLINICAL PARTICULARS

Therapeutic indication

Topical use
Minor inflammations of the mouth and throat such as stomatitis, gingivitis and pharyngitis [BHP 1996, Grünwald 2004; Schilcher 2010; Baumgartner 2011a; Blaschek 2016].

These indications are pharmacologically plausible and based on long-term use and experience.

Posology and method of administration

Dosage

Topical use
Crushed root, 1-2 g as a decoction or similar preparation as a rinse or gargle two to three times daily; tincture (1:5, ethanol 70% V/V), 5-10 drops in a glass of water two to three times daily; undiluted tincture painted on the affected surface two to three times daily [Grünwald 2004; Schilcher 2010; Blaschek 2016].

Method of administration
For local application.

Duration of administration
No restriction.

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

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Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
One case of contact dermatitis has been reported [Goday Buján 1998].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Radical scavenging effects
A methanolic extract (standardized to 15% neolignans) exhibited protective effects against haemolysis induced in red blood cells from rats by cumene hydroperoxide (IC_{50}: 0.28 µg/ml) or UVB radiation (IC_{50}: 0.78 µg/ml) and was much more effective than α-tocopherol (IC_{50}: 6.37 µg/ml). The extract (20 µg/ml) also showed protective effects on keratinocyte viability after UVB irradiation, increasing the survival rate to 96.5% ($p<0.001$) compared to 84.4% for the control [Carini 2002].

In a membrane model of lipid peroxidation (phosphatidylcholine liposomes), petroleum ether, chloroform and butyl acetate extracts inhibited lipid peroxidation during the propagation phase with IC_{50} values of 5.8, 3.7 and 0.23 µg/ml respectively [Maffei Facino 1997].

Anti-inflammatory activity
A dichloromethane extract (yield 5.5%) and various isolated lignan derivatives showed pronounced activity (>50% inhibition) at concentrations of 10 µg/mL in TNFα-stimulated HEK-293 cells stably transfected with a NF-kB-driven luciferase reporter gene. All compounds reduced NF-kB-dependent luciferase activity in a concentration-dependent manner. The extract (50 µg/mL) inhibited COX-1 by 82.5 ±8.9% and COX-2 by 83.9 ± 5.8%. Four of the lignans (50 µM) inhibited these enzymes to an extent of 57.2% to 83.3%. Moreover, the extract (50 µg/mL) and 3 of the lignans (50 µM) inhibited leukotriene formation by 69.5 ± 4.1% and between 80.2% and 94.6% respectively [Baumgartner 2011a].

Vasoprotective activity
In human umbilical vein endothelial cells (HUVECs) and HUVEC-derived EA.hy926 cells, the isolated compound 2-(2,4-dihydroxyphenyl)-5-(E)-propenylbenzofuran (DPPB) (1–10 µM) increased endothelial nitric oxide synthase (eNOS) activity. The effect was associated with an increase in Ca^{2+}, leading to the activation of eNOS via a signal transduction pathway involving Ca^{2+}/CaM, CaMKKb and AMPK [Ladurner 2012].

Antidiabetic activity
A dichloromethane extract (yield 5.5 %) at concentrations of 3-30 µg/mL concentration-dependently inhibited human recombinant PTP1B and enhanced insulin-stimulated glucose uptake in murine myocytes. The extract at a concentration of 30 µg/mL increased glucose uptake into murine C2C12 myocytes both in the absence and presence of insulin. Ratanhiaphenol III inhibited PTP1B with an IC_{50} of 20.2 µM and at concentrations of 10 and 30 µM concentration-dependently increased insulin receptor phosphorylation as well as insulin-stimulated glucose uptake in cultured myotubes [Heiss 2012].

Antiproliferative activity
An ethanolic extract (not further specified) showed a growth inhibition of Hep3B cells at concentrations of 50 and 100 µg/mL [Carras 2015].

Antimicrobial activity
Ethanol, methanol, hexane and ethyl acetate extracts inhibited the growth of various bacteria including *Escherichia coli* and *Staphylococcus aureus* [Neto 2002]. The MIC of a dry extract (obtained by percolation with acetone 80% and ethanol 80% followed by lyophilization of the combined extracts) against *S. aureus* was 20 µg/ml [Scholz 1989].

Certain neolignans and *nor*-neolignans isolated from rhatany root exhibited antibacterial and antifungal activity, inhibiting the growth of *E. coli*, *Aspergillus niger* and *Saccharomyces cerevisiae* [Arnone 1988; Bombardelli 1995].

No antimicrobial activity against *E. coli*, *S. aureus* or *A. niger* was observed with aqueous extracts [Anesini 1993].

Astringent activity
A tincture and a decoction of powdered rhatany root exhibited astringent activity, attributed mainly to proanthocyanidins with a degree of polymerization from 5-10; both preparations had a relative astringency of 0.3 (determined using freshly haemolyzed human blood) [Scholz 1989].

In vivo experiments

Anti-inflammatory activity
In a mouse ear model, a dichloromethane extract exhibited a dose-dependent inhibition of oedema which ranged from 24% at the lowest dose (30 µg/cm²) to 86% for the highest (300 µg/cm²) with an ID_{50} of 77 µg/cm². Eleven isolated compounds reduced the oedematous response from about 15% (0.1 µmol/cm²) to about 80% (1.0 µmol/cm²) in a dose-dependent manner (ID_{50} 0.31-0.60 µmol/cm²) comparable to the effect of indomethacin (ID_{50} 0.29 µmol/cm²) [Baumgartner 2011a].

Pharmacokinetic properties
No data available.

Preclinical safety data

Acute toxicity
No symptoms of toxicity were evident in rodents after a single dose of a dry extract (from combined acetone 80% and ethanol 80% percolates), administered orally at 300 mg/kg or intraperitoneally at 200 mg/kg [Scholz 1989].

Carcinogenicity
NIH black rats, 15 male and 15 female, 1-2 months old, were subcutaneously injected in the flank, weekly for a maximum of 75 weeks, with a total lyophilized extract (5 mg) or a tannin-containing fraction (2 mg) from rhatany root dissolved in 0.5 ml of saline solution; 30 control animals received saline only. The animals were observed for a period of 2 years. Both the extract and the tannin-containing fraction were shown to be carcinogenic in this experiment [Pradhan 1974]. However, in view of the high dose level and the duration and route of administration, the results do not appear to be relevant to the therapeutic use of rhatany root in humans [Simpson 1991].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
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ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Supplement 2009
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Supplement 2009
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
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HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
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HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Second Edition, 2003
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003

LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
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MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
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MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
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MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
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PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
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PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Second Edition, 2003
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
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SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
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SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
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SERPYLLI HERBA	Wild Thyme	Online Series, 2014
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TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
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TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
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URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
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ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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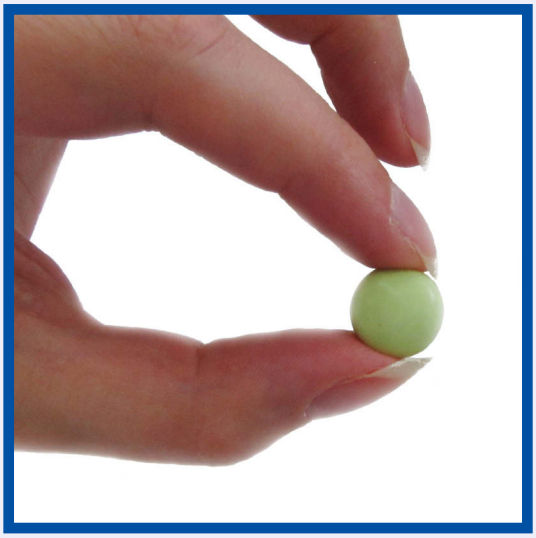

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

RHAMNI PURSHIANAE CORTEX

Cascara

DEFINITION

Cascara consists of the dried, whole or fragmented bark of *Rhamnus purshianus* D.C. (*Frangula purshiana* (D.C.) A. Gray ex J.C. Cooper). It contains not less than 8.0 per cent of hydroxyanthracene glycosides of which not less than 60 per cent consists of cascarosides, both expressed as cascaroside A ($C_{27}H_{32}O_{14}$; M_r 580.5) and calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Cascara].

CONSTITUENTS

The active constituents are hydroxyanthracene glycosides (8 – 10%) which can be subdivided into 3 groups [Staesche 2014; Sticher 2014; Manito 1995]:

- The mixed anthrone-C- and O-glycosides or the cascarosides (60 – 70%): cascarosides A - F and 10-hydroxycascaroside A,B,E and F. Cascarosides A and B are the 8-O-β-D-glucosides of 10-(S)-deoxyglucosyl aloe-emodin anthrone and of 10-(R)-deoxyglucosyl aloe-emodin anthrone (aloins A and B) respectively. Cascarosides C and D are the 8-O-β-D-glucosides of 10-(R)(S)-deoxyglucosyl chrysophanol anthrone (chrysaloins A and B); cascarosides E and F are 8-O-β-D-glucosides of 10-(R)(S)-deoxyglucosyl emodin anthrone.
- The pure anthrone-C-glycosides (10 – 30%): aloins A and B, 10-hydroxyaloins A and B together with chrysaloins A and B.
- The anthraquinone-O-glycosides (10-20%) including the monoglucosides of aloe-emodin, chrysophanol, emodin and physcion.

CLINICAL PARTICULARS

Therapeutic indications
For short term use in cases of occasional constipation [Staesche 2014; Veitch 2013; Wichtl 2009].

Posology and method of administration

Dosage
The correct individual dosage is the smallest required to produce a comfortable soft-formed motion.

Adults and children over 12 years
Preparations equivalent to 20-30 mg hydroxyanthracene derivatives, calculated as cascaroside A to be taken once daily at night [Martindale 2015; Staesche 2014; Veitch 2013; Wichtl 2009].

Elderly: dose as for adults.

Not recommended for use in children under 12 years of age.

The pharmaceutical form must allow for lower dosages.

Method of administration
For oral administration.

Duration of administration
Stimulant laxatives should not be used for periods of more than 2 weeks without medical advice. If symptoms persist after intake of the preparation medical advice should be sought [Tack 2011]

Contra-indications
Intestinal obstruction and stenosis, atony, inflammatory diseases of the colon (e.g. Crohn's disease, ulcerative colitis), appendicitis; abdominal pain of unknown origin; severe dehydration states with water and electrolyte depletion [Martindale 2015; Veitch 2013; Wichtl 2009; Pasricha 2006].

Special warnings and special precautions for use
As for all laxatives, cascara should not be given when any undiagnosed acute or persistent abdominal symptoms are present.

Chronic use may cause pigmentation of the colon (pseudomelanosis coli) which is harmless and reversible after drug discontinuation. Abuse with diarrhoea and consequent fluid and electrolyte losses may cause: dependence with possible need for increased dosages; disturbance of the water and electrolyte (mainly hypokalaemia) balance; an atonic colon with impaired function. Intake of anthranoid-containing laxatives for more than a short period of time may result in an aggravation of constipation. Hypokalaemia can result in cardiac and neuromuscular dysfunction, especially if cardiac glycosides, diuretics or corticosteroids are taken. Chronic use may result in albuminuria and haematuria.

If laxatives are needed every day the cause of the constipation should be investigated. Long term use of laxatives should be avoided. Use for more than 2 weeks requires medical supervision. In chronic constipation, stimulant laxatives are not an acceptable alternative to a change in dietary habits, physical activities and training for normal bowel evacuation [Martindale 2015; Veitch 2013; Keller 2011; Wichtl 2009; Pasricha 2006; Müller-Lissner 1993, 2005].

Interaction with other medicaments and other forms of interaction
Hypokalaemia (resulting from long term laxative abuse) potentiates the action of cardiac glycosides and interacts with antiarrhythmic drugs or with drugs which induce reversion to sinus rhythm (e.g. quinidine). Concomitant use with other drugs inducing hypokalaemia (e.g. thiazide diuretics, adrenocorticosteroids and liquorice root) may aggravate electrolyte imbalance [Staesche 2014; Veitch 2013; Wichtl 2009; Schulz 2004].

Pregnancy and lactation

Pregnancy
Not recommended during pregnancy.

Experimental studies as well as many years of experience do not indicate undesirable or damaging effects from anthranoid laxatives during pregnancy or on the foetus when used at the recommended dosage [Schmidt 1955; Westendorf 1993]. However, due to experimental data concerning a genotoxic risk from several anthranoids (e.g. aloe-emodin) a possible risk cannot be eliminated [Wichtl 2009].

Lactation
The use during breastfeeding is not recommended as there are insufficient data on the excretion of metabolites in breast milk. Small amounts of active metabolites (rhein) may appear in breast milk. However, a laxative effect in breast-fed babies has not been reported [Martindale 2015; Faber 1988].

Effects on ability to drive and use machines
None.

Undesirable effects
Abdominal spasms and pain, in particular with irritable colon; yellowish-brown or red (pH dependent) discolouration of urine by metabolites, which is not clinically significant [Martindale 2015; Tack 2011; Pasricha 2006; Schulz 2004; Tedesco 1985; Ewe 1986].

Overdose
The major symptoms are griping and severe diarrhoea with consequent losses of fluid and electrolytes, which should be replaced [Pasricha 2006]. Treatment should be supportive with generous amounts of fluid. Electrolytes, especially potassium, should be monitored; this is particularly important in the elderly and the young.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

Laxative activity
1,8-dihydroxyanthracene derivatives possess a laxative effect. The cascarosides and the anthrone-C-glycosides (aloins A and B, 10-hydroxyaloins A and B and chrysaloins A and B) are precursors which are not split by human digestive enzymes in the upper gut and therefore not absorbed. They are converted by the bacteria of the large intestine into the active metabolites mainly aloe-emodin-9-anthrone.

There are two different mechanisms of action:

(i) stimulation of the motility of the large intestine resulting in accelerated colonic transit and a decreased absorption of water and electrolytes (Na+, Cl-) into the colonic epithelial cells.

(ii) an influence on secretion processes by stimulation of secretion of water and electrolytes and increase of the leakage of the tight junctions resulting in enhanced fluid volume in the colon.

[Fairbairn 1965, 1970; Dreesen 1988; Hattori 1988; Che 1991; Akao 1996; Ishii 1990,1994a,1994b; 1998; Kai 2002; Alves 2004; Wanitschke 2003].

Possible mechanisms at cellular level are: inhibition of the sodium pump (Na^+/K^+-ATPase) [Ishii 1990; Rauwald 1992], Cl^--channels [Hönig 1992] and ATP-production [Rauwald 1992]; increase of NO [Izzo 1997; Park 2009a] and prostaglandins [Capasso 1983]; release of Ca^{2+}, histamine and serotonin [Park 2009a; Schulz 2004; Van Gorkom 1999].

Defaecation takes place after a delay of 6-12 hours due to the time taken for transport to the colon and transformation into the active compound [Staesche 2014; Martindale 2015; Müller-Lissner 2009; Pasricha 2006; Schulz 2004].

Antitumour activity
Aloe-emodin inhibited the growth of human neuroectodermal tumours in tissue cultures (ED_{50} 1-13 µg) and in mice with severe combined immunodeficiency (50 mg/kg b.w./day; $p<0.05$) [Pecere 2000].

Aloe-emodin inhibited cell proliferation in various hepatoma cell lines [Kuo 2002; Shieh 2003] and in Merkel carcinoma cell line [Wassermann 2002].

Furthermore, aloe-emodin demonstrated antiangiogenic activity by inhibiting NF-κB, basic fibroblast growth factors and iNOS [Srinivas 2007].

Other activities
Pretreatment of rats with aloe-emodin (2 x 50 mg/kg b.w. i.p.) significantly reduced acute liver injury induced by carbon tetrachloride ($p<0.05$). The changes in hepatic albumin and TNF-α mRNA were also normalized ($p<0.05$) [Arosio 2000].

Barbaloin and emodin showed a high affinity for phospholipids in different model membranes and exhibited antibacterial activity against *Escherichia coli* (MIC: 2.8 and 2.2 µM respectively) when incorporated into liposomes [Alves 2004].

Pharmacokinetic properties

No pharmacokinetic data have been directly obtained with cascara or its extracts. However, a human pharmacokinetic study using a mixture of aloins and their corresponding 3-rhamnosides (aloinosides A and B) has been reported. In this study, the equivalent of 16.4 mg of hydroxyanthracene derivatives was administered orally for 7 days during which aloe-emodin was detected only sporadically as a metabolite in the plasma, with maximum concentrations of less than 2 ng/mL. In the same study, rhein was detected in the plasma in concentrations ranging from 6-28 ng/mL (median C_{max} 13.5 ng/mL at median t_{max} 16 h) after single dose administration. In the 7-day administration there was no evidence of accumulation of rhein [Schulz 1993].

From another study it was concluded that free anthranoids absorbed systemically in humans are partly excreted in the urine as rhein or as conjugates even when rhein is not present in the administered drug (as in Cascara) [Vyth 1979]. It is not known to what extent aloe-emodin anthrone is absorbed. However, in the case of senna, animal experiments with radio-labelled rheinanthrone administered directly into the caecum show that only a very small proportion (less than 10%) of rhein anthrone is absorbed [De Witte 1988a].

Oral administration of emodin to rabbits at 10 mg/kg b.w. resulted in a very low serum concentration (approximately 2.5 µg/mL). Emodin was found to be highly bound (99.6%) to serum protein [Liang 1995].

An *in vitro* study using human intestinal cells (Caco-2) and the inverted rat gut sac showed an absorption of aloe-emodin of 6.6 to 11.3% at 5 to 50 µM [Park 2009b].

Systemic metabolism of free anthranoids depends upon their ring constituents [De Witte 1988b; Sendelbach 1989]. In the case of aloe-emodin, it was shown in animal experiments that at least 20-25 % of an oral dose will be absorbed. The bioavailability of aloe-emodin is much lower than the absorption, because it is quickly oxidized to rhein and an unknown metabolite, or conjugated. Maximum plasma values of aloe-emodin were reached 1.5-3 h after administration. Maximum concentrations in plasma were about 3 times higher than those in ovaries and 10 times higher than those in testes [Lang 1993].

Elimination of absorbed aglycones occurs via faeces and urine as glucuronides and sulfates [Sticher 2014].

Preclinical safety data

Acute toxicity
No mortality was observed in rats after a single oral administration of cascara at 6 g/kg and of aloin at 7.5 g/kg b.w. [Schmidt 1955]. Single oral doses of aloin at 50-500 mg/kg b.w. induced no toxic effects in rats [Bangel 1975]

Repeated dose toxicity
Cascara at a dose of 600 mg/kg b.w./day and aloin at a dose of 420 mg/kg b.w./day for 3 months showed low toxicity in rats [Schmidt 1955]. Aloin administered to rats at a dose of 200 mg/kg b.w./day, 2 days per week for 6 weeks also showed low toxicity [Bangel 1975]. Aloin at a dose of 100 mg/kg b.w./day for 20 weeks showed no specific toxic effects in mice [Siegers 1993].

Reproductive toxicity
Aloin at a dose of 200 mg/kg b.w. (for 1 day or 4 days during gestation) showed no evidence of any embryolethal, teratogenic or foetotoxic effects in rats [Bangel 1975].

Emodin, administered in feed to timed-mated rats (0, 425, 850 and 1700 ppm) and mice (0, 600, 2500 and 6000 ppm), was evaluated for potential effects on pregnancy outcome. Prenatal mortality, live litter size, foetal sex ratio and morphological development were unaffected in both rats and mice. At the highest dose, average foetal body weight per litter was unaffected in rats but was significantly ($p<0.001$) reduced in mice [Jahnke 2004].

Mutagenicity
No specific data are available for cascara or the cascarosides. Data for aloin derived from aloes indicate no genotoxic risk [Lang 1993; Bootman 1987a-b; CCR 1992a-c; Marquardt 1987]. Aloe-emodin showed positive and negative results *in vitro* but was clearly negative *in vivo* [Heidemann 1993]. Emodin was mutagenic in the Ames test [Brown 1976; Tikkanen 1983] but gave inconsistent results in gene mutation assays (V79 HGPRT) [Westendorf 1990; Bruggeman 1984], positive results in the UDS test with primary rat hepatocytes [Westendorf 1990] but negative results in the SCE assay [Bruggeman 1984].

Carcinogenicity
Data on the carcinogenicity of cascara or of the cascarosides are not available. Aloin fed to male NMRI mice in the diet at a level of 100 mg/kg/day for 20 weeks did not promote dimethylhydrazine-induced colorectal tumours [Siegers 1993].

In a 2-year study, male and female F344/N rats were exposed to 280, 830 or 2500 ppm emodin in the diet, corresponding to an average daily dose of emodin of 110, 320 or 1000 mg/kg b.w. in male rats and 120, 370 or 1100 mg/kg in female rats. No evidence of carcinogenic activity of emodin was observed in male rats. A marginal increase in the incidence of Zymbal's gland carcinoma occurred in the female rats treated with the high dosage but was interpreted as questionable [NTP 1999].

In a further 2-year study on $B6C3F_1$ mice, males were exposed to 160, 312 or 625 ppm emodin (equivalent to an average daily dose of 15, 35 or 70 mg/kg b.w.) and females to 312, 625 or 1250 ppm emodin (equivalent to an average daily dose of 30, 60 and 120 mg/kg b.w.). There was no evidence of carcinogenic activity in female mice. The low incidence of renal tubule neoplasms in exposed males was not considered relevant [NTP 1999].

Clinical safety data
In a case control study with retrospective and prospective evaluation, no causal relationship between anthranoid laxative use and colorectal cancer could be detected [Loew 1994a,1994b].

In one case report, cascara was reported to be associated with the development of cholestatic hepatitis, complicated by portal hypertension, after intake of 425 mg cascara (containing 5% cascarosides) 3 times daily for 3 days, but a causal relationship could not be demonstrated [Nadir 2000].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Second Edition, 2003
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
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ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Second Edition, 2003
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Second Edition, 2003
FUMARIAE HERBA	Fumitory	Supplement 2009
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Supplement 2009
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Second Edition, 2003
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
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LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
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MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
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MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Second Edition, 2003
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Second Edition, 2003
RATANHIAE RADIX	Rhatany Root	Supplement 2009
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Second Edition, 2003
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Second Edition, 2003
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Second Edition, 2003
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SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
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SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
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SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
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TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
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URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Second Edition, 2003
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Supplement 2009
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ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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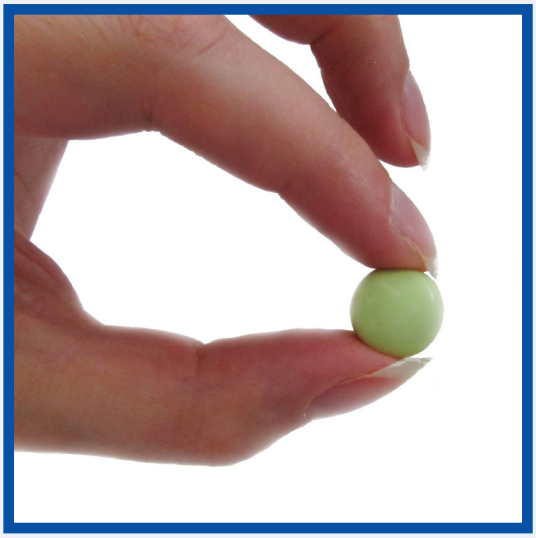
Rhei radix
Rhubarb

2019



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RHEI RADIX
Rhubarb

2019

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
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Notes for the Reader
Abbreviations
The monograph text
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Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
CCl_4	carbon tetrachloride
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase

INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

RHEI RADIX

Rhubarb

2019

DEFINITION

Rhubarb consists of the whole or cut, dried underground parts of *Rheum palmatum* L. or of *Rheum officinale* Baillon or of hybrids of these two species or of a mixture. The underground parts are often divided; the stem and most of the bark with the rootlets are removed. It contains not less than 2.2% of hydroxyanthracene derivatives, expressed as rhein ($C_{15}H_8O_6$; M_r 284.2), calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Rhubarb].

CONSTITUENTS

The main characteristic constituents are hydroxyanthracene derivatives (3-12%, depending on the method of determination) consisting mainly (60-80%) of mono- and diglucosides of rhein, chrysophanol, aloe-emodin, physcion and emodin, and only small amounts of the respective aglycones. Dianthrone glycosides (sennosides) are also present and small amounts of anthrone glycosides depending on the time of harvesting and the conditions of drying [van Os 1976; Chirikdjian 1983; Engelshowe 1985; Hänsel 1994, 1999].

Other constituents include gallotannins (ca. 5%) [Engelshowe 1985; Weiß 1990; Hänsel 1994, 1999], chromones, phenylbutanones and traces of volatile oil [Hänsel 1994, 1999; Miyazawa 1996].

CLINICAL PARTICULARS

Therapeutic indications
For short-term use in cases of occasional constipation [Weiß 1990; Reynolds 1996; Wichtl 1997; Schilcher 2000].

Although no published clinical data is currently available, the laxative effect of rhubarb is well-known, and is comparable to the other anthranoid containing laxatives.

Posology and method of administration

Dosage

The correct individual dose is the smallest required to produce a comfortable soft-formed motion.

Adults and children over 12 years: drug or preparations equivalent to 15-50 mg of hydroxyanthracene derivatives (calculated as rhein) daily, preferably taken in one dose at night [Hänsel 1994; Wichtl 1997; Schilcher 2000].

Not recommended for use in children under 12 years of age.

The pharmaceutical form must allow lower dosages.

Method of administration
For oral administration.

Duration of use
Stimulant laxatives should not be used for periods of more than 2 weeks without medical advice.

Contraindications
Pregnancy and lactation; children under 10 years of age [Hänsel 1994].

Not to be used in cases of intestinal obstruction and stenosis, atony, inflammatory bowel diseases (e.g. Crohn's disease, ulcerative colitis), appendicitis, abdominal

E/S/C/O/P MONOGRAPHS

pain of unknown origin [Hänsel 1994]; severe dehydration states with electrolyte depletion.

Special warnings and precautions for use
As for all laxatives, rhubarb should not be given when any undiagnosed acute or persistent abdominal symptoms are present.

If laxatives are needed every day the cause of the constipation should be investigated. Long term use of laxatives should be avoided. Use for more than 2 weeks requires medical supervision. Chronic use may cause pigmentation of the colon (pseudomelanosis coli) which is harmless and reversible after drug discontinuation. Abuse, resulting in loss of fluid and electrolytes, may cause [Leng-Peschlow 1992]: dependence with possible need for increased dosages; disturbance of the water and electrolyte (mainly hypokalaemia) balance; an atonic colon with impaired function. Intake of anthranoid-containing laxatives for more than a short period of time may result in aggravation of constipation. Hypokalaemia can result in cardiac and neuromuscular dysfunction, especially if cardiac glycosides, diuretics or corticosteroids are taken. Chronic use may result in albuminuria and haematuria.

In chronic constipation, stimulant laxatives are not an acceptable alternative to a change in diet.

Note: A detailed text with advice concerning changes in dietary habits, physical activities and training for normal bowel evacuation should be included on the package leaflet. An example is given in the booklet "Médicaments à base de plantes" published by the French health authority (Paris: Agence du Médicament).

Interaction with other medicinal products and other forms of interaction
Hypokalaemia (resulting from long term laxative abuse) potentiates the action of cardiac glycosides and interacts with anti-arrhythmic drugs or with drugs which induce reversion to sinus rhythm (e.g. quinidine). Concomitant use with other drugs inducing hypokalaemia (e.g. thiazide diuretics, adrenocorticosteroids and liquorice root) may aggravate electrolyte imbalance.

Pregnancy and lactation

Pregnancy
Not recommended during pregnancy.
There are no reports of undesirable or damaging effects during pregnancy or on the foetus when used in accordance with the recommended dosage schedule. However, experimental data concerning a genotoxic risk from several anthranoids (e.g. emodin) are not counterbalanced by sufficient studies to eliminate a possible risk.

Lactation
Breast-feeding is not recommended as there are insufficient data on the excretion of metabolites in breast milk. Excretion of active principles in breast milk has not been investigated. However, small amounts of active metabolites (e.g. rhein) are known to be excreted in breast milk. A laxative effect in breast-fed babies has not been reported [Faber 1988].

Effects on ability to drive and use machines
None known.

Undesirable effects
Abdominal spasms and pain, in particular in patients with irritable colon; yellow or red-brown (pH dependent) discoloration of urine by metabolites, which is not clinically significant [Cooke 1977; Tedesco 1985; Ewe 1986].

Overdose
The major symptoms are griping and severe diarrhoea with consequent losses of fluid and electrolytes, which should be replaced.

Treatment should be supportive with generous amounts of fluid. Electrolytes, especially potassium, should be monitored; this is particularly important in the elderly and the young.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

1,8-dihydroxyanthracene derivatives possess a laxative effect [Fairbairn 1970; Leng-Peschlow 1992]. The β-linked glucosides in rhubarb are not absorbed in the upper gut; they are converted by the bacteria of the large intestine into active metabolites (anthrones).

Based on experimental studies and studies in humans with Tinnevelly senna pods and isolated sennosides, two distinct mechanisms of action are assumed [Leng-Peschlow 1986]:

i. an influence on the motility of the large intestine (stimulation of peristaltic contractions and inhibition of local contractions) resulting in accelerated colonic transit, thus reducing fluid absorption [Garcia-Villar 1980; Bueno 1980], and

ii. an influence on secretion processes (stimulation of mucus and active chloride secretion) resulting in enhanced fluid secretion [Leng-Peschlow 1986, 1989].

Defecation takes place after a delay of 8-12 hours due to the time taken for transport to the colon and metabolic conversion of hydroxyanthracene glycosides to the active compounds.

In vitro experiments

Antimicrobial activity
In an agar plate assay, strong inhibition of *Helicobacter pylori* was observed with a water extract (MIC < 1 mg) [Bae 1998]. An ethanolic extract inhibited *Helicobacter pylori* growth with a MIC of 17.24 µg/ml; in this test the MICs of anthraquinone compounds isolated from rhubarb were 0.40 µg/ml (emodin), 0.60 µg/ml (rhein), 0.78 µg/ml (chrysophanol) and 0.85 µg/ml (aloe-emodin) [Gou 1997].

Rhein, in combination with ampicillin or oxacillin, had synergistic or partial synergistic effects against methicillin-resistant *Staphylococcus aureus* strains (MRSA). The MIC of the respective antimicrobial agent in combination with rhein, compared to the antimicrobial agent alone, ranged 0.28-1 for ampicillin and 0.18-1 for oxacicillin [Joung 2012].

An ethanolic extract exhibited antiviral activity against *Herpes simplex* by preventing virus attachment and penetration [Hsiang 2001].

The antimycotic activity of an aqueous extract against *Aspergillus fumigatus* and *Candida albicans* was comparable to that of nystatin. The growth of *Geotrichum candidum* and *Rhodotorula rubra* was inhibited to a lesser extent [Blaszcyk 2000].

Rhein isolated from rhubarb showed strong antimicrobial activity against *Candida albicans* and *Bacteroides fragilis* [Cyong 1987].

Anti-proliferative activity
A dried aqueous extract (8:1) had significant ($p<0.001$) dose- and time-dependent growth inhibitory effects on both human lung adenocarcinoma cells and human breast cancer cells with IC_{50} values of 620 ±12.7 and 515 ±10.1 µg/ml respectively [Li 2009].

Rhein inhibited growth of three cancer cell lines in a dose dependent manner. The IC_{50} values against human cervical cancer cells, breast adenocarcinoma cells (MCF-7) and hepatocellular carcinoma cells (HepG2) were 54.28 ± 0.17, 49.35 ± 0.23 and 36.34 ± 0.14 µMol respectively ($p<0.05$) [Al-Fatlawi 2014].

Aloe-emodin induced apoptosis of human nasopharyngeal carcinoma cells via caspase-8-mediated activation of the mitochondrial death pathway [Lin 2010].

Emodin exhibited antiproliferative, antimetastatic and apoptotic effects in human chronic myelocytic leukemia K562 cells, pancreatic cancer cells, cervical cancer hela cells, hepatoma cells, neuroblastoma cells and tongue squamous cancer cells [Chun-Guang 2010, Liu 2011, Yaoxian 2013, Hsu 2010, Huang 2013, Lin 2009].

Several phenolic compounds from rhubarb showed cytotoxicity against human oral squamous cell carcinoma and salivary gland tumour cell lines as well as human gingival fibroblasts [Shi 2001]. Aloe-emodin induced apoptotic cell death in human lung squamous cell carcinoma [Lee 2001].

Bioassay-guided fractionation of an ethyl acetate extract showed emodin to be a selective inhibitor of casein kinase II with an IC_{50} of 2 µM [Yim 1999].

However, methanolic extracts from *Rheum palmatum* and *Rheum officinalis* rhizomes were found to significantly ($p<0.01$) enhance proliferation of the oestrogen-sensitive MCF-7 cell line at concentrations of 100 and 30 µg/ml, respectively. This effect was mainly attributed to emodin and emodin-8-*O*-β-D-glucoside, which bound to human oestrogen receptors α and β [Matsuda 2001].

Other effects
Methanolic extracts from *Rheum palmatum* and *Rheum officinale* showed radical scavenging activity, reducing 40 µM α,α-diphenyl-β-picrylhydrazyl (DPPH) radical by 50% at concentrations of 5.2 and 3.3 µg/ml respectively. IC_{50} values on superoxide anion radical in the xanthine/xanthine oxidase system were 5.0 and 3.8 µg/ml [Matsuda 2001]. Pyrogallol autoxidation and hydroxyl radicals generated via the Fenton reaction were inhibited by anthraquinones from rhubarb [Yuan 1997].

A hot water extract inhibited rat squalene epoxidase, an enzyme that catalyzes a rate-limiting step of cholesterol biosynthesis, by 70% at a concentration of 50 µg/ml; several galloyl compounds isolated from rhubarb were found to be potent inhibitors of the enzyme [Abe 2000].

A 90% hydroethanolic dried extract (3.4:1) was administered intragastrically to normal and CCl_4-treated rats daily for 12 weeks at dosage levels equivalent to 2, 5.4, 14.69 and 40g crude drug/kg b.w. A decrease in the extent of cellular injury was observed in the two lowest dosage groups of CCl_4-treated rats. However, a significant increase in fibrosis indicating rhubarb-induced liver damage was observed in both normal rats at all dosage levels and CCl_4-treated rats at the two highest dosage levels [Wang 2011].

In vivo experiments
In rats with adenine-induced chronic renal failure a hot water extract decreased levels of serum urea nitrogen and creatinine, as well as the hepatic urea concentration, in a dose-dependent manner. The effects were significant at doses of 15 and 35 mg/rat/day ($p<0.01$ to $p<0.05$) and 55 mg/rat/day ($p<0.001$). Hypocalcaemia, hyperphosphataemia and the concentrations of guanidino compounds in the serum, liver and kidney were improved. Proanthocyanidin oligomers were shown to be the active substances [Yokozawa 1984, 1986].

Rats with streptozotocin-induced diabetic nephropathy were treated orally with 125 mg/kg b.w./day of a hot water extract (drug to extract ratio 4:1) over a period of 80 days. At the end of the experimental period treated animals showed decreases in blood glucose levels, serum triglycerides and total cholesterol, and increases in urinary excretion of urea nitrogen and creatinine. All the changes were significant ($p<0.01$) compared to untreated controls [Yokozawa 1997].

A hot water extract was administered orally to rats after subtotal nephrectomy (SN) at a dose of 150 mg/day from day 30 to day 120. The treated animals had significantly less proteinuria ($p<0.05$) compared to untreated SN controls on days 90 and 120 after SN. Renal function was similar in the two groups, but the severity of glomerulosclerosis was significantly ($p<0.5$) reduced by the treatment [Zhang 1996].

Anti-inflammatory activity of rhubarb was demonstrated in 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced mouse ear oedema. After single or multiple topical applications of TPA, an extract (50% ethanol, drug to extract ratio 3:1) applied topically at 0.5 mg/ear led to significant inhibition of oedema ($p<0.01$). Increased myeloperoxidase activity in the tissue after multiple applications of TPA was significantly reduced by the extract ($p<0.01$) [Cuéllar 2001].

Mice with streptozocin-induced diabetes were treated with emodin at 1.5 mg/kg i.p., daily for 3 weeks. The serum glucose level in the emodin-treated group was significantly ($p<0.01$) lowered compared to the diabetic control group. After 3 weeks glucose tolerance and insulin sensitivity in the emodin group were significantly ($p<0.05$) improved compared to control [Xue 2010].

In db/db mice treated daily for 2 weeks with either 100 mg/kg b.w. of rhein or emodin by oral gavage, total fat weight was significantly ($p<0.05$) decreased in the rhein group compared to an untreated control, whereas no effects were observed in the emodin group. In a further test, obesity was induced in C57BL/6 mice using a high fat diet. Mice were fed for 8 weeks with either a high fat diet mixed with 0.1% rhein or a high fat diet alone. At the end of treatment, body weight of the rhein-treated mice was significantly lower ($p<0.01$) than that of the high fat diet control group, indicating that rhein could block weight gain despite the amount of food intake in both groups being similar [Zhang 2012].

In mice on a high fat diet treated with rhein at 150 mg/kg b.w. by oral gavage, daily for 4 weeks, the expression of liver X target genes related to adipogenesis in white adipose tissue (regulating cholesterol homeostasis, lipid and energy metabolism) was significantly ($p<0.05$) suppressed compared to a high fat diet control [Sheng 2012].

Three groups of mice (n=10 each) were injected i.p. with myelomonocytic leukemia WEHI-3-cells and 2 weeks later began treatment for 14 days with a daily oral gavage of either 5 mg/kg or 10 mg/kg of emodin in olive oil or olive oil vehicle

only. Two further groups (n= 10 each) served as control (no treatment) or negative control (olive oil administration only). In comparison to the olive oil only treated leukemia mice group, CD19 and CD11b levels were significantly ($p<0.05$) increased in the 10 mg/kg and 5 mg/kg emodin groups respectively, whereas levels of CD3 were unaffected. The activity of peritoneal cavity phagocytes was significantly increased in the 5 mg/g emodin group ($p<0.05$) and in the 10 mg/kg emodin group ($p<0.001$) [Chang 2011].

Three weeks after transfection of nude mice with SW1990 pancreatic tumour cells, four groups (n=12 each) received ten i.p. injections (every three days) of either emodin (40 mg/kg), gemcitabine (125 mg/kg), emodin (40 mg/kg) plus gemcitabine (80 mg/kg) or isotonic saline. One week after the final injection (day 37), the tumour growth inhibition rate (difference of mean tumour volume between end and start of treatment in the treatment group divided by the respective difference in control group) was found to be high in the emodin and gemcitabine groups at 71.3 and 83.3% respectively, but highest in the combination group at 103.7% [Wei 2011].

Five groups of nude mice (n=5 each) were transfected with transplantable tumours induced by chronic myeloid leukemia K562 cells. After 12 days the groups were treated for 12 days with either 25, 50 or 100 mg/kg emodin, or 120 mg/kg hydroxy carbamide as positive control and isotonic saline as negative control. Tumour weight was reduced in the emodin groups, by 61.4% at 50 mg/kg ($p<0.05$) and by 75.6% at 100 mg/kg ($p<0.01$) and also in the positive control group by 78.3% ($p<0.01$) when compared to the negative control [Chun-Guang 2010].

Studies in humans

Although no published clinical data is currently available, the laxative effect of rhubarb is well-known, and is comparable to the other anthranoid containing laxatives.

Pharmacokinetic properties

No systematic data are available on rhubarb. It is assumed that aglycones present in the drug are absorbed in the upper gut, and that (by analogy with sennosides from senna) the β-linked glucosides are neither absorbed in the upper gut nor split by human digestive enzymes. They are converted by the bacteria of the large intestine into aglycones and subsequently to the active compounds, the anthrones [Kobashi 1980].

In rat liver microsomes emodin was metabolized to 6-hydroxy-aloe-emodin and 2-hydroxyemodin, and chrysophanol was converted to aloe-emodin [Mueller 1998].

Preclinical safety data

There are no studies on single dose toxicity, repeated dose toxicity, reproductive toxicity or *in vivo* tests on carcinogenicity of rhubarb or preparations from it. In the *Salmonella* microsome assay an ethanolic extract showed mutagenic effects against *S. typhimurium* strain TA 1537 [Paneitz 1999]. Some isolated anthraquinones (aloe-emodin, emodin, physcion and chrysophanol) gave positive results in *in vitro* genotoxicity studies [Bruggemann 1984; Westendorf 1990; Heidemann 1993, 1996]. All *in vivo* genotoxicity studies were negative [Heidemann 1993, 1996; Mengs 1997]. Sennosides A and B and rhein gave negative results in *in vitro* and *in vivo* mutagenicity tests [Heidemann 1993; Mengs 1993].

In a 2-year study, male and female F344/N rats were exposed to 280, 830 or 2500 ppm of emodin in the diet, corresponding to an average daily dose of emodin of 110, 320 or 1000 mg/kg b.w. in male rats and 120, 370 or 1100 mg/kg b.w. in female rats. No evidence of carcinogenic activity of emodin was observed in male rats. A marginal increase in the incidence of Zymbal's gland carcinoma occurred in female rats treated with the high dosage but was interpreted as questionable [NIH Publication 1999].

In a further 2-year study, on B6C3F1 mice, males were exposed to 160, 312 or 625 ppm of emodin (corresponding to an average daily dose of 15, 35 or 70 mg/kg b.w.) and females to 312, 625 or 1250 ppm of emodin (corresponding to an average daily dose of 30, 60 or 120 mg/kg b.w.). There was no evidence of carcinogenic activity in female mice. A low incidence of renal tubule neoplasms in exposed males was not considered relevant [NIH Publication 1999].

A 90% hydroethanolic dried extract (3.4:1) was administered intragastrically to normal and CCl_4-treated rats daily for 12 weeks at dosage levels equivalent to 2, 5.4, 14.69 and 40g crude drug/kg b.w. Although a decrease in the extent of cellular injury was observed in the two lowest dosage groups of CCl_4-treated rats, a significant increase in fibrosis indicating rhubarb-induced liver damage was observed in both normal rats at all dosage levels and CCl_4-treated rats at the two highest dosage levels [Wang 2011].

Clinical safety data

Despite a lack of formal preclinical data on rhubarb, epidemiological studies suggest that there is no carcinogenic risk to humans from the use of anthranoid laxatives [Siegers 1993; Nusko 1993; Sonnenberg 1993; Kune 1993, 1996; Loew 1996, 1997].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2019
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Supplement 2009
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003

LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2019
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

E/S/C/O/P Monographs

Online Series

The Scientific Foundation for Herbal Medicinal Products

The second edition of ESCOP Monographs, published as a hardback book in 2003 with a Supplement in 2009, has been widely acclaimed for its authoritative information on the therapeutic uses of herbal medicines. Monographs covering a total of 107 herbal substances include extensive summaries of pharmacological, clinical and toxicological data, and copious references to scientific literature form an important part of each text.

Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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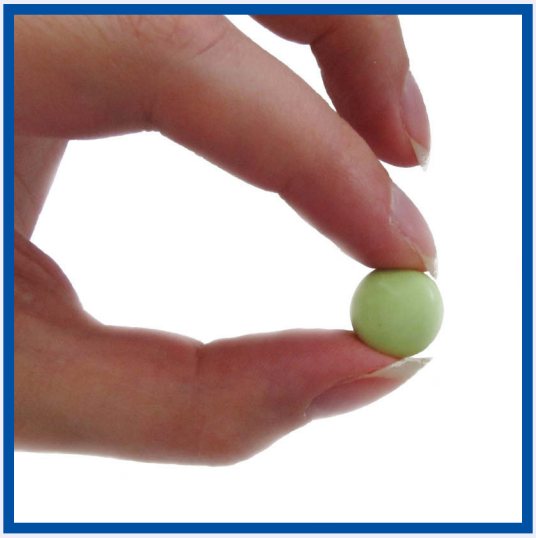
Ribis nigri folium
Black Currant Leaf

2017



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***The* Scientific Foundation for**
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2017

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

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Plant illustrated on the cover: *Ribes nigrum*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
CCl_4	carbon tetrachloride
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase

INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

RIBIS NIGRI FOLIUM 2017

Black Currant Leaf

DEFINITION

Blackcurrant leaf consists of the dried leaf of *Ribes nigrum* L. It contains not less than 1.0 per cent of flavonoids, expressed as isoquercitroside ($C_{21}H_{20}O_{12}$; M_r 464.4) and calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Blackcurrant leaf].

CONSTITUENTS

Characteristic constituents are: mono- and diglycosides of quercetin, kaempférol and isorhamnetin, mainly isoquercitroside and its malonic acid ester [Vagiri 2012, Vagiri 2015]; a flavanone, sakuranetin [Altkinson 1982]; monomeric flavanols (mainly gallocatechin and epigallocatechin) [Buzun 1978; Tits 1992a; Tits 1992b]; proanthocyanidins [Buzun 1978; Tits 1991; Tits 1992a; Tits 1992b], especially di- and trimeric prodelphinidins [Tits 1991; Tits 1992a; Tits 1992b]; hydroxycinnamic acid derivatives including chlorogenic, caffeic and *p*-coumaric acids [Trajkovski 1974]; phenolic acids as gallic and gentisic acids [Tabart 2011]; and traces of essential oil [Andersson 1963; Seitz 2014].

CLINICAL PARTICULARS

Therapeutic indications

Adjuvant in the treatment of rheumatic conditions [Decaux 1930; Garnier 1961; Cassis 1978; Seitz 2014; Leclerc 1983; Wichtl 2009].

In this indication, the efficacy is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Adults: Dried leaf as an infusion (20-50 g/litre, infused for 15 minutes), 250-500 ml daily [Cassis 1978; Leclerc 1983; Wichtl 2009]; fluid extract (1:1), 5 ml twice daily, taken before meals [Decaux 1930; Cassis 1978; Leclerc 1983; Wichtl 2009].

Method of administration
For oral administration.

Duration of use
No restriction. If symptoms persist or worsen, medical advice should be sought.

Contra-indications
None known.

Special warnings and precautions for use
None required.

Interaction with other medicinal products and other forms of interaction
None reported.

Pregnancy and lactation
In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No case of overdose reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Anti-inflammatory effects
A purified flavonoid extract obtained from blackcurrant leaf inhibited the biosynthesis and release of prostaglandins (IC_{30}: 1.03 mg/ml flavonoids) in isolated perfused rabbit heart. The flavonoid extract was 2.2 and 3.6 times more effective than isoquercitroside and rutin respectively [Pham Huu Chanh 1986].

Di- and trimeric prodelphinidins isolated from blackcurrant leaf were investigated for their effects on the metabolism of human chondrocytes and potential inhibition of COX-1 and COX-2. At the end of a 12-day incubation with 10 µg/ml of prodelphinidin, proteoglycans and type II collagen production were significantly increased in human chondrocytes ($p<0.025$ and $p<0.01$). Under the same conditions, inhibition of PGE_2 synthesis was observed in the presence of di- and trimeric prodelphinidins at a dose of 10 µg/ml ($p<0.025$ and $p<0.01$ respectively). An *in vitro* test on purified COX-1 and COX-2 showed a preferential inhibition of COX-2 (53.2±4.0%) compared to COX-1 (8.1±13.9%) at a concentration of 10^{-5} M dimeric procyanidin. However, no effect was observed on COX activity in the whole blood assay [Garbacki 2002].

In human endothelial LT2 cells, pre-treated for 24 h with a proanthocyanidin-enriched fraction from blackcurrant leaf (mixture of catechin, gallocatechin, di- and trimeric prodelphinidins) [Tits 1992 a] and activated with TNF-α, the cell adhesion immunoglobulin was dose dependently and significantly (10, 30 and 60 µg/ml) inhibited ($p<0.05$ or $p<0.01$). The same fraction did not significantly modify expression of IL-8 and vascular endothelial growth factor 165 (mediators involved in inflammatory processes associated with angiogenesis) [Garbacki 2005].

Antioxidant effects
Antioxidant properties of methanolic crude extracts from fresh blackcurrant leaf (2.4 g in 50 ml) of three different varieties were demonstrated by measuring the inhibition of lipid oxidation induced in rat liver microsomes by ferrous sulfate-ADP-ascorbic acid (IC_{50}: 6.44-7.29µl of methanolic extract per ml) or by *t*-butyl hydroperoxide (IC_{50}: 8.63-9.31 µl of methanolic extract per ml) [Costantino 1993].

A dry extract containing 11.9% flavonol glycosides (not further defined) suppressed the oxidation of erythrocyte membrane lipids induced by UV-C (IC_{50}: 22.8-24.2 µg/ml) and 2,2'-azobis (2-amidinopropane) dihydrochloride (IC_{50}: 7.15-7.25 µg/ml). The control substance, Trolox, showed IC_{50} values of 13.3-15.9 µg/ml and 3.6-4.2 µg/ml respectively [Bonarska-Kujawa 2014].

In vivo experiments

Anti-inflammatory effects
A 14%-ethanolic extract from blackcurrant leaf (60 g per litre), administered orally at 1 and 10 ml/kg b.w., produced dose-dependent anti-inflammatory effects corresponding respectively to 30% and 54% reductions in carrageenan-induced rat paw oedema compared to controls. Comparable activities were observed with reference substances: indometacin produced 63% reduction at 2.5 mg/kg and 66% at 5 mg/kg; niflumic acid produced 19% reduction at 25 mg/kg and 70% at 50 mg/kg. A 21-day oral treatment with the extract reduced oedema compared to control animals by 30% at 0.33 ml/kg, 42.5% at 1 ml/kg and 46% at 10 ml/kg, the last being statistically identical with the activities of indometacin at 1.66 mg/kg (49% reduction) and niflumic acid at 12.5 mg/kg (53% reduction). A 21-day oral treatment with a lyophilizate of the 14%-ethanolic extract (1 g of lyophilizate equivalent to 30 ml of extract or 1.8 g of leaf) gave an ED_{50} of 0.67 g/kg for the lyophilizate compared to 0.43 mg/kg for indometacin. The efficacy of the blackcurrant leaf extract was apparent in both the proliferative and exudative phases of inflammation [Declume 1989].

A lyophilizate prepared after maceration of blackcurrant leaf (100 g/litre) in 15% ethanol for 10 days at 20°C and administered intraperitoneally to rats exhibited potent anti-inflammatory activity in comparison with controls [Mongold 1993]. In the carrageenan-induced rat paw oedema test at 50 and 100 mg/kg b.w., the lyophilizate produced dose-dependent inhibition of inflammation ($p<0.01$), its effect at 100 mg/kg (70% inhibition) being similar to that of indometacin at 5 mg/kg (77% inhibition). In the cotton pellet-induced granuloma test, the lyophilizate at 150 mg/kg reduced inflammation by 18.6%, comparable to the 24% reduction with indometacin at 3 mg/kg. In the Freund adjuvant-induced arthritis test, the lyophilizate produced a dose-dependent reduction in inflammation of 18.7% at 150 mg/kg and 34.6% at 300 mg/kg, the latter being statistically identical to the 37.7% reduction obtained with indometacin at 3 mg/kg [Mongold 1993].

Prodelphinidins isolated from blackcurrant leaf, administered intraperitoneally, had a dose-dependent anti-inflammatory effect in the carrageenan-induced rat paw oedema model: 18%, 40% and 55% reductions in inflammation with 5, 10 and 40 mg/kg respectively. In a similar experiment, a crude aqueous extract from blackcurrant leaf produced 57% inhibition at 60 mg/kg, comparable to 44% with indometacin at 4 mg/kg and 47% with aspirin at 200 mg/kg [Tits 1992b].

Treatment of rats with a proanthocyanidin-enriched fraction from blackcurrant leaf [Tits 1992 a] (10, 30, 60 and 100 mg/kg, i.p.) significantly ($p<0.05$) reduced paw oedema induced by carrageenan in a dose and time-dependent (from 0 to 4 h) manner and also dose-dependently significantly inhibited ($p<0.05$) carrageenan-induced pleurisy in rats. The same fraction at 30 mg/kg also significantly lowered ($p<0.05$) TNF-α, IL-1β and nitrite/nitrate levels in pleural exudate [Garbacki 2004].

In rats treated with the same fraction (10, 30 and 60 mg/kg, i.p.) before intrapleural injection of carrageenan, exudate volume was significantly reduced ($p<0.05$ or $p<0.01$) in a dose-dependent manner (31, 37 and 55% respectively) 4 hours after injection. Infiltration of polymorphonuclear leucocytes was also significantly inhibited ($p<0.01$) in a dose-dependent manner (64, 73 and 75% respectively) [Garbacki 2005].

Analgesic effects
A lyophilizate prepared after maceration of blackcurrant leaf (100 g/litre) in 15% ethanol for 10 days exhibited potent analgesic activity, which may be of peripheral origin, in the acetic acid-induced writhing test after single dose i.p. administration to mice. The lyophilizate had an ED_{50} of 61.5 mg/kg and a therapeutic index (LD_{50}/ED_{50}) of 17.7. Paracetamol (acetaminophen) administered to the control group gave a higher ED_{50} of 132 mg/kg and a lower therapeutic index of 3.8 [Mongold 1993].

Diuretic activity
A fluid extract (1:1) of blackcurrant leaf showed a salidiuretic action (diuretic quotient 1.56) in rats when administered orally at a dose equivalent to 1500 mg dried leaf/kg; this was similar

to the effect of furosemide at 50 mg/kg (diuretic quotient 1.52) [Rácz-Kotilla 1977].

The potassium-sodium ratios in blackcurrant leaf and blackcurrant leaf decoction were found to be 128:1 and 242:1 respectively, which may contribute to a diuretic effect [Szentmihályi 1998].

Antihypertensive effects
A fluid extract (1:1) of blackcurrant leaf had an antihypertensive effect on cats, with an antihypertensive quotient of 1.82 at an oral dose equivalent to 400 mg dried leaf/kg, the effect lasting for 15-20 minutes; tolazoline had comparable antihypertensive quotients, 1.69 at 0.75 mg/kg and 2.12 at 1.0 mg/kg, but the effect lasted for only 5 minutes [Rácz-Kotilla 1977].

An infusion of blackcurrant leaf (20 g/litre), administered intravenously to normotensive rats at a dose equivalent to 360 mg dried leaf/kg b.w., produced a rapid fall of 45% in arterial blood pressure and the decrease was still 30% after 30 minutes [Lasserre 1983].

Pharmacokinetic properties
No data available.

Preclinical safety data
A lyophilized 14%-ethanolic extract (1 g of lyophilizate equivalent to 1.8 g of blackcurrant leaf), administered orally to rats at 2 g/kg/day for 21 days or 1.34 g/kg/day for 28 days, revealed no signs of toxicity and no gastric ulceration was observed [Declume 1989].

Rats treated orally for 28 days with a lyophilizate prepared after maceration of blackcurrant leaf (100 g/litre) in 15% ethanol for 10 days had no gastric ulceration. Compared to control animals no changes were apparent in food and fluid consumption or b.w., nor in results from blood analysis and histopathological evaluation of 14 different organs. In an acute toxicity study of the same lyophilizate in mice, the i.p. LD_{50} was 1.09 g/kg; oral doses up to 3 g/kg showed no overt toxicity [Mongold 1993].

The i.p. LD_0 and LD_{50} values of a blackcurrant leaf fluid extract (1:1) in mice were 22 and 49 g/kg respectively [Rácz-Kotilla 1977].

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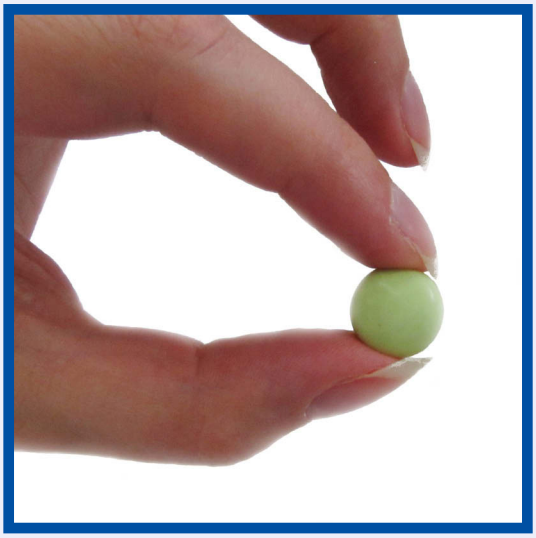

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Plant illustrated on the cover: *Ruscus aculeatus*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
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Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
CCl_4	carbon tetrachloride
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase

INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

RUSCI RHIZOMA

2017

Butcher's Broom

DEFINITION

Butcher's Broom consists of the dried, whole or fragmented underground parts of *Ruscus aculeatus* L. It contains not less than 1.0 per cent of total sapogenins, expressed as ruscogenins [a mixture of neoruscogenin ($C_{27}H_{40}O_4$; M_r 428.6) and ruscogenin ($C_{27}M_{42}O_4$; M_r 430.6)] and calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Butcher`s Broom].

CONSTITUENTS

The characteristic constituents are steroidal saponins based upon (25*R*)-spirost-5-ene-1β,3β-diol (ruscogenin) and spirosta-5,25(27)-diene-10,3β-diol (neoruscogenin), such as ruscoside, ruscin, deglucoruscoside and deglucoruscin. Minor constituents include flavonoids, anthraquinones, benzofurans, the coumarin esculin, essential oil (mainly monoterpenes) and sterols [Rauwald 1988; Schneider 1990; Mimaki 1998a, 1998b; Mari 2012; Barbic 2013; Sticher 2015].

CLINICAL PARTICULARS

Therapeutic indications
Supportive therapy for symptoms of chronic venous insufficiency, such as painful, tired and heavy legs, tingling and swelling [Vanscheidt 2002; Boyle 2003; Aguilar Peralta 2007; Lascasas Proto 2009; Guex 2010]. Supportive therapy for symptoms of haemorrhoids, such as itching and burning [Anger 1981; Willuhn 2009; Abascal 2005].

Posology and method of administration

Dosage
Adult daily dose: Solid or liquid extracts in amounts corresponding to 7-11 mg of total ruscogenins [Willuhn 2009].

Method of administration
For oral administration.

Duration of administration
No restriction; long-term administration may be advisable. If symptoms persist or worsen, medical advice should be sought.

Contraindications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

No adverse effects have been reported in mothers or newborn babies when used in late pregnancy [Anger 1981; Baudet 1991].

Effects on ability to drive and use machines
None known.

Undesirable effects
Contact allergy to ruscogenins might occur in rare cases [Elbadir 1998].

E/S/C/O/P
MONOGRAPHS

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In some studies, combinations have been used, consisting of an extract (not further specified) plus hesperidin methyl chalcone as well as in some cases ascorbic acid. In the text, these preparations are indicated by the term "a combination".

In vitro experiments

Vasoconstriction
In rings of saphenous vein from ovariectomized female rabbits, an extract (prepared using DMSO with further dilution in distilled water) (0.001-1 mg/mL) induced dose-dependent contractions. This effect was insensitive to blockade by either prazosin (3×10^{-7} M) or rauwolscine (10^{-7} M), but was partially inhibited by both substances in rings isolated from oestradiol-treated animals [Harker 1988].

A hydroalcoholic extract (not further specified) caused dose-dependent contraction in segments of isolated canine saphenous veins (effective dose: 30 µg/mL and higher). The maximal contraction caused by the extract averaged 80 ± 12% of the response to 10^{-4} M noradrenaline. Prazosin and rauwolscine given alone decreased, and the presence of both substances eliminated, the response to the extract (0.1-1 mg/mL, n = 5 each concentration). Cocaine reduced the contraction evoked by butcher's broom extract suggesting also an indirect sympathomimetic effect of the extract. The contractile response to the extract was also depressed by phentolamine, adenosine, verapamil and by sympathectomy with 6 hydroxydopamine. Tetrodotoxin, atropine, methysergide and indometacin did not significantly influence the effect of butcher's broom extract; acetylcholine enhanced the increase in tension [Marcelon 1983,1984,1988].

Femoral vein rings and coronary arterial rings were isolated from adult female mongrel dogs. An extract (not further specified; 0.001-1 mg/mL) induced dose-dependent contractions of the vein preparations. The effect was modulated by the integrity of the endothelial cells and the hormonal status of the animals. Treatment with prazosin plus rauwolscine reduced the contractions evoked by the extract following progesterone treatment; inhibition of α-adrenergic receptors in veins from oestrogen-treated animals augmented the contractions evoked by the combination [Miller 1991a,b]. In both the venous and arterial rings with endothelium, the extract (4-6 g/mL) relaxed contractions induced by noradrenaline (veins) or prostaglandin F2a (PGF2a; arteries) in a dose-dependent manner. The relaxing effect was inhibited by atropine and by inactivation of the endothelial relaxing factors [Miller 1991a].

An extract (not further specified; 0.001-1 mg/mL) induced dose-dependent contractions in rings from greater saphenous veins and varicose tributaries of patients (3 male, 17 female), who underwent vein stripping for primary varicosity (n = 5-7 for each experiment). Blocking of α- and β-adrenergic receptors with phentolamine and propranolol reduced the maximal tonus induced by butcher's broom extract in each type of vein [Miller 1994].

An extract (not further specified) caused comparable and moderate contraction of human saphenous veins with and without endothelium isolated from healthy volunteers (n = 8; 3 females, 5 males) and patients with primary varicosity (n = 14; 11 female, 3 male). The threshold dose ranged between 0.01 and 0.1 mg/mL. Contractions of the varicose tributaries were about two-fold greater than those of saphenous veins from the same patients. In veins from varicose patients, α_2-blockade with rauwolscine was more effective than α_1-blockade with prazosin in reducing contractions induced by butcher's broom in rings with and without endothelium [Miller 2000].

Maximal contractions induced by an extract (not further specified; 1 mg/mL) in veins taken from women undergoing varicectomy were independent from the oestrogen level during the menstrual cycle. Comparable effects were seen in veins from menopausal women [Marcelon 1988a].

A water-soluble extract (not further specified; 0.01, 0.1 and 1 mg/mL) caused dose-dependent reduction of noradrenaline accumulation in isolated normal and varicose human saphenous veins. The highest concentration caused a reduction of about 50% in the normal vein and reduced the formation of all metabolites of noradrenaline [Branco 1988].

Saponin mixtures and ruscogenin provoked remarkable vasoconstrictive effects in the isolated rabbit ear. The ED_{50} of ruscogenin was 20-25 fold lower than for the saponins [Capra 1972].

Effects on permeability
Damage to isolated pig ear vein induced by ethacrynic acid was diminished by pre-incubation with an extract (not further specified; 0.05%). This effect was related to reduced permeability to both water and protein [Hönig 1989].

Vasoprotection
In the presence of an extract (not further specified), the viability of human umbilical vein endothelial cells exposed to hypoxia increased by up to 60%. A pronounced effect was observed when cells under hypoxia were incubated for 48 hours with concentrations above 330 µg/mL [Baurain 1994]. The extract at 0.05 µg/mL provided only slight protection from the hypoxia-induced decrease in ATP, while 50 µg/mL totally prevented the effect of hypoxia (n=9 for each concentration). Inhibition of phospholipase A2 activation by 50%, observed with the extract at 0.05 µg/mL, increased only slightly with higher concentrations (0.5 µg/mL, 5 µg/mL and 50 µg/mL). The extract also dose-dependently inhibited hypoxia-induced adherence of neutrophils to the endothelial cells: 0% at 0.05 µg/mL and 88% at 50 µg/mL [Bouaziz 1999].

Effects on lymphatic vessels
Noradrenaline (10^{-8} to 10^{-4} M) and a combination (0.01-1 mg/mL) caused dose-dependent contractions in isolated canine lymphatic thoracic duct. The activity of the combination was partially inhibited by prazosin and rauwolscine, whereas phentolamine completely eliminated the contractile response [Marcelon 1988b].

Addition of an extract (not further specified; 30 µg/mL) increased the contraction frequency caused by electrical field stimulation in isolated bovine mesenteric lymphatic vessels (n = 8) by about 50%. Noradrenaline blocked the response to the field stimulation (n = 6) [McHale 1991].

Antioxidant effect
Ruscogenin showed antioxidative effects against Formyl-Met-Leu-Phe (FMLP)-induced extra- and intracellular superoxide generation in mouse bone marrow neutrophils, with IC_{50} values of 1.07 ± 0.32 µM and 1.77 ± 0.46 µM respectively. Phorbol myristate acetate (PMA)-elicited extra- and intracellular superoxide generation were also suppressed by ruscogenin, with IC_{50} values of 1.56 ± 0.46 µM and 1.29 ± 0.49 µM

respectively. Furthermore, it inhibited the membrane translocation of p47phox and p67phox and reduced FMLP-induced phosphorylation of cytosolic phospholipase A2 (cPLA2) and p21-activated kinase (PAK). The cAMP levels and PKA expression were increased by ruscogenin. Ruscogenin inhibited phosphorylation of protein kinase B (Akt), p38 mitogen-activated protein kinase (p38MAPK), extracellular signal-regulated kinase 1 and 2 (ERK1/2), and c-Jun N-terminal kinase (JNK). The inhibitory effects of ruscogenin on superoxide production and the phosphorylation of Akt, p38MAPK, and ERK1/2 were reversed by PKA inhibitor (H89) [Lin 2015].

Anti-inflammatory effect
Ruscogenin significantly suppressed zymosan A-evoked peritoneal total leukocyte migration in mice in a dose-dependent manner (1 and 3 mg/kg), while these concentrations had no effect on PGE2 content in peritoneal exudate. At a concentration of 1μM, ruscogenin also inhibited TNFα-induced over-expression of ICAM-1 both at mRNA and protein levels and considerably suppressed NF-κB activation by decreasing NF-κB p65 translocation and DNA binding activity [Huang 2008].

Elastase activity
Ruscogenins inhibited the activity of porcine pancreatic elastase (IC_{50}: 119 μM; competitive inhibition) [Maffei Facino 1995].

Antimicrobial effect
A dry extract (70% methanol) was active against 8 bacteria and 5 fungi (MIC between 0.2 and 1.00 mg/mL). The antifungal activity against *Trichoderma viride* exceeded the one of the positive controls, ketoconazole [Hadžifejzović 2013].

In vivo experiments

Vasoconstriction
An extract (not further specified) was added to the superfusion solution applied to cheek pouch preparations of male hamsters. The venules constricted with doses above 0.05 mg/mL/min, while arterioles remained unchanged. Venular constriction evoked by the extract at 0.2 mg/mL/min was blocked by prazosin (10^{-9} M), by diltiazem (10^{-9} M) and by high concentrations of rauwolscine (10^{-6} M) [Bouskela 1994a].

The vasoconstrictive effects of an extract (not further specified) applied topically to hamster cheek pouch preparations were found to be temperature-dependent. At 25°C venules and arterioles dilated. At 36.5°C venules constricted, whereas arterioles either dilated with extract concentrations of up to 0.05 mg/mL or remained unchanged even with higher concentrations of the extract. At 40°C constriction of venules was more pronounced than at the lower temperatures, whereas arterioles only constricted with higher concentrations of the extract (above 0.01 mg/mL) [Bouskela 1991].

Intravenous administration of an extract (not further specified) at 5 mg/kg b.w. caused venular constriction in the cheek pouch of male hamsters, but did not affect the arteriolar diameter. Mean arterial pressure was not affected at this dose level [Bouskela 1991].

Oral administration of a solution of an extract (not further specified) at 150 mg/kg b.w. for 28 days resulted in constriction of the venules by 30% and dilatation of the arterioles by 37% in the hamster cheek pouch (n = 6) compared to water-treated controls (n = 6). Arteriolar and venular side branches were not affected [Bouskela 1991].

The vasoconstrictive effects of an extract (not further specified) were investigated in lower leg muscle preparations of 12 anaesthetized young adult cats. Following intra-arterial administration of 0.4 mg/min/kg b.w., arterial, arteriolar and venular resistance increased by 30-35% within 90 seconds, while higher doses (0.8 mg/min/kg and 3 mg/min/kg) produced sustained dilator effects. This pattern of response was consistent in all experiments (n = 24). Intravenous administration of the extract at 1-9 mg/min/kg resulted in a dose-dependent venoconstrictive response (n = 10) [Mellander 1991].

In dogs (n = 4), constant infusion of an extract (not further specified) at 50 μg/hour/kg b.w. for 5 days (total doses: 60-84 mg) had beneficial effects on alterations caused by denervation of the lateral saphenous vein segments, with the increase in smooth muscle cell diameter being completely prevented. The extract increased the O-methylating capacity of the denervated and the non-denervated tissues [Teixeira 1988].

Effect on permeability
Topical application of an extract (not further specified; 0.002-2.0 mg/mL/min) to male hamster cheek pouch preparations dose-dependently inhibited the increase in macromolecular permeability caused by histamine. A concentration of 0.2 mg/mL/min elicited 50% inhibition of the histamine-induced permeability. This effect was blocked by prazosin and by diltiazem, but not by rauwolscine (all applied topically) [Bouskela1994b].

An intravenously administered extract (not further specified) at 5 mg/kg b.w. showed protective effects against leakage of dextran in the hamster cheek pouch after topical administration of various permeability-increasing substances such as bradykinin, leukotriene B_4 or histamine [Bouskela 1993].

Two isolated saponins and esculin were tested for their activity against hyperpermeability in endothelial cells induced by thrombin. The saponins reduced permeability to 41.9% and 42.6% whereas esculin reduced it to 53.3% [Barbic 2013].

Oedema-protective effect
The oedema-protective effects of an extract (containing 2.5% of ruscogenin) and ruscogenin were demonstrated in a perfusion model of the hindleg of anaesthetized cats. Oedema was induced by perfusion with 0.1% of ethacrynic acid for 10 minutes. The extract (200 or 400 mg/kg b.w.) or ruscogenin (20 or 80 mg/kg b.w.) were administered orally 4 hours before induction of oedema. Alternatively, the extract at 10 or 20 mg/kg or ruscogenin at 4 mg/kg were administered intravenously 1 hour prior to oedema induction. Each treatment group comprised 5 or 6 animals and 16 animals served as controls. After an additional 45-minute perfusion with 0.9 % sodium chloride solution, the blood, protein and water contents in the oedema of the skin and muscle were measured. The optimal protective dose of the extract was 20 mg/kg b.w. when administered intravenously, whereas an oral dose 10- to 20-fold higher was necessary. An intravenous dose of ruscogenin at 4 mg/kg b.w. was as effective as an oral dose of 20 mg/kg [Felix 1983].

Anti-inflammatory effect
An extract (not further specified) showed anti-inflammatory activity in rats when administered by the intraperitoneal (100, 200 or 300 mg/kg b.w.) or the rectal route (suppositories, 275 mg/kg b.w.). The effect of 300 mg/kg of the extract (i.p.) was equivalent to 72% of the activity of phenylbutazone (165 mg/kg i.p). Two other purified extracts with higher concentrations of ruscogenins were effective at lower doses [Chevillard 1965].

In rats oedema was induced by injection of dextran, histamine, serotonin or hyaluronidase. Intraperitoneal administration of purified sapogenins from butcher's broom at 20, 40 or 80 mg/

kg b.w. reduced limb oedema; this antiphlogistic activity was suppressed by adrenalectomy. Slight anti-inflammatory activity was observed against granulomas induced by subcutaneous implantation of cotton pellets. When the purified sapogenins were administered orally neither antiphlogistic nor anti-inflammatory activity was observed [Cahn 1965].

Saponins and ruscogenins isolated from butcher's broom showed anti-inflammatory activity in carrageenan- and brewer's yeast-induced rat paw oedema when administered intraperitoneally or intravenously. In the rabbit, only the ruscogenins (80 mg/kg b.w. i.p.) decreased capillary permeability significantly ($p = 0.05$). These substances were not active against capillary fragility [Capra 1972].

Effect on lymph vessels
Intravenous administration of a water-soluble extract (not further specified) at 1, 2 or 5 mg/kg b.w. dose-dependently enhanced flow duration and intensity in isolated lymph vessels of anaesthetized dogs. A similar effect was seen in the veins. The activity of the extract was not influenced by injection of nifedipine [Pouget 1991].

Pharmacological studies in humans
In a randomized, double-blind, crossover, 4-armed study, 20 healthy volunteers (11 men and 9 women aged between 20 and 43 years) took a single oral dose of four different treatments, separated by 1-week wash-out periods: 450 mg of an extract, 450 mg of trimethylhesperidin chalcone (TMHC), a combination of both substances, or a placebo. Venous function, capillary filtration rate, tissue and blood volume were monitored before intake and after 50, 70, 90, 120 and 150 minutes. Compared to placebo, butcher's broom extract significantly decreased venous capacity ($p<0.01$), reduced the blood pool in the lower leg ($p< 0.05$) and reduced tissue volumes in the foot and ankle ($p<0.01$). TMHC did not influence venous capacity and its influence on tissue volume was about 3 times weaker than that of the extract [Rudofsky 1989].

In a double-blind, placebo-controlled study the influence of venous stasis on several microrheological factors and the effects of taking a combination for 30 days were investigated in 25 patients suffering from chronic venous insufficiency (13 verum, 12 placebo). All parameters were also measured in 20 untreated healthy controls. In patients treated with the combination, haematocrit values after stasis did not increase, while they rose in the placebo group. The combination prevented an increase in plasma viscosity after stasis and improved red cell deformability. The red blood cell aggregation index after stasis was similar to that of control subjects, while it increased after stasis in the placebo group [Le Devehat 1991].

Application of 4-6 g of a cream containing 1.6 % of an extract twice daily to the legs of healthy volunteers (n=18) led to constriction of the femoral vein, demonstrated by a decrease in diameter in 8 out of 10 of the verum group by 0.5-3 mm (median=1.25), compared to no change or a dilatation by 0.5 mm (median) in 7 out of 8 of the placebo group (significant between group difference: $p = 0.0139$). In a group of 9 women suffering from pregnancy-related varicosis, application of the preparation to 3 of the women demonstrated that increases in the femoral vein diameter of the painful leg were significantly ($p = 0.047$) lower with treatment with the cream compared to without, and pain in the leg was reduced [Berg 1991].

Clinical studies

Chronic venous insufficiency, varicosis
A meta-analysis of studies using an oral combination to treat chronic venous insufficiency included 20 randomised, placebo-controlled, double blind studies, 5 randomised studies against a positive control, and 6 single arm studies (without placebo) including a total of 10,246 patients. In all studies the response to the preparation was compared to baseline values. On a 4 point symptom severity scale, where 0 corresponds to no symptoms and 3 to severe symptoms, the combination significantly reduced the severity of pain by 0.44 points ($p = 0.02$), cramps by 0.26 ($p = 0.025$), heaviness by 0.53 ($p = 0.001$) and paraesthesia by 0.29 points ($p = 0.031$) compared to placebo. There was also a significant ($p = 0.014$) reduction in venous capacity of 0.7 ml/100 ml with the combination compared to placebo. Non-significant decreases in calf and ankle circumference (0.72 and 1.17 cm respectively) and in the severity of oedema (0.43 points), were found in the treated patients compared to placebo [Boyle 2003].

The efficacy of an orally administered dry extract (15-20:1; methanol 60%) was evaluated in a randomized, double-blind, placebo-controlled study involving 166 women suffering from chronic venous insufficiency (Widmer grades I and II; CEAP 3-4). After a placebo run-in period of 2 weeks, the patients were randomly assigned to either 72-75 mg of the extract or placebo daily for 12 weeks. Data relating to 148 patients (30-89 years, 150-182 cm height, 49-97 kg weight), with mean disease durations of 14.6 years in the extract group ($n = 77$) and 15.1 years in the placebo group ($n = 71$), were eligible for efficacy analysis. Significant differences in favour of the verum treatment were found for reductions in lower leg volume and ankle circumference after 8 and 12 weeks ($p<0.001$), and in leg circumference ($p<0.001$) and the main subjective symptoms ($p<0.05$) after 12 weeks. There was a clear relationship between the changes in lower leg volume and the subjective symptoms "tired, heavy legs", "sensation of tension" and "tingling sensation" ($p<0.05$). Global assessment by the investigators also confirmed the superiority of the extract treatment ($p<0.05$) compared to placebo [Vanscheidt 2002].

In a prospective, multicentre, open clinical study, an oral combination was tested for 8 weeks in 124 patients suffering from chronic venous insufficiency. Initial reports of intensity were 79% for pain, 85% for heaviness, 74% for cramps and 82% for oedema, decreasing within two weeks to 20%, 12%, 8% and 14% respectively. The authors state that symptoms were absent at the end of treatment. Capillaroscopy changes at treatment completion were: 98% to 20% inter-capillary fluid decrease; 80% to 20% efferent loop thickening; 5% to 2% peri-capillary bed, and 5% to 4% mega-capillaries [Aguilar Peralta 2007].

Fifty-two female chronic venous disorder (CVD) patients were allocated consecutively to one of three groups: a combination, elastic compression stockings and no treatment. After 4-weeks treatment, the combination significantly decreased venous diameters (popliteal vein parameters: right $p< 0.04$ and left $p<0.01$) and great saphenous vein diameters (bilaterally $p<0.01$) compared pre-treatment, effects which were comparable to compression stockings [Lascasas Porto 2009].

In an observational, single arm, multicentre, prospective trial, 192 patients suffering from CVD were treated with a combination. Patient profiles, risk factors, clinical symptomatology and quality of life (QoL) assessed by SF-12 and CIVIQ questionnaires were evaluated at inclusion and after 12 weeks of treatment. Clinical symptoms such as feeling of heaviness, pain, swelling of the lower limbs and cramps improved with treatment and ankle circumferences significantly ($p<0.001$) decreased over time. The physical and psychological dimensions of the SF-12 score as well as the CIVIQ score significantly ($p<0.001$) improved over time [Guex 2010].

Haemorrhoids
In observational studies more than 1800 patients (including 26 pregnant women) suffering from haemorrhoids and anorectal complaints have been treated topically with isolated ruscogenins in suppositories (each containing 8 mg) and/or creams (0.8%) for 2 days to 13 weeks. Improvements were reported with respect to symptoms and objective evaluation by the physicians. The efficacy of treatment was estimated as very good or good in 85-93% of cases [Salzmann 1977; Bärmig 1978; Anger 1981].

Retinopathy
In 60 type 2 diabetic patients, oral treatment for 3 months with 37.5 mg of an extract (15-20:1; methanol 60%) twice a day showed an increased amplitude of oscillating potentials by 15% and an increase in visual acuity from 0.83 to 0.93 (not statistically significant results). Furthermore, it led to improvement of the fundus of the eyeball in 23% of the patients (n=20) and prevented progression of retinopathy in all cases [Archimowicz-Cyryłowska 1996].

Pharmacokinetic properties

Pharmacokinetics in animals
The pharmacokinetic characteristics of a radiolabelled extract (not further specified) were investigated in two male Wistar rats. The activity of the extract was 2.8 mCi/mg, with 94% of the total radioactivity bound to saponins. Each animal received 1 mL of extract orally. Radioactivity was detected in the blood 15 minutes after administration. After 2 hours, 39% of the radioactivity was found in the blood samples. After 3.5 hours, in 10 mL samples of blood a maximum radioactivity of 0.41% and 0.45% was observed and was maintained until the end of the 24-hour observation period. The extract was eliminated mainly in the faeces but also renally in a ratio of 2:1, 46% of the administered radioactivity being excreted within 96 hours. Biliary excretion within 24 hours was 8.5 and 10.8% [Chanal 1978].

The bioavailability of a ^{3}H-labelled extract (not further specified) after oral, topical and intravenous administration was studied in rats. Male Wistar rats were treated orally with 1 mL of an aqueous solution containing 0.1 mL of a fluid extract (2% saponins, 70 µCi). Male Atrichi rats were treated topically with 250 mg of a combination of labelled butcher's broom extract, melilotus extract and dextran sulphate, corresponding to 25µCi. By the intravenous route, male Wistar rats received 0.5 mL of an aqueous solution containing 50 mg of the extract (56 µCi). Based on radioactivity in the blood and urine, absorption by the oral route was estimated at 65% and by the local route at 25%. After intravenous administration the existence of an enterohepatic cycle was demonstrated and one-third of the radioactivity was eliminated via the faeces. Following oral administration maximum radioactivity was found in the blood after 2 hours and was maintained for 24 hours. In similar experiments with orally-treated Atrichi rats 80% of the radioactivity was eliminated within 72 hours, with 35% of the dose being detected in the urine [Chanal 1981].

Macaca monkeys (3 females, 3 males) were treated with a tritiated extract (not further specified; 1.5 mCi/kg b.w.) by oral (n = 4) or intravenous (n = 1) routes, or topically in a fixed combination (n = 1). Thin layer chromatography showed that 80-83% of the labelling was fixed in the sapogenin fraction of the extract. Most of the radioactivity was found in the bile, the digestive tract and the urinary tract 2 hours after oral administration, with less in the liver and kidneys. After 7 hours, radioactivity had further increased only in the urinary tract, the bile and the faeces. Radioactivity after 24 hours was markedly lower in all measured tissues. Percutaneous absorption was about 20% compared to absorption after oral administration. Maximum urinary excretion occurred after about 7.5 hours and sapogenins were identified as the major urinary metabolites. A considerable proportion of the radioactivity was excreted in urine (26%, 20% and 4% respectively) and faeces (6.5%, 23% and 9% respectively) within 24 hours after i.v., oral or topical administration [Bernard 1985].

Pharmacokinetics in humans
The presence of butcher's broom saponins in the blood was clearly demonstrated after oral administration of 1 g of butcher's broom extract in three volunteers. Plasma levels of the spirostanol glycosides degluconeoruscin and deglucoruscin were measured by HPLC for 4 hours at 30-minute intervals. The T_{max} for degluconeoruscin was 90-120 minutes after dosing, with a C_{max} of 2.5 µg/mL [Rauwald 1991].

Preclinical safety data

Acute toxicity
The acute toxicity of an ethanolic extract (not further specified) was investigated in dogs and guinea pigs. In 6 male and female dogs death occurred within 1 hour following intravenous infusion of the extract at doses between 0.83 and 1.8 g/kg. By the intraperitoneal route no toxic signs were found in guinea pigs at doses lower than 1.5 g/kg b.w.; animals receiving doses of 2 g/kg b.w. and above died. Toxic and subtoxic doses affected the respiratory function, and lethal doses resulted in hyperventilation followed by fatal apnoea [Moscarella 1953; Caujolle 1953].

Estimation of the oral and intraperitoneal LD_{50} values of a fluid extract (ethanol 50% V/V) in rats and mice revealed differences depending on the harvest time of the plant, the route of administration and the use of roots or rhizomes. The oral LD_{50} of the rhizome extracts was 2.07-2.39 mL/kg b.w. in rats and 24.69-33.73 mL/kg in mice; after intraperitoneal administration the toxicity was 10-20 fold higher. Root extract was found to be more toxic than rhizome extract (1.4-1.8 fold in rats and 4-5 fold in mice). The observed symptoms of intoxication were convulsion, paralysis and gastro-intestinal inflammation with dysentery. Animals died following respiratory failure. Autopsies revealed pronounced irritation of the mucosa and strong visceral congestion [Boucard 1967].

Oral LD_{50} values of ruscogenin and Ruscus saponins were estimated to be greater than 3g/kg b.w. in mice and rats [Capra 1972].

Repeated dose toxicity
An extract (not further specified) was given to male rabbits in their diet for 26 weeks, 17 animals received 2g/kg b.w., 19 received 5g/kg and 16 animals served as controls. Body weights and blood counts did not reveal any differences between treated animals and controls [Roux 1969].

Rats were treated with 300 mg/kg of ruscogenin or *Ruscus* saponins by gavage daily for 8 weeks (10 animals per group, with 10 untreated rats as controls). No toxic signs or differences in body and organ weight were observed. Histological examination did not reveal any pathological changes in any group. Blood glucose and liver function did not differ significantly between the groups. An increase in diuresis and excretion of electrolytes was observed in the ruscogenin group but not in animals treated with saponins [Capra 1972].

Reproductive toxicity
Reproductive toxicity was studied with a combination containing an ethanolic extract (10%), TMHC, citric acid, methyl-4-esculetol

and ascorbic acid; 5 mL of the preparation contained 0.5 mL of the butcher's broom extract. Twenty female Wistar rats received a daily dose of 2.4 mL of the preparation, equivalent to 25 times the recommended dose for humans; 20 untreated animals served as controls. Treatment started one week before conception and continued until delivery. The animals tolerated the drug without any signs of intoxication. The fertility of females in the treatment group was comparable to that in the control group, and the offspring did not show any teratogenic signs [Labie 1984].

Clinical safety data

A dry extract (15-20:1, methanol 60%) taken orally by 77 women at a dosage of 72-75 mg daily for 12 weeks was well tolerated. Overall tolerability was assessed as very good by 76.8% and good by 23.2% of the patients and was comparable to that of placebo treatment. Laboratory data, comparison of vital signs and physical examination before and after treatment did not reveal any treatment-related changes [Vanscheidt 2002].

An early report stated that contact allergy to ruscogenins is a rare adverse effect [Elbadir 1998]. However, this was not confirmed in more recent studies using preparations of ruscogenins, apart from one report of a case of contact dermatitis in a patient using a topical preparation (not further specified) for haemorrhoidal disease. Patch testing revealed positive results for ruscogenins [Ramirez-Hernandez 2006].

In a case report, the development of ketoacidosis in a diabetic patient after consumption of an extract (not further specified) was described. A causal relationship could not be established [Sadarmin 2013].

Combination preparations

Placebo-controlled and open studies with combinations containing butcher's broom extracts have not revealed any specific side effects. Moderate gastric complaints were observed [Monteil-Seurin 1991; Parrodo 1999].

Single cases of diarrhoea and lymphocytic colitis have been reported following the use of combinations containing butcher's broom extract [Beaugerie 1994; Maechel 1992; Rassiat 2001].

In a prospective, multicentre, open study in 124 patients who were treated with a combination containing 300 mg of an extract (not further specified) for 8 weeks, mild headache and vertigo were reported in 24% of the patients during the first week which disappeared in the second week. Mild transient epigastric complaints were reported in 19% of the patients in the fourth week [Aguilar Peralta 2007].

Reproductive toxicity

Ruscogenin-containing suppositories were well tolerated by 30 pregnant women without any apparent effects on the newborn babies [Anger 1981].

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E/S/C/O/P
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MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Second Edition, 2003
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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Online Series

The Scientific Foundation for Herbal Medicinal Products

Salicis cortex
Willow Bark

2017

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MONOGRAPHS

***The* Scientific Foundation for**
Herbal Medicinal Products

SALICIS CORTEX
Willow Bark

2017

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
CCl_4	carbon tetrachloride
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase

INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

SALICIS CORTEX

Willow Bark

2017

DEFINITION

Willow bark consists of the whole or fragmented dried bark of young branches or whole dried pieces of current year twigs of various species of the genus *Salix* including *S. purpurea* L., *S. daphnoides* Vill. and *S. fragilis* L. The drug contains not less than 1.5 per cent of total salicylic derivatives, expressed as salicin ($C_{13}H_{18}O_7$; M_r 286.3) and calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Willow Bark].

CONSTITUENTS

The characteristic constituents are derivatives of salicin, mainly salicortin, 2'-*O*-acetylsalicortin and/or tremulacin [Meier 2014]. Young twigs (bark plus wood) contain the same constituents in lower concentrations, when compared to the bark alone. Further constituents, the polyphenols, flavanone and chalcone glycosides, including procyanidins are now also considered to be characteristic of willow bark, as they have been shown to attribute to its beneficial effects [Fiebich 2004; Narstedt2007; Freischmidt 2015].

The bark of *Salix purpurea* L. may contain 6-8.5%, and the bark of *S. fragilis* 4-10%, of total salicin (determined after hydrolysis) [Meier 1985a]. Phenol glucosides present include salicortin (up to 9%), tremulacin (rarely more than 1%) and salireposide (0.1-1.2%), with small amounts of syringin and purpurein (up to 0.4%) [Egloff 1982; Shao 1991; Meier 1985b]. Other constituents include the yellow chalcone isosalipurposide (0.15-2.2%), the flavanones eriodictyol-7-glucoside (0.18-0.4%) and (+)- and (–)-naringenin-5-glucoside (0.4-1.5% each) [Meier 1985b/1988; Freischmidt 2015], approximately 0.5% of (+)-catechin and other polyphenols [Shao 1991], as well as flavan-3-ols and dimeric and trimeric procyanidins [Jürgenliemk 2007; Kaufeld 2014]. Phenolic acids, *p*-hydroxybenzoic, vanillic, cinnamic, *p*-coumaric, ferulic, and caffeic acids were confirmed for the genus of *Salix*. Pyrocatechol (2.25 mg/g) was detected in *S.purpurea* bark [Poblocka 2010]. A lignan, sisymbrifolin, occurred in *S. alba* [Du 2007]. Procyanidin B1 occurs in *S. purpurea* and in *S. alba* clones (0.26-2.24 mg/g) [Poblocka 2008].

The bark of *Salix daphnoides* Villars contains over 4% of total salicin [Meier 1985a]. Phenol glucosides present include salicortin (3-11%), tremulacin (up to 1.5%) and salicin (up to 1%); a small amount of syringin (up to 0.2%) also occurs [Egloff 1982; Julkunen 1989; Shao 1991; Meier 1985b]. Other constituents include the yellow chalcone isosalipurposide (0.2-1.5%), the flavanones (+)- and (–)-naringenin-5-glucoside (0.3-1.0% each) and naringenin-7-glucoside (0.3-1.5%) [Meier 1987/1985b], approximately 0.5% of (+)-catechin and other polyphenols [Shao 1991]. Thirteen characteristic constituents were confirmed in willow bark extracts from commercial products (*S. purpurea x daphnoides*). Picein was only detected in *S. daphnoides* [Kammerer 2005].

CLINICAL PARTICULARS

Therapeutic indications

For the relief of low back pain [Chrubasik 2000a; 2001a; 2001b] and of mild rheumatic conditions [Mayer 1949; Wagner 1995; Schaffner 1997; Meier 1998; Blumenthal 2000; Schmid 2000/2001a; Lardos 2004; Beer 2008; Wichtl 2009; Schilcher 2010].

For the relief of fever associated with common cold and headache [Mayer 1949; Bradley 1992; Wagner 1995; Meier 1998; Kaul 1999; Blumenthal 2000; März 2002; Wichtl 2009; Schilcher 2010]. Efficacy in these indications is plausible on the basis of human experience and long-standing use.

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Posology and method of administration

Dosage
Adult dose, oral use for low back pain and mild rheumatic conditions:
Daily dose of dry hydroalcoholic or aqueous extracts, equivalent to 120-240 mg of total salicin [Schmid 2000/2001a; Chrubasik 2000a; 2001a; 2001b; Lardos 2004; Beer 2008; Wichtl 2009].

Adult dose, oral use for fever, common cold and headache:
Dried bark in decoction, 1-3 g (3-4 times daily);
Liquid extract (1:1, 25% ethanol), 1-3 ml (3-4 times daily);
Tincture (1:5, 25% ethanol), 5-12 ml (3-4 times daily)
[Schaffner 1997; Meier 1998; Blumenthal 2000].

Elderly: dose as for adults.
Children: generally not recommended for children and adolescents under 18 years of age. From age 12 to 17 years the product should not be used without medical advice.

Method of administration
For oral administration.

Duration of use
No restriction. No severe adverse events were reported in investigations of long term treatment with willow bark extracts, including eight clinical studies with a duration of use of 6-8 weeks involving a total of 5871 patients, and a 6 month observational study including 436 patients [Uehleke 2013; Vlachojannis 2014].

Contra-indications
A history of aspirin allergy. In cases of sensitivity to salicylates, the use of willow bark preparations should be avoided [Boullata 2003; Wagner 2003; Clauson 2005; Vasquez 2005].

Special warnings and special precautions for use
The treatment of children with willow bark extracts is not recommended, because of the structural similarity of salicylic derivatives in willow bark to acetylsalicylic acid. The use of synthetic acetylsalicylic acid (aspirin) in children is still associated with the so-called Reye's syndrome, although the number of reported cases appears to be declining with the availability of better diagnostic tools [Clark 2001; McGovern 2001].

Interaction with other medicinal products and other forms of interaction
Following a daily oral dose of willow bark extract, corresponding to 240 mg salicin, patients' platelet aggregation was minimally inhibited, when compared with inhibition by a cardioprotective dose of 100 mg acetylsalicylic acid. Therefore it is possible that willow bark may slightly increase the effects of oral anticoagulants [Krivoy 2001].

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
Allergic reactions were attributed to willow bark in 4 patients, including skin rash, swollen eyes and pruritus [Chrubasik 2000a, 2001b].

Overdose
No case of overdose reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties
Recent reviews describe the pharmacological and pharmacokinetic properties of willow bark extracts, and compare the medicinal properties of willow bark with other herbal drugs [Chrubasik 2000b; März 2002; Wagner 2003; Gagnier 2007; Wegener 2009; Vlachojannis 2009].

In vitro experiments

Anti-inflammatory effects and mechanism of action
The hen's egg chorioallantoic membrane test system [Luepke 1985; Luepke 1986] has been used to study the anti-inflammatory effect of the willow bark constituents salicin and tremulacin. Onset of their anti-inflammatory effect is delayed in comparison with saligenin (salicyl alcohol), sodium salicylate and acetylsalicylic acid, indicating that the active principles may be metabolites of salicin and tremulacin [Steineggger 1972].

An extract inhibited LPS-induced COX-2-mediated PGE2 release and weakly inhibited TNFα, IL1β and IL6 release from human monocytes, but had no direct effect on COX-1 or COX-2. The controls, salicin and acetylsalicylic acid, had no effect on any of the parameters [Fiebich 2004].

An extract inhibited LPS-activated monocyte mRNA expression of TNFα, COX-2 and transcription factor NF-kappaB [Bonaterra 2010].

Catechol, a bioactive degradation product of salicortin, reduced TNFα-induced ICAM-1 expression in human endothelial cells [Knuth 2011]. Flavonoids and catechol in a willow bark extract contributed to the reduction of ICAM-1 expression in endothelial cells [Freischmidt 2012].

A purified 2,3-trans procyanidin fraction from willow bark demonstrated redox-sensitive endothelium-dependent relaxation in porcine coronary arteries [Kaufeld 2014].

As flavanone and chalcone glycosides, including procyanidins, in willow bark extracts are considered to contribute to the efficacy of willow bark, as has been demonstrated in various experiments [Fiebich 2004; Narstedt 2007; Vlachojannis 2009; Freischmidt 2012, Kaufeld 2014], a quantitative determination method using hydrolysis of the aglycones, naringenin, eriodictyol and chalconaringenin has been developed for use in willow bark preparations [Freischmidt 2015].

Anti-angiogenesis, apoptotic and cytotoxic activity
In endothelial cells (ECV304) treated with salicin (2-5mM) for 24h, migratory properties were reduced, tubular formation was inhibited and the mRNA expression of vascular endothelial growth factor (VEGF) was decreased. Angiogenesis was suppressed by blocking the reactive oxygen species production and extracellular signal-regulated kinase (ROS-ERK) activation. This indicates that salicin significantly inhibits angiogenic activities of endothelial cells [Kong 2014]. Flavonoids, proanthocyanidins (GI50 at 33-103 µg/ml) and salicyl alcohols (GI50 at 50-243 µg/ml) in willow bark extract fractions, inhibited cancer cell growth and promoted apoptosis of human colon and lung cancer cell lines [Hostanska 2007].

In vivo experiments

Anti-inflammatory effects
Isolated tremulacin, injected subcutaneously at 100 mg/kg b.w., significantly inhibited carrageenan-induced paw oedema ($p<0.001$ in hours 3-6 after carrageenan injection) and peritoneal

leucocyte migration (by 50%, p<0.01) in rats, and croton oil-induced ear oedema (by 43%, p<0.001) and acetic-acid induced writhing (p<0.01 to p<0.001) in mice. Inhibition of leukotriene B_4 biosynthesis in pleural leucocytes (obtained *ex vivo* from rats 4 hours after intra-pleural injection of carrageenan) also supported the activity of tremulacin in acute inflammatory animal models [Cheng 1994].

In the rat 6d air pouch model, a willow bark extract showed a significant increase of reduced glutathione (GSH) levels, *in vitro* and *in vivo*. It also raised superoxide dismutase activity. The extract was more potent than acetylsalicylic acid or celecoxib [Khayyal 2005].

The contribution of polyphenols and flavonoids to the mechanism of action and overall effect of aqueous extracts of *S. purpurea* and *S. daphnoides* has been shown. However, *in vivo* and *in vitro* studies suggest that the clinical efficacy of willow bark cannot be entirely explained by the total salicin fraction alone [Narstedt 2007].

Antipyretic effects
Salicin administered orally to rats at 5 mmol/kg b.w. significantly reduced yeast-induced fever, producing a normal temperature, and completely preventing fever when administered simultaneously with yeast. However, salicin at this dose level did not affect the rectal body temperature of afebrile rats. On the other hand, both sodium salicylate and saligenin at 5 mmol/kg lowered body temperature significantly in afebrile rats [Akao 2002].

Antitumour activity, inhibition of tumour angiogenesis and apoptosis
In a mouse model, salicin at 10 mg/kg/day i.p. significantly inhibited tumour growth. Angiogenesis during tumour growth was reduced by the salicin treatment, as measured by two markers, the level of haemoglobin and the expression of CD31 on endothelial cells. This indicates that salicin might inhibit tumour progression by reducing angiogenesis within the tumour [Kong 2014].

Pharmacological studies in humans

Platelet aggregation
In a randomized, placebo-controlled, double-blind study, patients with acute exacerbations of chronic low back pain were treated with either oral willow bark extract corresponding to 240 mg of salicin (n = 19) or placebo (n = 16) daily for 28 days; a third group of patients suffering from chronic ischaemic heart disease (n = 16) received 100 mg of acetylsalicylic acid daily during the study period. Arachidonic acid-induced platelet aggregation, measured in blood samples drawn from the patients after 28 days of treatment, was minimally inhibited by the willow bark extract, but significantly less when compared to the inhibition by acetylsalicylic acid. The mean percentages of maximal arachidonic acid-induced platelet aggregation were 61%, 78% and 13% in the willow bark extract, placebo and acetylsalicylic acid groups respectively [Krivoy 2001].

Clinical Studies

Controlled studies
In a randomized, double-blind, placebo-controlled pilot study, a total of 21 patients with degenerative rheumatic conditions were treated daily for 14 days, either with a standardized extract of willow bark (n = 11, 240 mg salicin/d) or with placebo (n = 10). Pain intensity was decreased in 8/10 patients in the willow bark group and in 5/10 patients in the placebo group. No severe adverse reactions were reported in 10 of 11 patients receiving willow bark; 1 patient dropped out due to stomach ache, which appeared to be unrelated to taking the preparation. A similar number of slight adverse events (tiredness, stomach ache, head ache) were reported in both groups [Schaffner 1997].

A standardized willow bark extract in coated tablets was evaluated in patients with osteoarthritis in a randomized, double-blind, placebo-controlled study at an oral dosage corresponding to 240 mg of salicin (n = 39) or placebo (n = 39) daily for 2 weeks following a washout phase with placebo for 4-6 days. Efficacy was assessed by means of the WOMAC Osteoarthritis Index. The WOMAC pain score in the willow bark group decreased significantly (-14%, p<0.047) compared to that of the placebo group (+ 2%). The final overall assessments showed significant superiority of the willow bark extract over placebo (physicians' assessment, p=0.0073; patients' assessment, p=0.0002) and demonstrated a moderate analgesic effect in osteoarthritis [Schmid 2000 / 2001a].

In a randomized, double-blind, placebo-controlled, three-arm study, patients with an exacerbation of chronic low back pain were assigned to one of three treatments: a standardized willow bark extract in coated tablets corresponding to either 2 × 120 mg of salicin (n = 70) or 2 × 60 mg of salicin (n = 70), or placebo (n = 70), daily for 4 weeks. Efficacy was assessed using the Arhus Low Back Pain Index, and the opioid analgesic tramadol was the sole rescue medication. From intention-to-treat analysis, the number of pain-free patients without rescue medication (responders) in the last week of the study was significantly higher (p<0.001) in the verum groups and also dose-dependent: 27 out of 70 (39%) in the 240 mg group and 15 out of 70 (21%) in the 120 mg group, compared to 4 out of 70 (6%) in the placebo group. Significantly more patients in the placebo group required tramadol (p<0.001) during each week of the study. Willow bark extract thus appeared to be a useful and safe treatment for low back pain [Chrubasik 2000a].

Two randomized, placebo-controlled clinical trials, one in hip or knee osteoarthritis and the other in active rheumatoid arthritis, were conducted for 6 weeks, comparing a standardized willow bark extract (240 mg salicin/d), with placebo, and in one of the studies also with diclofenac. Neither study found significant efficacy for willow bark extract when compared with placebo. However, as was expected, the difference between placebo and diclofenac was highly significant (p=0.0002) [Biegert 2004].

In a randomized, double-blind, parallel group trial, the efficacy and tolerability of 2 doses of willow bark extract (90 or 180 mg salicin/d) were compared to diclofenac sodium (150 mg/d) for 3 weeks in patients with knee or hip osteoarthritis. No other NSAIDs were allowed. Analgesic activity and pain intensity of both groups treated with the extract were statistically comparable to that of the diclofenac sodium group. Tolerance of the extract was considered as good [Lardos 2004].

Open studies
An older, open, uncontrolled clinical study for various acute and chronic rheumatic conditions and respiratory tract infections (n = unknown), reported anti-rheumatic, anti-neuralgic and anti-pyretic activity in 70% of the treated cases [Mayer 1949].

In an open, randomized, controlled, post-marketing study, patients with acute exacerbations of low back pain were assigned to treatment with a willow bark extract (corresponding to 240 mg of salicin, n = 114) or the synthetic anti-rheumatic rofecoxib, a selective COX-2 inhibitor (12.5 mg, n = 114) daily for 4 weeks. All patients were free to use additional conventional treatments if necessary. About 20 patients were pain-free (visual analogue

Table 1. Summary of clinical studies with willow bark extract

Lit. reference First Author Year	Clinical study design ** No. patients n = verum: placebo: positive control	Diagnosis	Verum medication Placebo and/or Reference therapy Duration of treatment	Outcome Safety
Controlled studies				
Schaffner 1997	Pilot study R / D-B / P-C n = 11:10	Painful arthritis (degenerative disease)	Extract (240 mg salicin/d) 2 weeks	*n.s. Better pain reduction for the extract ADE: 30:30
Schmid 2000/2001a	R / D-B / P-C n = 39:39	Osteoarthritis	Extract (240 mg salicin/d) 2 weeks	*** Sign. decrease of WOMAC-score ($p<0.047$)
Chrubasik 2000a	R / D-B / P-C n = 70:70:70	Exacerbation of chronic lower back pain	Extract (120 mg salicin/d) Extract (240 mg salicin/d) Placebo 4 weeks Rescue medication, tramadol, allowed for all groups.	*** Sign. Number of responders to the extract requiring no rescue medication was significantly higher than with placebo ($p<0.001$). Sign. more use of tramadol in the placebo-group ($p<0.001$).
Biegert 2004 (first trial)	R / D-B / P-C / P-R-C n = 43:43:41	Cox- or gonarthrosis (hip or knee)	Extract (240 mg salicin/d) Diclofenac (100 mg/d) Placebo 6 weeks	*n.s. decrease of WOMAC score for extract vs. placebo. Significant for diclofenac vs. placebo ($p=0.0002$).
Biegert 2004 (second trial)	Pilot study R / D-B / P-C n = 13:13	Active rheumatoid arthritis	Extract (240 mg salicin/d) Placebo 6 weeks	*n.s. decrease of VAS-Pain score for extract (15%) vs. placebo (4%)
Lardos 2004	R / D-B / P-R-C, 3-arm n = 17:22:21	Cox- or gonarthrosis (hip or knee)	Extract (90 mg salicin/d), Extract (180 mg salicin/d) Diclofenac (150 mg/d) 3 weeks	*** Sign. Extract pain score statistically comparable to diclofenac Extract well tolerated
Open studies				
Mayer 1949	open study, uncontrolled n = 120	Among others, acute-chronic rheumatic conditions	3-6 g/d powdered drug	70% of these conditions showed a tendency to lower disease activity.
Chrubasik 2001a	open, P-R-C, post-marketing +rescue therapy n= 114:114	Acute exacerbations of low back pain	Extract (240 mg salicin/d) Rofecoxib (12.4 mg)	*** Sign Comparable number of pain-free patients in both groups. ADEs comparable
Chrubasik 2001b	open, non-randomized, 3- arm/ P-R-C Post-marketing surveillance n=115:112:224	Acute exacerbations of low back pain	Extract (120 mg salicin/d) Extract (240 mg salicin/d) Conventional therapy 4 weeks	* n.s. Relatively better pain relief with willow bark extract at 240 mg/d, compared to 120 mg/d.
Beer 2008	open, P-R-C, prospective cohort study n= 88:40	Degenerative cox- or gonarthrosis (mild to fairly severe cases)	Extract (120-240 mg salicin/d) Standard therapy (= pos. ref.) 6 weeks	Extract has comparable efficacy to positive ref., at 6 weeks. Tolerability better for extract.

Saller 2008	open, uncontrolled, observational study n = 763 (completed the study)	Rheumatologic pain (dorsopathies, soft tissue disorders, inflammatory polyarthropathies, arthrosis)	1-4 tabs/d (1 tab equiv. to 60 mg salicin); co-medication was possible 6-8 weeks	* n.s. Moderate reduction of pain; 3-4 tabs better than 1-2 tabs. The extract was well tolerated. More ADE's in co-medication-treated patients.
Uehleke 2013	open, uncontrolled, observational study n = 436	Chronic rheumatic pain (osteoarthritis and back pain)	Extract (240 mg salicin/ 2 tabs/d) co-medication allowed 6 months	***sign pain reduction ($p<0.01$) after 3 weeks 176 ADEs were regarded as unrelated to the extract

* n.s. = data for willow bark are not significant;
** R = randomized; D-B = double-blind; P-C = placebo-controlled; P-R-C = positive-reference-controlled; C = controlled.
*** Sign. = data for willow bark are significantly better than placebo or comparable to a positive reference.
ADE = Adverse drug event; sign. = significant; pos. ref. = positive reference drug.

scale score < 2) in each group after 4 weeks of treatment. About 60% of patients in each group responded well to treatment, as judged by improvement of ≥ 30% in the Total Pain Index. Only a few patients resorted to additional conventional treatment options. The incidence of adverse events was similar in the two groups and there was no significant difference in efficacy between willow bark extract and rofecoxib [Chrubasik 2001a].

In an open, non-randomized, controlled post-marketing study, groups of patients with acute exacerbations of low back pain received willow bark extract corresponding to either 120 mg (n = 115) or 240 mg (n = 112) of salicin daily or, as controls, no treatment with willow bark (n = 224). All patients had access to conventional treatments according to the budget of the general practitioner. Better pain relief and less reliance on supplementary conventional treatments was evident in the group treated with 240 mg of salicin, compared to the group treated with 120 mg. A cost reduction of 14-40% was reported for the willow bark groups [Chrubasik 2001b].

An open, multi-centre, observational study was conducted in patients with gonarthrosis and coxarthrosis. They were treated for 6 weeks with a standardized willow bark extract (dose equivalent to 120-240 mg salicin/d; n=88) or with standard reference medications (n=40; positive control). The WOMAC score was used for evaluation. Willow bark treatment was comparable to that of standard therapies in mild or fairly severe cases of gonarthrosis and coxarthrosis, and had fewer side effects [Beer 2008]. Further open, uncontrolled studies [Werner 2004; Saller 2008; Uehleke 2013], are presented in table 1.

Pharmacokinetic properties

Pharmacokinetics in vitro

Hydrolysis of salicin
Salicin was stable under acidic conditions (0.5% hydrochloric acid, with or without pepsin) and produced no saligenin (salicyl alcohol), even after incubation with human saliva at pH 7.2 [Steinegger 1972; Fötsch 1989a].

Hydrolysis of salicortin
Salicortin was unchanged after 1 hour of incubation in artificial gastric juice (pH 1.0). After 6 hours of incubation with artificial intestinal juice (pH 7.4-7.6 at 37°C) salicortin was degraded to salicin at $t_{0.5}$ = 4.02 hours [Meier 1987; Meier 1990].

Enzymatic hydrolysis and esterification
Both β-glucosidase extracted from almonds (EC 3.2.1.21) and β-glucosidase derived from guinea pig liver, converted salicin and salicortin to saligenin [Julkunen 1992; Gopalan 1992]. However, salicin derivatives acetylated on the sugar moiety (2'-*O*-acetylsalicin and 2'-*O*-acetylsalicortin) and tremulacin were not decomposed by β-glucosidase. Non-specific esterases (EC 3.1.1.1) from rabbit and porcine liver transformed salicortin to salicin (98.1%), acetylsalicortin to acetylsalicin (75.5%) and tremulacin to tremuloidin (63.9%) [Julkunen 1992]. Pancreatic proteases degraded salicortin to salicin and tremulacin to tremuloidin [Wutzke 1991].

Erythrocyte membrane permeability and protein binding
Transport of salicin and saligenin into erythrocytes was rapid for saligenin (1 minute to saturation) and delayed for salicin (4 hours to saturation). The process was reversible, release being rapid for saligenin and slower for salicin. Saligenin and salicin both bind to human serum albumin but saligenin has a significantly higher affinity [Matsumoto 1993].

Metabolic transformation by homogenized kidney, liver and lung
Saligenin was transformed into salicylic acid by homogenized liver, kidney and lung. Gentisic acid was qualitatively detectable in homogenized liver after incubation with saligenin [Fötsch 1989a]. Salicin was partially metabolised to saligenin and salicylic acid after incubation with homogenized kidney from rats [Adamkiewicz 1961].

Intestinal metabolism
Salicin injected into an isolated, closed-off section of the male rat intestine, appendix and colon was hydrolysed by intestinal bacteria to its main metabolite saligenin [Fötsch 1989b].

Transport through intestinal wall
Transport of salicin and saligenin through the isolated intestinal wall was confirmed using the closed-off posterior section of the male rat intestine. When salicin and saligenin were injected into the closed intestine, both passed the ileum wall unchanged. Saligenin appeared to penetrate the intestinal wall faster than salicin [Adamkiewicz 1961].

Pharmacokinetics in animals

Serum concentration-time curve and metabolism in rats
After oral administration of salicin to 4 male Wistar rats at 1 mmol/kg (0.268 g/kg) the urinary metabolites were: unchanged salicin (ca. 15% of the dose), saligenin (ca. 0.1%), salicylic acid (ca. 30%), conjugated salicylic acid (ca. 5%) and gentisic acid (ca. 2%). After oral administration of a total of 2.5 g of salicin in successive daily doses of 1 mmol/kg, only unchanged salicin was identified in the faeces [Fötsch 1989a].

The concentration of the metabolite salicylic acid was determined in the serum of rats after oral administration of salicin (400 mg/kg) or sodium salicylate (29 mg/kg). With salicin, no salicylic acid appeared during the first 2 hours but then it appeared in the serum, gradually increased and peaked at 5 hours with C_{max} = 82.4 µg/ml. After administration of sodium salicylate, salicylic acid appeared rapidly and reached a maximum concentration at 1.5 hours with C_{max} = 104.2 µg/ml. Elimination was slower after administration of sodium salicylate than after salicin. The relative bioavailability of salicylic acid from salicin was only 3.25% of that from sodium salicylate [Fötsch 1990].

Plasma levels of salicin, saligenin and salicylic acid were measured and compared to sodium salicylate after oral administration of each compound to rats at 1, 2.5 and 5 mmol/kg. Salicin appeared to be a prodrug, which was gradually transported to the lower part of the intestine, hydrolysed to saligenin by intestinal bacteria and converted to salicylic acid after absorption. Salicin absorption is slow compared to that of saligenin or salicylic acid [Akao 2002].

In rats administered 100 mg/kg of oral salicortin, catechol and salicylic acid were detected in serum, after serum processing with metabolizing enzymes. The predominant metabolite was catechol sulfate. Unconjugated catechol could not be detected [Knuth 2013].

Metabolism of radioactively-labelled salicin in mice
^{14}C-labelled salicin (8.9 mg/mouse) was rapidly and completely metabolised after oral administration to mice. Free salicylic acid was detected in the blood. Elimination was mainly by the renal route. Salicortin (4 mg/mouse) and tremulacin (11 mg/mouse) were partially metabolised. The metabolite gentisic acid was transiently detected in the small intestine [Wutzke 1991].

Pharmacokinetics in humans

Absorption, distribution, metabolism and elimination
Salicin (4.0 g, corresponding to 1730 mg of saligenin) and, in a separate experiment, 2.0 g of sodium salicylate were taken as self-medication by the investigator to study and compare the kinetics. The maximum plasma concentration of free salicylates was reached about 2 hours after administration of salicin with C_{max} = 100 µg/ml. In comparison, sodium salicylate yielded C_{max} = 150 µg/ml, also after about 2 hours. Metabolites equivalent to more than 86% of the administered salicin were recovered in 24-hour urine: salicyluric acid (51%), salicyl glucuronide (14%), salicylic acid (12%), gentisic acid (5%) and saligenin (4%), together with a small amount of unchanged salicin [Steinegger 1972]. The urinary metabolite spectrum of oral acetylsalicylic acid in man [Dinnendahl 1982] is very similar to that of salicin taken orally [Meier 1990]. Populin (6'-*O*-benzoyl salicin) is not metabolised after oral administration in man [Steinegger 1972].

Absorption and metabolism of willow bark extract
Twelve male volunteers took 3 tablets containing willow bark extract (standardized to provide a total dose of 55 mg salicin) and cola nut extract. The plasma C_{max} of salicylic acid was 130 ng/ml, reached 3 hours after administration, and the plasma half-life was calculated as 2.5 hours. An exceptional increase in the plasma level of salicylic acid observed in one volunteer 4-6 hours after administration was attributed to the effects of eating lunch during this period. Administration of a second dose of 3 tablets four hours after the first dose produced a C_{max} of 311 ng/ml six hours after the first dose. This level was not increased further by a third dose of 3 tablets eight hours after the first dose [Pentz 1989].

A standardized willow bark extract preparation corresponding to 240 mg of salicin was administered to 10 healthy volunteers in two equal doses at 0 and 3 hours. Urine and serum collected over a 24-hour period showed that salicylic acid was the major metabolite in serum (86% of total salicylates). Peak levels of salicylic acid were reached after 2 hours (1.2 mg/L) and the area under the curve (AUC) was equivalent to that expected from an intake of 87 mg of acetylsalicylic acid. From this study it was concluded that willow bark extract leads to much lower serum salicylate levels than observed after analgesic doses of synthetic salicylates. The formation of salicylic acid alone does not therefore explain the analgesic or anti-rheumatic effects of willow bark [Schmid 2001b].

Quantities of gentisic, salicyluric and salicylic acids found in human plasma, from persons not taking aspirin drugs, were assumed to be derived from vegetarian food containing salicylic acid-derived compounds, such as can be found in cucumber, melon, cherry and others [Pirker 2004].

Eight healthy volunteers received a single oral dose of 4 tablets of willow bark extract, corresponding to 240 mg salicin (2 controls did not receive willow bark). Catechol and salicylic acid were detected in serum, after processing with the metabolizing enzymes glucuronidase and sulphatase. The predominant metabolite was catechol sulfate. Unconjugated catechol did not occur in serum [Knuth 2013].

Preclinical safety data

Acute toxicity
A hydroethanolic (30%) willow bark extract, administered to mice, yielded an LD_{50} of 28 ml/kg. With a dry extract of willow bark the LD_{50} could not be reached [Leslie 1978; März 2002].

Repeated dose, chronic and reproductive toxicity
A hydroethanolic (30%) extract of willow bark (35% of a combination product, including primula root extract and a plasmolysate of *Candida utilis*) was administered by gavage to rats for 13 weeks, at a dose of 1.6 ml/kg. No adverse events were reported during this period, and there was no negative effect on reproduction in female rats. Teratogenic effects were not observed in rabbits during this period [Leslie 1979; März 2002].

Mutagenicity and carcinogenicity
No data available.

Induction of gastric lesions
Oral administration of salicin did not induce gastric lesions in rats even at a dose of 5 mmol/kg. However, saligenin and sodium salicylate did induce severe gastric lesions in a dose-dependent manner in the range of 1-5 mmol/kg [Akao 2002].

Prediction of adverse events
Using an *in vivo* screening tool in rats (gene expression profiling) to predict possible adverse events due to herbal medicinal products containing salicylates and the antidepressant imipramine, it was found that imipramine crossed the threshold level for adverse events several times, whereas the willow

bark extract reached the threshold level only once [Ulrich-Merzenich 2012].

Clinical safety data
In studies involving 6993 patients and healthy volunteers treated with various preparations containing willow bark extracts, only mild adverse events were reported [Mayer 1949; Pentz 1989; Schaffner 1997; Krivoy 2001; Schmid 2000/2001a; Chrubasik 2000a; 2001a; 2001b; Biegert 2004; Lardos 2004; Werner 2004; Beer 2008; Saller 2008; Knuth 2013; Uehleke 2013].

No significant adverse reactions were reported in 10 out of 11 patients treated daily for 14 days with a standardized extract of willow bark (240 mg salicin/d). One patient dropped out due to stomach ache, which appeared to be unrelated to taking the preparation [Schaffner 1997].

In a 2-week randomized study involving osteoarthritis patients, the number of patients experiencing adverse events was the same in the placebo group (16 out of 39) as in those treated with willow bark extract corresponding to 240 mg of salicin per day (16 out of 39). However, more adverse events were reported in the placebo group (28) than in the verum group (17), the most frequent being allergic skin reactions and gastrointestinal upsets. The most important adverse event in the verum group was a skin rash starting on day 10, however the patient did have a medical history of frequent allergic reactions [Schmid 2000; 2001a].

In a 4-week randomized, three-arm study in patients with low back pain (three groups of 70), an allergic reaction (skin rash, swollen eyes, pruritus) reported by 1 patient receiving low-dose willow bark extract (equivalent to 2 × 60 mg of salicin / d) was attributed to the extract; dizziness reported by 2 patients taking the extract at the higher dose (2 × 120 mg of salicin/d) was attributed to tramadol rescue medication. In the placebo group, 3 patients reported dizziness and other symptoms attributed to tramadol, and 3 reported mild gastrointestinal ailments [Chrubasik 2000a].

In 227 patients treated with willow bark extract (corresponding to 120 or 240 mg of salicin daily), occasional mild adverse events occurred as well as 3 cases of allergy which could be attributed to the willow bark [Chrubasik 2001b].

During a 2-day treatment of 12 male volunteers with 12 tablets, containing a combination of willow bark extract (166.6 mg, standardized to 11% total salicin) and a dry extract of cola nut (38 mg, standardized to 25% caffeine), no adverse events were reported [Pentz 1989].

In a 2-month double-blind placebo-controlled study involving 41 patients with chronic arthritic pain, treated daily with 2 tablets of a combination product containing 200 mg of powdered willow bark and four other herbs, 3 adverse events were reported, that could not be directly related to willow bark: one of dyspeptic symptoms, one of diarrhoea and one of headache [Mills 1996].

An extensive review of willow bark studies supported the hypothesis that the extract is less prone to cause adverse reactions in the stomach than are known to be caused by acetylsalicylic acid. This appears to be because willow bark does not inhibit cyclooxygenase in the stomach wall, since its active metabolites are generated in the intestine after passing through the stomach as intact glycosides; the development of stomach lesions is therefore unlikely [Kaul 1999; März 2002]. Another review of the safety of willow bark extract treatments concluded that the incidence of adverse effects is low [Chrubasik 2000b].

Peak levels of salicylic acid in the serum of 10 healthy volunteers who received a willow bark extract corresponding to 240 mg of salicin per day were approximately 1.4 mg/L. In contrast, peak levels of 35-50 mg/L have been reported after the intake of 500 mg of acetylsalicylic acid. Therefore, the observed analgesic effect of willow bark may not be attributed to salicylic acid alone. It can be postulated that other constituents (e.g. polyphenols, tannins, flavonoids, salicin, salicin esters or others) may contribute to the overall effects of willow bark [Schmid 2000/2001a; Narstedt 2007]; and it may consequently be considered to have a broader mechanism of action and to be safer, than aspirin [Chrubasik 2000a; Akao 2002; Fiebich 2005; Clauson 2005; Narstedt 2007; Vlachojannis 2011].

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MONOGRAPHS

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ALOE CAPENSIS	Cape Aloes	Online Series, 2014
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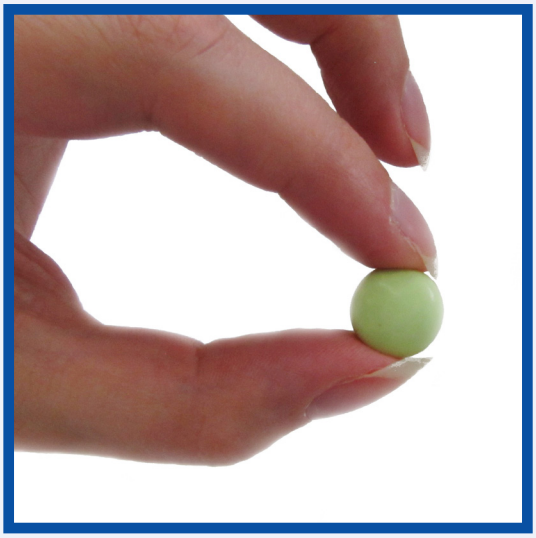

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FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

SALVIAE FOLIUM

2020

Sage Leaf

DEFINITION

Sage leaf consists of the whole or cut dried leaves of *Salvia officinalis* L. The whole drug contains not less than 12 mL/kg of essential oil and the cut drug not less than 10 mL/kg of essential oil, both calculated with reference to the anhydrous drug.

The material complies with the monograph of the European Pharmacopoeia [European Pharmacopoeia].

Fresh material may also be used, provided that when dried it complies with the monograph of the European Pharmacopoeia.

CONSTITUENTS

Essential oil, up to 2.5% [Sticher 2015], containing monoterpenes such as α- and β-thujone (up to 63% and 13%, respectively), camphor, 1,8-cineol, borneol and manool [Müller 1992; Lawrence 1998; Nicolella 2014]. Monoterpene glycosides [Croteau 1984; van den Dries 1989]. Diterpenoids such as carnosic acid and its derivatives, e.g. carnosol [Brieskorn 1969; Rutherford 1992], and a quinone methide [Tada 1997]. Triterpenoids including ursolic acid, oleanolic acid and their derivatives [Brieskorn 1980,1991]. Flavonoids, e.g. hispidulin, 5-methoxysalvigenin [Brieskorn 1971,1979; Kavvadias 2003]. Phenolic compounds, e.g. rosmarinic acid and derivatives [Gracza 1984; Lu 1999a], a caffeic acid trimer [Lu 1999b], and flavonoid and phenolic glycosides [Lu 2000; Wang 1999,2000].

CLINICAL PARTICULARS

Therapeutic Indications

Dyspeptic complaints such as heartburn and bloating [Schilcher 2016; Blaschek 2016], hyperhidrosis [Rösing 1989; Brieskorn 1991; Weiss 2000; Blaschek 2016], hot flushes [Bommer 2011], supportive treatment of hyperglycaemia and hyperlipidaemia [Sà 2009; Kianbakht 2011, 2013, 2016].
Inflammations and infections of the mouth and throat such as stomatitis, gingivitis and pharyngitis [Brieskorn 1991; Weiss 2000; Hubbert 2006].

Posology and method of administration

Dosage

Oral use

Dyspeptic complaints
1 - 2.5 g cut herbal drug as an infusion, up to 3 times daily [Van Hellemont 1988, Bradley 2006; Schilcher 2010; Blaschek 2016].

Liquid extract (ethanol 50%, 1:4-5) 30 drops 3-5 times daily or liquid extract (ethanol 96%, 1:4-6) 10-15 drops several times daily [Schilcher 2016].

Hyperhidrosis
2 - 4.5 g cut herbal drug as a cooled tea in the evening [Rösing 1989; Blaschek 2016].

Dried aqueous extract (4-7:1) 80 – 160 mg 3 times daily after a meal. For treatment of nocturnal sweating 160 – 320 mg before bedtime [Rösing 1989; Schilcher 2016].

Hot Flushes
Thujone-free soft extract 280 mg (equivalent to 3400 mg tincture of fresh leaves, DER 1:17) once daily [Bommer 2011].

E/S/C/O/P MONOGRAPHS

Supportive treatment of hyperglycaemia and hyperlipidaemia
4 g cut herbal drug twice daily as an infusion [Sà 2009].
Dry extract (4:1; ethanol 80%) 500 mg 3 times daily [Kianbakht 2011, 2013, 2016].

Topical use

Inflammation of the mouth or throat
2.5 – 3 g cut herbal drug as an infusion for a mouthwash or gargle 3 times daily [Van Hellemont 1988; Blaschek 2016; Schilcher 2016].

Extract (1:1, ethanol 70%) as a spray 3 times daily [Hubbert 2006].

Extract (1:4-5; ethanol 50%): 1-2 teaspoons in 150 mL warm water, gargle for 1 minute every 2 hours [Schilcher 2016].

Extract (1:4-6; ethanol 96%): dilute ½ teaspoon in 150 mL warm water, gargle several times daily [Schilcher 2016].

1% hydroethanolic mouthwash applied for 60 seconds twice daily [Behesthi-Rouy 2015].

Method of administration
For oral administration or topical application.

Duration of administration
If the symptoms persist or worsen, medical advice should be sought. In hyperhidrosis, treatment for 2-4 weeks, using an aqueous preparation, is recommended [Rösing 1989].

Contra-indications
None known.

Special warnings and special precautions for use
The daily dose of 6.0 mg thujone should not be exceeded [EMA 2012].

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
Due to the potential toxicity of some constituents of the essential oil [von Skramlik 1959; Pinto-Scognamiglio 1967; Gessner 1974; Millet 1979], the use of sage leaf is not recommended during pregnancy or lactation.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No effects are to be expected at a daily dose up to 6.0 mg thujone [EMA 2012].

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antimicrobial effects
An extract enriched in diterpene phenols (35% w/w) was tested against various aerobic bacteria and Candida species in an agar dilution test. The extract had an effect against *Corynebacterium pseudodiptheric*um (MIC = 10 µg/mL, MBC = 20 µg/mL) and *B. subtilis* (MIC = 20 µg/mL, MBC = 20 µg/mL). Carnosic acid was effective against *C. pseudodiphtericum* (MIC = 32 µg/mL, MBC = 128 µg/mL) and *S. aureus* (MIC = 64 µg/mL, MBC = 64 µg/mL) [Weckesser 2006].

An extract (acetone 70%, not further specified) showed activity against vancomycin-resistant Enterococci. Oleanolic acid and ursolic acid were identified as the active compounds with MICs of 8 and 4 µg/mL respectively. The MICs against MRSA were 32 and 16 µg/mL respectively, and against *S. pneumoniae* 16 and 8 µg/mL respectively. There was no effect against gram negative bacteria such as *S. marcescens* [Horiuchi 2007].

The anti-herpetic effect of extracts (ethanol 20, 40, 60 or 80% V/V) of plants from two different locations was investigated in RC-37 cells (African green monkey kidney cells). Either cells were pretreated with extracts before viral infections, or viruses were treated with extracts before cell infection, or the extract was applied after the virus had penetrated the cell. All extracts showed a high virucidal activity against HSV-1 and 2 as measured by plaque reduction. The extracts of the sample of origin 1 resulted in dose-dependent IC_{50} values ranging from 0.03-7.5 µg/mL for HSV-1 and 0.02 to 0.75 µg/ml for HSV-2, the extracts of the sample of origin 2 from 0.12-11.18µg/mL for HSV-1 and 0.21-3.20 µg/mL for HSV-2. The antiviral activity of extracts prepared with higher ethanol concentrations was more pronounced. Pretreatment of HSV-1 and 2 with the extracts also reduced plaque formation. The extracts were not able to reduce plaque formation in cells already infected with the virus [Schnitzler 2008].

The antibacterial activities of two dilutions (1:25 and 1:100) of an extract (1:2, ethanol 60%) were examined on *Helicobacter pylori* and *Campylobacter jejuni*. Both dilutions showed >99% inhibition of bacterial growth for both species at both concentrations [Cwikla 2010].

The activity of different extracts and synergistic effects with antibiotics were tested against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Proteus mirabilis*. An acetone dry extract from 30 g leaves was most effective with MIC values between 0.019 mg/mL and 1.25 mg/mL. The combination with amoxicillin showed a higher synergistic capacity in comparison to a combination with chloramphenicol. This was shown by an increased activity from 2- to 10-fold depending on bacterial strain (mean fractional inhibitory concentration FIC 0.35 to 1.37) [Stefanovic 2012].

A dried extract (80% methanol) showed significant ($p<0.001$) inhibitory effects against leishmanial promastigotes (IC_{50} =184.32 µg/mL) and amastigotes. A 60% lethality of parasites internalised in macrophages was shown [Nikmehr 2014].

Sage oil exhibited bactericidal and fungicidal properties against Gram-positive and Gram-negative bacteria, filamentous fungi and yeasts, e.g. *Candida albicans* [Jalsenjak 1987; Janssen 1989]. An aqueous and a 50%-ethanolic extract of sage leaf exhibited strong inhibitory effects on the collagenolytic activity of *Porphyromonas gingivalis* [Osawa 1991]. Aerial parts of sage contain diterpenes with activity against vesicular stomatitis virus [Tada 1990; Chiba 1992; Tada 1994].

The essential oil exhibited activity against *E. coli*, *B. subtilis*, *Micrococcus luteus*, *S. aureus*, *P. aeruginosa*, *Enterobacter aerogenes*, *K. pneumoniae* and *Salmonella spp.* The undiluted oil showed the strongest effects against *M. luteus* and *E. aerogenes*.

Dilutions of the oil (1/2, 1/5, 1/10, 1/20 and 1/100) were less effective, indicating a dose-dependent effect [Fadil 2015].

Synergistic effects of the essential oils of young and old leaves with the antibiotics ceftriaxone, ciprofloxacin and gentamicin against MRSA were assessed. The essential oil of young leaves intensified the antibiotic activity of ceftriaxone and ciprofloxacin more than the oil of old leaves [Milenkovic 2015].

The effect of the essential oil in 50% ethanol (V/V) at a final concentration of 10 mg/mL was examined on six fungal strains (*Aspergillus niger*, *Aspergillus flavus*, *Botrytis cinerea*, *Rhizoctonia solani*, *Fusarium oxysporum*, and *Alternaria alternata*), using amphotericin B (20 µg/well) as a positive control. *Fusarium oxysporum* was the most sensitive strain with an MIC value of 0.156 mg/mL [Khedher 2017].

The essential oil reduced biofilm formation of *Streptococcus pyogenes* in a concentration-dependent manner, significant ($p \leq 0.05$) for all strains at 0.5 mg/mL. Growth inhibition of *S. pyogenes* resulted in an MIC of 0.5 mg/mL [Wijesundara 2018].

Antioxidant effects
A 50%-methanolic extract from aerial parts of sage considerably inhibited lipid peroxidation in both enzyme-dependent and enzyme-independent test systems [Hohmann 1999; Zupkó 2001].

The effects of an extract (85% ethanol, not further specified) on the antioxidant system including CAT, SOD, GST and GSH were investigated by determining the reduction capability on the DPPH radical. The extract (3, 10, 30, 100 and 300 µg) prevented the increase of GST, SOD and CAT, but did not prevent a decrease in GSH levels [Mayer 2009].

An aqueous and an 80% methanolic extract, at concentrations of 0.5 and 0.033% respectively, showed significant ($p<0.05$) antioxidant activity including free radical scavenging activity, reducing power, β-carotene bleaching inhibition and lipid peroxidation inhibition in brain cell homogenates. The methanolic extract exhibited greater activity than the aqueous extract [Martins 2015].

Anti-inflammatory effects
The anti-inflammatory effect of a dried ethyl acetate extract (94 g drug/600 mL; containing 4.3 % carnosol, 20.1% carnosic acid) was investigated in a cell-free mPGES-1 activity assay. Carnosol and carnosic acid were the main active constituents in inhibiting mPGES-1. Carnosol did not show any effect in LPS-stimulated human whole blood, while carnosic acid (1, 3 and 30 µM) inhibited PGE_2 formation (IC_{50} = 9.3 µM). The formation of 6-keto $PGF_{1\alpha}$, 12-HHT, PGH_2 and thromboxane B_2 was not affected [Bauer 2012].

Sage infusion (1.5 g dried leaves/150 mL), fractions thereof (aqueous distillate and non-volatile dry matter) as well as several isolated compounds (at concentrations corresponding to the infusion) were examined for their anti-inflammatory effects using human gingival fibroblasts. A significant ($p<0.05$) inhibition of PMA/I-induced IL-8 and IL-6 release was demonstrated for the infusion (99.2 ± 0.1% and 98.5 ± 0.1% respectively) as well as for the fractions. A significant ($p<0.05$) more than 50% mean inhibition of both IL-8 and IL-6 release was demonstrated for 1,8-cineole, borneol, camphor and thujone [Ehrnhöfer-Ressler 2013].

Carnosic acid dose-dependently (2.5-20 µM) suppressed the LPS-induced inflammatory response in RAW264.7 macrophages and reduced the LPS-elicited overexpression through restraining transcript levels and gene expression of Nos2, TNF-α, COX2 and monocyte chemotactic protein (MCP-1). It was shown by western blot analysis that carnosic acid downregulates the MAPK pathway by blocking the phosphorylation of ERK ½, JNK and p38. LPS-induced IKKβ/IκB-α/NF-κB pathway activation was also inhibited by carnosic acid, based on decreasing phosphorylation of IKKβ, IκB-α and NF-κB p65 subunit and hence preventing nuclear translocation. Carnosic acid suppressed the FoxO pathway by blocking the nuclear translocation of FoxO1 and 3 in a concentration-dependent (5-20 µM) manner. The FoxO pathway regulates pro-inflammatory genes and mediators such as IL6, TNF-α and MCP-1 [Wang 2018].

Antigenotoxic effects
An extract (ethanol 40%) inhibited H_2O_2- and 2,3-dimethoxy-1,4-naphthoquinone (DMNQ)-induced DNA damage in HepG2 cells at concentrations of 0.01-100 mg/mL. Concentrations of 1 and 2 mg/mL significantly ($p<0.001$) decreased the damage, assessed as the amount of DNA strand breaks. The extract significantly ($p<0.001$) increased GPx, decreased SOD and had no effect on iGSH [Kozics 2013].

Two extracts (ethanol 95%, yield 1.73%; and acetone 75%, yield 3.87%) showed dose- and time-dependent inhibitory effects on the viability of HepG2 cells and WRL-68 cells. The ethanolic extract exhibited a higher activity towards HepG2 cells and provoked a significant ($p<0.001$) increase in LDH at 50 and 150 µg/mL, significantly ($p<0.001$) decreased ATP in a dose-dependent manner and changed the morphology of HepG2 cells [Jiang 2017].

Hypoglycaemic effects
Carnosol and carnosic acid, as well as an extract (ethanol 80%, DER 1:10), selectively activated Gal4-PPARγ fusion receptor in a concentration-dependent manner (EC_{50} = 41.2 ± 5.9µM, 19.6 ± 2.0µM and 33.7 ± 7.3mg/L, respectively). PPARα and δ were not affected [Rau 2006].

Several compounds were tested for PPARγ-activating properties at three concentrations (0.1, 1.0, 10 µM). Carnosol, carnosic acid, oleanolic acid, α-linolenic acid and 12-O-methyl carnosic acid at 10 µM provoked PPARγ-activation. 20-hydroxyferruginol did not activate PPARγ [Christensen 2010].

Other effects
The influence of a dried ethanol extract (55.8 mg/mL) on the activity of CYP2D6 was investigated by measuring the reaction rate of dextromethorphan-O-demethylation from dextromethorphan to dextrorphan at concentrations of 815, 81.5 and 8.15 µg/mL in the incubation medium. The extract resulted in an inhibition of CYP2D6 with an IC_{50} of 800 µg/mL [Hellum 2007].

Reaction mixtures containing 300 µg of gastric vesicular protein, 1 mM ATP, 20 mM $MgCl_2$ and 50mM Tris-HCl (pH 7.4) with and without hydroethanolic extract (0.03, 0.1 and 0.3 mg/mL) were used for colorimetric determination of the release of inorganic phosphate from ATP as an indicator for ATPase activity, with 0.2 mg/mL omeprazole as positive control. An extract (85% ethanol) inhibited the activity of the ATPase by 46-66% and dose-dependently showed higher results than omeprazole (49%) [Mayer 2009].

An extract (ethanol 70%, not further specified) inhibited angiogenesis in HUVEC and rat aorta models at 300 µg/mL and higher concentrations, it also induced wound repair by endothelial cells at 100-200 µg/mL in a dose-dependent manner. The hexane fraction showed an angiogenesis-inhibiting activity at concentrations above 50 µg/mL (HUVEC) and 50-100 µg/mL (wound repair) [Keshavarz 2010].

In isolated tracheal preparations, a dry extract (methanol 70%, yield 15%) at 1-10 mg/kg dose-dependently inhibited K^+ (25 mM)-induced contractions. Pretreatment of the tissues with 4-aminopyridine reversed the inhibitory effect of the extract against K^+, whereas glibenclamide did not show any effect. When tested against carbachol-induced pre-contractions the extract showed a dose-dependent (0.03-0.1 mg/mL) inhibitory effect and shifted the inhibitory concentration response curves of isoprenaline to the left, similar to that of papaverine [Gilani 2015].

Cells from twelve immature female mice were grown to measure the oestradiol and progesterone concentration under the influence of varioust concentrations (0, 10, 50, 100, 500 and 1000 mg/mL) of an extract (70% ethanol, yield 17%) using radioimmunoassay (RIA) for oestradiol and immunoradiometric assays (IRMA) for progesterone determination. Oestradiol and progesterone concentrations, as well as ALP activity, increased significantly ($p<0.001$) at high concentrations (500 and 100 mg/mL) of the extract. The *in vitro* maturation of oocytes (obtained from 25-35 days old Balb/C female mice after i.p. injection of 17 IU pregnant mare serum gonadotropin and 17 IU human chorionic gonadotropin) was investigated in seven experimental groups with various extract concentrations (5, 10, 50, 100, 250, 500 and 1000 mg/mL) and one control group. Cell viability assays showed that at higher concentrations the percentage of living cells decreased. At 500 and 1000 mg/mL of extract the amount of granulosa cells was reduced significantly ($p=0.02$, $p=0.01$, respectively), while at a concentration of 100 mg/mL of the extract the cell cultures showed an early stage of apoptosis. Lower doses had no effect on the growth of granulosa cells or on function and maturation of oocytes [Monsefi 2017].

An aqueous extract (not further specified) demonstrated a cytoprotective and renoprotective effect against CCl_4-induced nephrotoxicity using Vero cells in an MTT and biosensor assay. After extract exposure, the LC_{50} of CCl_4 increased from 6.06 mM to 8.08 mM [Flampouri 2017].

The essential oil showed cytoprotective activity against UMSCC1 cells with an IC_{50} of 135 µg/mL [Sertel 2011].

The effects of isolated phenolic diterpenes on melanin biosynthesis in B16 melanoma cell lines as well as on AChE were examined. Isorosmanol showed a melanin-inhibiting activity comparable to arbutin. At 500 µM, 7α-methoxyrosmanol and isorosmanol inhibited AChE activity by 50% and 65% respectively [Sallam 2016].

In vivo experiments

Antinociceptive and anti-inflammatory effects

Topical application of various extracts (n-hexane, chloroform, methanol) and essential oil were investigated in Croton oil-induced ear oedema in mice. Chloroform extracts exhibited the strongest effect (ID_{50} = 105.9 µg), followed by n-hexane extracts (ID_{50} = 683.2 µg). The methanolic extract had a weaker effect (ID_{50} > 1000 µg). The essential oil showed no effect. The anti-inflammatory activity of ursolic acid, the main component of the chloroform extract, was evaluated in comparison to oleanolic acid and indomethacin. The oedema-inhibiting effect of ursolic acid was in a comparable range with indomethacin and higher than oleanolic acid [Baricevic 2001].

A tincture (ethanol 70%, 1:10) adminstered intraperitoneally to male rats (no information on dosage) showed anti-inflammatory effects on acute inflammation induced by turpentine oil. The tincture reduced total leukocyte count, differential leukocyte count ($p<0.0001$) and nitrite levels ($p<0.05$) [Oniga 2007].

An aqueous extract (yield 15%; 10, 31.6, 100, 316, 1000 mg/kg) and a butanol fraction (10, 31.6, 100, 316 mg/kg) demonstrated analgesic effects in rats after i.p. administration in the hot-plate and the formalin-induced paw licking tests. The effect was inhibited by the opioid-receptor-antagonist naloxone (5 mg/kg). The extract and the fraction exhibited dose-dependent anti-inflammatory activity by reducing the degree of swelling in carrageenan-induced paw oedema as well as of cotton pellet granuloma. The butanol fraction (ED_{50} 29 ± 4 mg/kg) showed greater activity than the aqueous extract (ED_{50} 90 ± 5 mg/kg) [Qnais 2010].

In the acetic acid model in mice, an extract (ethanol 85%, not further specified) at doses of 10, 30 and 100 mg/kg b.w. p.o. reduced writhing, total leukocytes and plasma extravasation. Mice treated orally with the extract, carnosol (10 mg/kg b.w.), ursolic or oleanolic acid (each 30 mg/kg b.w.) one hour before formalin injection, exhibited a reduction of neurogenic and inflammatory responses. Pretreatment with naloxone (1 mg) reversed the antinociceptive effect of the extract at 100 mg/kg. Administration of the extract one hour before injection of glutamate into the paw reduced oedema and demonstrated antinociceptive activity at each dose [Rodrigues 2012].

The effects of an extract (approx. 20:1; ethanol 80%) on nociception and on prevention of morphine-induced tolerance and dependence were studied in rats using the tail-flick and naloxone precipitation withdrawal tests. Intragastric (i.g.) administration of 600 and 800 mg/kg of extract exhibited a significantly ($p<0.001$) greater antinociceptive effect than vehicle alone, while 400 mg/kg demonstrated a non-significant benefit. Morphine tolerance and dependence respectively were induced by injection of morphine (10 mg/kg, s.c.) twice a day for 7 days or escalating doses of morphine (2.5 mg/kg s.c. on days 1 and 2, followed by an increased dose each day up to 5, 10, 20, 40 and 50 mg/kg) twice daily for 7 days, followed by administration of naloxone (3 mg/kg s.c.) after the last injection of morphine on day 7. The extract was given at doses of 400, 600 or 800 mg/kg i.g. 30 minutes before each dose of morphine in both experiments. The highest dose of extract prevented development of significant tolerance to the antinociceptive activity of morphine while the lowest dose did not. In the morphine dependence experiment, the extract at 600 and 800 mg/kg attenuated most of the naloxone-induced withdrawal signs including weight loss, jumping, penis licking, teeth chattering, wet dog shakes, rearing, standing, sniffing, face grooming and paw tremor and increased sleep duration. Furthermore, the extract at the highest dose significantly ($p<0.001$) increased pentobarbital-induced sleeping time and decreased sleep latency ($p<0.05$) [Hasanein 2015].

The effects of an extract (5:1, ethanol 80%; 100 and 200 mg/kg, p.o.) as well as rosmarinic acid (10 and 20 mg/kg, i.p.) and caffeic acids (30 and 40 mg/kg, i.p.) on peripheral neuropathic pain after chronic constriction injury were investigated in mice. These substances, as well as clomipramine (5 mg/kg, i.p.) as positive control, were administered on days 0, 1, 7, 14 and 21 following surgery. All treatments significantly ($p<0.05$) increased mechanical sensitivity, cold and thermal withdrawal latency ($p<0.001$) and enhanced functional recovery of the injured sciatic nerve. The same treatments ameliorated haematological parameters and did not alter biochemical levels. Histopathological findings revealed a protective effect of the extract, rosmarinic acid and caffeic acids against the induced damage [El Gabbas 2019].

Larvicidal effect

An extract (ethanol 80%, yield 12.3 ± 1.2%) demonstrated larvicidal activity against *Culex pipiens*, transmitting diseases

like Japanese encephalitis and Yellow fever, resulting in an LC_{50}, LC_{90} and LC_{100} of 287, 487 and 600 ppm respectively [Abdelhakim 2016].

Hypoglycaemic effects
In streptozotocin-induced diabetic rats, administration of a methanolic extract (1:5; 100, 250, 400 and 500 mg/kg b.w., i.p.) produced a slight decrease in serum glucose. No effect was observed in healthy fasted rats [Eidi 2005].

Oral administration of an ethanolic extract (1:5, yield 16%; 0.1, 0.2 and 0.4 g/kg) for 14 days demonstrated a hypoglycaemic effect and also significantly ($p<0.001$) reduced triglycerides, total cholesterol, urea, uric acid, creatinine, AST and ALT in streptozotocin-induced diabetic rats as compared to 600 µg/kg glibenclamide [Eidi 2009].

An extract (methanol 80%, yield: 23%; 250 mg/kg p.o. for 21 days) showed an increase in SOD activity (33%) as well as a decrease in triglycerides (40%, $p<0.0001$), LDL- (30%, $p<0.001$) and VLDL cholesterol (22%, $p<0.001$) in alloxan-induced diabetic rats [Sani 2012].

The effects of an extract (ethanol 80%, 1:10) on learning and memory in streptozocin-induced diabetic rats, as well as on the antioxidant capability and hypoglycaemic properties, were evaluated after administration of 400, 600 and 800 mg/kg p.o. for 30 days. In the passive avoidance learning test the higher doses (600 and 800 mg/kg) improved learning and memory deficits in diabetic rats and also cognition of healthy rats. Significant hypoglycaemic activity ($p<0.001$), lipid peroxidation inhibition and enhanced activity of antioxidant enzymes such as SOD ($p<0.05$) and catalase ($p<0.05$) were observed [Hasanein 2016].

Obese mice (n=32; on a high fat diet) were treated for five weeks with a high (400 mg/kg/day) or a low (100 mg/kg/day) dose of an extract (methanol 80%, not further specified). An oral glucose tolerance test (OGTT) resulted in significantly lowered blood glucose levels 30 minutes after the glucose load ($p<0.05$ and $p<0.001$ after high and low doses respectively). The effect was similar to that of rosiglitazone (3 mg/kg/day; positive control). Insulin sensitivity improved, as demonstrated by a reduction in fasting plasma insulin levels and a reduced insulin sensitivity index by 39% and 60% respectively. The low dose significantly decreased concentrations of plasma insulin, triglycerides and non-esterified fatty acid levels ($p<0.05$). Anti-inflammatory cytokines (IL-2, IL-4 and IL-10) increased ($p=0.003$, $p=0.036$, $p=0.006$, respectively), while pro-inflammatory cytokines (TNF-α, KC/GRO and IL-12) decreased ($p=0.030$, $p=0.004$, $p=0.034$, respectively) [Khedher 2018].

Lipid-lowering effects
The inhibitory effect of single doses of a methanolic extract (yield: 30.4%; 500 and1000 mg/kg p.o.), carnosol (200 mg/kg) and carnosic acid (5, 10 and 20 mg/kg) on serum triglyceride elevation was investigated in olive oil-loaded mice. The extract significantly ($p<0.01$) suppressed triglyceride elevation at both doses. Carnosic acid exhibited an effect equivalent to that of the pancreatic lipase inhibitor orlistat, while carnisol had no significant effect. Carnosic acid was subsequently administered to mice on a high fat diet at 5, 10 and 20 mg/kg/day p.o. for 14 days. Carnosic acid at 20 mg/kg significantly ($p<0.05$) reduced body weight gain compared to the high fat diet control mice and at all doses significantly ($p<0.01$) suppressed the increase of the epididymal fat pad [Ninomiya 2004].

The effects of the essential oil and simvastatin were studied in hyperlipidaemic mice. Animals were randomly divided into four groups which received a standard diet (control), a high fat diet or a high fat diet combined either with simvastatin (2.5 mg/kg b.w./day) or with the essential oil (4 mg/kg b.w./day) for 8 weeks. The essential oil and simvastatin reduced body weight gain, hyperlipidaemia, disruption of liver and renal functions and reactive oxygen species production in mice on the high-fat diet. Total cholesterol, triglycerides, total lipids and LDL cholesterol levels as well as aspartate transaminase, alanine transaminase, GGTP and LDH activities were reduced, while faecal lipids increased compared to those of high fat diet only mice. The reductions in triglycerides, LDL and VLDL were significantly ($p<0.01$) greater for the essential oil than simvastatin [Koubaa-Ghorbel 2020].

Gastroprotective effects
The gastroprotective effect of 30, 100, 300 and 1000 mg/kg of an extract (85% ethanol) was investigated in rats. Omeprazole (40 mg/kg) was used as positive control. Gastric lesions were induced by oral administration of 0.5 ml 80% ethanol per animal one hour after the administration of the test substances. The extract decreased the area of gastric lesions in a dose-dependent manner, with an ED_{50} of 84.0 mg/kg.

Acetic acid (20%) was used to induce chronic gastric ulcers in a further group of rats. Seven days after the induction procedure, rats were treated orally for a further 7 days with either vehicle, 20 mg/kg omeprazole or 10, 30 or 100 mg/kg of the extract. The extract dose-dependently reduced ulcer volume by 59, 66 and 76% respectively, as compared to 75% by omeprazole.

In a futher group of rats, either vehicle (control), 40 mg/kg omeprazole or 300, 600 or 1000 mg/kg of the extract were administered after pylorus ligature, 4 h later the rats were killed and gastric secretions collected. The extract at the highest dose reduced the volume of gastric juice from 6.4 to 3.2 mL ($p<0.001$) and the total acidity [mEq[H^+]/L] from 47.9 to 13.7 ($p<0.001$) compared to control, while omeprazole decreased the volume to 4.9 mL ($p<0.05$) and the total acidity to 15.6 mEq[H^+]/L ($p<0.001$) [Mayer 2009].

Memory-improving effects
An ethanolic extract (1:5; 80% ethanol; 5, 10, 50, 100 mg/kg i.p.) demonstrated significantly ($p<0.001$) increased memory retention in rats in a passive avoidance test. The extract at 50 mg/kg significantly ($p<0.0001$) potentiated the memory retaining effects of nicotine and pilocarpine. While the the nicotinic receptor antagonist mecamylamine and the muscarinic receptor antagonist scopolamine significantly ($p<0.0001$) attenuated the response [Eidi 2006].

Antimutagenic effects
Acute (24h), chronic (48h) and two combined treatments (one acute, one chronic) with an infusion (15 mg/mL; 200mL) and/or the mutagen MMS were analysed in the somatic mutation and recombination test on *Drosophila melanogaster*. The infusion reduced the frequency of mutations indicated by a decreased number of large, small, single and twin spots on the chapped wings of D. melanogaster but did not lower the mutagenic effect to the level of spontaneous mutagenesis [Patenkovic 2009].

The chemoprotective properties of an aqueous extract (13.3 g/L boiling water) were investigated in 3-week-old rats. Azoxymethane (AOM; 15 mg/kg) was used as a mutagen. Two groups received the extract with their drinking water for two weeks before the administration of AOM or vehicle, two groups for 4 weeks after the application of AOM or vehicle, two control groups received water and water+AOM respectively. The extract significantly ($p<0.05$) decreased the number of aberrant crypt foci when administered two weeks before AOM but showed no effect when applied for 4 weeks after AOM [Pedro 2016].

Oral treatment of mice with 1 mg/kg of mitomycin C followed

by 25, 50 or 100 µL/kg of the essential oil resulted in a dose-dependent decrease of aberrations induced by mitomycin C. The concentrations of 25 and 50 µL/kg significantly ($p<0.001$) reduced the frequency of metaphases [Vujosevic 2004].

Wound healing effects
Topical application of a hydroethanolic extract (150 g leaves/600 mL; 1, 3 and 5% in an ointment consisting of 25% eucerin and 75% vaseline) demonstrated wound healing properties in rats. Wound contraction was significantly ($p<0.05$) and dose-dependently increased on days 12, 15, 18 and 21 as compared to placebo and untreated controls. Duration of re-epithelialisation ($p<0.05$), new vessel formation and fibroblast distribution ($p<0.05$) were significantly improved [Karimzadeh 2017].

Antioxidative effects
An extract (ethanol 40%) increased the resistance to oxidative stress and reduced H_2O_2- and 2,3-dimethoxy-1,4-naphthoquinone-generated DNA damage in liver cells of rats. Administration of 3.33, 6.67 and 13.33 mg/kg p.o. for 14 days provoked a significant increase in glutathione peroxidase (GPx) ($p<0.05$, $p<0.01$ and $p<0.001$ respectively) and in GSH ($p=0.01$) in rat hepatocytes. A change in SOD activity was not observed [Horváthová 2016].

To investigate the effect of an aqueous extract (not further specified) on bleomycin-induced lung fibrosis, male Wistar rats were given a single dose of bleomycin (4 mg/kg, intratracheal) followed by the extract (50, 100 and 150 mg/kg, i.p.) 3 days later for 4 weeks. The extract at 150 mg/kg normalized increased lipid peroxidation and decreased SOD as well as CAT levels. The fibrosis score was significantly decreased by the extract at 100 mg/kg (2.8 ± 0.34 vs. 3.9 ± 0.3; $p<0.05$) and 150 mg/kg (1.9 ± 0.27 vs. 3.9 ± 0.3; $p<0.01$) as compared to the bleomycin group [Bahri 2019].

Antiulcerogenic effects
Male Wistars rats were treated with 50, 75, 100 or 150 µg/kg b.w. of an ethanolic extract or received distilled water or omeprazole (30 mg/kg) one hour before ulcer induction with ethanol. The total area of lesion (mm^2) was significantly reduced to 109.14, 35.25, 29.66 and 29.55 in all groups receiving the extract (all $p<0.05$) compared to negative control (151.4) and omeprazole (50.76). All extract doses and omeprazole reduced the ulcerative lesion index significantly ($p<0.05$ or <0.001 respectively). Omeprazole ($p<0.01$) and the extract at doses of 50 µg/kg and 75 µg/kg (both $p<0.05$) as well as at 100 µg/kg and 150 µg/kg (both $p<0.001$) led to a significant increase in the curative ratio [Fiorentin 2013].

Other effects
The locomotor activity of mice assessed in an open-field test was not affected by single oral doses of 10, 30 and 100 mg/kg of an ethanolic extract (85% ethanol, not further specified) [Rodrigues 2012].

A dry extract (methanol 70%, yield 15%) at 1-10 mg/kg dose-dependently inhibited carbachol-induced bronchospasms in anaesthetized rats with three-fold greater potency than aminophylline at 3-30 mg/kg [Gilani 2015].

The essential oil and thujone (89.5% α- and 10.5% β-thujone) were examined in the light/dark (L/D) test in rats providing an estimation of the degree of animal anxiety. Thujone at 5, 10 and 25 mg/kg b.w. as well as the oil at 10, 25 and 50 mg/kg b.w. significantly ($p<0.001$) increased the time that the animals spent in the illuminated part of the apparatus and the number of transitions between the light and dark compartments, in a similar manner to diazepam (2 mg/kg b.w.). Diazepam-induced sleeping time was significantly ($p<0.001$) prolonged by the higher doses of thujone and of the oil [Radulović 2017].

α- or β-thujone (78 nmol injected intracerebrally) affected the open field behaviour of three-day old chicks, inducing anxiogenic behaviour as shown by the reduction of locomotor activity and an increase in latency. Moreover, α-thujone eliminated the stress-induced flunitrazepam sensitive $GABA_A$-receptor recruitment [Rivera 2014].

Pharmacological studies in humans
In a randomized, placebo-controlled, double-blind, five-period crossover study, the effects of a dried ethanolic extract (7.5:1; ethanol 70%) on cognitive performance in twenty healthy adults (65 to 90 years, mean age 72.95) were assessed using the Cognitive Drug Research computerised assessment battery. Volunteers attended six laboratory visits, one practice session and five subsequent visits when they received a single dose each of either placebo or extract at 167, 333, 666 and 1332 mg with a 7 day washout period between each visit. Cognitive performance was assessed before each treatment and at 1, 2.5, 4 and 6 h post treatment. Secondary memory performance was significantly enhanced by the extract at 333 mg at all testing sessions, by the 167 mg dose at 2.5 and 4 h and by the 1332 mg dose only at 4h compared to placebo. Accuracy of attention was significantly improved by the 333 mg dose at 1, 4 and 6h and by the two highest doses at 4h, when compared to placebo. There were no significant effects on speed of memory and speed of attention. Working memory was significantly improved at 1h with the 333 mg dose [Scholey 2008].

A non-randomized crossover trial with six healthy female volunteers (40-50 years) for four weeks showed that consumption of a tea (4 g leaves/300 mL twice a day) improved lipid profiles and antioxidant defences. LDL cholesterol and TC levels decreased by 12.4% and 5.4% respectively, while HDL values increased by 50.6%. An increase in SOD and CAT activities indicated antioxidant activity. Hsp72, an endogenous stress modulator, was increased (its reduction correlates with reduced insulin sensitivity in type 2 diabetes) [Sà 2009].

Clinical studies

Antihidrotic effects
In an open study, patients with idiopathic hyperhidrosis were treated daily for 4 weeks with either 440 mg of a dry aqueous extract corresponding to 2.6 g of leaf (n=40) or an infusion from 4.5 g of leaf (n=40). The achieved reduction in sweat secretion (less than 50%) was comparable in the two groups, although slightly greater in the group treated with extract [Rösing 1989].

Antimicrobial and anti-inflammatory effects
A randomized, double-blind, placebo-controlled, parallel group study with adaptive two-stage design and interim analysis assessed the efficacy and tolerability of a spray containing an extract (1:1, ethanol 70%) in patients with pharyngitis (n=82 per group). Throat pain intensity was evaluated using a VAS before treatment and at intervals of 15 minutes for the first 2 hours after the first spray application. The treatments continued with two more applications on treatment day one, three applications on day two and the last application on day three in the morning before the final visit. The patients took three puffs (140 µl/puff) per single application. Significant ($p<0.001$) pain reduction was observed in the verum group as compared to placebo [Hubbert 2006].

In a randomized, double-blind, placebo controlled clinical trial, the antibacterial activity of a 5% mouthwash (0.5 g ethanolic extract (48%) in 100 ml distilled water) against *Streptococcus*

mutans in dental plaque was investigated in 70 female children (11-14 years, n=35 per group) for three weeks. In the verum group (rinsing for 60 seconds twice a day, with continued usual oral hygiene measures) a significant (p=0.001) reduction in colony count from 3900 to 600 units was observed. The use of placebo mouthwash resulted in a non-signficant decrease from 4071 to 3174 colonies only [Beheshti-Rouy 2015].

In a controlled trial, patients (aged 20 to 40 years) suffering from minor aphthous ulceration (n=15) or gingivitis (n=15) applied a mouthwash (1 tsp of dried herb steeped for 20 mins in 1 cup of boiling water) for 60 seconds twice daily for 6 days, while the control group (n =15 for both complaints) used a saline mouthwash. Gingival index scores decreased from 2.05±0.5 to 0.82±0.2 ($p<0.05$) in the sage group and from 2.02±0.7 to 1.35±0.6 ($p<0.05$) in the saline group after 6 days. The patients with aphthous ulceration underwent observation of the ulcer on days 1, 3 and 6. Significant reduction in pain intensity (p=0.001 to p=0.005) was observed in the sage group, and complete healing of ulceration was seen in three patients (p=0.02) on day 6 after treatment with the sage mouthwash [Fawzi 2017].

Hypoglycaemic and lipid-lowering properties
The efficacy of 500 mg of an extract (yield 23%; not further specified) given three times daily for two months was investigated in a randomized, double-blind, placebo-controlled study including female and male patients with newly diagnosed primary hyperlipidaemia (hypercholesterolaemia, hypertriglyceridaemia or both, triglyceride and/or total cholesterol levels between 240-300 mg/dL). The extract (n=34) significantly lowered blood levels of TC (19.6%, $p<0.001$), triglycerides (22.8%, $p<0.001$), LDL (19.7%, p=0.004) and VLDL (13.3%, p=0.001) and increased HDL (20.2%, p=0.001) as compared to placebo (n=33), but had no significant effect on SGOT, SGPT and creatinine [Kianbakht 2011].

In a randomised, double-blind, placebo-controlled study, 80 type II diabetic patients who had not reached ideal control of the disease received either tablets containing 150 mg of an extract (not further specified) or placebo three times daily day for three months. Fasting blood sugar and 2-hours postprandial glucose levels were checked at the beginning and every two weeks. Glycosylated haemoglobin (HbA1c), lipid profile, liver and kidney function were tested at the beginning and at the end of the trial. After 12 weeks, the 2-hours postprandial glucose levels were significantly ($p<0.05$) decreased from 222.0 ± 58.4 mg/dL to 174.5 ± 21.0 mg/dL in the verum group compared to a decrease from 214.6 ± 42.8 mg/dL to 207.7 ± 49.2 mg/dL in the placebo group. The fasting blood sugar levels decreased from 144.2 ± 43.6 mg/dL to 115.0 ± 45.9 mg/dL in the verum group and from 142.9 ± 34.7 mg/dL to 143.4 ±39.5 mg/dL in the placebo group. No differences were observed in any other parameters [Behradmanesh 2013].

In a randomized, triple-blind, placebo-controlled trial, type 2 diabetic patients aged 40 to 60 years (fasting glucose 150-200 mg/dL, HbA1c 7-9%, LDL 100-150 mg/dL, triglycerides 200-300 mg/dL; medication: 2 x 5 mg glyburide and 2 x 500 mg metformin per day), received additional treatment with either placebo (n=40) or 500 mg of an extract (4:1, ethanol 80%; n=40) 3 times a day for 3 months. The extract signficantly (all $p<0.05$) lowered levels of fasting glucose (25.8%), HbA1c (14.4%), total cholesterol (17.7%), triglycerides (32.2%) and LDL (19.2%) and increased HDL (34.8%) when compared to placebo [Kianbakht 2013].

In a randomized, double-blind, placebo-controlled clinical trial including hypercholesterolaemic type 2 diabetes patients (n=50 per group; 40 to 60 years, HbA1c < 8%, LDL 100-130 mg/dL; medication: 15 mg glyburide, 2000 mg metformin and 10 mg atorvastatin), the effects of 500 mg extract (4:1; ethanol 80%) three times a day as add-on therapy for two weeks were evaluated. Fasting glucose, 2 h postprandial glucose, HbA1c, TC, LDL and triglycerides decreased, while HDL increased, when compared to placebo ($p<0.05$) [Kianbakht 2016].

Effect on postmenopausal symptoms
The effect of an extract on postmenopausal women (n=69; aged 50 to 65 years (mean age 56.4 ± 4.7), at least 12 months since last menstruation) experiencing at least five flushes daily was investigated in an open, multicentre trial using the Menopause Rating Scale (MRS), the change in intensity and frequency of hot flushes and the total score of the mean number of intensity rated hot flushes (TSIRHF). Each patient received 280 mg thujone-free spissum extract (equivalent to 3400 mg tincture of fresh leaves, DER 1:17) daily for 8 weeks, to be taken before their strongest flush of the day. The TSIRHF decreased significantly (p=0.0001) from 9.3±12.2 to 4.8±3.6 (48%) after 4 weeks, and to 3.8±3.5 (64%) after 8 weeks. The mean number of mild, moderate, severe and very severe flushes showed reductions of 46% ($p>0.05$), 62% (p=0.0001), 79% (p=0.0001) and 100% ($p<0.05$) respectively at week 8. The mean global MRS score and all subscales decreased: MRS (43%, $p<0.0001$), somato-vegetative subscale (43%, $p<0.0001$), psychological subscale (47%, $p<0.0001$) and urogenital subscale (20%, $p<0.01$). A positive effect was seen for MRS symptoms such as heart discomfort, irritability, anxiety, physical and mental exhaustion, joint and muscular discomfort and especially hot flushes and sleep problems [Bommer 2011].

In a randomized, double-blind, placebo-controlled trial postmenopausal women with hot flushes and nocturnal sweating (n=50 per group) received 3 x 100 mg sage tablets (not further specified) per day or placebo for 8 weeks. Menopasual symptoms were evaluated using the MRS 1 week before treatment and after 8 weeks, while duration, frequency and intensity of hot flushes and night sweats were evaluated every week during the trial. The mean duration of hot flushes in the verum group (3.6±1.6 at baseline), declined each week and was reduced to 1.4±0.7 at week eight ($p<0.001$). The mean number of hot flushes significantly ($p<0.001$) decreased from 5.7±2.6 to 2.3±0.8 in the verum group at the end of the intervention, and the mean severity of hot flushes from 2.35 to 1, with the latter remaining almost unchanged in the placebo group. The number of nocturnal sweats was significantly ($p<0.001$) reduced in the verum group. The MRS total score was significantly ($p<0.001$) reduced by 25% after eight weeksin the verum group. No significant differences were observed between the groups with regard to oestradiol levels, although the verum group's oestradiol levels did significantly (p=0.014) increase [Forouhari 2016].

In a randomized, double-blind, placebo-controlled clinical trial, 66 postmenopausal women suffering from menopausal symptoms received either 300 mg extract (not further specified) daily for 3 months or placebo. Menopause Rating Scale (MRS) and Pittsburgh Sleep Quality Index (PSQI) questionnaires were completed at the beginning and at the end of the study. The checklists for hot flushes and night sweats were completed at weeks 0, 2, 4, 6, 8, 10 and 12. In the verum group, the mean score of flushing, palpitation, sleeping disorders, muscle and joint aches, depression, nervousness, anxiety and sexual desire and satisfaction significantly decreased by 1.6, 0.4, 1.6, 2.1, 1.4, 1.2, 1.6, and 0.8 units, respectively, in the verum group compared to the placebo group ($p<0.001$). The mean score of PSQI significantly ($p<0.05$) decreased by 3.8 units in the verum group (9.4 ± 3.7 vs 5.6 ± 1.9) while no change was observed in the placebo group (Zeidabadi 2020).

Effects on premenstrual syndrome
In a randomized, triple-blind, placebo-controlled clinical trial, 110 women aged 18 to 35 years with premenstrual syndrome (PMS) received either 500 mg of an extract (20:1; ethanol 96%; n=47) or placebo (n=43) daily from day 21 of their cycle until day 4 of the next cycle, for two consecutive months. Data were collected from 90 subjects who completed the study, using a demographic and menstrual cycle history questionnaire as well as a reporting form for daily PMS symptoms, containing a checklist of 20 physical items as well as mood and behavioural symptoms. The severity of symptoms decreased significantly ($p<0.001$) in the verum group during the first and second month of treatment by 19.8% and 23.4% respectively. Physical symptoms of PMS such as breast tenderness, oedema, bloating and fatigue as well as psychological symptoms including tension, irritability and depressive mood also decreased significantly ($p<0.001$) in the verum group compared to placebo [Abdnezhad 2019].

Effects on cognition
The effect of an extract (ethanol 45%, 1:1) on the cognition of patients with mild to moderate Alzheimer's disease was examined in a randomized, double-blind, placebo-controlled trial involving forty patients aged between 65 and 80 years with a score of ≥12 on the cognitive subscale of Alzheimer's Disease Assessment Scale (ADAS-cog) and ≤2 on the Clinical Dementia Rating (CDR). Each patient was given 60 drops/day of the extract or placebo for 16 weeks. The ADAS-cog ($p<0.0001$) and CDR ($p<0.0001$) values were significantly improved compared to placebo after 16 weeks. Patients in the placebo group showed increased agitation compared to verum [Akhondzadeh 2003].

Pharmacokinetic properties

Absorption
In single-dose toxicokinetic studies in rats and mice, α-thujone was rapidly absorbed without any species or sex difference and rapidly distributed into tissues including the brain. Females showed a higher brain-plasma ratio and had a higher sensitivity to thujone-induced toxicity [NTP 2011, Waidyanatha 2013].

Metabolism
α-Thujone incubated with rabbit liver cytosol in the presence of NADPH gave thujol and neothujol in low yield. On incubation with mouse liver cytosol alone, α-thujone was stable, but in the presence of NADPH it was rapidly metabolized, the major product being 7-hydroxy-α-thujone together with 4-hydroxy-α-thujone, 4-hydroxy-β-thujone and other minor metabolites [Höld 2000].

In mice treated intraperitoneally with α-thujone, the brain levels of α-thujone and 7-hydroxy-α-thujone were dose- and time-dependent, but α-thujone appeared at much lower levels and was less persistent than 7-hydroxy-α-thujone [Höld 2000].

Human liver microsomes produced two major (7- and 4-hydroxy-thujone) and two minor (2-hydroxy-thujone and carvacrol) metabolites. Glutathione and cysteine conjugates were detected in human liver homogenates. No glucuronide or sulphate conjugates were detected. Major hydroxylations accounted for more than 90% of the primary microsomal metabolism of α-thujone [Abass 2011].

After consumption of 900 mL of an infusion from 6 g sage (containing 0.64 mg/L α-thujone corresponding to 4.6–14.3 µg/kg b.w.), the α-thujone metabolites 2-hydroxythujone, 4-hydroxythujone and 7-hydroxythujone were found in the urine. After consumption of 575 µg α-thujone, 4-hydroxythujone and 7-hydroxythujone were detected in the urine of the volunteers. 2-Hydroxythujone was not detected in any of the samples. α-Thujone was found in a low amount, with a maximum concentration of 94 ng/L for the volunteer with the highest dose of 14.3 µg/kg b.w. [Thamm et al 2018].

Bioavailability
After single administration by gavage of 25 mg/kg (rats) or 40 mg/kg (mice) of α-thujone and 50 mg/kg (rats) or 80 mg/kg (mice) of α,β-thujone, the bioavailability in rats was generally higher than in mice [NTP 2011, Waidyanatha 2013].

Preclinical safety data

Acute toxicity
The oral LD_{50} of the essential oil in rats was 2.6 g/kg [von Skramlik 1959]. After i.p. administration to rats, cortical phenomena remained subclinical below 0.3 g/kg whereas convulsions appeared above 0.5 g/kg and became lethal above 3.2 g/kg [Millet 1979].

In rats, the oral LD_{50} of thujone (α + β) was 192 mg/kg. I.p. administration led to both convulsant and lethal effects at a dose of 180 mg/kg b.w. [Pinto-Scognamiglio 1967].

The i.p. LD_{50} of α-thujone in mice was 45 mg/kg b.w. [Höld 2000]. It is a reversible modulator of the γ-aminobutyric acid (GABA) type A receptor, but it is rapidly metabolized and detoxified to 7-hydroxy-α-thujone and other metabolites [Höld 2000]. The i.v. LD_{50} for α-thujone in rats was 0.031 mg/kg b.w. [NTP 2011].

The subcutaneous LD_{50} values in mice were determined as 87.5 mg/kg b.w. for α-thujone and 442.4 mg/kg for β-thujone [Rice 1976, Lawrence 1998, Höld 2000].

Subchronic toxicity
In subchronic toxicity tests in rats, oral administration at 10 mg/kg/day produced convulsions in only 1 out of 20 animals by the 38th day [Pinto-Scognamiglio 1967].

Administration of α-thujone and α,β-thujone by gavage to B6C3F1 mice and to Fischer 344 rats at doses of 0, 1, 3, 10, 30 or 100 mg/kg for 14 days produced increased mortality in the group receiving the highest dose associated with neurotoxic effects such as hyperactivity, tremors or tonic seizures [NTP 2011].

Chronic toxicity
α,β-thujone was administered by gavage to B6C3F1 mice at doses of 0, 3, 6, 12, and 25 mg/kg b.w./day and to Fischer 344 rats at doses of 0, 12.5, 25, and 50 mg/kg b.w./day for 2 years. In both species, increased mortality was observed in the group receiving the highest dose and in rats also in the 25 mg/kg group. Clonic and tonic seizures were observed with the middle and the highest dose in rats and with the highest dose in mice. A small increase in clonic seizures was also observed in rats receiving 12.5 mg/kg. The administration of α,β-thujone resulted in increased incidences of non-neoplastic lesions in the brain and spleen of male and female rats, the kidney of male rats and the pituitary gland of female rats, usually at the two highest dose levels. In the rat, the NOAEL value was 12.5 mg/kg for mortality and tonic seizures (no NOAEL for clonic seizures). In the mouse, the NOAEL was 12 mg/kg b.w. for seizures and mortality [NTP 2011].

Mutagenicity
A sage leaf tincture at doses up to 200 µl/plate showed no mutagenic activity in the Ames test using *Salmonella typhimurium* strains TA98 and TA100 with or without S9 metabolic activation [Schimmer 1994]. Sage oil showed no

mutagenic or DNA-damaging potential in the Ames test or *Bacillus subtilis* rec-assay [Zani 1991].

Clinical safety data
In a non-randomized, crossover trial involving six healthy female volunteers, consumption of a tea (4 g/ 300 mL twice a day) for four weeks did not cause any hepatotoxicity or other adverse effects [Sà 2009].

In an open, multicentre trial involving 69 menopausal women given 280 mg thujone-free spissum extract (equivalent to 3.4 g tincture of fresh leaves, DER 1:17, thujone < 10 ppm) for 8 weeks, only mild adverse effects (mild abdominal pain and mild diarrhoea in one patient) were reported. Tolerability was rated as very good by 90% of physicians and patients. A high degree of safety was observed regarding haematological parameters including leukocyte, erythrocyte and thrombocyte counts, as well as creatinine and bilirubin levels [Bommer 2011].

In a randomized, triple-blind, placebo-controlled trial involving 86 women and men with type 2 diabetes taking 500 mg of an extract (4:1; ethanol 80%) every 8h for 3 months, no significant ($p>0.05$) effects on hepatic and renal function parameters compared to the placebo group were reported [Kianbakht 2013].

In a randomized, triple-blind clinical trial, 90 volunteers received either 500 mg of an extract (20:1; ethanol 96%) or placebo daily, from day 21 of their cycle until day 4 of the next cycle, for two consecutive months. A questionnaire to assess side effects included 9 items and revealed increased bleeding and skin allergy in one case in the verum group as well as one case of allergy and gastrointestinal problems in the placebo group [Abdnezhad 2019].

Two cases of intoxication in children due to accidental use of sage oil have been reported: Shortly after accidental topical exposure, a 33-day-old boy showed recurrent generalized tonic-clonic convulsion, nystagmus, hyperreflexia and irritability. After the second day of hospitalisation seizures stopped. No sustained or irreversible neurologic effect were observed. A 5 ½ year-old girl manifested tonic-clonic seizures that lasted 10 minutes shortly after accidental intake of approximately 5 mL of sage oil [Halicioglu 2011, Lachenmeier 2012].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Online Series, 2019
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Online Series, 2019
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Online Series, 2019
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHAMOMILLAE ROMANAE FLOS	Roman Chamomile Flower	Online Series, 2019
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HELICHRYSI FLOS	Sandy Everlastin Flower	Online Series, 2019
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013

HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	LiquoriceRoot	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Online Series, 2020
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Online Series, 2020
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Online Series, 2020
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Online Series, 2021
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Online Series, 2020
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Online Series, 2020
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

E/S/C/O/P Monographs

Online Series

The Scientific Foundation for Herbal Medicinal Products

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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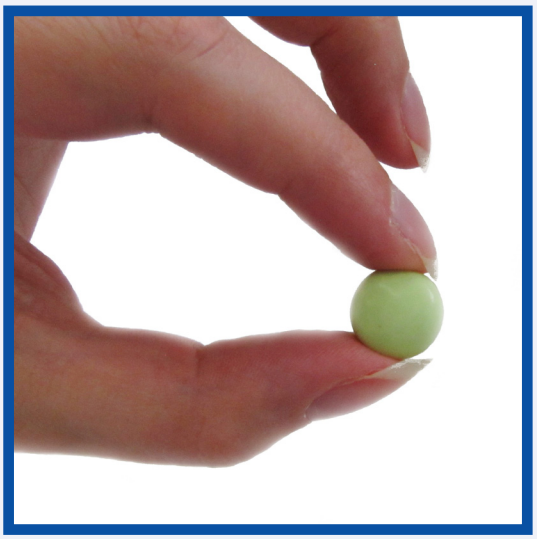
The Scientific Foundation for Herbal Medicinal Products

Salviae trilobae folium

Sage Leaf, Three-lobed

2014



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MONOGRAPHS

***The* Scientific Foundation for**
Herbal Medicinal Products

SALVIAE TRILOBAE FOLIUM
Sage Leaf, Three-lobed

2014

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

Edited by Simon Mills and Roberta Hutchins
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Plant illustrated on the cover: *Salvia fruticosa*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
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Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

SALVIAE TRILOBAE FOLIUM

2014

Sage Leaf, Three-lobed

DEFINITION

Three-lobed sage leaf consists of the whole or cut, dried leaves of *Salvia fruticosa* Mill. [syn. *S. triloba* L.fil.]. It contains not less than 18 ml/kg of essential oil in the whole drug, and not less than 12 ml/kg of essential oil in the cut drug, both calculated with reference to the dried material.

The material complies with the monograph of the European Pharmacopoeia [Sage leaf, three-lobed].

CONSTITUENTS

1.5-3.5% of essential oil consisting mainly of terpenes with cineole (40-67%), α- and β-thujone (2-6%), camphor (1-24%), camphene (0.3-5%), borneol (1%), β-caryophyllene, α- and β-pinene, bornyl acetate, limonene, linalyl acetate and viridoflorol [Länger 1996; Stahl-Biskup 2008; Pitarokili 2003; Brand 2008; Papageorgiou 2008].

Other characteristic constituents include phenolic acids (1-4%) such as 3,4-dihydroxybenzoic, rosmarinic and caffeic acids [Brand 2008; Papageorgiou 2008]; flavonoids such as salvigenin, glucosides and glucuronides of apigenin, chrysoeriol, hispidulin, luteolin, 6-methoxyluteolin and jaceosidin [Abdalla 1983; Brand 2008]; diterpenes such as carnosol and triterpenes such as ursolic and oleanolic acids (< 8%) [Stahl-Biskup 2008; Brand 2008].

CLINICAL PARTICULARS

Therapeutic indications

Inflammations and infections of the mouth and throat such as stomatitis, gingivitis and pharyngitis [Stahl-Biskup 2008; Benedum 2006]; digestive disorders such as dyspepsia [Benedum 2006].

Efficacy for these indications is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Topical use
Adult daily dose: An infusion of 3 g of dried drug in 125 ml of water as a mouthwash or gargle [Stahl-Biskup 2008; Böhme 2010].

Oral use
Adult dose: 1-1.5 g of dried drug as an infusion in 150 ml of water, once or several times daily [Stahl-Biskup 2008; Böhme 2010].

Method of administration
For oral administration.

Duration of administration
No restriction. If symptoms persist or worsen, medical advice should be sought.

Contraindications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported [Williamson 2009].

E/S/C/O/P MONOGRAPHS

Pregnancy and lactation
No data available. In accordance with normal medical practice, three-lobed sage leaf should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Data relating to the essential oil and lipophilic extracts are included below for completeness, although their components are present to only a minor extent in aqueous preparations such as those recommended under Dosage.

Pharmacodynamic properties

In vitro experiments

Antioxidant and radical-scavenging activity
An aqueous extract (0.25 g/10 ml, 1.4% polyphenols) showed antioxidant activity of 53.5 µmol trolox equivalents per g of dry plant material and an 80% methanolic extract (0.25 g/10 ml, 2.4% polyphenols) 175 µmol trolox equivalents per g of dry plant material in the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical cation decolourisation test [Tawaha 2007].

In the deoxyribose assay an infusion (1 g/25 ml) exhibited antioxidant activity in the presence or absence of digestive enzymes and bile salts. The amount of total dialysable iron was increased compared to the Fe(III) control under simulated gastric conditions [Matsingou 2000].

An acidic aqueous methanolic dry extract (30% V/V, 3.4-5.2:1) had an IC_{50} value of 0.021-0.046 g/litre in the DPPH assay and was also active in the ABTS cation decolourisation assay. The free radical scavenging activity in both assays was correlated with the total phenolic content (6.4-14.4%) determined by a colorimetric method [Papageorgiou 2008].

An ethanolic dry extract (1.6:1) and an acetone dry extract (4.2:1) reduced DPPH by 98.5% and 97.6% respectively. Antioxidant activity of these extracts was also observed in the phosphatidylcholine liposome assay. The protective effect of the extracts against oxidation of sunflower oil under accelerated conditions (120°C) was evaluated in a Rancimat apparatus using 0.02% (w/w) of extract in bulk oil; the ethanolic and acetone extracts protected the oil by factors of 1.7 and 2.2 compared to a blank control [Exarchou 2002]. In another study using the Rancimat method a methanolic dry extract (3.8:1) protected sunflower oil from oxidation by a factor of 1.08 at 0.02% and 3.03 at 1% [Bozan 2002].

Free radical scavenging activity of the essential oil of three-lobed sage leaf harvested during different seasons, and some of its components, was determined in the DPPH assay. IC_{50} values of the essential oils were between 2.4 and 5.2 g/litre, while that of β-caryophyllene was 18.6 g/litre, and 1,8-cineole and α-pinene were inactive; IC_{50} values of the positive controls, ascorbic acid and butylated hydroxytoluene, were 0.06 g/litre and 0.08 g/litre respectively. A positive correlation was found between free radical scavenging activity and concentrations of oxygenated sesqui- and diterpenes [Papageorgiou 2008].

Acetyl- and butyrylcholinesterase inhibitory activity
A dichloromethane dry extract (13.4:1) inhibited acetylcholinesterase activity by 27% at 25 µg/ml and 51% at 100 µg/ml [Senol 2010].

In another study, a 75%-hydroethanolic dry extract (1 g/60 ml; 1% total phenols) inhibited acetylcholinesterase activity with an IC_{50} of 0.71 mg/ml [Orhan 2009].

An ethyl acetate dry extract (1 g/20 ml) slightly inhibited acetylcholinesterase activity, by 28% at 1.0 mg/ml and 11% at 0.25 mg/ml, while a dry aqueous extract (1 g/20 ml) was inactive [Salah 2005].

In an assessment of the inhibition of human cholinesterases by three-lobed sage leaf essential oil, contradictory results were obtained in the case of butyrylcholinesterase, whereas IC_{50} values of 0.04-0.06 mg/ml were reported for inhibition of acetylcholinesterase. The most pronounced acetylcholinesterase inhibition by individual components of the oil was observed for 1,8-cineole (IC_{50}: 0.06 mg/ml), β-caryophyllene (IC_{50}: 0.03 mg/ml), α-pinene (IC_{50}: 0.1 mg/ml) and β-pinene (IC_{50}: 0.2 mg/ml); α-thujone and camphor at 0.5 mg/ml inhibited the enzyme by 37% and 13% respectively. No or only low butyrylcholinesterase inhibition was observed with all tested compounds [Savelev 2004].

Cytotoxic activity
An aqueous dry extract (2 g/150 ml, 71.5 µg/ml rosmarinic acid, 28.6 µg/ml 6-hydroxyluteolin-7-glucoside, 29.4 µg/ml other phenolic compounds, lyophilised) at 50 µg/ml significantly inhibited cell proliferation in the human colon cancer cell line HCT15 (from 26.2% in the control to 4.7%, $p \leq 0.001$). No significant effect was observed in CO115 cells; rosmarinic acid at 10-100 µg/ml was inactive in both cell lines. The extract and rosmarinic acid induced apoptosis in a concentration-dependent manner. In HCT15 cells the extract increased the proportion of apoptotic cells from 0.4% in the control to 6.6% at 50 µg/ml, rosmarinic acid increased numbers to 2.5% ($p \leq 0.001$). In CO115 cells, the proportion of apoptotic cells increased from 1.8% in the control to 6.8% after treatment with the extract and to 3.6% by rosmarinic acid at the same concentrations. No cleaved caspase-9 and caspase-3 were observed 24 hours after treatment.

Both the extract and rosmarinic acid inhibited the mitogen-activated protein kinase / extracellular signal-regulated kinase (MAPK/ERK) pathway in HCT15 cells by 50% ($p \leq 0.001$) and had no effects in CO115 cells. No inhibition on Akt (a serine/threonine kinase) phosphorylation was observed. Both pathways are commonly altered in colorectal carcinoma, and lead to increased proliferation and inhibition of apoptosis [Xavier 2009].

Three-lobed sage leaf essential oil and its main components exhibited cytotoxic activity against African green monkey kidney (Vero) cells. The essential oil caused complete cell death within 24 hours of exposure at a dilution of 1/500 (V/V) and within 48 hours at 1/1000. At 1/2000 a transient decrease in cell viability was initially observed, but this had been overcome after 48 hours. Among the main components at dilutions of 1/1000 (i.e. 0.10%), thujone was the most cytotoxic causing 95% reduction in viability, and camphor showed moderate activity (40% reduction), whereas 1,8-cineole was less active (5% reduction) [Sivropoulou 1997].

In another evaluation of cytotoxic activity, the IC_{50} values for

an undefined dichloromethane-methanol dry extract were 180 µg/ml in mouse fibrosarcoma cells, 250 µg/ml in a benign human breast carcinoma cell line and 290 µg/ml in a metastatic human breast carcinoma cell line [Kaileh 2007].

Spasmolytic activity
A decoction and an infusion from three-lobed sage leaf, and corresponding fractions obtained by partitioning these between benzene and water, as well as a fluid extract (1:2, 70% ethanol), had varying effects on the tone and phasic contractile activity of isolated segments from guinea pig ileum. The decoction, and the aqueous fraction of the infusion, caused contraction of the smooth muscle preparation without affecting phasic contractions, the aqueous fraction of the decoction decreased the tone and caused relaxation, while the fluid extract potentiated phasic activity but did not affect the tone. The infusion caused contraction of the intestinal segments and intensified phasic contractions. All the extracts inhibited contractions induced by acetylcholine (10^{-7} M), barium chloride (10^{-4} M), histamine (10^{-5} M) and serotonin (10^{-5} M) by 20-90% [Todorov 1984].

Antimicrobial and antiviral activity of the essential oil
The essential oil of three-lobed sage leaf exhibited slight inhibitory activity against growth of *Fusarium moniliforme*, *Rhizoctonia solani*, *Phytophthora capsici* [Müller-Riebau 1997], *Botrytis cinerea*, *Fusarium solani* var. *coeruleum* [Daferera 2003], *Trichophyton rubrum* and *Trichosporon beigelii* [Adam 1998]. In tests of the antifungal activity of the essential oil, 1,8-cineole and camphor against 13 other fungal species (type cultures and isolates), such as *Aspergillus* sp., *Penicillium* sp., *Phomopsis*, *Alternaria*, *Microsporum* and *Epidermophyton* sp., minimium inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) respectively were found to be: 5.0-15.0 µl/ml and 5.0-20.0 µl/ml for the essential oil, 4.0-9.0 µl/ml and 5.0-15.0 µl/ml for 1,8-cineole, and 3.0-5.0 µl/ml and 3.0-10.0 µl/ml for camphor [Sokovic 2002].

Three-lobed sage leaf essential oil exhibited low activity against *Erwinia herbicola* (MIC: 2 mg/ml) and was inactive against *Pseudomonas syringae*, whereas leaf surface phenolics extracted with dichloromethane inhibited both bacterial strains. Camphor, with an MIC and MBC (minimum bactericidal concentration) of 2 mg/ml, was more effective than 1,8-cineole (both values ≥ 2 mg/ml) [Karamanoli 2000].

Essential oil of three-lobed sage leaf cultivated in Brazil inhibited the growth of *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhimurium*, *Aeromonas* sp., *Klebsiella oxytoca*, *Citrobacter* sp., *Serratia marcescens*, *Bacillus* sp., *Pseudomonas* sp. and *Staphylococcus* sp. The MICs (0.1 to 10 mg/ml) and MBCs (0.1 to >10 mg/ml) indicated that the essential oil of *Salvia triloba* was generally more effective than that of *S. officinalis* against these bacterial strains. The activity was more pronounced against Gram-negative bacteria [Delamare 2007].

In the disk diffusion assay the essential oil, 1,8-cineole and thujone showed moderate activity against a selection of eight bacteria, *Salmonella typhimurium* being the most resistant and *Pseudomonas aeruginosa* being the most sensitive, while camphor was almost inactive. The essential oil was bactericidal against *Staphylococcus aureus* at 1/4000 dilution, while dilutions up to 1/10000 caused a considerable decrease in bacterial growth rates [Sivropoulou 1997].

Hydrosols of three-lobed sage leaf (the aqueous phases obtained by 1 hour of steam distillation) were inactive against fifteen bacterial strains such as *E. coli* or *S. aureus* [Sagdic 2003].

The virucidal activity of three-lobed sage leaf essential oil and its main components was evaluated against HSV-1, an ubiquitous human pathogen. At a concentration of 0.2% the essential oil inactivated 80% of infectious virus within 30 minutes and thujone at 0.1% inactivated 95%, whereas 1,8-cineole and camphor at 0.1% inactivated 35% and 0% respectively [Sivropoulou 1997].

Other effects
Pretreatment of murine fibrosarcoma cells with a dichloromethane-methanol dry extract at 62.5 µg/ml revealed a weak inhibitory effect on the IL-6 promoter and the recombinant NFκB promoter construct [Kaileh 2007].

An ethanolic extract showed moderate binding affinity at the $GABA_A$-benzodiazepine receptor site [Salah 2005].

In vivo experiments

Antidiabetic activity
The hypoglycaemic activity of a 10% infusion was evaluated in normoglycaemic rabbits and rabbits with alloxan-induced diabetes. A single oral dose of 2.5 ml/kg b.w. (corresponding to 0.25 g/kg of dried leaf) had no acute effect on blood glucose levels in either group. Repeated oral administration at 2.5 ml/kg/day over a period of 7 days produced a significant reduction of blood glucose levels in hyperglycaemic rabbits but had no effect in normoglycaemic animals. Compared to hyperglycaemic control animals, blood glucose levels in treated hyperglycaemic rabbits were 20.2% lower 60 minutes after the last administration of infusion ($p<0.05$), 24.9% lower after 90 minutes ($p<0.01$) and 25.7% lower after 120 minutes ($p<0.01$).

After oral glucose loading at 1 g/kg, administration of a single oral dose of the infusion at 0.25 ml/kg significantly reduced blood glucose levels in both normo- and hyperglycaemic animals, the effect being more evident in the latter. In treated hyperglycaemic rabbits the increase in blood glucose was only 19.2% ($p<0.05$), 7.8% ($p<0.01$) and 7.1% ($p<0.01$) after 60, 90 and 120 minutes, compared to 78.2%, 73.1% and 55.5% in hyperglycaemic control animals receiving water only.

A single oral administration of the infusion had no significant effects on plasma insulin levels in any group of rabbits following oral glucose loading, nor on blood glucose levels in normoglycaemic animals after intravenous glucose loading. The results suggested that three-lobed sage leaf does not influence glucose metabolism; the hypoglycaemic activity might be due to inhibition of intestinal absorption of glucose [Perfumi 1991].

In another study, the effect of an infusion (5:1) in streptozotocin (STZ)-induced diabetic rats was investigated. One week after i.p. injection of a single dose of 45 mg/kg b.w. STZ, the glucose level was 311 ± 25 mg/dL in diabetic rats (n=12) compared to 119 ± 4 mg/dL in healthy rats (n=12). In water drinking STZ-diabetic animals (n=6), plasma glucose levels continued to increase throughout the 14-day experimental period but remained stable in STZ-diabetic animals (n=6) drinking the infusion (0.28% w/v, ad libidum) ($p<0.01$ on day 14). In non-diabetic animals glucose levels were not influenced by drinking the infusion (n=6). The administration of the infusion did not significantly change the liver glycogen content or induce liver toxicity or increase the regeneration of β-cell mass. The increase in intestinal Na^+/glucose cotransporter-1 (SGLT1), localized to the enterocyte brush-border membrane, was significantly abrogated by treatment with the infusion (30% compared to untreated animals, $p<0.01$) without changes in total cellular transporter protein levels. Comparable results were obtained in rats with high carbohydrate diet-induced diabetes (n=6) drinking the infusion (32%, $p<0.05$) when compared to a water drinking group (n=6). Rosmarinic acid (0.58 mg/mL) in the drinking water inhibited SGTL1 to 50% ($p<0.01$). No effects

were observed on glucose transporter 2, Na^+/K^+-ATPase or glucagon-like peptide-1 levels by the treatment [Azevedo 2011].

Antihypertensive activity
A dry decoction, administered intraperitoneally to nembutal-anaesthetized, spontaneously hypertensive rats as a single dose of 50 mg/kg b.w., reduced blood pressure by 31% compared to saline controls after 120 minutes [Todorov 1984].

Anti-inflammatory activity
Three-lobed sage leaf was successively extracted with chloroform (dry yield: 6.75%), ethanol (4%), butanol (1%) and water (> 20%, viscous extract). Oral administration of the chloroform fraction at 25 mg/kg b.w. significantly inhibited carrageenan-induced rat paw oedema by 17% ($p<0.01$) compared to 47% by diclofenac at 10 mg/kg as positive control ($p<0.01$); the other fractions were less effective. In the cotton pellet granuloma assay the chloroform fraction inhibited chronic mean gain in pellet weight by 18 mg compared to 29 mg by diclofenac ($p<0.01$) [El-Sayed 2006].

Central effects
A dry decoction and the dry benzene fraction from an infusion, administered subcutaneously to mice at 100 mg/kg b.w. 10 minutes before administration of hexobarbital (80 mg/kg b.w.), delayed the onset of sleep [Todorov 1984].

In another study, a 75%-hydroethanolic dry extract (1g/60 ml; total phenolic content: 10.9 mg/g, expressed as gallic acid equivalent) exerted dose-dependent anti-amnesic activity in mice. A passive avoidance apparatus (step-through type) was used to investigate long-term memory; amnesia was induced by scopolamine (1 mg/kg, given intraperitoneally 30 minutes after oral administration of the extract) and donezepil was used as a positive control. The extract had a relative memory-enhancing effect of 57.1 and 71.4% at 200 and 400 mg/kg b.w. respectively (control group 0%, donezepil group set to 100%) [Orhan 2009].

Clinical studies
No clinical studies have been published on mono-preparations of three-lobed sage leaf.

Pharmacokinetic properties
No data available.

Preclinical safety data

Mutagenicity
An aqueous dry extract (1 g/100 ml, extracted at 50°C) at 40 and 80 µg per plate showed no mutagenic potential in the Ames test using *Salmonella typhimurium* strains TA98 and TA100 with and without metabolic activation. In the alkaline single cell gel electrophoresis (COMET) assay in human lymphocytes treatment with 40 µg of the aqueous dry extract produced a significant increase in COMET tail moments when compared to the positive control, 4 mM hydrogen peroxide (22.0 vs 13.2, $p<0.01$) [Basaran 1996].

Essential oil of three-lobed sage leaf at concentrations of 250-2000 ppm did not exhibit any mutagenic activity in the Ames test using *S. typhimurium* strains TA97, TA98, TA100 and TA102 in [Adam 1998].

Effects on fertility
Ingestion of an aqueous dry extract by female rats at 200, 400 and 800 mg/kg b.w. from days 1 to day 6 of pregnancy had no significant effects on the number of pregnancies, implantations, viable fetuses or resorptions when compared to the control. Intragastric administration of a 95%-ethanolic dry extract at 400 mg/kg also had no effect on the number of pregnancies or implantations; however, the number of viable fetuses decreased ($p<0.005$) and the number of resorptions increased ($p<0.005$).

In contrast, intragastric administration of the aqueous dry extract at 800 mg/kg or the ethanolic dry extract at 400 mg/kg to adult female rats for 30 consecutive days had no effect on the occurrence of pregnancy, but the number of implantations ($p<0.05$) and viable fetuses ($p<0.01$) decreased and the number of resorptions increased ($p<0.01$ for aqueous dry extract, $p<0.005$ for ethanolic dry extract). Ingestion of the same extracts at the same dosage levels for 30 consecutive days by adult male rats had no effect on the final proportion of pregnant females impregnated by these males; however the number of implantations and viable fetuses were reduced in such females ($p<0.01$ for aqueous dry extract, $p<0.001$ for ethanolic dry extract), whereas the number of resorptions increased ($p<0.005$). Prenatal exposure of male and female rat offspring to the ethanolic dry extract at 400 mg/kg had no effect on the timing of testicular descent or vaginal opening [Al-Hamood 1998].

Ulcerogenicity
Three-lobed sage leaf was successively extracted with chloroform (dry yield: 6.75%), ethanol (4%), butanol (1%) and water (> 20%, viscous extract), and each fraction was orally administered to rats at 25 mg/kg b.w. The ulcer indices were 0 for the ethanol fraction, 1.2 for the water fraction ($p<0.01$) and 1.3 for the chloroform fraction ($p<0.01$), compared to 12.3 for acetylsalicylic acid at 1 mg/kg ($p<0.01$) [El-Sayed 2006].

Toxicity of thujone
Although α-thujone is present in the essential oil of three-lobed sage leaf, the proportion is far less than in essential oils of *Salvia officinalis* or *Artemisia absinthium*; aqueous preparations are unlikely to contain toxic amounts of α-thujone.

Clinical safety data
No data available.

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MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Second Edition, 2003
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Second Edition, 2003
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Second Edition, 2003
RATANHIAE RADIX	Rhatany Root	Supplement 2009
RHAMNI PURSHIANI CORTEX	Cascara	Second Edition, 2003
RHEI RADIX	Rhubarb	Second Edition, 2003
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Second Edition, 2003
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Second Edition, 2003
SALICIS CORTEX	Willow Bark	Second Edition, 2003
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Second Edition, 2003
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VIOLAE HERBA CUM FLORE	Wild Pansy	Supplement 2009
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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The second edition of ESCOP Monographs, published as a hardback book in 2003 with a Supplement in 2009, has been widely acclaimed for its authoritative information on the therapeutic uses of herbal medicines. Monographs covering a total of 107 herbal substances include extensive summaries of pharmacological, clinical and toxicological data, and copious references to scientific literature form an important part of each text.

Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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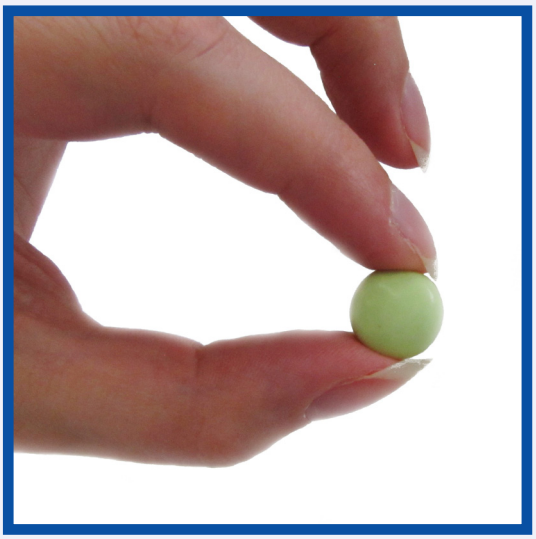
The Scientific Foundation for Herbal Medicinal Products

Sambuci flos

Elder flower

2013



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Herbal Medicinal Products

SAMBUCI FLOS
Elder Flower

2013

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

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Plant illustrated on the cover: *Sambucus nigra*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

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Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
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Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

SAMBUCI FLOS

2013

Elder Flower

DEFINITION

Elder flower consists of the dried flowers of *Sambucus nigra* L. It contains not less than 0.8% of flavonoids, expressed as isoquercitrin ($C_{21}H_{20}O_{12}$; M_r 464.4) and calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Elder flower].

CONSTITUENTS

The characteristic constituents are flavonoids (0.7-3.5%) such as rutin, isoquercitrin, hyperoside, astragalin and glycosides of isorhamnetin [Dawidowicz 2006, Leifertova 1971, Lin 2007, Males 1999, Petitjean-Freytet 1991]; about 3% of phenolic acids (chlorogenic, ferulic and $_p$-coumaric acids) and their glycosides [Leifertova 1971, Lin 2007, Males1999, Petitjean-Freytet 1991]; and 0.1-0.3% of essential oil consisting mainly of monoterpenes such as linalool and its oxides, citronellol and hotrienol [Eberhardt 1985a, Eberhardt 1985b, Jørgensen 2000, Kaack 2006, Velísek 1981].

Other constituents include fatty acids and alkanes [Richter 1974, Toulemonde 1983], triterpenes such as α- and β-amyrin and ursolic acid, sterols [Makarova 1997, Willuhn 1977], *N*-phenylpropenoyl-L-amino acid amides (0.01%) such as N-(*E*)-caffeoyl-L-aspartic acid amide [Hensel 2007], and about 2% of potassium [Szentmihályi 1998].

CLINICAL PARTICULARS

Therapeutic indications

As a diaphoretic in the treatment of common cold, fever and chills [Bradley 1992, Hiermann 1994, Willuhn 2002].

Efficacy is plausible for this indication on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Adult dose, three times daily: 3-5 g of dried flowers as an infusion; 3-5 ml of liquid extract (1:1, ethanol 25% V/V); 10-25 ml of tincture (1:5, ethanol 25% V/V) [Bradley 1992, Hiermann 1994, Willuhn 2002].

Method of administration
For oral administration.

Duration of administration
No restriction. If symptoms persist or worsen, medical advice should be sought.

Contraindications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with normal medical practice, elder flower should not be used during pregnancy and lactation without medical advice.

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Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antioxidant and radical scavenging activity
Free radical scavenging and antioxidant activities of hydroethanolic (80% V/V) extracts of elder flower obtained by pressurized liquid extraction at varying temperatures (20-200°C) were determined in the DPPH assay. The extract obtained at 20°C (1.4% flavonoids) reduced DPPH by 92% after 120 minutes, and the extract obtained at 100°C (2.1% flavonoids) by 94% [Dawidowicz 2006].

Antiadhesive activity
Preincubation of *Helicobacter pylori* with N-(*E*)-caffeoyl-L-aspartic acid amide (1 mg/ml) led to strong and reproducible inhibition of adhesion of the bacteria to sections of human stomach mucosa. No bactericidal or bacteriostatic activities were observed [Hensel 2007].

Anti-inflammatory activity
A dry aqueous extract of elder flower (0.25 mg/ml) inhibited PAF-induced exocytosis of elastase from human neutrophils by 57% in a spectrophotometric assay compared to distilled water as a blank. The positive control (ginkgolide B) inhibited exocytosis by 60% [Tunon 1995].

A dry methanolic extract of elder flower (30 µg/ml in DMSO) inhibited release of the cytokines IL-1α, IL-1β and TNF-α from human peripheral mononuclear cells by 44, 49 and 50% respectively, compared to a solvent blank. A dry hexane fraction (38, 45 and 31% inhibition respectively) and a dry chloroform fraction (31, 44 and 57% inhibition respectively) from the methanolic extract showed comparable activity, whereas a dry *n*-butanol fraction and the residual water showed lower or no activity at this concentration [Yesilada 1997].

An infusion of elder flower (≥0.005 g/100 ml) significantly inhibited induction of TNF-α release ($p<0.05$) from LPS- and fimbriae-stimulated human monocytic THP-1 cells (cells differentiated into macrophages). Complete inhibition was achieved at 0.16 g/100 ml. Release of the pro-inflammatory cytokines TNF-α, IL-1β and IL-6 using additional bacterial stimuli was also potently inhibited in the monocytic THP-1 cells, as well as in mouse macrophages, at the same concentrations. In contrast, release of the anti-inflammatory cytokine IL-10 was only moderately inhibited. The infusion significantly inhibited activation of NF-κB, a central factor in pro-inflammatory gene expression, by several stimuli ($p<0.05$) at 0.08 g/100 ml in both human and mouse macrophages. Bacterial activation (by *Porphyromonas gingivalis*) of integrin, a possible effector of pathological inflammation, in human neutrophils was significantly suppressed by the same infusion ($p<0.05$ at 0.08-0.3 g/100 ml). The greatest inhibition of 73-76%, observed at a dilution of 0.3 g/100 ml, was at least as effective as a positive control. The oxidative burst of PMA- and bacteria-activated human neutrophils, monitored by a dichlorofluorescein assay, were significantly and concentration-dependently inhibited by the same elder flower infusion ($p<0.05$). Almost complete inhibition was observed at 0.16 and 0.3 g/100 ml respectively [Harokopakis 2006].

N-(*E*)-caffeoyl-L-aspartic acid amide (10 µg/ml) significantly increased mitochondrial dehydrogenase activity ($p<0.05$) in HepG2 human liver cells by 52%, estimated by the MTT assay after 48 hours. In human keratinocytes, mitochondrial activity and the mitotic cell proliferation rate both increased by about 55% after 60 hours of incubation ($p<0.01$) at 10 µg/ml. In HepG2 cells proliferation was slightly enhanced. No necrotic cell toxicity was observed [Hensel 2007].

Antidiabetic activity
An aqueous extract of elder flower (1 g/litre) increased glucose uptake by 70% ($p<0.05$), glucose oxidation by 50% ($p<0.01$) and glycogenesis by 70% ($p<0.05$) in the absence of insulin in an isolated insulin-sensitive mouse abdominal muscle preparation, but did not significantly alter the insulin stimulus. The extract (0.25-1 g/litre) exerted a significant stimulatory effect on insulin secretion from clonal pancreatic β-cells in a concentration-dependent manner (4.5 pmol insulin/million cells after 20 minutes at 1 g/litre, $p<0.01$). This insulin-releasing effect was significantly potentiated in the presence of 16.7 mM glucose ($p<0.001$). The activity decreased by 41% after the removal of constituents of MW < 2000 Da. Consecutive extraction of elder flower with *n*-hexane, ethyl acetate, methanol and water showed that only the methanol and water extracts increased insulin release ($p<0.001$). Known elder flower constituents such as rutin, lupeol and β-sitosterol had no effect on insulin release [Gray 2000].

In vivo experiments

Diuretic activity
A dry aqueous elder flower extract (approximately 5:1), administered intraperitoneally in saline to rats as a single dose of 50 mg/kg b.w., significantly increased urinary volumes compared to a saline control in the period from 2 to 24 hours after administration (after 2 hours, 2.4 ml urine/100 g vs. 1.6 ml urine/100 g, $p<0.05$; after 24 hours, 7.5 ml urine/100 g vs. 5.8 ml urine/100 g, $p<0.01$). Urinary volumes obtained with hydrochlorothiazide (10 mg/kg) were 2.8 ml/100 g after 2 hours and 7.1 ml/100 g after 24 hours ($p<0.05$). Increases in urinary excretion of sodium and potassium were observed after 8 hours, while the pH remained unchanged (pH 8.4-8.8). The saline overload was eliminated within 5.0 hours in the case of the elder flower extract and within 5.3 hours in the case of hydrochlorothiazide [Beaux 1999].

Anti-inflammatory activity
Intragastric administration of an elder flower dry extract (ethanol 80% V/V) at 100 mg/kg b.w. inhibited carrageenan-induced rat paw oedema by 27% ($p<0.01$) compared to 45% by indomethacin at 5 mg/kg [Mascolo 1987].

In another study, intraperitoneal administration of an unsaponifiable fraction of elder flower to mice at 0.5 ml/animal moderately enhanced phagocytosis [Delaveau 1980].

Secretolytic activity
Daily intragastric administration of a hydroethanolic extract (19% V/V, corresponding to 0.6 g elder flower/100 ml) to rabbits at 6.5 ml/kg b.w. for 3 days significantly increased bronchial secretion by 43% compared to the control (19% V/V ethanol) and by 111% compared to physiological saline ($p<0.01$) [Chibanguza 1984].

Pharmacokinetic properties
No data available.

Preclinical safety data

Acute toxicity
After daily intragastric administration of a hydroethanolic extract (19% V/V, corresponding to 0.6 g elder flower/100 ml) to rabbits at 6.5 ml/kg b.w. for 3 days, no significant changes in breathing and pulse rates, number of erythrocytes or sodium, calcium and potassium concentrations were observed, compared to the control (19% V/V ethanol) [Chibanguza 1984].

Clinical safety data
No data available.

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Supplement 2009
ALOE CAPENSIS	Cape Aloes	Second Edition, 2003
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Second Edition, 2003
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
BALLOTAE NIGRAE HERBA	Black Horehound	Supplement 2009
BETULAE FOLIUM	Birch Leaf	Second Edition, 2003
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Second Edition, 2003
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Supplement 2009
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Second Edition, 2003
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Second Edition, 2003
FUMARIAE HERBA	Fumitory	Supplement 2009
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Supplement 2009
GRINDELIAE HERBA	Grindelia	Supplement 2009
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Second Edition, 2003
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Second Edition, 2003
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003

LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Supplement 2009
MARRUBII HERBA	White horehound	Online Series, 2013
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MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
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MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Second Edition, 2003
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Second Edition, 2003
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Second Edition, 2003
RATANHIAE RADIX	Rhatany Root	Supplement 2009
RHAMNI PURSHIANI CORTEX	Cascara	Second Edition, 2003
RHEI RADIX	Rhubarb	Second Edition, 2003
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Second Edition, 2003
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Second Edition, 2003
SALICIS CORTEX	Willow Bark	Second Edition, 2003
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Second Edition, 2003
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VIOLAE HERBA CUM FLORE	Wild Pansy	Supplement 2009
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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The second edition of ESCOP Monographs, published as a hardback book in 2003 with a Supplement in 2009, has been widely acclaimed for its authoritative information on the therapeutic uses of herbal medicines. Monographs covering a total of 107 herbal substances include extensive summaries of pharmacological, clinical and toxicological data, and copious references to scientific literature form an important part of each text.

Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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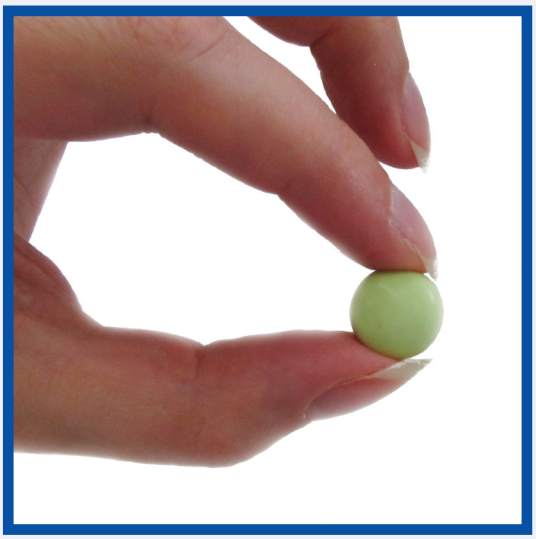
The Scientific Foundation for Herbal Medicinal Products

Serpylli herba
Wild Thyme

2014



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MONOGRAPHS

***The* Scientific Foundation for**
Herbal Medicinal Products

SERPYLLI HERBA
Wild Thyme

2014

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Second Edition, completely revised and expanded

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

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Plant illustrated on the cover: *Thymus serpyllum*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
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Notes for the Reader
Abbreviations
The monograph text
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Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

SERPYLLI HERBA

2014

Wild Thyme

DEFINITION

Serpylli herba consists of the whole or cut, dried, flowering aerial parts of *Thymus serpyllum* L.s.l. It contains not less than 3.0 mL/kg of essential oil (dried drug).

The material complies with the European Pharmacopoeia [Wild thyme].

CONSTITUENTS

Essential oil: 0.1 to 0.6% with carvacrol as major compound and variable amounts of thymol, linalool, linalyl acetate, borneol, 1,8-cineole, germacrene D, p-cymene, γ-terpinene, myrcene, β-caryophyllene [Loziene 1998; Mockute 2004; Raal 2004; Sefidkon 2004; Stahl-Biskup 2007; Paaver 2008; Cavar 2009, Schilcher 2010, Nedorostova 2011, Hussain 2013]. The composition depends largely on the species, genotype, ontogenic development, origin and growth conditions [Rasooli 2002].

Triterpenoids such as ursolic acid (0.9 to 1.4%) and oleanolic acid (0.37 to 0.48%), and low amounts of 3β-hydroxyolean-12-en-28-oic acid and dihydroursolic acid [Janicsak 2006; Aziz 2008; Stahl-Biskup 2009].

Hydroxycinnamic acids (1.3 to 6.8%) with rosmarinic acid (0.76 to 2.44%) and lithospermic acid (0.31 to 4.28%) as main components [Ziakova 2003; Kulisic 2007; Stahl-Biskup 2007; Fecka 2008, Boros 2010].

Flavonoids (0.95 to 2.99%) such as luteolin-7-O-glucuronide, luteolin-7-O-glucoside, luteolin-7-O-rutinoside, eriocitrin or scutellarin-7-O-β-D-glucopyranosyl(1→4)-O-α-L-rhamnopyranoside [Washington 1986; Kulisic 2007; Fecka 2008, Miron 2011].

Tannins (3.4 to 7.4 %) [Stahl-Biskup 2009, Komes 2011]; amino acids [Mazulin 2002].

CLINICAL PARTICULARS

Therapeutic indications

Internal use:
Catarrh of the upper respiratory tract [Jänicke 2003; Schilcher 2010; Stahl-Biskup 2007; Stahl-Biskup 2009].

External use:
As a bath additive in the supportive treatment of acute or chronic diseases of the respiratory tract [Jänicke 2003; Stahl-Biskup 2007].

Efficacy in these indications is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Internal use:
Adult daily dose: 4-6 g of the drug [Jänicke 2003; Schilcher 2010; Stahl-Biskup 2007; Stahl-Biskup 2009].
As an infusion, 2 g (1 - 2 teaspoons) in 150 mL of water, 1 cup before meals 2 to 3 times daily [Jänicke 2003; Stahl-Biskup 2007; Stahl-Biskup 2009].

External use:
Decoction: 1 g of the drug per litre of water [Jänicke 2003].

Method of administration
For oral administration and local application.

E/S/C/O/P MONOGRAPHS

Duration of administration
No restriction. If symptoms persist or worsen, medical advice should be sought.

Contraindications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No case of overdose reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antioxidant activity
An extract (80% methanol; 5:1) exhibited antioxidant activity in the ferrylmyoglobin/$ABTS^{\bullet+}$ assay with a Trolox equivalent antioxidant capacity of 14.7 mM/g dry weight. The effect correlated with the content of total phenolics [Alzoreky 2001].

A ferric reducing/antioxidant power (FRAP) of 10.9 mmol/L was determined for an infusion (3 g/200 mL); this correlated with the total phenol content; a phenol antioxidant coefficient (FRAP/ total phenolics) of 3.7 was found [Katalinic 2006].

In a broad screening for antioxidant activity, an infusion (15 g/150 mL dried and residue redissolved to 60 g/L) had an IC_{50} of 0.45 g/L in the diphenylpicrylhydrazyl (DPPH) assay and an antioxidant activity index of 1.90 in the Rancimat assay. The activity in the carotene bleaching assay was low and the infusion remained without effect in the TBARS assay [Kulisic 2006].

An infusion (2 g/200 mL) showed antioxidant activity in the ABTS assay (equivalent to 2.44 mM Trolox after 5 min extraction). After 15 min extraction the activity was equivalent to approximately 3.6 mM Trolox and after hydrolysis of the extract to approximately 4.8 mM Trolox. In the FRAP assay the capacity was equivalent to 7.48 mM Fe(II) after 5 min extraction, 6.49 mM Fe(II) after 15 min extraction and approximately 16 mM Fe(II) after hydrolysis [Komes 2011].

In the carotene/linoleic acid assay extracts from wild thyme inhibited lipid peroxidation with IC_{50} values of 261 µg/mL (water extract), 41.2 µg/mL (ethanolic extract) and 96.9 µg/mL (essential oil), whereas the IC_{50} of butylated hydroxytoluene (BHT) as positive control was 12 µg/mL. The IC_{50} values in the DPPH assay were 31.6 µg/mL for the water extract, 13.2 µg/mL for the ethanolic extract and 15.7 µg/mL for BHT [Mata 2007].

Copper-induced LDL oxidation was inhibited by an infusion as well as by the essential oil of wild thyme at concentrations of 0.02 g/L [Kulisic2007].

The relative inhibition of copper-catalyzed oxidation of human LDL by the essential oil was 45.5% [Teissedre 2000].

In the DPPH assay the essential oil showed a radical scavenging activity equivalent to 87% of the activity of BHT [Topal 2008].

Antimicrobial activity

In a disc diffusion assay the essential oil showed strong bactericidal activity against *Staphylococcus aureus* and *Bacillus subtilis* [Rasooli 2002].

S. aureus, Yersinia enterocolitica and two strains of *E. coli* were inhibited by the essential oil as shown in a disc diffusion assay and by serial dilution method [Sagdic 2003].

Wild thyme essential oil was in the group with the highest inhibitory activity against infectious vaginal microorganisms such as *Candida albicans, Streptococcus agalactiae* and *Enterococcus faecalis* [Arnal-Schnebelen 2004].

In an agar dilution test the essential oil was found to have MICs of <0.05 to <0.2% and maximal tolerated concentrations of <0.006 to <0.05% (v/v) against *E. coli*, *Salmonella typhimurium*, *S. aureus* and *Listeria monocytogenes* [Oussalah 2007].

The antibacterial activity of the essential oil against different strains of *S. aureus* was also tested in the vapour phase. MICs for two methicillin-sensitive strains were 33 µL/L, for MRSA 260 µL/L and for several clinical isolates of *S. aureus* between 66 and 130 µL/L [Nedorostova 2012].

In another study using the agar diffusion method, the essential oil of wild thyme was the most active against *E. coli, S. aureus, Klebsiella pneumoniae* and *Staphylococcus epidermidis* as compared to 18 other essential oils [Bulut 2009].

Spore germination and mycelial growth of different *Aspergillus* species were inhibited by the essential oil in a dose-dependent manner. The inhibition of sporulation was less pronounced [Rahman 2003].

In a box vapour assay for antifungal activity the essential oil was tested and showed high potency against *Trichophyton mentagrophytes*. The activity was attributed to the content of carvacrol. The results correlated with those of an agar diffusion assay [Inouye 2006].

Acetylcholinesterase inhibitory activity
The essential oil of wild thyme showed the strongest inhibition of acetylcholinesterase (IC_{50} 190 µg/mL) as compared to an ethanolic (IC_{50} 252 µg/mL) and an aqueous extract (IC_{50} 348 µg/mL) of the same plant material [Mata 2007].

Antiproliferative effects
A methanolic dry extract (not further specified) showed cytotoxic activity against MCF-7 and MDA-MB-231 breast cancer cells with IC_{50} values of 509 and 276 µg/mL. The extract induced apoptosis as shown via analysis of DNA fragmentation and caspase 3/7 activity. The activities of DNA methyltransferase and histonedeacetylase were significantly reduced by 250 and 500 µg/mL of the extract ($p<0.05$) [Bozkurt 2012].

The essential oil exhibited an antiproliferative activity in MCF-7 cells (IC_{50} 95.8 µg/mL), LN-CaP prostate cancer cells (IC_{50}

105.0 µg/mL) and NIH-3T3 fibroblasts (IC_{50} 105.0 µg/mL) [Hussain 2013].

In vivo experiments
Administration of a freeze dried aqueous extract of wild thyme to euthyroid rats (2.5 mg/100 g b.w. i.v.) decreased serum thyroid stimulating hormone (TSH) concentration by about 50% within 3 hours compared to the control group. The pituitary TSH concentration was significantly reduced to approximately 40% of control ($p<0.005$-0.001). The concentrations of thyroxine (T4) remained unchanged while triiodothyronine (T3) was slightly decreased. After a high dose of 40 mg/100 g b.w. the serum TSH level and thyroidal secretions decreased rapidly and remained suppressed during the 12 hours observation period. There was also a significant reduction ($p<0.005$-0.001) in the T4 and T3 levels at 3 and 6 hours post-injection. Serum prolactin levels were significantly increased after the low dose (2.5mg/100g; $p<0.05$); but significantly decreased after the high dose (40mg/100g; $p<0.005$-0.001), this effect vanished between 6 and 12 hours after injection. In goitrous rats, a 70% ethanolic extract (5 mg/kg b.w. i.v.) led to a significant decrease ($p<0.05$-0.01) in the serum TSH concentration. However, the pituitary TSH concentration was increased significantly ($p<0.05$) only by an ethanolic extract oxidized with KMnO4 [Sourgens 1982].

Clinical studies
No data available.

Pharmacokinetic properties
No data available.

Preclinical safety data
No data available.

Clinical safety data
No data available.

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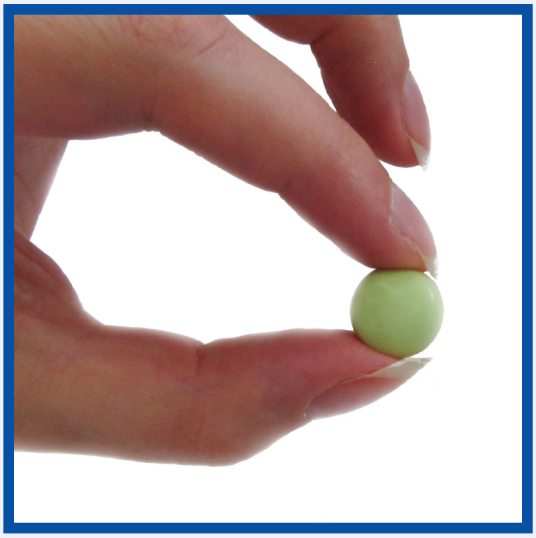

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
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Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
CCl_4	carbon tetrachloride
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase

INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

SOLIDAGINIS VIRGAUREAE HERBA 2018

European Goldenrod

DEFINITION

European Goldenrod consists of the whole or fragmented, dried, flowering aerial parts of *Solidago virgaurea* L. It contains minimum 0.5 per cent and maximum 1.5 per cent of flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried drug).

The material complies with the monograph of the European Pharmacopoeia [Goldenrod, European].

CONSTITUENTS

Flavonoids (approx. 1.5%) including astragalin, hyperoside, isoquercitrin, nicotiflorin, quercitrin and rutin; triterpene saponins of the oleanane type (0.2 – 0.3%) mainly bisdesmosidic glycosides of polygalacic acid; phenol diglucosides (0.2-1%) leiocarposide and virgaureoside A; caffeic acid derivatives, predominantly 3,5-di-*O*-caffeoylquinic acid (2.8%) and chlorogenic acid (1.1%); phenolic acids such as salicylic and caffeic acid; essential oil (0.4 – 0.6%) [Hiller 1975, 1979, 1985, 1986; 1991; Bader 1990a, 1991, 1992, 1995, 1999; Bradley 2006; Fötsch 1988, Inose 1991, 1992; Kalemba 1992, Miyase 1994; Poetsch 1997; Wittig 1999; Starks 2010].

CLINICAL PARTICULARS

Therapeutic indications
Irrigation of the urinary tract, especially in cases of inflammation and renal gravel, and as an adjuvant in the treatment of bacterial infections of the urinary tract [Schilcher 1990, 1992, 2001; Schmitt 1996; Laszig 1999a, 1999b; Weiß 1999, Bader 2008].

Posology and method of administration

Dosage

Adults: An infusion of 3-5 g of comminuted herbal substance in 150 ml of water, 2-4 times daily. Dry extract: 350-450 mg, 3 times daily [Weiß 1999; Schilcher 2000; Edwards 2015; Volk 2015].

Children: 1-4 years of age, 1-2 g of dried herb daily as an infusion; 4-10 years of age, 2-5 g; 10-16 years of age: 4-8 g [Dorsch 1998].

Method of administration
For oral administration.

Duration of administration
No restriction.

Contra-indications
Known hypersensitivity to plants of the Asteraceae (Compositae) [Lundh 2006; Mills 2005; Schätzle 1998; Zeller 1985].

Special warnings and special precautions for use
European golden rod should not be used in patients with oedema due to impaired heart or kidney function [Mills 2005].

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

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Effects on ability to drive and use machines
None known.

Undesirable effects
Rare cases of minor gastrointestinal complaints [Mills 2005].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties
European goldenrod has shown diuretic, anti-inflammatory, antimicrobial, antispasmodic and analgesic properties.

In vitro experiments

Spasmolytic activity
A 60% hydroethanolic extract inhibited acetylcholine-induced spasms in isolated guinea pig ileum with 15% of the activity of papaverine [Westendorf 1981]. The extract also inhibited acetylcholine-induced spasms in isolated rat bladder [Westendorf 1983].

An aqueous leaf extract (not further specified) inhibited muscarinic M2 and M3 receptor-mediated contraction of rat and human urinary bladder muscle strips. Radioligand binding and inositol phosphate accumulation showed that the extract at a concentration of 0.01% inhibited muscle contraction through a non-competitive muscarinic receptor antagonism, whereas at a concentration of 0.1% a non-specific inhibitory effect was observed [Borchert 2004].

Diuretic activity
Acylated triterpenoid saponins (especially virgaureasaponin B) transiently changed the cell membrane permeability and induced alterations in ionic homeostasis with enhanced permeability between the intracellular and extracellular compartments [Melzig 2001].

Quercetin and its derivatives inhibited neutral endopeptidase, which regulates the formation of urine via the excretion of sodium ions [Apati 2003].

A flavonoid fraction from a methanolic extract increased urinary calcium excretion [Chodera 1991].

Anti-inflammatory activity
A hydroethanolic extract (containing 0.52 mg/mL of rutin, 0.64 mg/mL of flavonoids and 45.6%V/V of ethanol) at concentrations from 0.01 to 5% inhibited biochemical model reactions (including xanthine oxidase, diaphorase in the presence of the auto-oxidizable quinone juglone, lipoxygenase, dihydrofolate reductase) and photodynamic reactions driven by riboflavin or rose bengal, representing inflammatory situations [Meyer 1990, 1995; Strehl 1995].

A tincture (70% v/v ethanol) and an infusion (1/40) showed *in vitro* radical scavenging activity at 1 mg/mL; as well as inhibition of hydrogen-donating ability and elevated reducing power [Apáti 2003]. A hydromethanolic (50% v/v) extract dose-dependently increased glutathione-S-transferase (GST) activity. Isolated rutin and quercitrin (250 mM) increased dose-dependent GST activity, by 50% and 24.5% respectively, while the aglycone quercetin inhibited the enzyme by 30% at 250 mM. Fractions containing flavonoids showed activating effects while those containing caffeic acid derivatives were inhibitory [Apáti 2006]. The compounds 2'-methoxybenzyl-2-methoxy-6-hydroxy-benzoate and 2'-methoxy-benzyl-2,6-dimethoxybenzoate isolated from a 90% ethanolic extract inhibited the release of TNF-α and IL-6 release from LPS-induced RAW264.7 murine monocytes [Li 2014].

Antibacterial activity
A methanolic extract was active against *Staphylococcus aureus*, *Enterobacter faecalis*, *Escherichia coli* and *Bacillus cereus*. The MIC value against bacteria was 50 µg/mL) [Demir 2009].

Nine clerodane diterpenes, solidagoic acids C-I, cleroda-3,13(14)-dien-16,15:18,19-diolide and cleroda-3,13(14)-dien-15,16:18,19-diolide displayed moderate antibacterial activity against *Staphylococcus aureus* with a MIC from 20 to 60 µg/mL [Starks 2010].

Antifungal activity
Deacylated triterpenoid saponins (virgaureasaponins 1, 2 and 3), showed higher activity against several Candida species (*C. albicans*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, *C. pseudotropicalis*, *C. guilliermondi*, *C. glabrata*) and *Cryptococcus neoformans* than a mixture of genuine ester saponins [Bader 1990b].

A 75% ethanolic extract (1:1) showed antimycotic activity against dermatophytes, especially against *Trichophyton mentagrophytes*, *Microsporum gypseum* and *Microsporum canis* [Pepeljnjak 1998].

An aqueous extract containing 0.7 mg/mL saponins did not inhibit the growth of *Candida albicans* but did decrease the yeast-hyphal transition, biofilm formation and pre-formed biofilms which are key virulence factors of *Candida albicans* [Chevalier 2012].

A hydromethanolic (50%) extract revealed activity against *Candida albicans* hyphae, the pathogenic form of this yeast. In an activity-guided fractionation from an active saponin fraction six oleanane-type triterpenoid saponins were isolated, of which four showed significant ($p<0.05$) inhibition of *C. albicans* yeast-hyphal conversion [Laurençon 2013].

Immunobiological activity
Mitogenic effects on murine spleen and thymus cells as well as on human mononuclear cells were demonstrated for triterpenoid saponins from European goldenrod. The activity of murine bone marrow macrophages was stimulated and induction of cytotoxic macrophages and TNF-α release from murine macrophages were observed [Plohmann 1997].

Treatment of mouse peritoneal macrophages with isolated 2-methoxybenzyl-2-hydroxybenzoate and benzyl-2-hydroxy-6-methoxybenzoate at concentrations from 1 to 100 µg/mL resulted in a stimulation of macrophage function and TNF-α production [Choi 2005].

Cytotoxic activity
Aqueous and ethanolic extracts exhibited cytotoxic activity on various tumour cell lines, including human prostate (PC3), breast (MDA435), melanoma (C8161) and small cell lung carcinoma (H520) [Gross 2002].

Isolated terpenoids and phenolics showed cytotoxicity against human tumour cell lines (A549-non small cell lung adenocarcinoma, SK-OV-3-ovarian, SK-MEL-2 - skin melanoma, XF498 - CNS and HCT15 - colon). The ED_{50} values ranged from 1.52 to 18.57 µg/mL [Choi 2004].

Erythrodiol-3-acetate, α-tocopherol-quinone and trans-phytol,

isolated from the n-hexane fraction demonstrated cytotoxicity against 5 human cell lines (A-549-lung carcinoma, XF498-CNS carcinoma, SK-MEL-2-melanoma, SH-VO-3-ovarian adenocarcinoma and HCT15 colon adenocarcinoma) [Sung 1999].

In vivo experiments

Diuretic activity
Significant increases ($p<0.01$) in diuresis and excretion of sodium, potassium and chloride were demonstrated in rats after oral administration of an aqueous infusion of European goldenrod containing 0.3% of flavonoids at 4.64 and 10.0 mL/kg b.w.. The increases in urinary sodium, potassium and chloride were considered to be due to the electrolyte content of the administered extracts. The increase in urine volume was more pronounced at the lower dose level [Schilcher 1988].

Isolated leiocarposide had a diuretic effect (20% weaker than that of furosemide at 6 mg/kg) after oral or intraperitoneal administration to rats at 25 mg/kg b.w., but did not influence urinary mineral excretion [Chodera 1985, 1986, 1988; Budzianowski 1999]. Furthermore, leiocarposide had an inhibitory effect on the growth of human urinary calculi implanted into the rat bladder. The growth of renal calculi was significantly decreased after 6 weeks of leiocarposide (25 mg/kg p.o.) administration [Chodera 1988].

A flavonoid fraction from European goldenrod administered to rats resulted in an increase of diuresis (88% after 24h), a decrease of overnight excretion of urine potassium and sodium and an increase of excretion of calcium [Chodera 1991].

Several fractions from a methanol/water extract (70:30) showed significant diuretic and saluretic activity in Sprague Dawley rats. The hydroxycinnamic acid fraction (100 mg/kg p.o.) significantly increased sodium and potassium excretion in urine comparably to furosemide at 10 mg/kg. There was no influence on calcium ion excretion in either the hydroxycinnamic acid or furosemide groups. The flavonoid fraction (100 mg/kg p.o.) did not elevate urine volume or ion excretion. The saponin fraction (25 mg/kg – 100 mg/kg p.o.) increased urine volume and saluretic activity for sodium and potassium ions, comparable to furosemide at 10 mg/kg [Kaspers 1998].

Anti-inflammatory and analgesic activity
Anti-inflammatory and analgesic effects of a hydroalcoholic extract have been demonstrated in various models of inflammation. The activity of the extract was similar to that of salicyl alcohol and indomethacin [Okpanyi 1989].

In Freund's adjuvant-induced arthritis of rat paw, a 46%-ethanolic extract, administered orally at 5 mL/kg on days 15-21 after injection of the adjuvant, significantly ($p<0.05$) reduced the intensity of inflammation to nearly 40% of the control value on day 21 [El-Ghazaly 1992].

A terpene fraction and its components exhibited antiulcer activity in HCl/ethanol induced gastric lesions in Swiss albino mice. At 100 mg/kg p.o., solidagenone demonstrated a significant gastroprotective effect ($p<0.05$) comparable to lansoprazole at 20 mg/kg. Solidagen-6β-ol and 3α-hydroxysolidagenone showed higher activity than solidagenone, while its epimers were inactive [Schmeda-Hirschmann 2002].

Leiocarposide showed anti-inflammatory and analgesic effects after subcutaneous administration to rodents. It inhibited carrageenan-induced rat paw oedema by 20% at 100 mg/kg and 27% at 200 mg/kg ($p<0.05$) after 5 hours, compared to 54% by phenylbutazone at 50 mg/kg. The analgesic effect of leiocarposide at 200 mg/kg in the hot plate test in mice was similar to that of aminophenazone at 50 mg/kg after 0.5 and 1 hour, but considerably weaker after 2 hours [Metzner 1984]. A mixture of saponins from European goldenrod, administered i.v. at 1.25-2.5 mg/kg b.w., had an inhibitory effect on sodium nucleinate-induced rat paw oedema [Jacker 1982].

Antitumour activity
A fraction of an aqueous extract was administered i.p. or s.c. every third day for 25 days in an experimental prostatic tumour model in mice. The growth of the tumours was inhibited at 5 mg/kg b.w. without any apparent side effects [Gross 2002].

Virgaureasaponin E (1 mg/kg/day) showed antitumour activity in the allogeneic sarcoma 180 tumour model and in the syngeneic DBA/2-MC.SC-1 fibrosarcoma tumour model. In mice treated with virgaureasaponin E, phagocytosis of bone marrow cells and proliferation of spleen and bone marrow cells were stimulated in an ex vivo assay; the TNF-α concentration in blood increased considerably compared to the control group [Bader 1996, 1998; Plohmann 1997, 1999].

Other effects
Hypotensive and sedative effects of extracts have been demonstrated in rats (180 and 360 mg/kg of a 2% aqueous extract administered i.v.) [Lasserre 1983] and in dogs (150 mg/kg of a non-specified extract of the leaves) [Ràcz-Kotilla 1977].

A significant increase in ACE activity and serum lactate dehydrogenase, creatine phospokinase, ALT, AST, TC, triglycerides, free fatty acids, cardiac MDA and NO levels, and a significant decrease in levels of GSH and SOD in cardiac tissue ($p < 0.05$) was provoked by s.c. injection of isoproterenol (30 mg/kg; twice at an interval of 24 h, for two consecutive days) in albino rats, when compared to a normal control group. Pretreatment with a methanolic extract (not further specified) for 5 weeks at a dose of 250 mg/kg significantly ($p<0.05$) prevented these observed changes [El-Tantawy 2014].

Pharmacological studies in humans
In a double-blind, crossover study with 22 healthy volunteers, a single oral dose of 5 mL of a tincture (65% ethanol) made from fresh plants (0.57 g per g of tincture) produced a 30% rise in the amount of urine compared to placebo [Brühwiler 1992].

Clinical studies
In an open study in 53 patients suffering from urinary tract inflammation, symptoms disappeared in 70-73% of cases after treatment with a daily dose of 5 mL of a tincture (65% ethanol) made from fresh plants [Brühwiler 1992].

In an open, post-marketing study, 745 patients with irritable bladder were treated daily with 3 x 380 mg of a dry extract (5.4:1). After 2 weeks of treatment, micturition frequency had decreased, and symptom-related responder rates were between 69 and 85% [Schmitt 1996].

The efficacy of a dry extract (5.0-7.1:1, ethanol 30% m/m; 3 × 425 mg of extract daily for 4 weeks on average) was investigated in an open, multi-centre, post-marketing study involving a total of 1487 patients, with subgroups for urinary tract infections, irritable bladder and urinary calculi/renal gravel. For patients with recurrent urinary tract infections (n = 555) the extract alone was as effective as when combined with initial antibiotic treatment [Laszig 1999a, b]. In patients suffering from chronic or recurrent irritable bladder symptoms (n = 512), even incontinence could be improved or eliminated in 2 out of 3 cases. The subgroup with "urinary calculi/renal gravel" comprised 427 patients

(32% of whom had additional urinary tract infections and 11% had additional symptoms of irritable bladder); for the typical symptoms, responder rates of 81% (feeling of pressure in the region of the bladder) to 98% (colics) were determined. Global improvement (CGI-scale) under treatment was evaluated by the physicians as "very much or much better" in 79% of the cases [Laszig 1999b].

In a subgroup analysis of the above study, a subgroup of 512 patients with chronic recurrent irritable bladder condition (aged 13 - 96 years, 77% female) was treated for five weeks with 425 mg of a dry extract (5.0-7.1:1) 3 times per day. 96% of the patients showed improvement recorded by the CGI scale, and in 80% of patients' estimations, effectiveness was good or very good [Pfannkuch 2002].

Case reports on 10 patients treated with the above extract during extracorporeal shock wave lithotripsy in hospital and during 4-week after-care indicated a positive spasmolytic effect from treatment with the extract: no colics occurred and additional spasmolytic drugs were unnecessary [Laszig 1999b].

Pharmacokinetic properties

A 60% ethanolic extract (100 µg/mL) did not influence the expression of CYP1A2 and the transporter protein MDR1 expression in LS180 cells. However, it did induce a 1.9-fold expression of CYP3A4 gene [Brandin 2007].

After oral administration of leiocarposide and salicin to rats, the glycosides were mostly excreted unchanged in urine. Hydrolysis of the ester and glycosidic bonds from leiocarposide to leiocarpic acid and saligenin as well as from salicin to saligenin mainly occurred in the caecum and colon. Saligenin was oxidized to salicylic acid in liver, kidney and lung homogenates. Gentisic acid, product of further oxidation of salicylic acid, was detected only in liver homogenate [Fötsch 1989a].

Experiments in rats demonstrated that leiocarposide is poorly absorbed after oral administration and is mostly excreted unchanged in the faeces, with less than 10% of metabolites found in urine. Of the administered dose, 2% was leiocarpic acid, 3-conjugates (2%), salicylic acid (0.5%), 5-conjugates (0.1%) and salicyluric acid (0.5%). Salicin was well-absorbed and excreted in the urine as unchanged drug (15%) and the following metabolites: 0.1% saligenin, 30% salicylic acid, 5% 5-conjugates, 0.1% salicyluric acid, 2% gentisic acid and 0.1% 2,3-dihydroxy-benzoic acid. The different metabolic rates were explained by the high stability of the ester bond of leiocarposide, which is hydrolyzed in artificial intestinal fluid only very slowly with a $t_{1/2}$ of 41.7 h [Fötsch 1989b].

Preclinical safety data

The oral LD_{50} of leiocarposide in rats was determined as 1.55 g/kg b.w. [Chodera 1985].

The mutagenic potential of NaN3 in AMES assay with *Salmonella typhimurium* TA1535 was inhibited by a n-hexane extract at 2.5 mg/mL, whereas ethanolic extracts (> 5 mg/mL) did not show antimutagenic activity [Kolodziej 2011].

Clinical safety data

No side effects were reported in an open multicentre study involving 1487 patients treated for five weeks with 425 mg of a dry extract (5.0-7.1:1) 3 times daily [Pfannkuch 2002].

Hypersensitivity reactions and mild gastrointestinal complaints (in 0.07%-0.3% patients) were the only adverse reactions observed after intake of European goldenrod [Schätzle 1998; Gardner 2013; Edwards 2015].

Two post-marketing studies showed very good or good tolerability in 97-98% of patients (n = 745 and 1487) during 2-4 weeks of treatment. One suspected case of a minor adverse drug reaction (heart burn) was reported [Schmitt 1996; Laszig 1999a,b].

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ALOE CAPENSIS	Cape Aloes	Online Series, 2014
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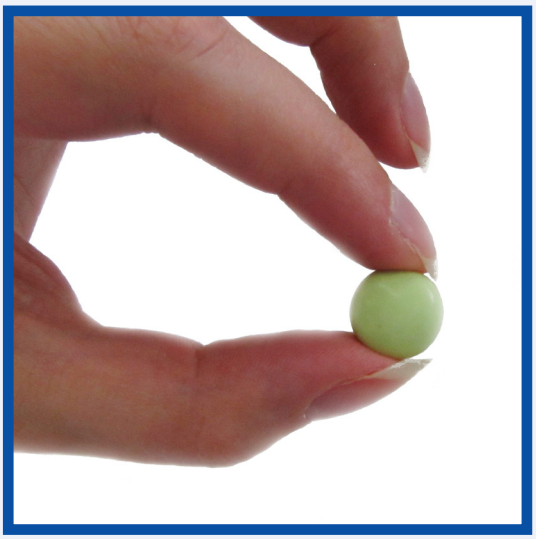

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Plant illustrated on the cover: *Symphytum officinale*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

SYMPHYTI RADIX

2012

Comfrey Root

DEFINITION

Comfrey root consists of the dried rhizomes and roots of *Symphytum officinale* L.

The material complies with the monograph of the Deutscher Arzneimittel-Codex [Beinwellwurzel – Symphyti radix. In: DAC 1979] or the British Herbal Pharmacopoeia [Comfrey Root - Symphyti radix. In: British Herbal Pharmacopoeia 1996].

Fresh material may be used provided that when dried it complies with one of the above monographs.

CONSTITUENTS

Characteristic constituents of comfrey root include: 0.6-4.7% of allantoin [Dennis 1987]; abundant mucilage polysaccharides (about 29%) composed of fructose and glucose units [Franz 1969]; phenolic acids such as cinnamic acid, chlorogenic acid (0.012 %), caffeic acid (0.004%), α-hydroxycaffeic acid and rosmarinic acid (up to 0.02%) [Andres 1991; Grabias 1998; Staiger 2009]; glycopeptides and amino acids [Hiermann 1998]; and triterpene saponins in the form of monodesmosidic and bidesmosidic glycosides based on the aglycones hederagenin (e.g. symphytoxide A), oleanolic acid [Aftab 1996] and lithospermic acid [Wagner 1970].

Pyrrolizidine alkaloids (0.05-0.08%) with 1,2-unsaturated necine ring structures are also present, almost entirely in the form of their *N*-oxides, the main ones being 7-acetylintermedine and 7-acetyllycopsamine together with smaller amounts of intermedine, lycopsamine and symphytine [Brauchli 1982; Mütterlein 1993].

CLINICAL PARTICULARS

Therapeutic indications

External use only
Pain and swelling in muscles and joints [Koll 2002; Koll 2004; Tschaikin 2004; Pabst 2004; Predel 2005; Staiger 2007; Grube 2007; Giannetti 2010], osteoarthritis [Grube 2007; Schulz 2007], acute myalgia in the back [Giannetti 2010], strains, contusions and distortions [Koll 2002; Koll 2004; Tschaikin 2004; Pabst 2004; Predel 2005; Staiger 2007]; epicondylitis, tendovaginitis and periarthritis [Petersen 1993].

Also used for tendinitis syndrome, knee joint injuries, non-active gonarthrosis, insect bites, mastitis, fractures, wound healing and skin inflammation. In these indications, the efficacy is plausible on the basis of long-standing use [Häberle 1952; Briel 1953; Ziolkowski 1957; Korte 1958; Büzberger 1960; Prinzing 1960; Deister 1963; Awang 1987; Koehler 1987; Kothmann 2003; Barnes 2007, Staiger 2009].

Posology and method of administration

Dosage

External use only
Adults and children from 3 years: ointments or other preparations containing up to 35% of root extract (1:2, ethanol 60% V/V), applied 3-4 times daily [Koll 2002; Koll 2004; Predel 2005; Staiger 2005; Staiger 2007; Grube 2007; Staiger 2008; Giannetti 2010].

Some national authorities restrict the external use of comfrey root. The German health authorities, for example, limit the content of pyrrolizidine alkaloids (PA) (including *N*-oxides) with 1,2-unsaturated necine structures in the amount of a preparation to be applied daily to not more than 100 µg. There are no restrictions for preparations containing less than 10 µg PA [Schilcher 2010].

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Method of administration
For topical application only.

Duration of use
The German health authorities recommend not more than 4-6 weeks per year if the daily application contains between 10 µg and 100 µg of pyrrolizidine alkaloids (including their *N*-oxides) with 1,2-unsaturated necine structures [Teuscher 2009; Schilcher 2010].

Contraindications
None known.

Special warnings and special precautions for use
In cases of wound healing and skin inflammation, PA-free comfrey root preparations should be used.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
As no human data are available, the potential risk is unknown. In accordance with general medical practice the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Anti-inflammatory activity
Rosmarinic acid has been shown to possess anti-inflammatory activity in various test systems. At concentrations ranging from 0.1 to about 10 mM it significantly inhibited the formation of malondialdehyde in human platelets ($p \leq 0.001$ at 1 mM) with an IC_{50} of 3.37 mM [Gracza 1985]. In another study rosmarinic acid inhibited prostaglandin synthesis and carrageenan- and gelatine-induced erythrocyte aggregation. Allantoin had no effect, or no significant effect, on erythrocyte aggregation in these tests [Gracza 1987].

In preparations of rat stomach a glycopeptide isolated from comfrey root dose-dependently inhibited the release of prostaglandins PGE_2 (IC_{50}: 1.47 ng/ml) and PGI_2 (IC_{50}: 1.0 ng/ml), and of 12-HETE (IC_{50}: 3 ng/ml) and arachidonic acid (AA; IC_{50}: 27.0 ng/ml) too. AA- and ADP-induced platelet aggregation was not inhibited by 3 or 10 µg/ml of the glycopeptide [Hiermann 1998].

In a study of the influence of a 60%-ethanolic comfrey root extract on different elements of the human immune system, the extract was found to exert dose-dependent anti-complementary effects on the classical pathway of complement activation (IC_{50}: 1.8 µg/ml), the alternative pathway of complement activation (IC_{50}: 77.8 µg/ml) and the terminal route of complement activation (IC_{50}: 24.0 µg/ml). These activities were correlated with a fraction of molecular weight (MW) > 1,000 kD (IC_{50} values in µg/ml: 0.4, 13.6 and 2.6 respectively). It appears that this fraction interfered with the activity of the complement cascade at the levels of C1 and C3. A dose-dependent decrease in the luminol-dependent chemiluminescence produced by polymorphonuclear leukocytes upon stimulation with opsonized zymosan was also observed (IC_{50} of the root extract: 162.5 µg/ml, IC_{50} of the fraction of MW > 1,000 kD: 163.8 µg/ml) [van den Dungen 1993].

Wound-healing effects
A 40% ethanolic extract from comfrey root and its high MW fraction (> 1,000 kD) were investigated in a test model of wound healing (fibroblasts in a collagen matrix). Both the extract and the fraction dose-dependently inhibited shrinkage of the collagen matrix. Mechanistic and kinetic studies showed a direct interaction of this fraction with collagen and involvement in an early stage of collagen gel contraction. Furthermore, the high MW fraction modified the binding of fibroblasts [van den Dungen 1990; van den Dungen 1993].

Other effects
An 80% ethanolic extract from comfrey root was reported to have antibacterial activity against Gram-positive and Gram-negative bacteria. The effect was 2- to 12-fold weaker than that of gentamicin or tetracycline [Izzo 1995].

An aqueous extract from comfrey root, as well as protein- and carbohydrate-rich fractions isolated from it, stimulated proliferation of neoplastic cells and exerted an antimitotic effect on PHA-stimulated human T-lymphocytes [Olinescu 1993].

Symphytoxide A at 30-300 µg/ml reduced the force and rate of contractions in isolated guinea-pig atria but produced stimulant effects in smooth muscle preparations such as guinea-pig ileum and rat uterus, producing a concentration-dependent contractile response at 10-100 µg/ml. All these effects were abolished in the presence of atropine (80 ng/ml). In frog skeletal muscle preparations symphytoxide A at 100-300 µg/ml produced concentration-dependent contractile responses. No contraction was seen after pre-treatment with d-tubocurarine [Gilani 1994].

In vivo experiments

Anti-inflammatory activity
An orally-administered aqueous extract from comfrey root inhibited carrageenan-induced rat paw oedema, indicating anti-inflammatory activity, and bioactivity-guided fractionation of the extract led to the isolation of a glycopeptide which dose-dependently inhibited oedema formation. After oral administration to rats the ED_{50} of the glycopeptide was 61 µg/kg, whereas the ED_{50} of indometacin was 10 mg/kg under the same conditions [Hiermann 1998].

In another study, oral administration of an 80%-ethanolic extract from comfrey root at 100 mg/kg body weight inhibited carrageenan-induced rat paw oedema by 42%, comparable to the 45% inhibition produced by oral indometacin at 5 mg/kg [Mascolo 1987].

Anti-inflammatory properties of a dry extract from comfrey root were also demonstrated in rats with paw oedema induced by simultaneous injection of carrageenan and prostaglandin E1. The extract (administered intraperitoneally) did not prevent inflammation but it suppressed leukocyte infiltration 3 and 4 hours after induction of inflammation [Shipochliev 1981].

Wound-healing effect
Minced fresh root and an acidic precipitate from an ethanolic

(40% V/V) extract were tested for wound-healing properties in a pig model; both preparations were applied daily on deep dermal burns. Treatment with either preparation resulted in a rigid crust, strongly adherent to the wound bed and the wound-dressing material [van den Dungen 1993].

Pharmacological studies in humans

The effects of dermatological preparations containing 5 or 10% of a comfrey root extract (2:7, 50% ethanol) on the process of healing of experimentally induced UV-B erythema were studied in 29 volunteers in a controlled trial. The anti-inflammatory potency of the extract was found to be equal to or greater than that of diclofenac (no further details available). A positive correlation could be demonstrated between efficacy and the concentration of a caffeic acid derivative in the extract, but not for allantoin [Andres 1989; Andres 1991].

Clinical studies

Five randomized, controlled studies support the therapeutic efficacy of comfrey extracts in the treatment of ankle sprains and complaints of the skeletal and locomotor system, such as osteoarthritis, epicondylitis, tendovaginitis and periarthritis [Petersen 1993; Koll 2004; Predel 2005; Grube 2007; Giannetti 2010]. The results of post-marketing surveillance studies [Koll 2002; Tschaikin 2004; Pabst 2004; Staiger 2008] accord with those of the controlled studies [Staiger 2005; Staiger 2007]. All of the included studies were performed with a comfrey root extract (1:2, ethanol 60%V/V) with less than 0.35 ppm of pyrrolizidine alkaloids in the finished product.

Placebo-controlled studies

The efficacy of an ointment containing 35% of the comfrey root extract was evaluated in a randomized, double-blind, placebo-controlled multicentre study involving 142 patients with a unilateral ankle sprain. Treatment was started within the first 6 hours after injury and continued for 8 days with assessments on days 0, 4 and 7. The affected ankle joint was treated locally with about 2 g of the ointment four times a day. The primary target parameter, tenderness of the ankle joint, was measured by tonometry (difference in tolerated pressure between injured and healthy ankles). During the course of treatment pain in the comfrey extract group regressed significantly more than in the placebo group ($p = 0.0001$). At the final assessment the reductions in tenderness compared to initial values were 2.44 kp/cm^2 in the verum group compared to 0.95 kp/cm^2 in the placebo group. Compared to placebo, the verum treatment showed significant reductions in pressure pain (tonometric method, $p<0.0001$), ankle oedema (figure-of-eight method, $p = 0.0001$), ankle mobility (dorsiflexion, $p = 0.002$; plantar flexion, $p = 0.0116$) and global efficacy ($p<0.0001$) [Koll 2004].

Another randomized, double-blind, placebo-controlled study investigated the effect of the same ointment containing 35% of a comfrey root extract over a 3-week period on 220 patients suffering from painful osteoarthritis of the knee. The primary target parameter was pain relief, assessed by the patient using a visual analogue scale (VAS) to record the reduction in total score from pain at rest and in movement. During the study the total score significantly ($p<0.001$) decreased by 51.6 mm (54.7%) in the verum group and 10.1 mm (10.7%) in the placebo group. The secondary target was improvement in pain, stiffness and functional symptoms as determined by total scores in the WOMAC test (Western Ontario and McMaster Universities). At the end of the study the reduction in the verum group (60.4 mm, 58.0%) and in the placebo group (14.7 mm, 14.1%) was significant ($p<0.001$). Significant improvement in the verum group was also evident with respect to four explorative secondary parameters: SF-36 (quality of life), angle measurement (mobility of the knee), CGI (clinical global impression) and global assessment of efficacy by physicians and patients ($p<0.001$ for each parameter) [Grube 2007, D'Anchise 2007].

The efficacy of the same comfrey ointment was investigated in a multicentre, randomised, double-blind, placebo-controlled clinical trial in patients with acute upper and lower back pain. Over a period of 5 days, 120 patients were treated with 4 g verum or placebo three times daily. The primary efficacy variable was the area under the curve (AUC) of the visual analog scale (VAS) on active standardised movement values assessed on 4 visits. During the treatment, pain intensity on active standardised movement decreased on average (median) by approximately 95.2 % in the comfrey extract group and by 37.8 % in the placebo group ($p<0.001$). Compared to placebo, verum significantly improved secondary efficacy variables (each $p<0.001$): the AUC of the reported back pain at rest, the AUC of the pressure algometry in the trigger point, the global assessment of the efficacy by the patients and investigators, and the functional impaiment measured with the Oswestry disability index (functional impairment)[Giannetti 2010].

In a 4-week pilot study, 41 patients with musculoskeletal disorders (mainly epicondylitis, tendovaginitis and periarthritis) were topically treated with the same ointment ($n = 20$) or placebo ($n = 21$). Efficacy was assessed using several pain parameters: tenderness when pressure applied, pain at rest and during exercise. With respect to "tenderness when pressure applied", the ointment proved superior to placebo in patients with epicondylitis ($n = 7/8$) and tendovaginitis ($n = 6/5$), but not in patients with periarthritis [Petersen 1993].

Comparison with reference drug

The same ointment as above was compared to a gel preparation containing 1% of diclofenac in a randomized, single-blind multicentre study. Patients suffering from acute unilateral ankle sprain (distortion) applied 2 g of either the comfrey ointment ($n = 82$) or the diclofenac gel ($n = 82$) four times daily for 7 ± 1 days. The primary efficacy variable was pain arising from pressure on the injured area, measured with a calibrated caliper (tonometer) on days 0, 4 and 7 and evaluated by the AUC of the pain-time curve; the results indicated that the comfrey ointment was statistically non-inferior, and possibly superior, to the diclofenac gel for the treatment of acute ankle sprain. Results for secondary variables, including the circumference of the joint (swelling, figure-of-eight method), individual spontaneous sensations of pain at rest and during movement (assessed using a visual analogue scale), the use of rescue medication (paracetamol), and evaluations of global efficacy and tolerability by both physicians and patients, also showed equivalence of the comfrey and diclofenac treatments [Predel 2005].

Post-marketing surveillance studies

In a surveillance study, use of the same comfrey ointment was examined in 306 children aged 3 to 12 years. The preparation was applied to children with various conditions such as contusions (61.4%), strains (14.1%), distortions (30.4%) and other indications (6.9%). In the majority of cases the ointment was applied three times daily (57.8%), in others four times daily (26.1%) or twice a day (13.4%). The majority ($n= 281$) were treated for 5 to 8 days. During the observation period symptoms of pain at rest and during the night, pain during motion, tenderness pressure, impaired mobility and general condition improved considerably. The physicians assessed the global efficacy as excellent in 52.3% of cases and good in 44.5 % [Staiger 2008].

In another surveillance study 163 patients applied the same ointment for a variety of conditions, the most frequent being contusions (33% of patients), painful joint complaints (28%),

sprains (26%) and painful muscle complaints (23%). Most patients applied the preparation two (38%) or three (48.5%) times daily and the median duration of treatment was 11.5 days. During the observation period symptoms of pain at rest and during the night, pain during motion, tenderness when pressure applied, impaired mobility, painful muscle complaints and swellings were improved markedly. Morning stiffness of the joints decreased (94%) from 17 minutes initially to 1 minute. The use of non-steroidal anti-inflammatory drugs (NSAIDs) was reduced or discontinued by 13.5% of patients. The physicians assessed global efficacy as excellent in 38.7% of cases and good in 54.6% [Tschaikin 2004].

In a similar surveillance study 162 patients applied a preparation containing 30% of the same comfrey root extract to several conditions such as painful joint complaints (34%), contusions (26.5%) or painful muscle complaints (21.6%). Most patients applied the preparation once (23.5%) or twice (52.5%) daily and the median duration of treatment was 11.8 days. Symptoms of pain at rest and pain during movement, impaired mobility, swelling and painful muscle complaints improved markedly during the observation period. Morning stiffness of investigated joints was decreased by 90% from 20 minutes initially to 2 minutes. The use of NSAIDs was reduced or discontinued by 21% of patients. Global efficacy was assessed by the physicians as excellent in 65.4% of cases and good in 32.7% [Pabst 2004].

Pharmacokinetic properties
No data available.

Preclinical safety data
No data available for the external use of comfrey root.

The pyrrolizidine alkaloids (PAs) in comfrey root are considered to be toxic, especially after oral ingestion [Schoental 1968; Hirono 1978; Schilcher 1982; Brauchli 1982; Schimmer 1983; Mattocks 1986; Behninger 1989; Jaspersen-Schib 1990; Forth 1991; Winship 1991; Röder 1992; Roeder 1995; Michler 1996; Yarnell 1999; Yeong 1990; Yeong 1991; Yeong 1993; Stickel 2000; Mei 2005; Györik 2009; Teuscher 2009; Chen 2010a; Chen 2010b; Liu 2010; Mei 2010; He 2010; Ji 2010; Wiedenfeld 2011].

However, processes are available to remove over 99% of PAs during the production of extracts from comfrey root, enabling preparations for topical application to contain less than 0.35 ppm of pyrrolizidine alkaloids [Grube 2007]. The use of such preparations, as exemplified by the clinical studies summarized above, is considered to have an acceptable level of safety [Schilcher 1982: Schilcher 2010].

Mutagenic activity
A comfrey root extract (1:2, ethanol 60%V/V) was investigated for its ability to induce gene mutations in *Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1535 and TA 1537 with and without metabolic activation using the mammalian microsomal fraction S9 mix. The extract showed *no* biologically relevant increases in revertant colony numbers and was not mutagenic in the bacterial reverse mutation assay [Benedek 2010].

Clinical safety data
No major side effects were reported from the clinical studies summarized above involving a total of 1318 patients. The tolerability of topical preparations containing comfrey root extracts was judged to be very good or good in more than 90% of patients The adverse effects related to the study medication were limited to skin reactions (skin redness, eczema, pruritus) and were of mild and moderate severity. [Petersen 1993; Koll 2002; Tschaikin 2004; Pabst 2004; Koll 2004; Predel 2005; Grube 2007; Staiger 2008; Giannetti 2010].

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Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
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ALOE CAPENSIS	Cape Aloes	Second Edition, 2003
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BALLOTAE NIGRAE HERBA	Black Horehound	Supplement 2009
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The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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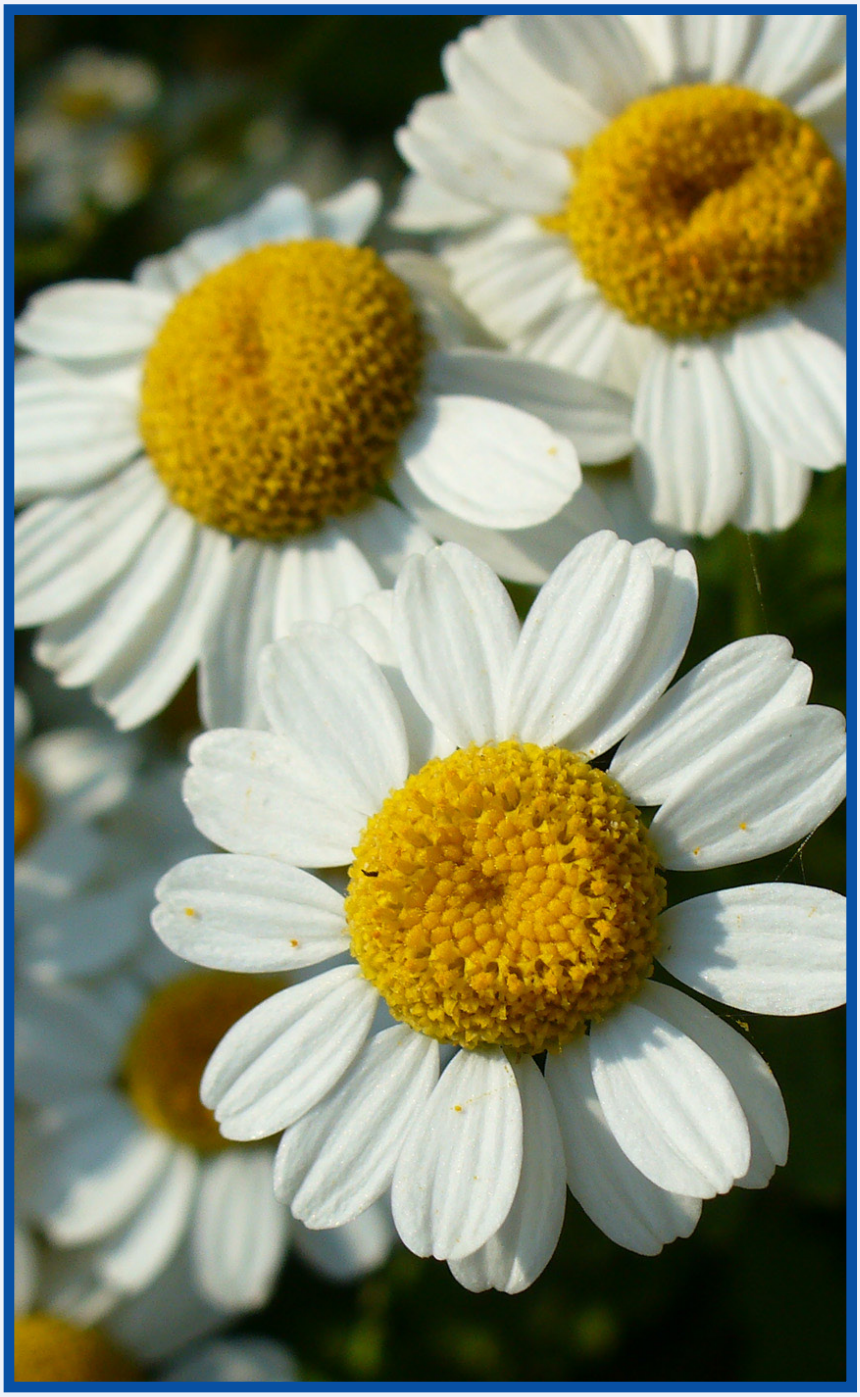

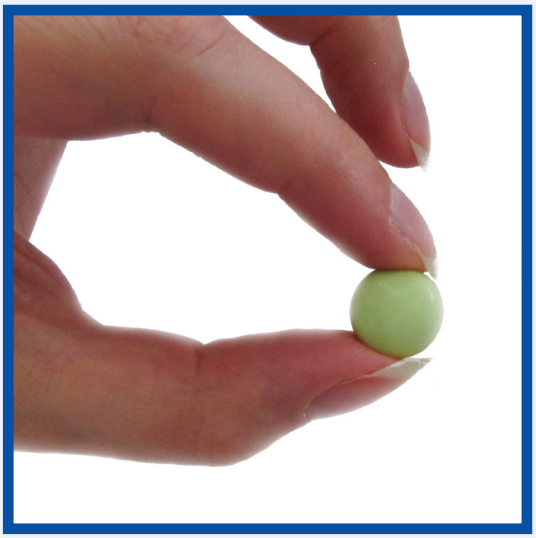

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

TANACETI PARTHENII HERBA 2014

Feverfew

DEFINITION

Feverfew consists of the dried, whole or fragmented aerial parts of *Tanacetum parthenium* (L.) Schultz Bip. It contains not less than 0.2 per cent of parthenolide ($C_{15}H_{20}O_3$; M_r 248.3), calculated with reference to the dried drug.

The material complies with the European Pharmacopoeia [Feverfew].

Fresh material may also be used provided that, when dried, it complies with the European Pharmacopoeia.

CONSTITUENTS

The characteristic constituents are sesquiterpene lactones with an α-methylenebutyrolactone structure of which parthenolide, a germacranolide, is the major component. The others, which occur in significantly smaller amounts, are mainly guaianolides including canin, 10-*epi*-canin, tanaparthin-α- and -β-peroxides, artecanin and 8-hydroxyestafiatin [Quick 1976; Bohlmann 1982; Begley 1989; Heptinstall 1992; Dolman 1992].

Other constituents include monoterpenes, principally camphor and trans-chrysanthenyl acetate and polyacetylene compounds (spiroketal enol ethers) [Bohlmann 1982; Knight 1995], lipophilic flavonoids such as 6-hydroxy-kaempferol-3,6-dimethylether and 6-hydroxykaempferol-3,6,4'-trimethylether (= santin), quercetagetin-3,6,3'-trimethylether and quercetagetin-3,6,4'-trimethylether, hydrophilic flavonoids principally apigenin-7-glucuronide and luteolin-7-glucuronide [Williams 1999a; Williams 1999b; Long 2003] and dicaffeoylquinic acids [Wu 2007].

CLINICAL PARTICULARS

Therapeutic indications

Prophylaxis of migraine [Berry 1984; Johnson 1985; Murphy 1988; Heptinstall 1988; Groenewegen 1992; Palevitsch 1997; Ernst 2000; Pfaffenrath 2002; Diener 2005].

Posology and method of administration

Dosage

Adult daily dose: 50 – 120 mg of powdered feverfew [Johnson 1985; Murphy 1988; Ernst 2000; Palevitsch 1997]; 18.75 mg of a supercritical CO_2 extract corresponding to 3 g fresh feverfew [Pfaffenrath 2002; Diener 2005].

Method of administration
For oral administration.

Duration of administration
Treatment for at least 12 weeks is recommended [Groenewegen 1992; Cady 2002; Diener 2005]. If symptoms persist or worsen after 12 weeks, medical advice should be sought.

Contraindications
Hypersensitivity to feverfew or other members of the Compositae [Hausen 1992].

Special warnings and special precautions for use
Abrupt ending of a long-term treatment can provoke withdrawal symptoms, including a rebound of migraine symptoms, anxiety, insomnia as well as muscle and joint stiffness [Johnson 1985].

Interactions with other medicaments and other forms of interaction
None reported.

E/S/C/O/P MONOGRAPHS

No adverse side effects were noted in a large number of individuals taking feverfew together with other medications [Groenewegen 1992].

Pregnancy and lactation
No data available.

In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
In rare cases, allergic contact dermatitis, mouth ulceration or tongue irritation and inflammation may occur [Mitchell 1971; Hausen 1983; De Corres 1984; Turner 1985; Senff 1986; Guin 1987; Mattes 1987; Talaga 1989; Groenewegen 1992; Hausen 1992; Paulsen 2002; Paulsen 2007; Sharma 2007; Killoran 2007; Agarwal 2008; Lakshmi 2008].

Cases of abdominal pain and indigestion in patients who have taken feverfew for long periods have been reported. Rare cases of diarrhoea, flatulence, nausea or vomiting have been noted [Turner 1985; Baldwin 1987; Murphy 1988; Hausen 1992].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Much of the biological activity of parthenolide (and the other sesquiterpene lactones) can be explained by the presence of the α-methylenebutyrolactone functional group and its possible interaction with biologically active thiol (sulphydryl) groups [Groenewegen 1986; Heptinstall 1987; Heptinstall 1987b; Groenewegen 1992; Barsby 1992; Sumner 1992; Barsby 1993, Kwok 2001].

There is no firmly established link between the constituents of feverfew and its migraine prophylactic properties. However, many experimental observations, mainly *in vitro*, suggest such a link. Most of these results have been obtained using crude plant extracts although some have been obtained using pure parthenolide or other sesquiterpene lactones from feverfew [Groenewegen 1986; Heptinstall 1987a; Heptinstall 1987b].

Anti-inflammatory effects
Feverfew extracts or pure parthenolide inhibit the production of prostaglandins, which are mediators of inflammation [Groenewegen 1992]. Sesquiterpene lactones having an α-methylenebutyrolactone group are known to have anti-inflammatory activity [Hall 1979].

Feverfew extracts have been shown to inhibit prostaglandin biosynthesis in bovine seminal vesicular mitochondrial fraction, probably by interfering with the action of phospholipase A_2 at the beginning of the arachidonic acid cascade rather than at the cyclo-oxygenase step [Collier 1980; Thakkar 1983; Pugh 1988; Sumner 1992].

Feverfew extracts inhibited thromboxane B_2 and leukotriene B_4 production in leukocytes indicating that they are presumably inhibitors of both 5-lipoxygenase and cyclo-oxygenase [Capasso 1986; Sumner 1992].

An acetone extract of feverfew inhibited phorbol myristate acetate–induced chemiluminescence of human polymorphonuclear leucocytes with an IC_{50} of 0.79±0.19 mg leaf/ml whole blood [Brown 1997].

Parthenolide suppressed inducible nitric oxide synthase (iNOS) promoter activity dose-dependently from 2.5 μM with an IC_{50} of 10μM, thus reducing the formation of NO-radicals. The TPA-induced increase in iNOS promoter activity was also suppressed with an IC_{50} of 2 μM [Fukuda 2000].

A 70% ethanolic extract of feverfew (1:80) and parthenolide (2 μg/ml) inhibited the expression of intercellular adhesion molecule-1 induced by cytokines IL-1 (up to 95% suppression), TNF-α (up to 93% suppression) and IFN-γ (up to 39% suppression) [Piela-Smith 2001].

The LPS-stimulated release of TNF-α and MCP-1 (monocyte chemoattractant protein) in THP-1 human monocytes was significantly inhibited by a 90% ethanolic extract ($p<0.01$) and by a CO_2 supercritical fluid extract ($p<0.05$) [Chen 2007].

Parthenolide was shown to bind to IκB kinase β, the kinase subunit that plays an important role in cytokine-mediated signalling. A biotinylated parthenolide derivative directly inhibited IκB kinase β [Kwok 2001].

Pre-treatment of A549 cells with parthenolide inhibited TNF-α mediated interleukin 8 (IL-8) gene expression ($p<0.05$), activation of the IL-8 promoter ($p<0.05$), translocation of NF-κB and degradation of the NF-κB inhibitory protein [Mazor 2000].

Parthenolide significantly inhibited LPS-induced production of IL-6 and TNF-α by RAW 264.7 cells at 4 μM ($p<0.05$) [Smolinsky 2003].

Parthenolide at 20 μM significantly ($p<0.01$) decreased LPS-induced release of TNF-α (IC_{50} 6.2 μM), IL-1β (IC_{50} 2.5 μM), IL-6 (IC_{50} 5.2 μM) and PGE_2 from peripheral blood mononuclear cells [Shah 2010].

A parthenolide-depleted extract of feverfew (0.026% parthenolide) did not inhibit TNF-α induced NF-κB activity but did inhibit [Sur 2009]:
- the activity of pro-inflammatory enzymes 5-LO, phosphodiesterase-3 and phosphodiesterase-4;
- the release of pro-inflammatory mediators NO, PGE_2 and TNF-α from macrophages and TNF-α, IL-2, IFN-γ and IL-4 from human mononuclear cells;
- the TPA-induced release of PGE_2 from human skin equivalents.

The extract also showed free radical scavenging activity against a wide range of reactive oxygen species. It restored cigarette smoke-mediated depletion of cellular thiols and attenuated the formation of UV-induced hydrogen peroxide [Martin 2008].

In rat peritoneal leucocytes, 6-hydroxykaempferol-3,6-dimethylether and santin inhibited cyclo-oxygenase (CO; IC_{50} 182 and 27 μM respectively) as well as 5-lipoxygenase (5-LO; IC_{50} 182 and 58 μM respectively) in the same way. In contrast, quercetagetin-3,6,3′-trimethylether showed preferential activity against CO, with IC_{50} values of 22 μM for CO and 167 μM for 5-LO [Williams 1999a].

Serotonergic effects
Feverfew extracts inhibit the secretion of serotonin (5-HT)

from blood platelets and also prevent aggregation in response to external chemical stimuli [Groenewegen 1986; Makheja 1981; Makheja 1982]; it is unclear whether phospholipase A2 inhibition is involved in these processes [Groenewegen 1992; Heptinstall 1987a]. The effects on platelets are probably due to the Michael addition mechanism since prior addition of a thiol to the extracts or pure parthenolide removes this bioactivity [Groenewegen 1992; Heptinstall 1987b].

Parthenolide displaced [^{3}H] ketanserin from 5-HT_{2A} receptors from rat and rabbit brain and from cloned 5-HT_{2A} receptors [Weber 1997].

Parthenolide did not show agonistic or antagonistic effects towards serotonin on isolated rat stomach fundus at concentrations from 1 to 10 µM. However, parthenolide non-competitively antagonised the effects of fenfluramine and dextroamphetamine, two indirect acting serotonergics, at a concentration of 10 µM [Béjar 1996].

Similar results were also obtained in another study. Furthermore it was shown that in contrast to parthenolide, a dichloromethane extract of feverfew blocked 5-HT_{2A} and 5-HT_{2B} receptors and neuronally released serotonin at a concentration of 10 µM [Mittra 2000].

Influence on cytochrome P450
An 80% methanolic extract of feverfew inhibited the activity of CYP1A2 (tacrine), CYP2C9 (tolbutamide), CYP2C19 (imipramine), CYP2C8 (paclitaxel), CYP3A4 (midazolam) and CYP2D6 (dextromethorphan) with IC50 values from 53 to 299 µg/ml [Unger 2004].

Antitumour activity
Sesquiterpene lactones with an α-methylenebutyrolactone group, including parthenolide, have been shown to be cytotoxic towards different human tumour cell lines [Lee 1971; Hoffmann 1977]. Parthenolide inhibits the incorporation of thymidine into DNA and thus the cytotoxic activity may occur at the DNA replication level [Woynarowski 1981a, 1981b].

Various studies have demonstrated an antitumour activity for parthenolide by:
- irreversible inhibition of cell growth (mouse fibrosarcoma and human lymphoma) after 24h exposure at concentrations above 5 µM [Ross 1999];
- stimulation of 1,25-dihydroxyvitamin D3-induced differentiation of HL-60 cells via inhibition of NF-κB activity [Kang 2002];
- stimulatory activity on tubulin assembly and by dose-dependent inhibition of the growth of MCF-7 cells [Miglietta 2004];
- induction of mitochondrial dysfunction and apoptosis via Bcl-2 family proteins [Zhang 2004];
- inhibition of proliferation of different cell lines [Wu 2006; Parada-Turska 2007; Parada-Turska 2008; Oghuchi 2009; Weng 2009].

Studies on parthenolide-induced apoptosis and molecular mechanisms confirmed this activity [Won 2005; Zunino 2007; Pajak 2008; Anderson 2008].

Other effects
Parthenolide interferes markedly with both contractile and relaxant mechanisms in blood vessels, almost certainly due to the presence of the α-methylenebutyrolactone functional group; when this is blocked, these activities are lost [Barsby 1992; Barsby 1993].

The lactones and an ethanolic extract showed antimicrobial activity *in vitro*, inhibiting the growth of Gram-positive (but not Gram-negative) bacteria, yeasts and filamentous fungi [Fukuda 2000; Kalodera 1996]. The essential oil was bactericidal and fungicidal against mainly Gram-negative bacteria, yeasts and fungi [Kalodera 1997].

Parthenolide inhibited promastigote growth of *Leishmania amazonensis* with an IC_{50} of 0.37 µg/ml. Parthenolide also reduced the survival index of the amastigote forms by 50% at 0.8 µg/ml [Tiuman 2005].

The epimastigote growth of *Trypanosoma cruzi* was inhibited significantly ($p<0.05$) more by parthenolide than the reference benznidazole. The $IC_{50/96h}$ for parthenolide was 0.5 µg/ml compared to 1.8 µg/ml for benznidazole. The internalization index (number of infected cells x mean of amastigotes per cell) of the amastigote forms was reduced by 50% at 2 µg/ml of parthenolide [Izumi 2008].

An ethyl acetate extract and parthenolide exhibited antiviral activity against *Herpes simplex* 1 with an IC_{50} of 40 µg/ml and 0.3 µg/ml respectively [Onozato 2009].

In vivo experiments

Anti-inflammatory activity
Significant inhibition ($p<0.01$) of collagen-induced broncho-constriction in guinea pigs was obtained after intravenous administration of 1 ml feverfew extract (1 g of chopped fresh leaves in 2.5 ml distilled water) [Keery 1986]. It has been interpreted as a consequence of phospholipase A_2 inhibition. A similar inhibition of prostanoid biosynthesis suggests that parthenolide may suppress the induction of nephrocalcinosis in rats [Groenewegen 1992].

An extract of feverfew, administered orally (10–40 mg/kg b.w.), and parthenolide, administered intraperitoneally (1–2 mg/kg), led to significant anti-nociceptive ($p<0.05$ to $p<0.001$) and anti-inflammatory ($p<0.05$) effects against acetic acid-induced writhing in mice and carrageenan-induced paw oedema in rats respectively. Naloxone failed to reverse the induced antinociception. Higher doses of the extract (up to 60 mg/kg orally) did not alter the locomotor activity, potentiate the pentobarbital-induced sleep time nor change the rectal temperature in rats [Jain 1999].

Parthenolide reduced the phorbol myristate acetate-induced contact sensitivity in the murine ear oedema assay (0.5 mg/ear). Reduced parthenolide lost its activity completely [Kwok 2001].

Mice were exposed daily to UVB light for 10 weeks and treated topically with a 1% solution of parthenolide-depleted feverfew extract pre- and post-UV exposure. The UV-induced epidermal hyperplasia was significantly reduced in the verum group (62.3 µm to 45.4 µm; $p<0.05$). The same solution also reduced UV-induced DNA and cellular damage in porcine skin [Martin 2008].

Topical application of a parthenolide-depleted feverfew extract significantly reduced TPA-induced oedema in mice with an ED_{50} of 0.09%. In another experiment mice were sensitized to oxazolone and one hour after the challenge a parthenolide-depleted feverfew extract was applied at 0.1% and 0.01%. In the verum group the mean biopsy weight was reduced by 70% and 52% respectively (compared to hydrocortisone at 0.1% and 0.01% with a reduction of 77% and 65% respectively). The release of the pro-inflammatory cytokines TNF-α, IL-2 and IFN-γ was also significantly reduced (all reductions $p<0.05$) [Sur 2009].

I.p. administration of parthenolide in rats at 10 mg/kg b.w. for 6 days significantly reduced nitroglycerin-induced neuronal activation in the following brain nuclei: paraventricular nucleus of the hypothalamus ($p<0.03$), parabrachial nucleus ($p<0.05$), nucleus tractus solitarius ($p<0.03$) and nucleus trigeminalis caudalis ($p<0.03$) [Tassorelli 2005].

Anti-ulcerogenic activity
Gastric ulcers induced by oral administration of absolute ethanol to rats were dose-dependently reduced by oral pre-treatment with a chloroform extract of feverfew (2.5–80 mg/kg) or parthenolide (5-40 mg/kg). The protection ranged between 34 and 100% for the extract and 27 and 100% for parthenolide. The mean ulcer index was reduced from 4.8±0.3 (control) to 1.4±0.2 (extract 40 mg/kg) and 0.5±0.1 (parthenolide 40 mg/kg) [Tournier 1999].

Pharmacological studies in humans

Platelet aggregation responses in 10 patients taking feverfew for 3.5 to 8 years were indistinguishable from those of a control group of 4 patients who had stopped taking feverfew at least 6 months previously [Groenewegen 1992; Biggs 1982].

In a double-blind, randomised, placebo-controlled study 12 volunteers were treated topically with a preparation containing parthenolide-depleted feverfew extract (1%) once daily for 2 days before and after exposure to UVB irradiation at varying minimal erythema doses (MED). In the verum group the UV-induced erythema was significantly reduced as shown by cross-polarized photography, by the expert grader assessment ($p<0.05$ at 1 and 1.5 MED UVB dose) and by chromameter readings ($p<0.05$ at 0.5 and 1 MED) [Martin 2008].

Topical treatment of 8 volunteers with a preparation of parthenolide-depleted feverfew extract 30 minutes prior to challenge by methyl nicotinate resulted in a dose-dependent reduction in erythema as measured by apparent haemoglobin content: reduction of 28% ($p<0.01$), 39% ($p<0.05$) and 68% ($p<0.05$) for 0.5%, 0.75% and 1% extract, respectively [Sur 2009].

Clinical studies

In a systematic review, six randomised, placebo-controlled, double-blind trials of feverfew mono-preparations for the prevention of migraine in human subjects were evaluated by the Jadad score [Johnson 1985; Murphy 1988; Palevitch 1997; De Weerdt 1996; Kuritzky 1994; Pfaffenrath 1999]. The review concluded that feverfew is likely to be effective in the prevention of migraine [Ernst 2000].

In a further systematic review by the same authors, 5 randomised, placebo-controlled, double-blind trials (343 patients) met the inclusion criteria [Johnson 1985; Murphy 1988; De Weerdt 1996; Palevitch 1997; Pfaffenrath 2002]. Kuritzky 1994 was excluded from this review and Diener 2005 is not mentioned. Pfaffenrath 2002 describes the same study as Pfaffenrath 1999, cited in the previous review. The authors concluded that there is not sufficient evidence to convincingly suggest an effect of feverfew for preventing migraine [Pittler 2009].

In a double-blind, randomised, placebo-controlled study, an oral dose of 2 x 25 mg of dried feverfew leaf was administered for 6 months to 8 migraine patients, while 9 patients took placebo. All patients had previously treated themselves daily with fresh feverfew leaf for 3-4 years, with substantial alleviation of symptoms. The mean frequency of headaches in the verum group was 1.7 per month during the 6 months and 1.5 per month during the final 3 months, compared with 3.13 and 3.43 respectively in the placebo group ($p<0.02$). Nausea or vomiting, or both, occurred on only 39 occasions in the verum group compared with 116 occasions in the placebo group ($p<0.05$) [Johnson 1985].

In a double-blind, randomised, placebo-controlled, crossover trial in 59 migraine patients, after a 1-month run-in period on placebo (dried cabbage leaf) each patient received feverfew for 4 months then placebo for 4 months (or vice versa). The verum groups received one capsule daily containing 70-114 mg of dried feverfew leaf, equivalent to 0.545 mg of parthenolide. There was a 24% reduction in the number of migraine attacks ($p<0.005$) during feverfew treatment (compared with the placebo phase) and, although no significant change in the duration of individual attacks, a significant reduction ($p<0.002$) in nausea and vomiting during attacks. Global assessments also showed that feverfew was significantly ($p<0.01$) superior to placebo [Murphy 1988].

In another crossover study 57 migraine patients were treated in 3 phases. In the first phase (open) all patients received 100 mg of powdered feverfew leaf per day for 2 months. In the second and third phases, with a randomized, double-blind, crossover design, the patients received feverfew (same dosage) or placebo consecutively, each for an additional month. Compared to placebo treatment, feverfew caused a significant reduction in pain intensity ($p<0.01$) and in the severity of typical symptoms such as nausea, vomiting, sensitivity to light (all $p<0.001$) and sensitivity to noise ($p<0.03$) [Palevitch 1997].

In a randomized, double-blind, placebo-controlled, crossover study, 50 migraine patients received placebo daily for 1 month. Over the following 4 months the patients received 1 capsule daily containing a 90% ethanolic extract of feverfew standardized to 0.5 mg of parthenolide and subsequently placebo for 4 months (or vice versa). No significant prophylactic effect could be demonstrated for the feverfew preparation, but the patients tended to use less of other medications [De Weerdt 1996].

In a double-blind, randomised, placebo-controlled study 147 patients suffering from migraine, with and without aura, were treated for 12 weeks after a 4-week baseline period. Three dosages (2.08 mg, 6.25 mg and 18.75 mg) of a CO_2 feverfew extract (8% parthenolide) administered 3 times daily, were compared with placebo. The primary efficacy parameter was the number of migraine attacks during the last 28 days of treatment (= migraine frequency) compared to baseline. Secondary endpoints included total and average duration and intensity of migraine attacks, and number of days with accompanying migraine symptoms. No significant effect was found for either primary or secondary outcomes. Subgroup analysis including patients with at least four migraine attacks during the baseline period ($n=49$) showed a significant ($p=0.02$) reduction in migraine frequency for the 6.25 mg verum group compared to placebo; with an average of 1.8 less attacks in the final 28 days than during the baseline period [Pfaffenrath 2002].

In a double-blind, randomised, placebo-controlled study, 170 migraine patients (with 4 to 6 attacks within a 4-week baseline period) were treated for 16 weeks with 6.25 mg CO_2 feverfew extract (8% parthenolide) three times daily or placebo. Migraine frequency decreased significantly ($p=0.046$) from 4.8 attacks (baseline period) to 2.9 attacks (mean of second and third 28-day treatment period) compared to 3.5 attacks for placebo [Diener 2005].

Prophylactic effects of feverfew have also been claimed with regard to various forms of arthritis [Groenewegen 1992; Berry

1984; Hausen 1992]. However, a double-blind, placebo-controlled, 6-week study, involving 40 female patients with rheumatoid arthritis receiving either 70-86 mg of dried leaf per day or placebo, showed no beneficial effects [Pattrick 1989].

Pharmacokinetic properties

An *in vitro* study with human intestinal cells (Caco-2) demonstrated a substantial linear transport of parthenolide at 250 µM (compared with propranolol and losartan). This transport was not affected by MK-571, an inhibitor of P-glycoprotein, showing that there is no efflux of parthenolide and suggesting a good bioavailability [Khan 2003].

Parthenolide given to patients at doses of up to 4 mg per day could not be detected in plasma [Curry 2004].

Preclinical safety data

Reproductive toxicity
The theoretical maximum tolerated dose of 12.9 ml/kg/day of a 65.3 mg/ml feverfew solution (20% ethanol; 0.23% parthenolide) was confirmed as non-toxic in a preliminary experiment using incremental dosing by oral gavage in 2 non-pregnant, female rats for 8 days. This dose of feverfew (839 mg/kg/day) was administered to female rats on either gestation days (GD) 1-8 or GD 8-15. Controls received an equivalent dose of ethanol or distilled water. On GD 20, foetuses were weighed and examined for signs of external, internal or skeletal malformations: the liver weight of the verum group in GD 1-8 was significantly ($p<0.05$) lower compared to the ethanol group, but not to the water controls. When rat embryos were explanted and cultured with the extract at concentrations from 1 to 4 µl/ml, malformations and embryotoxicity were demonstrated in a dose-dependent manner [Yao 2006].

Clinical safety data

A systematic review of 6 clinical trials using feverfew mono-preparations for the prevention of migraine led to the conclusion that there are no major safety problems. In two clinical trials, the incidence of adverse effects was actually higher in the placebo groups than in the verum groups [Ernst 2000; Pittler 2009].

Thirty female migraine patients who had taken feverfew daily (12.5-250 mg) for more than 11 consecutive months were compared to a matched control group of 30 female migraine patients who had not used feverfew. No statistically significant difference in the mean frequency of chromosomal aberrations and sister chromatid exchanges in lymphocytes were observed over a period of several months. Urine from feverfew-using migraine patients did not induce a significant increase in the mean number of revertants in the Ames mutagenicity assay (30 ml of urine, concentrated and dosed in 50 ml of DMSO), with or without metabolic activation, in comparison with urine from matched non-using migraine patients [Johnson 1987; Anderson 1988].

Parthenolide given to cancer patients at doses of up to 4 mg per day for 28 days was well tolerated without dose-limiting toxicity [Curry 2004].

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MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Second Edition, 2003
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Second Edition, 2003
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Second Edition, 2003
RATANHIAE RADIX	Rhatany Root	Supplement 2009
RHAMNI PURSHIANI CORTEX	Cascara	Second Edition, 2003
RHEI RADIX	Rhubarb	Second Edition, 2003
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Second Edition, 2003
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Second Edition, 2003
SALICIS CORTEX	Willow Bark	Second Edition, 2003
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Second Edition, 2003
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VIOLAE HERBA CUM FLORE	Wild Pansy	Supplement 2009
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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Online Series

The Scientific Foundation for Herbal Medicinal Products

The second edition of ESCOP Monographs, published as a hardback book in 2003 with a Supplement in 2009, has been widely acclaimed for its authoritative information on the therapeutic uses of herbal medicines. Monographs covering a total of 107 herbal substances include extensive summaries of pharmacological, clinical and toxicological data, and copious references to scientific literature form an important part of each text.

Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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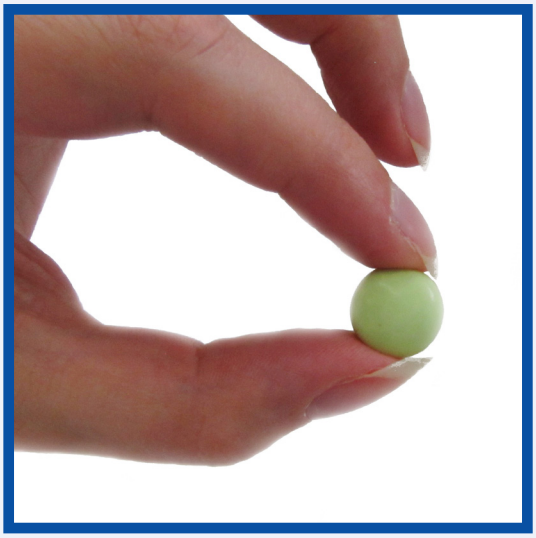
Online Series

The Scientific Foundation for Herbal Medicinal Products

Taraxaci radix

Dandelion root

2024



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E/S/C/O/P
MONOGRAPHS

The Scientific Foundation for
Herbal Medicinal Products

TARAXACI RADIX
Dandelion root

2024

E/S/C/O/P
European Scientific Cooperative
On Phytotherapy

ESCOP Monographs were first published in loose-leaf form progressively
from 1996 to 1999 as Fascicules 1-6, each of 10 monographs

Second Edition, completely revised and expanded

Second Edition, Supplement 2009

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

Edited by Roberta Hutchins and Simon Mills
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Plant illustrated on the cover: *Taraxacum officinale*

FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books*, ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009,* but are not available online for copyright reasons.

After purchase of a single monograph*,* the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in the production of ESCOP monographs and other activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-kB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-b	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

TARAXACI RADIX

2024

Dandelion root

DEFINITION

Dandelion root consists of the whole or cut, dried underground parts of *Taraxacum officinale* F.H.Wigg.

The material complies with the European Pharmacopoeia [Dandelion root].

Fresh material may also be used provided that, when dried, it complies with the European Pharmacopoeia.

CONSTITUENTS

Sesquiterpene lactones including eudesmanolides (tetrahydroridentin B and taraxacolide-1-O- β-D-glucopyranoside), guaianolides (11 β,13-dihydrolactucin and ixerin D) and germacranolides, taraxinic acid and 11,13-dihydrotaraxinic acid, their β-D-glucopyranosyl esters and β-malonylglucopyranosyl esters, as well as ainslioside and ainsliolide; lupane- and euphane-type triterpenes such as 18α,19α-epoxy-21β-hydroxylupan-3-one, officinatrione, 3β-acetoxyeupha-7,24-dien-6-one and others; benzyl glucoside and the phenylpropanoid glycosides dihydroconiferin, syringin and dihydrosyringin; triterpene alcohols and phytosterols, including taraxasterol [Hänsel 1980, Kisiel 2000a, 2000b, Kashiwada 2001, Saeki 2013, Kikuchi 2016, Esatbeyoglu 2017, Jedrejek 2019]; the γ-butyrolactone glucoside ester taraxacoside [Rauwald 1985]; phenols and phenol carbonic acids such as pyrogallol, pyrocatechol, epicatechin gallate, gallic acid, syringic, vanillic and protocatechuic acid; chlorogenic acids, hydroxycinnamic acids e.g. caffeic, ferulic, cichoric, 3-coumaric and 4-coumaric acid; di- and tri-4-hydroxyphenylacetic acid derivatives of inositol [Clifford 1987, Kenny 2014a, 2014b, Jedrejek 2019]; low amounts of flavonoids [Kenny 2014a]; potassium [Vogel 1977], amino acids [Petlevski 2001] and linear inulin-type polysaccharides consisting of β-(2→1) bonded fructofuranose [Schütz 2006, Olennikov 2009, Savych 2021]. The latex was found to contain a serine proteinase (taraxalisin) [Rudenskaya 1998].

All constituent types and amounts are subject to seasonal variation [Hook 1993, Wilson 2001, Petkova 2015, Huang 2020].

CLINICAL PARTICULARS

Therapeutic indications

Restoration of hepatic and biliary function, dyspepsia, loss of appetite, as an adjuvant in minor urinary complaints, increasing the amount of urine to achieve flushing of the urinary tract [Vogel 1977, Weiss 1991, Leung 1996, Teuscher 2020].

Posology and method of administration

Dosage
Adults: 3-5 g of the drug or 5-10 mL of tincture (1:5, ethanol 25% v/v), three times daily [Bradley 1992].

Method of administration
For oral administration.

Duration of use
If symptoms persist or worsen, medical advice should be sought.

Contraindications

Occlusion of the bile ducts, gall-bladder empyema, obstructive ileus [Weiss 1991, Leung 1996].

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Special warnings and special precautions for use

None required.

Interaction with other medicaments and other forms of interaction

None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
Dandelion root can produce allergic contact dermatitis [Lovell 1991] due to the presence of the sesquiterpene lactone taraxinic acid β-glucopyranosyl ester [Hausen 1982].

Overdose
No case of overdose reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antioxidant and radical scavenging effects
An 80% ethanolic extract dose-dependently inhibited ADP-induced platelet aggregation with a maximal inhibition of 85% at a concentration equivalent to 0.04 g of dried root per mL of human platelet-rich plasma. A fraction of the extract containing low molecular weight polysaccharides caused 91% inhibition, and a fraction enriched in triterpenes/steroids 80% inhibition, of platelet aggregation, both at a concentration equivalent to 0.04 g of dried root per mL of plasma [Neef 1996].

An ethyl acetate fraction from a methanolic extract scavenged DPPH radicals with an IC_{50} of 33 µg/mL; in the FRAP assay it had an antioxidant capacity of 433.1 Trolox equivalents/g [Kenny 2014a].

A fraction from a methanolic extract at a concentration of 50 µg/mL significantly ($p<0.05$) induced the transcription factor Nrf2 in Huh7 human hepatoma cells [Esatbeyoglu 2017].

Several fractions from an 80% methanolic extract at concentrations from 0.5 to 50 µg/mL significantly ($p<0.05$ to $p<0.001$) prevented lipid peroxidation in plasma treated with hydrogen peroxide/iron and the oxidative damage to plasma protein as well as the changes in the levels of thiol groups and protein carbonylation. These effects were not dose-dependent [Jedrejek 2019].

Anti-proliferative and anti-cancer effects
Apoptosis was induced in a dose dependent manner in Jurkat human leukaemia cells following treatment with an aqueous extract (1:2) at concentrations between 25 and 150 µL/mL with an IC_{50} value of approximately 100 µL/mL [Ovadje 2011].

An aqueous dry extract (yield 7.34%) induced apoptosis and autophagy in chronic myelomonocytic leukaemia cell lines (MV-4-11, HL-60, U-937) in a dose- and time-dependent manner via late DNA fragmentation and early activation of initiator caspase-8 respectively [Ovadje 2012].

At 200 µg/mL, a 70% methanolic extract significantly ($p<0.01$) reduced cell viability of HepG2 cells and activated AMPK. Apoptosis was proven via downregulation of Bcl-2, up-regulation of BAX and a decrease in the Bcl-2/BAX ratio. A reduction of procaspase 3 and PARP1 cleavage was observed [Rehman 2017].

Anti-diabetic and anti-adipogenic effects
Treatment of differentiating human primary visceral adipocytes with an aqueous extract (5-70 µg/mL) for 10 and 20 days resulted in a dose-dependent reduction of cell viability. At 70 µg/mL the inhibition was 52.5% after 10 days and 44.7% after 20 days. At 30 µg/mL, triglyceride accumulation in the cells was reduced [Colitti 2016].

In HepG2 cells, an aqueous extract (yield 12.1%) at a concentration of 50 µg/mL significantly ($p<0.05$) increased the uptake of the fluorescent glucose analogue 2-(n-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino)-2-deoxy¬glucose as compared to control [Juee 2020].

An aqueous extract (yield 24.9%; 64 mg/g polysaccharides) at 500 µg/mL inhibited α-glucosidase by 78.3% and α-amylase by 77.0%. In insulin-resistant HepG2 cells, the reduced glucose consumption and synthesis of intracellular glycogen were significantly ($p<0.05$) improved. The decreased activity of hexokinase and pyruvate kinase in these cells was significantly ($p<0.05$) increased [Li 2021].

Other effects
A methanolic extract (not further specified) showed strong inhibitory activity towards the formation of leukotriene B_4 from activated human neutrophils (90% inhibition at 3 µg/mL). The butanol-soluble fraction of the extract inhibited leukotriene B_4 formation by 86% at 3 µg/mL, while ethyl acetate- and water-soluble fractions displayed only weak activity (32 and 21% inhibition at 3 µg/mL respectively) [Kashiwada 2001].

Addition of 10% of an infusion (30 g root in 100 mL boiling water) to a broth medium of *Bifidobacteria* resulted in a significantly ($p<0.05$) better growth of six *Bifidobacteria* strains, the growth of two strains was slightly inhibited and one of six strains was not changed [Trojanová 2004].

In human embryonic kidney cells (HEK 293), the intracellular Ca^{2+} levels were dose-dependently increased by an ethanolic extract (not further specified) at concentrations of 10-400 µg/mL in the presence of extracellular Ca^{2+}. The extract also induced the release of Ca^{2+} from the endoplasmic reticulum and stimulated the activity of phospholipase C [Gerbino 2018].

An aqueous extract (yield 11.5%) at 5 µg/mL significantly ($p<0.05$) reversed the heavy metal (Cd, Ni, As, Cu)-induced antibiotic resistance of kanamycin and ampicillin against *E. coli*. The Cd-induced increased activity of β-lactamase and of acetyltransferase was significantly reduced ($p<0.05$) by the extract [Yang 2020].

Different fractions from an 80% methanolic extract enriched with sesquiterpene lactones (A and B), hydroxyphenylacetic acids (C), chlorogenic acids (D) or hydroxycinnamic acid derivatives (E) at 10 and 50 µg/mL significantly ($p<0.001$) inhibited adhesion of resting platelets to collagen, and lipid peroxidation in platelets treated with hydrogen peroxide/iron. Adhesion of thrombin-activated platelets to collagen or fibrinogen and of ADP-activated platelets to fibrinogen was most potently reduced by fractions C, D and E. In contrast, the most potent inhibitory effect on the aggregation of ADP-stimulated platelets was observed with fractions A, B and C [Lis 2019].

Taraxinic acid β-D-glucopyranosyl ester inhibited HIV-1 replication in acutely infected H9 cells with an IC_{50} of 1.68 µg/mL. The compound was slightly toxic against uninfected H9 cell growth with an IC_{50} of 7.94 µg/mL [Kashiwada 2001].

In vivo experiments

Hepatoprotective effects
In male Swiss mice exhibiting liver damage induced by permethrin (96 mg/kg/day p.o.), the effects of supplementing their diet with 2% root powder (corresponding to 5 g/kg b.w./day) for 14 days were studied. Increased liver weights and water intake were noted

after permethrin ingestion, which were significantly ($p<0.001$) reduced towards normal by the supplementation. Significant ($p<0.01$ to $p<0.001$) ameliorating effects were also observed in the plasma against the increases of ALT, AST, LDH and ALP activities and the bilirubin level. In the liver, supplementation normalised the increased MDA, GSH and GPx levels, and also reduced SOD levels (all $p<0.001$) and improved the vacuolisation, congestion and condensed nuclei induced by permethrin [Ghorbel Koubaa 2020].

For 7 days Wistar rats were orally administered with a 70% ethanolic extract (not further specified) at 50 and 100 mg/kg b.w./day or a hepatoprotective herbal combination consisting of 8 components at 56 mg/kg/day as control. Liver damage was induced by 1.5 mL CCl_4 i.p. 6 hours after the last dose and the animals were sacrificed on day 8. The CCl_4-induced decreased concentrations of SOD, CAT, glutathione and peroxidase in the liver were significantly ($p<0.001$) restored by the higher dose of extract when compared to control and comparable to the herbal combination. The increased MDA concentration was significantly ($p<0.01$) attenuated by the higher extract dose as compared to control and was also comparable with the herbal combination [Sumanth 2006].

In male ICR mice, liver damage was induced with ethanol (5 g/kg b.w./day by gavage for 8 days). One group received 1 g/kg/day of a lyophilised hot water extract (1:20) by gavage 30 min before ethanol administration. The extract significantly reduced the ethanol-induced elevated levels of ALT, AST, ALP and LDH in the serum (all $p<0.05$). The reduction of the activities of CAT, GST, GPx and glutathione reductase as well as of the concentration of GSH in the liver due to ethanol were normalised by the extract (all $p<0.05$). The increase of MDA in the liver was also significantly ($p<0.05$) attenuated [You 2010].

Hepatic fibrosis was induced by i.p. administration of CCl_4 (20% in olive oil, 2 mL/kg) twice a week for 4 weeks in male BALB/c mice. For the following 10 days, the animals received a tincture (4:1; 12% ethanol) at i.p. doses of 200 and 600 mg/kg b.w./day. The tincture significantly ($p<0.05$) reduced the CCl_4-induced increases in weight gain, relative liver weights and hydroxyproline content (as a marker of the degree of fibrosis in the liver). The elevated serum ALP activity was decreased with the high dose and the reduced SOD activity was normalised at both doses. In contrast, increased AST and ALT activities remained unchanged. The tincture reduced hepatic fibrinous deposits, restored the histoarchitecture of the livers, modulated the expression of glial fibrillary acidic protein and α-smooth muscle actin and increased metallothionein I/II in the liver [Domitrović 2010].

The effects on CCl_4-induced hepatic injuries of a crude ethanolic extract (yield 22%), and an ethyl acetate (0.42%) fraction enriched in two sesquiterpene lactones, were studied. Male Swiss albino mice received either the extract at 150 mg/kg b.w. or the fraction at 20 mg/kg b.w. for 7 seven days as pre-treatment before intoxication (pre-treatment; single dose of 20 µg/kg i.p. CCl_4) or for 7 days after intoxication (post-treatment; single dose of 20 µg/kg i.p.CCl_4). The increase of total bilirubin and the decrease in total protein, as well as the elevated activity of AST, ALT and ALP in the serum, due to CCl_4 were significantly ($p<0.05$) improved by all treatments compared to control. CCl_4-induced increases in liver weight, liver protein and lipid peroxidation were significantly ($p<0.05$) reduced, and the depletion of reduced glutathione in the liver was restored ($p<0.05$) by both pre- and post-treatment; post-treatment was slightly more effective. Histological examination showed significant liver protection in mice post-treatment with the extract and in both pre- and posttreatment with the fraction [Mahesh 2010].

In male Wistar rats, injuries in hepatic and testicular tissues were induced by irradiation with 8.5 Gy γ-rays, resulting in increases in oxidative markers (MDA, protein carbonylation) and decreases in antioxidant markers (GSH, SOD, GPx, CAT) in both tissues compared to control (all $p<0.001$). Oral treatment with 200 mg/kg b.w./day of an aqueous extract (not further specified) for 14 days before irradiation or 14 days after irradiation led to significant ($p<0.001$) improvements of these parameters as compared to only irradiated animals. The extract also positively modulated the irradiation-disturbed activities of purine nucleoside phosphorylase, glutamate dehydrogenase and glutathione-S-transferases in serum as well as of ALT, AST and ALP in the liver; levels of LDH and albumin in the liver were largely restored, and those of IL-1β, TNF-α and caspase-3 were reduced (all $p<0.001$ as compared to irradiated rats). A significant improvement of serum levels of testosterone, inhibin B and FSH, as well as testicular Zn and steroidogenic acute regulatory protein, and cholesterol side-chain cleavage enzyme *P450scc* gene expression were observed (all $p<0.001$). Histopathological changes in the hepatic and testicular tissues following irradiation were ameliorated. The improvements of most parameters were slightly better by pre-treatment with the extract as compared to the treatment after irradiation [Abdel-Magied 2019].

Chronic liver failure was induced in male Wistar rats by s.c. administration of human serum albumin, this was followed by oral treatment with an extract (70% ethanol; yield 15.2%) at doses corresponding to 50, 100 and 200 mg of the root/kg b.w. daily for 7 days. Acute on chronic liver failure (ACLF) was induced on day 8 with D-galactosamine (700 mg/kg i.p.) and LPS (10 µg/kg i.p.). The extract significantly decreased the elevated markers of liver injury (AST, ALT, ALP, γ-glutamyltransferase, total bilirubin; all $p<0.001$), renal markers (creatinine, urea; $p<0.001$) and parameters of oxidative stress (total oxidative stress, oxidative stress index, MDA, NO, 3-nitrotyrosine: all $p<0.001$; total thiols; $p<0.01$) in serum as compared to untreated ACLF rats. Histopathology showed significantly ($p<0.001$) reduced hepatic injuries in all extract groups compared to the untreated ACLF group [Pfingstgraf 2021].

In male ICR mice, liver damage was provoked by an i.p. injection of 400 mg/kg b.w. of paracetamol. Six hours after paracetamol the animals were treated with polysaccharide fractions DPR1 or DPR2 from the root (50, 100 or 200 mg/kg i.p. three times daily for two days). N-Acetylcysteine (120 mg/kg) served as positive control. The paracetamol-induced injuries in liver tissue (haemorrhages, necrosis, neutrophil infiltration) were attenuated by all doses of both fractions to an extent comparable with the positive control. Paracetamol-induced elevated serum AST levels and oxidative stress markers in the liver (ROS, MDA) were significantly ($p<0.01$) and dose-dependently reduced by DRP1 and DRP2. Both fractions at 100 and 200 mg/kg significantly ($p<0.01$) reversed the paracetamol-induced changes in liver antioxidant enzyme activities. These effects were mediated by the Nrf2-Keap pathway [Cai 2017].

Metabolic effects

Male rabbits were fed a high-cholesterol diet (250 g/day) with or without 1% dried root for 4 weeks. The high fat diet increased liver weight and the serum concentrations of AST and ALT as well as plasma concentrations of TC, LDL-C, triglycerides and phospholipids. Only the elevated ALT and triglyceride concentrations were significantly ($p<0.05$) reduced by the supplementation. Increased LDL and phospholipids were further augmented by the root. No effect on decreased HDL was observed. The supplementation significantly ($p<0.05$) attenuated the effects of the high fat diet on GSH, GST, GPx, CAT, SOD and TBARS in the liver. In the aorta, atherosclerotic lesions were reduced and increased wall thickness was significantly ($p<0.05$) decreased [Choi 2010].

Crossbred (PL x PLW) x Duroc pigs with an initial body weight of 25.0 kg received a diet containing 4% root powder until reaching a body weight of 115 kg. As compared to controls without supplementation, the animals at a weight of 50 and 100 kg showed significantly ($p<0.05$) lower TC and LDL plasma concentrations and the highest percentage of the HDL fraction at both fattening periods. Significantly ($p<0.05$) less cholesterol was observed in backfat, the Longissimus dorsi muscle and the liver. Beneficial effects on several parameters of the fatty acid composition of the heart, liver and Longissimus dorsi reached significance as well ($p<0.005$) [Grela 2014].

Aqueous and 80% ethanolic extracts, administered orally to mice at the equivalent to 25 g of dried root per kg b.w., showed no hypoglycaemic activity in oral glucose tolerance tests [Neef 1995].

In alloxan-induced diabetic Swiss albino mice, oral administration of an aqueous extract (yield 12.1%) at doses of 200 and 400 mg/kg b.w. dose- and time-dependently reduced blood glucose levels. The strongest effect was observed after 5 hours with 40.4% and 62.3% reduction respectively. No significant effects were observed in a s.c. glucose tolerance test [Juee 2020].

Anti-inflammatory effects
A dry 80%-ethanolic extract inhibited carrageenan-induced rat paw oedema by 25% when administered orally at 100 mg/kg b.w. one hour before oedema elicitation, compared to 45% inhibition by indomethacin at 5 mg/kg [Mascolo 1987].

In another experiment, carrageenan-induced rat paw oedema was partially inhibited following i.p. administration of 100 mg/kg b.w. of an extract (42% after 3 hours and 29% after 5 hours) [Tita 1993].

A dry methanolic extract (not further specified), at a concentration of 1 mg per ear topically applied 30 min before challenge with TPA, inhibited mouse ear oedema by 51% as compared to indomethacin with 96% inhibition at the same dose [Yasukawa 1998].

Female C57BL/6 mice with ulcerative colitis induced by 2% dextran sodium sulphate (DSS) in their drinking water received a hot water extract (yield 15%; 20 mg/kg b.w. p.o.) twice daily for 10 days. The extract improved the progressive body weight loss and disease severity index from day 3, significantly on day 10 ($p<0.01$) as compared to DSS. The shortening of colon length after DSS was restored ($p<0.01$). The higher histological scores for several parameters of tissue damage and inflammation were ameliorated ($p<0.01$). Elevated inflammatory cytokines (TNF-α, IL-6, IL-1β), MPO activity, ROS (all $p<0.001$) and MDA ($p<0.05$) in the colon were significantly reduced. The decreased SOD activity was improved [Ding 2018].

Ulcerative colitis was induced in male and female C57BL/6 mice by addition of 2% DSS to their drinking water for 10 days. In parallel, mice were treated by gavage with a dry extract (ethanol; 1:10) at doses of 0.5, 1 or 2 mg/mouse/day. Sulfasalazine (100 mg/kg b.w./day) served as positive control. The extract dose-dependently reduced the colon mucosal damage index, significant at the highest dose ($p=0.03$). In histopathology, only mild epithelial cell degeneration and necrosis, as well as low numbers of inflammatory cells, were observed in colonic sections of animals receiving sulfasalazine or the high extract dose. Both these treatments also attenuated the DSS-induced changes in TNF-α, IL-6, IL-1β and IL-10 as well as oxidative injuries in serum and colon tissues. The reduced SOD activity was increased and the enhanced PAF, PGE_2 and MPO levels were decreased. The DSS-induced high phosphorylation level of ERK, JNK or p38 in colon tissues was blocked. The extract at the highest dose also completely reversed all alterations in the phosphorylation levels of NF-κB p65 and IκBα, in the abundances of IκBα and p65 in the cytoplasm and in the production of NF-κB and p65 in nuclei. All effects of the extract were reversed by the MAPK agonist, BIM-23A760 [Zhou 2022].

Anti-tumour effects
Female Wistar albino rats aged 50 days received a single oral dose of 7,12-dimethylbenz(α)anthracene (DMBA) to induce breast cancer. Four months after the administration of DMBA, one group was orally treated with 500 mg/kg b.w./day of an aqueous dry extract (not further specified) for 4 weeks. The animals were killed 5 months after DMBA administration. The elevated levels of serum cancer antigen 15-3 after DMBA were almost normalized by the extract. The DMBA-induced up-regulation of the mRNA expression of *Pdk1*, *Akt1*, *Pik3r1*, *Erbb2*, *Plk3ca* and *Map3kr1* was significantly ($p<0.05$) reduced by the extract as compared to the DMBA group. The excessive proliferation of the lining epithelium of acini and ductules with hyperchromatic nuclei and excessive immunostaining of Bcl2 in the mammary gland tissues after DMBA was ameliorated by the extract. The average tumour weight was reduced to approximately one third by the extract in comparison to DMBA [Nassan 2018].

Immunocompromised 6-week old CD1 nu/nu mice were s.c. injected with prostate cancer cells (DU-145, PC-3) in the hind flanks. After tumour formation, mice received an aqueous extract (not further specified) at a dose equivalent to 40 mg/kg/day in their drinking water for 8 weeks. Tumour volume and weight were significantly reduced as compared to controls receiving water ($p<0.0001$ for DU-145 xenografts and $p<0.01$ for PC-3 xenografts). Body weight gain measured twice a week during the 8 weeks did not differ from control [Nguyen 2019].

Choleretic effect
A decoction from fresh root (equivalent to 5 g of dried plant), administered intravenously to dogs, doubled the volume of bile secreted from the liver during a 30-minute period [Chabrol 1931].

Other effects
Male Swiss albino mice infected with cercariae of *Schistosoma mansoni* were left untreated or received orally either praziquantel (I-PQZ; 500 mg/kg b.w. for 2 days) or root powder (I-Dn; 300 mg/kg for 7 weeks) or the combination of the two medications (I-PQZ+Dn), all starting 45 days after infection. At the end of the experiment, the worm burden in the treated groups was significantly ($p<0.001$) reduced by 93.3% (I-PQZ), 53.1% (I-Dn) and 96.2% (I-PQZ+Dn) as compared to untreated controls. All treatments also resulted in significant decreases in the mean egg counts in the liver and the intestine, in mature and immature eggs, as well as an increase in dead eggs and a reduction in the number and diameter of hepatic granulomas (all $p<0.001$). The elevated levels of IL-6 and TNF-α as well as AST and ALT activities in infected mice were significantly ($p<0.05$) reduced by all treatments. All parameters produced better results with the combination than with PQZ alone [Atwa 2022].

No consistent pattern of diuresis was observed with various extracts (petroleum ether, chloroform or methanol) and their fractions when administered to mice at a dose of 50 mg/kg. Some extracts showed natriuretic and kaliuretic effects related to the potassium content of the roots [Hook 1993, Tita 1993].

Golden Pompano fish (*Trachinotus ovatus*) were fed a diet supplemented with an extract containing 50% polysaccharides at doses of 0, 0.5, 1, 2, 4 and 10 g/kg b.w. for 8 weeks. The highest final body weight, weight gain rate, specific growth rate, feed intake and protein deposit rate were observed with 1 g/kg and the highest feed and protein efficiency ratio with 0.5 g/kg supplementation (all $p<0.05$). Significant increases in plasma total

protein, ALP, complement 4, lysozyme and IgM content as well as decreases in triglycerides and LDL cholesterol were determined in all groups (all $p<0.05$) as compared to control. Hepatic SOD, total antioxidant capacity, CAT, GPx and glutathione reductase were augmented and MDA reduced, whereas the influence on these parameters in the plasma was less. After challenge with *Vibrio harveyi*, significantly ($p<0.05$) higher survival rates were observed in fish receiving supplementation [Tan 2017].

Using the same experimental set-up, increases of intestinal antioxidant parameters (SOD, CAT, total antioxidant activity) and a reduction of intestinal MDA were observed. Intestinal morphology was improved as shown by increased muscle thickness, villus length, width and number in the fore- and hindgut as well as muscle thickness and villus width in the midgut. An enhancement of the intestinal barrier function was observed by the increase of zo-1 and occludin mRNA levels and the decrease of claudin-12 and claudin-15 mRNA levels. Improved intestinal immunity was deduced from increased goblet cell numbers and the regulation of the expression of immune-related genes [Tan 2018].

In another study, Golden Pompano fish received a diet supplemented with 0, 0.5 or 1 g/kg b.w. of the same extract for 8 weeks. Weight gain rate and feed intake as well as transcription levels of GPx and IL-10 in the spleen were significantly ($p<0.05$) increased by the higher dose. No significant effects on growth factor β-1, glutathione reductase and CAT in the spleen were observed. The supplementation led to an augmentation of the relative abundance of *Actinobacteria* and *Firmicutes* and a reduction of pathogenic bacteria such as *Mycoplasma*, *Acinetobacter* or *Vibrio* in the gut microbiota. Intestinal goblet cell numbers were increased and epithelial lymphocyte numbers were decreased [Tan 2020].

The effects of an extract (80% ethanol; yield 13%; 200 mg/kg b.w./day p.o.) against doxorubicin (DXR)-induced cardiotoxicity and haematological changes were studied in male Wistar albino rats for four weeks. In the third and fourth week DXR (2.5 mg/kg) was injected 3 times weekly. The DXR-induced changes in red blood cells, total leukocytic count, monocytes, granulocytes and lymphocytes as well as in cardiac biomarkers (creatinine kinase MB, LDH, troponin I, atrial and B-type natriuretic peptide) and cardiac oxidative parameters (MDA, GSH, SOD) were attenuated by co-administration of the extract. Semiquantitative scoring of histopathological lesions due to DXR showed ameliorative effects of the extract [Gad El-Karim 2019].

To evaluate the potential of a 70% ethanolic extract (not further specified) against adverse effects of sodium benzoate (SB), Wistar rats were treated i.p. daily for 14 days with the following: (i) saline (control), (ii) 200 mg/kg b.w. SB, (iii) 200 mg/kg/day SB + 40 mg/kg extract 30 min after SB, (iv) 600 mg/kg SB or (v) 600 mg/kg SB + 40 mg/kg extract 30 min before SB. Messy fur, anxiety and mortality were observed in groups (iv) and (v) (20 and 10% respectively) as well as apathy in group (iv) and aggression in group (v). Group (ii) showed no behavioural changes, but in some animals of group (iii) aggression and anxiety occurred. Analysis of urinary parameters resulted in decreased pH as well as increased nitrite, proteinuria, ketonuria and bilirubin excretion in groups (ii) and (iv) as compared to control. Co-treatment with the extract improved all parameters. The high SB dose caused anaemia, neutropenia and thrombocytopenia as shown by decreased red blood cell count, haemoglobin concentration, number of segmented and unsegmented neutrophils among numerous other parameters. Addition of the extract ameliorated the effects of 600 mg/kg SB. Structural changes in hepatocytes were only induced in group (iv) and improved by the extract in group (v) [Suljević 2022].

Wistar albino rats consumed on average 39.4 mL/day of a decoction (3 g root/300 mL water) or drinking water for 4 weeks. In a perfusion model using isolated hearts, no differences were observed in the minimum and maximum rate of left ventricular pressure development, systolic and diastolic left ventricular pressure, heart rate and coronary flow. Markers of oxidative status in the blood differed significantly ($p<0.05$) from the water control: the H_2O_2 concentration, lipid peroxidation index and reduced glutathione activity were increased whereas superoxide anion radical, SOD and CAT concentrations were reduced [Radoman 2023].

The cardioprotective effects of a methanolic extract were studied in male Wistar rats in a model of ischaemia-reperfusion (I/R) injury-induced myocardial dysfunction. The extract was administered i.p. at daily doses of 50, 100 or 200 mg/kg b.w. for 7 days, and 24 hours after the last dose, in the perfusate 10 min before and during ischaemia and during 40 min of reperfusion. Two other groups received the extract for 7 days and either 30 µM L-NAME or 10 µM glibenclamide in the perfusate 10 min before and during ischaemia and during 40 min of reperfusion. Pretreatment with the extract significantly ($p<0.001$) and dose-dependently attenuated I/R injury-induced increases in the release of LDH and creatine kinase in the coronary effluent and of TBARS in heart tissue, as well as the reduced levels of GSH, total protein and CAT. The significant ($p<0.01$ to $p<0.001$) reduction of myocardial infarction weight and volume was also dose-dependent. In isolated hearts, the reduced coronary flow rate and heart rate were improved by the extract. The NOS inhibitor L-NAME reversed all effects of the extract whereas the K(ATP) channel blocker glibenclamide exhibited only a weak effect [Sharma 2023].

Clinical studies

No published clinical data are available.

Pharmacokinetic properties

The effects of the root on plasma levels of the irreversible tyrosine kinase inhibitors dasatinib, imatinib and nilotinib were studied in male Wistar rats. The animals were treated by gavage with the inhibitors at doses of 25 mg/kg b.w. or the suspension of root powder at 500 mg/kg plus 25 mg/kg dasatinib, imatinib or nilotinib 30 min later. No differences in the main pharmacokinetic parameters were observed in the co-treatment with imatinib or nilotinib. Co-administration of the root and dasatinib resulted in a decrease of the bioavailability of the inhibitor as compared to dasatinib alone. This was shown by significant ($p<0.05$) reductions of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ by approximately 50% via an inhibitory effect on CYP4A3 and P-gp. In addition, the clearance of dasatinib nearly doubled [Alzoman 2019].

Preclinical safety data

The intraperitoneal LD_{50} of a fluid extract (1:1) was determined as 36.6 g/kg b.w. in mice [Rácz-Kotilla 1974].

A dry ethanolic extract showed low toxicity in mice and rats at doses up to the equivalent of 10 g (oral) and 4 g (intraperitoneal) of dried drug per kg b.w. [Tita 1993].

I.p. administration of a tincture (4:1; 12% ethanol) at a dose of 600 mg/kg b.w./day to BALB/c mice for ten days did not result in any differences in body weight gain, liver weights, serum AST, ALT, ALP, SOD, liver hydroxyproline content, liver histopathology and tissue expression of glial fibrillary acidic protein, alpha-smooth muscle actin and metallothionein I/II [Domitrović 2010].

In Wistar rats of both sexes, no signs of toxicity were observed in behaviour and physical appearance for 30 min after a single oral dose of 5 g/kg of a 70% ethanolic extract and during an additional observation period of 14 days [Aremu 2019].

Single oral doses of 2 g/kg b.w. of a petroleum ether, a chloroform,

a methanol and an aqueous extract (yields 1.5, 0.6, 19.4 and 12.1% respectively) were administered to female albino mice. The extracts did not cause any changes in general behaviour or physical activity over 7 days [Juee 2020].

Male Wistar rats were treated orally with a dry aqueous extract at doses equivalent to 1.84 or 3.68 g root/kg b.w. for 60 days. The extract at the higher dose significantly ($p<0.001$) reduced body weight gain. The weight of testes ($p<0.01$) and of seminal vesicles as well as the concentration and motility of sperms (all $p<0.001$) were significantly decreased at both doses as compared to control. Serum testosterone concentration and the percentage of pregnancies after mating were also decreased by both doses when compared to control, with the high dose reducing the number of foetuses to 0. The rate of morphological abnormalities of sperms significantly increased ($p<0.001$). Rat testicular histology showed late maturation arrest with reduced germinal epithelium thickness and interstitial cells after the low dose and severe disruptions in the architecture of seminiferous tubules, early mutation arrest and germ cell hypoplasia after the high dose. The spermatogonial stem cell self-renewal and proliferation genes *gfr1*, *mcsf* and *plzf* were also influenced by both doses [Tahtamouni 2017].

Clinical safety data

In a patch test, seven patients (2F;5M) with suspected dandelion dermatitis reacted positive to a root extract (equivalent to 1% of plant material in yellow petrolatum) [Lovell 1991].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Online Series, 2019
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Online Series, 2021
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Online Series, 2019
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Online Series, 2019
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHAMOMILLAE ROMANAE FLOS	Roman Chamomile Flower	Online Series, 2019
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CORIANDRI FRUCTUS	Coriander fruit	Online Series, 2024
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Online Series, 2021
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Online Series, 2021
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EPILOBII HERBA	Willow Herb	Online Series, 2024
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Online Series, 2023
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil´s Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HELICHRYSI FLOS	Sandy Everlastin Flower	Online Series, 2019
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John´s Wort	Online Series, 2018

JUGLANDIS FOLIUM	Walnut Leaf	Online Series, 2024
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Online Series, 2020
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Online Series, 2021
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passionflower Herb	Online Series, 2023
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Online Series, 2020
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Online Series, 2021
PRUNI AFRICANAE CORTEX	Pygeum Bark	Online Series, 2020
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher´s Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Online Series, 2021
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Online Series, 2024
THYMI HERBA	Thyme	Second Edition, 2003
TILIAE FLOS	Lime Flower	Online Series, 2022
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Online Series, 2020
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Online Series, 2020
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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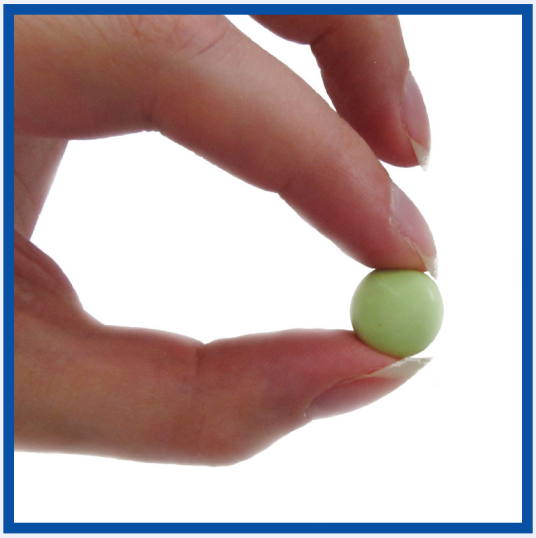

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FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

TILIAE FLOS

Lime Flower

2022

DEFINITION

Lime flower consists of the whole, dried inflorescence of *Tilia cordata* Miller, *Tilia platyphyllos* Scop., *Tilia × vulgaris* Hayne or a mixture of these.

The material complies with the monograph of the European Pharmacopoeia [Lime flower, Ph. Eur.].

Tilia x europaea L. is the currently accepted synonym of *Tilia x vulgaris* Hayne [Tutin 1968, Medicinal Plant Names Services 2021].

CONSTITUENTS

The characteristic constituents include:

- Flavonoids (ca. 1%), principally glycosides of quercetin, such as isoquercitrin, hyperoside and rutin, and of kaempferol, especially tiliroside among others [Toker 2001; Karioti 2014; Ziaja 2020].
- Proanthocyanidins of various degrees of polymerisation (DP) including dimeric procyanidins B2 and B4, a prodelphinidin trimer and procyanidin oligomers and polymers with DP 3 to 14 [Karioti 2014; Ropiak 2016; Symma 2020; Ziaja 2020].
- Phenolic acids such as chlorogenic acid, caffeic acid, p-coumaric acid and protocatechuic acid [Karioti 2014; Teuscher 2016; Ziaja 2020].
- Mucilage polysaccharides (3-10%) composed of D-galactose, L-rhamnose, L-arabinose, minor amounts of D-xylose, D-mannose and D-glucose as well as D-galacturonic acid and D-glucuronic acid [Kram 1983, 1985; Schmidgall 2000].
- Essential oil (0.02-0.08%) consisting of up to 100 compounds including monoterpenes such as linalool and geraniol, phenylpropanes like eugenol and anethole, sesquiterpenes e.g. farnesyl acetate and hexahydrofarnesyl acetone, aliphatic hydrocarbons including heneicosane, tricosane, pentacosane as well as 2-phenylethanol, its esters and nonanal and nonanoic acid [Buchbauer 1992a, 1992c; Toker 1999; Fitsiou 2007; Kosakowska 2015; Kowalski 2017; Kelmendi 2020].
- Alkaloids of the 3,4-dihydro-2H-pyrrole-type (tiliines A and B), piperidine-type (tiliamines A and B) and 3-O-acetylated piperidine-type (tilacetines A and B) [Symma 2021].
- Coumarins (e.g. scopoletin, fraxetin-8-β-D-glucoside) [Teuscher 2016].

CLINICAL PARTICULARS

Therapeutic indications

Feverish colds and cold-related cough, upper respiratory catarrh, restlessness [Bradley 1992; Barnes 2007; Teuscher 2016; Bertram 2018].

Efficacy in these indications is plausible on the basis of human experience and longstanding use.

Posology and method of administration

Dosage

Adults and children over 4 years
Three times daily: 2-4 g dried drug in infusion or equivalent preparations [Bradley 1992; Teuscher 2016; Bertram 2018]; liquid extract (1:1, 25% ethanol) 2-4 mL; tincture (1:5, 25% ethanol) 4-10 mL [Bradley 1992].

Method of administration
For oral administration.

E/S/C/O/P
MONOGRAPHS

Duration of administration
No restriction.

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available.

In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
Some allergic reactions have been reported including allergic rhinitis after consuming a tea. In topical application, urticaria occurred after using a shampoo containing lime flower extract and contact dermatitis in cosmeticians preparing herbal beauty products. In skin prick tests to aeroallergens, sensitisation prevalence was described [Picardo 1988; De Smet 1993; Mur 2001; Rudzki 2003; Krakowiak 2004; Yazicioglu 2004; Loureiro 2005].

Overdose
No case of overdose reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antioxidant and radical scavenging activities
In various assays including DPPH, ABTS, Fe^{2+}/ascorbate-induced lipid peroxidation and carotene bleaching, radical scavenging or antioxidant activities were shown for several aqueous, methanolic or hydroethanolic extracts [Choi 2002; Katalinic 2006; Duda-Chodak 2011; Albayrak 2012; Aralbaeva 2017; Jabeur 2017].

Effects on enzyme inhibition
A dry extract (methanol 50%) exhibited lipase inhibition of more than 70% in two assays and retained the effect in one of the assays after removal of tannins [Slanc 2009].

A dose-dependent inhibition of α-glucosidase and α-amylase was shown for a hot water extract (5 g drug/100 mL). α-Glucosidase was inhibited by 75% at 0.5 mg/mL and by 100% at 2.5 mg/mL. At a dose of 25 mg/mL α-amylase was inhibited by 71% [Ranilla 2010].

Inhibitory effects on α-amylase, α-glucosidase, acetyl-cholinesterase and butyrylcholinesterase were demonstrated for an extract (methanol 80% containing 1% HCl), with EC_{50} values of 8.2, 10.8, 73.8 and 199.9 mg/mL respectively [Nowicka 2019].

At 50 µg/mL, an extract (2 g drug/100 mL, 50% ethanol) reduced tyrosinase activity to 34% and elastase activity to 24% [Lianza 2020].

Antimicrobial activity
A methanolic dry extract (1:10) showed antimicrobial activity against *Bacillus subtilis, B. cereus, Klebsiella pneumoniae, Morganella morganii, Pseudomonas aeruginosa* and *Staphylococcus aureus* [Albayrak 2012].

Extracts (80% ethanol) from numerous samples of lime flowers were tested in a screening for antimicrobial activities. Among the panel of human and plant pathogenic microorganisms tested, *Streptococcus pyogenes* and *Bacillus subtilis* were the most sensitive bacteria to some of the samples, with MICs of 160 and 120 µg/mL respectively [Pavlović 2020].

Effect on cell proliferation
An aqueous dry extract (1.5 g/20 mL) had a significant ($p \leq 0.05$) stimulatory effect on the proliferation of lymphocytes with maximum activity at a concentration of 20 µg/mL. This effect was comparable with the benzodiazepine agonist Ro 5-4864 suggesting that the extract exerted the stimulatory action via peripheral type benzodiazepine binding sites [Anesini 1999].

Dry extracts of varying polarity (water, ethanol, dichloromethane) showed antiproliferative effects on the tumour lymphocyte cell line BW 5147. The dichloromethane extract was the most active with an EC_{50} value of 4.8 µg/mL (ethanol extract: 52.5 µg/mL, aqueous extract: 4987 µg/mL). On normal lymphocytes stimulated with concanavalin A, the dichloromethane extract exhibited the greatest antiproliferative activity with an EC_{50} value of 14 µg/mL (ethanol extract: 359 µg/mL, aqueous extract: 500 µg/mL) [Barreiro Arcos 2006].

Other effects
The interaction of an aqueous extract (3 g drug in 100 mL water, containing 0.84 mg caffeic acid, 0.11 mg rutin/mL and about 100 µM GABA) with the $GABA_A$ receptor complex in rat brain was investigated. The extract inhibited [^{3}H]muscimol binding with an IC_{50} value of 2.7 ± 0.3 µg/mL. Furthermore, it caused 44.0% inhibition of [^{3}H]flunitrazepam specific binding at the highest concentration tested (10 mg extract/mL) and stimulated ^{36}Cl uptake by the synaptoneurosomes [Cavadas 1997].

An ethanolic extract (100 g lime flower/L) from 5 to 1000 µg/mL caused a concentration-dependent and significant ($p \leq 0.05$) contractile effect on dispersed smooth muscle cells from guinea pig small intestine. The contraction was inhibited by atropine [Al-Essa 2007].

A dichloromethane extract at 10 µg/mL caused a moderate (50-75%) transactivation of the peroxisome proliferator-activated receptors α and γ. A detannified methanol extract at the same concentration moderately inhibited NF-κB [Vogl 2013].

A moderate bioadhesive effect of a polysaccharide fraction was demonstrated in the porcine buccal membrane model by the reduction of the carbohydrate titre in the supernatant of the incubation solution by approximately 40% [Schmidgall 2000].

Several procyanidins from lime flowers at concentrations of 1 and 5 µM had no effect on the viability of human neutrophils as compared to LPS-stimulated and -unstimu-lated controls. At these concentrations, most compounds significantly ($p<0.05$) reduced the IL-8 secretion of LPS-stimulated neutrophils, but did not influence TNF-α, MMP-9 and MIP-1α production [Czerwińska 2018].

Fractions enriched in alkaloids from a dry extract (water-acetone

7:3; yield 17%) significantly ($p<0.05$) increased acetylcholine-induced contractions of mouse trachea slices by approximately 30%, an effect which was reversed by atropine [Hake 2021].

In vivo experiments

Sedative effects
A hot water dry extract (not further specified), administered i.p. to male mice at 25, 50 and 100 mg/kg b.w., significantly ($p\leq0.01$) decreased mobility times and number of rearings in the open-field test. In the elevated plus maze test there was a significant ($p\leq0.01$) decrease in the total number of entries into the different sections of the maze [Coleta 2001].

Inhalation of an essential oil caused a 45% decrease in the motility of mice. The effect of the essential oil in caffeine-induced over-agitation of the animals was limited [Buchbauer 1992b].

Other effects
The hepatoprotective effect of an infusion was studied in Wistar rats for 50 days. The animals received either 20% ethanol or 20% ethanol plus 2% of an infusion with their drinking water. The ethanol-induced elevated serum levels of AST and LDH, as well as total albumin and total cholesterol, were reduced significantly ($p<0.05$) by the infusion and a trend towards a reduction was observed for ALT. With respect to markers of the antioxidant system such as reduced glutathione, glutathione reductase, SOD, GST, CAT, GPx and MDA in various organs (liver, spleen, kidney, brain, heart, erythrocytes) no restoration of ethanol-induced changes to control levels was seen [Yayalaci 2014].

Anthelmintic effects of a hydroacetonic extract enriched in tannins was tested against *Oesophagostomum dentatum*, a helminth parasite of pigs. Concentrations of 125 and 250 µg/mL strongly inhibited the development of eggs (stage L1) to the infective third stage larvae (L3), with complete suppression at 0.5 and 1 mg/mL. Overnight incubation with 1 mg/mL significantly ($p<0.01$) reduced the migratory ability of L3 larvae. Moulting of L3 to L4 larvae was decreased after 14 days of incubation (1 mg/mL; $p<0.01$), while reduced mobility of L4 larvae was noted after 24 hours [Williams 2014].

Pharmacokinetic properties
After inhalation of an essential oil, benzylalcohol and traces of benzaldehyde were detected in blood samples of mice [Buchbauer 1992b].

Preclinical safety data
A hot water extract (yield 9.2%) at a concentration of 640 µg/mL of the medium did not result in toxic effects on Vero cells in the MTT assay or in cell morphology [Sotiropoulou 2020].

Repeated dose toxicity studies
After supplementation of the drinking water of rats with 2% of an infusion for 50 days, no changes in serum ALT, total proteins, total albumin and total cholesterol were observed. Serum AST and LDH increased significantly ($p<0.05$) [Yayalaci 2014].

A hydroethanolic extract enriched in phenolics was added to the drinking water of K14HPV16 mice (wildtype HPV16- and haemozygotic HPV16+) at a concentration of 450 µg/mL for 33 days. No mortalities were observed. No significant differences in serum biochemical parameters (microhaematocrit, creatinine, urea, ALT, AST), in organ weights (heart, lung, spleen, left kidney and thymus), in oxidative stress parameters in the liver and kidneys (ROS, SOD, CAT, glutathione-S-transferase, MDA) as well as in liver and kidney lesions were observed between exposed groups and the respective controls receiving water. Only in male wildtype mice was an increase in CAT in the kidney significant ($p<0.05$) as compared to water control [Ferreira 2021].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 200
AGRIMONIAE HERBA	Agrimony	Online Series, 2019
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Online Series, 2021
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Online Series, 2019
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Online Series, 2019
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHAMOMILLAE ROMANAE FLOS	Roman Chamomile Flower	Online Series, 2019
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Online Series, 2021
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Online Series, 2021
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HELICHRYSI FLOS	Sandy Everlastin Flower	Online Series, 2019
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Online Series, 2018

JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Online Series, 2020
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Online Series, 2021
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Online Series, 2020
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Online Series, 2021
PRUNI AFRICANAE CORTEX	Pygeum Bark	Online Series, 2020
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Online Series, 2021
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TILIAE FLOS	Lime Flower	Online Series, 2022
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Online Series, 2020
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Online Series, 2020
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

E/S/C/O/P Monographs

Online Series

The Scientific Foundation for Herbal Medicinal Products

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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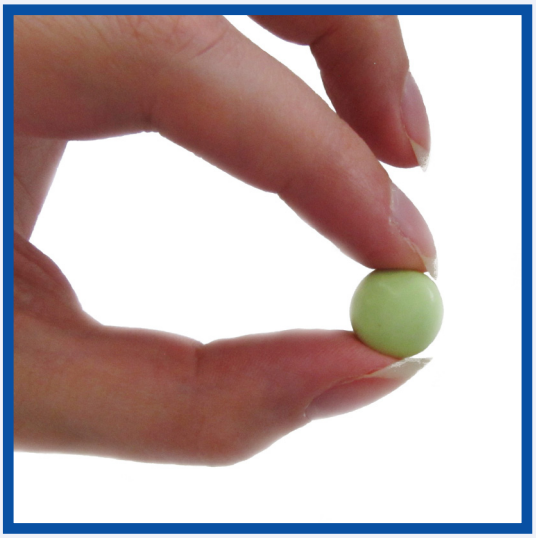
The Scientific Foundation for Herbal Medicinal Products

Tormentillae rhizoma

Tormentil

2013



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E/S/C/O/P
MONOGRAPHS

***The* Scientific Foundation for**
Herbal Medicinal Products

TORMENTILLAE RHIZOMA
Tormentil

2013

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On Phytotherapy

ESCOP Monographs were first published in loose-leaf form progressively
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Second Edition, completely revised and expanded

Second Edition, Supplement 2009

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

Edited by Simon Mills and Roberta Hutchins
Cover photograph by em. Univ. Prof. Dr. Wolfgang Kubelka,
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Plant illustrated on the cover: *Potentilla erecta (P. tormentilla)*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
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Notes for the Reader
Abbreviations
The monograph text
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Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

TORMENTILLAE RHIZOMA 2013

Tormentil

DEFINITION

Tormentil consists of the whole or cut, dried rhizome, freed from the roots, of *Potentilla erecta* (L.) Raeusch. (*P. tormentilla* Stokes). The dried drug contains minimum 7 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1).

The material complies with the European Pharmacopoeia [Tormentil].

CONSTITUENTS

Approximately 15 to 22% tannins consisting mostly of condensed tannins (changing to phlobaphenes during storage, "tormentil red"). Monomers such as catechin and epicatechin, dimers such as procyanidins B3 and B6, trimers as well as pentameric and hexameric derivatives [Hermann 1957, Ahn 1973, Glasl 1983, Lund 1985, Vennat 1992, Kolodziej 1992, Horz 1994; Scholz 1994, Frohne 2002].

Ellagitannins, mainly the dimer agrimoniin (1%) [Lund 1985], in addition to pedunculagin and 2,3-hexahydroxydiphenic acid glucoside as well as laevigatin B and F [Geiger 1990, Geiger 1991, Geiger 1994, Horz 1994, Frohne 2002].

p-Coumaric, sinapic and other phenolic acids such as caffeic and gallic acid as well as catechin gallates [Frohne 2002]. Flavonoids e.g. kaempferol [Vennat 1992, Horz 1994, Frohne 2002] and quercetin glucuronides [Kolodziej 1992].

Triterpene saponins such as chinovic, tormentic, euscaphic, ursolic, oleanoic and 3-epi-pomolic acids, as well as their glycosides e.g. the tormentic acid monoglucoside tormentoside and its isomers kaji-ichigoside F1 and arjunetin [Bilia 1994, Horz 1994, Stachurski 1995, Frohne 2002, Kite 2007].

CLINICAL PARTICULARS

Therapeutic indications

Non-specific, acute diarrhoea [Braun 1953, Hoffmann 1954, Horz 1994, Schulz 1999, Frohne 2002, Fintelmann 2002, Subbotina 2003, Schilcher 2007]. Adjuvant treatment of acute and subacute enteritis and colitis [Fintelmann 2002, Huber 2007, Moss 2007, Schilcher 2007].
Slight irritations of the mucosa of mouth and throat [Braun 1953, Hoffmann 1954, 1955, Horz 1994, Volodina 1997, Fintelmann 2002, Frohne 2002, Schilcher 2007].

Efficacy in these indications is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Internal use:

Adults:
Daily dose: 4-6 g herbal drug [Horz 1994, Frohne 2002, Schilcher 2007].
Decoction: 1 teaspoon per cup 3-4 times daily [Schilcher 2007].
Tincture (1:5): 30-50 drops 3-4 times daily [Van Hellemont 1988].
Tincture (1:10): 10-30 drops per small glass of water (ca. 50 ml) several times a day, in acute conditions every hour [Braun 1953, Schilcher 2007].
Dry extract (3.5–4.5:1; ethanol 60%): 1200 to 3000 mg daily [Huber 2007].

Children (from 3 months onwards):
Liquid extract (1:10, ethanol 40%): 3 drops daily per year of life [Subbotina 2003].

External use:
Decoction: 3-4 tablespoons per cup for gargling or rinsing several times daily [Schilcher 2007].
Tincture (1:5): Undiluted for brushing the gingiva several times daily [Van Hellemont 1988].
Tincture (1:10): 10-20 drops per glass of water for gargling or rinsing or undiluted for brushing the gingiva several times daily [Schilcher 2007].

Method of administration
For oral administration and local application.

Duration of administration
No restriction. If symptoms persist or worsen, medical advice should be sought.

Contraindications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported, but due to the high content of tannins, in sensitive persons slight gastric complaints may occur [Schulz 1999, Schilcher 2007].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Astringent activity
Due to its high tannin content, tormentil has astringent effects [Horz 1994].

The astringency of ellagitannins isolated from tormentil was determined by haemanalysis with fresh human blood and by their relative affinity to methylene blue. Agrimoniin, pedunculagin and laevigatin F showed the highest activity [Geiger 1991, 1994].

Antisecretory activity
A lyophilized hot water extract (3 g herbal drug/150 ml) was examined on isolated rabbit colon. Mucosal application of 200-800 µg/ml of the extract showed a dose-dependent increase in the PGE_2-stimulated chloride secretion of 4-32% [Geiger 1994].

Antibacterial activity
A water-soluble fraction of tormentil was tested for its bacteriostatic activity on various cultures. At concentrations of 2.5-10 mg/ml, inhibition of *Staphylococcus aureus*, *Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli* (2 strains), *Shigella Boydii, Sh. Flexneri* type 1, *Sh. Sonnei* type 1, *Streptococcus faecalis* and *Pasteurella pseudotuberculosis* type 1 was observed. The same fraction showed agglutinating properties on suspensions of *Salmonella paratyphi* type A and B, *S. typhi, S. typhimurium, Brucella, Shigella Flexneri* type 1, *Sh. Sonnei* type 1 and *Pasteurella pseudotuberculosis* type 1 [Pourrat 1963].

Isolated polyphenolic compounds from tormentil showed bactericidal activity against *Bacillus subtilis, B. polymyxa* and *Azotobacter* [Nikitina 2007].

Antiviral activity
A decoction of tormentil was examined for its virustatic properties using HeLa cells incubated with a suspension of the respective virus. The decoction showed strong virustatic effects on *Herpes* virus [May 1978].

Antioxidant activity
The water-soluble fraction of a tormentil extract (prepared from 60 g powder, 500 ml ethanol 80% v/v), as well as procyanidins fractionated according to their degree of polymerisation, were tested for their antioxidant properties towards superoxide anion. The extract, an ethanolic fraction (containing monomers, dimers and trimers), a methanolic fraction (containing tetramers) and an acetone/water (7:3) fraction (containing pentamers and hexamers) showed superoxide anion-scavenging properties with an IC_{50} of 24, 56, 13 and 1.25 µg/ml, respectively [Vennat 1994].

The same preparations were tested for their effect on lipoperoxidation and anti-elastase activity. The extract, and the ethanolic, methanolic and acetone/water fractions, inhibited lipoperoxidation in rat liver microsomes induced by an Fe^{2+}/ADP complex and sodium ascorbate with an IC_{50} of 33, 6, 19 and 14 µg/ml, respectively. The extract inhibited elastase activity with an IC_{50} of 44 mg/ml and the methanolic and acetone/water fractions with an IC_{50} of 194 and 4 µg/ml, respectively, whereas the ethanolic fraction showed weak activity [Bos 1996].

In another experiment, the antioxidant effect of an extract (70% ethanol) was measured by its influence on photochemiluminescence of Gly-Trp using riboflavin for superoxide generation. A strong anti-oxidant effect was seen as compared to other plant extracts [Bol'shakova 1998].

Antioxidant effects of a tormentil preparation (single dose dissolved in the smallest possible volume of water, no further information available) were examined in cell-free oxidant-generating systems and inflamed human colorectal biopsies from patients with active ulcerative colitis who were treated with 5-aminosalicylate, prednisolone or azathioprine. In a xanthine/xanthine oxidase system which was used to detect superoxide scavenging by luminol-enhanced chemiluminescence, tormentil showed dose-dependent antioxidant activity (IC_{50} with a dilution of 10^{-6} parts per volume, ppv). In a phycoerythrin degradation assay which determined radical scavenging properties by fluorimetry, tormentil had an IC_{50} at a concentration of 10^{-5} ppv. In a chemiluminescence assay which was used to detect effects on the generation of oxygen radicals by sigmoid or rectal mucosal biopsies, tormentil significantly ($p < 0.05$) reduced chemiluminescence when biopsies were incubated with a 1:1000 dilution [Langmead 2002].

Anti-inflammatory activity
An extract (25 µg/ml, ethanol 50% V/V) of tormentil inhibited by 88 % the activity of porcine pancreatic elastase against the substrate N-succinyl-L-alanyl-L-alanine-L-valine-p-nitroanilide (SAAVNA) [Lamaison 1990].

An aqueous lyophilized extract of tormentil was tested for its

inhibitory activity on prostaglandin biosynthesis in bovine seminal vesicle microsomes with ^{14}C-arachidonic acid. Indomethacin was used as a reference. An inhibitory effect of 78 ± 9 % was shown. The same extract was tested for its platelet-activating factor (PAF)-induced exocytosis in neutrophils using SAAVNA (0.2 mM) as a substrate and produced an inhibition of 92 ± 2% [Tunon 1995].

Cytotoxic activity
An extract prepared with 40 % ethanol was tested on human lymphoblastoid Raji cells in the presence of 5 % fetal bovine serum. Methothrexate, fluorouracil, cyclophosphamide and vinblastine were used as reference substances. Tormentil suppressed the cell growth at 10 and 50 µg/ml. The final cell density was 30 ± 6 % and 2 ± 2 %, respectively, as compared to controls. In addition, tormentil was found to be cytotoxic and disrupted the process of cytokinesis which led to the formation of giant polyploid cells [Spiridonov 2005].

Agrimoniin showed strong cytotoxicity on MM2 cells in vitro (IC_{50}: 2.6µg/ml), but the activity decreased to about 4 % of the initial activity (62.5 µg/ml) after addition of foetal calf serum to the culture. I.p. injection of 10 mg/kg agrimoniin increased the number of peripheral white blood cells and the percentage of monocytes [Miyamoto 1987].

In vivo experiments

Antitumour effects
Isolated agrimoniin (from *Agrimonia pilosa*) administered i.p. before or after i.p. inoculation with MM2 mammary tumour cells effectively blocked tumour growth in C3H/He mice at doses higher than 10mg/kg. I.v. (3 mg/kg) and p.o. (300 mg/kg) application did not block the tumour growth but did cause cell regression, even 14 days before the tumour cell inoculation. Agrimoniin was also effective post-inoculation by each administration route and prolonged the life span. MH134-bearing and C3H/He mice were given agrimoniin i.p. once daily for 12 days or i.v. on days 7 and 10. In both cases tumour growth was suppressed but the animals died due to lymph node and lung metastasis. On Meth-A-bearing BALB/c mice, 3 and 10 mg/kg of agrimoniin markedly inhibited tumour growth after daily i.p. administration from day 10 to 18 [Miyamoto 1987].

Hypoglycaemic effects
The effect of isolated tormentic acid (from *Poterium ancistroides*) was studied in normoglycaemic, hyperglycaemic and streptozotocin-induced diabetic rats. Oral administration of 30 mg/kg of tormentic acid for up to 120 min, or intravenous application for up to 60 min, when compared to control produced a significant decrease in blood glucose levels ($p<0.05$ to 0.001 and $p<0.01$ to 0.001, respectively) with a corresponding significant increase in insulin levels ($p<0.01$ to 0.001 and $p<0.05$ to 0.01, respectively) when compared to control. In addition, tormentic acid at an oral dose of 30 mg/kg significantly improved glucose tolerance ($p<0.01$ to 0.001) and significantly increased the insulin secretory response to glucose ($p<0.05$ to 0.001) in glucose-induced hyperglycaemia (glucose load administration at 30, 60, 90, and 120 min). In rats with a severe state of streptozotocin-induced diabetes, neither oral administration of tormentic acid (30 mg/kg) nor glibenclamide (10 mg/kg) caused any significant decrease in plasma glucose concentration or any significant modification in plasma insulin levels [Ivorra 1988]

Other effects
A dried methanolic extract and a lyophilized aqueous extract (each prepared from 5g) were tested for their molluscicidal activity on *Biomphalaria glabrata*, the intermediate host of schistosomiasis. The snails were exposed to a distilled water solution containing the extract, tannic acid was used as control. The methanolic extract was 100% lethal from a concentration of 100 ppm onwards, the aqueous extract from 400 ppm, and tannic acid from 50 ppm [Schaufelberger 1983].

A tormentil extract (4:1; acetone 75 %) was tested for its antihypertensive, antiallergic, immunostimulating, interferon-inducing and antiviral effects in different *in vivo* models. Compared to chemical substances the tormentil extract did not demonstrate effects [Lund 1985].

Clinical data

Sixteen patients (15 completed the study) between 18 and 65 years suffering from active ulcerative colitis received a tormentil dry extract (3.5–4.5:1; ethanol 60%) at increasing doses of 1.2, 1.8, 2.4 and 3.0 g/d for 3 weeks each. Each treatment phase was followed by a 4-week washout phase. Aminosalicylate and steroid medication was permitted to be continued. For the assessment of efficacy, the outcome parameters were clinical activity index (CAI) and C-reactive protein. During therapy with 2.4 g extract per day, median CAI improved from 8 (6 to 10.75) mg/L at baseline to 4.5 (1.75 to 6); and median C-reactive protein from 8 (3 to 17.75) to 3 (3 to 6) mg/L. Mean CAI decreased during treatment phases whereas it increased during the washout phases. The therapeutic effects of tormentil extract could not be explained by concomitant medications and the use of steroids could be reduced [Huber 2007].

A randomized, double-blind, placebo-controlled trial was conducted in 40 children aged between 3 months and 7 years suffering from rotavirus diarrhoea. The treatment group (n=20) received 3 drops of tormentil extract (1:10, ethanol 40 %) per year of age, three times daily until discontinuation of diarrhoea, or a maximum of 5 days. A significant reduction of the stool output was seen on the second day (25.9 ml/kg vs. 31.5 ml/kg; $p=0.029$), and in total duration of diarrhoea (3 days vs. 5 days; $p<0.0001$) in the treatment group compared to the control group. After 48 h diarrhoea decreased in 8 (40 %; $p<0.0001$) children in the verum group compared to 1 child (5 %) in the placebo group. The volume of parenteral and oral rehydration was comparable in both groups on day 1. On day 2, patients in the treatment group required less parenteral rehydration solutions than patients in the control group (22.9 ml/kg vs. 46.6 ml/kg; $p=0.0009$). In addition, stool consistency in children in the treatment group was normalized within 4 days, compared to 6 days in the placebo group ($p<0.0001$). The length of hospitalisation was significantly shorter for the treatment group (3.5 days vs. 5.5 days; $p<0.0001$) [Subbotina 2003].

Pharmacokinetic properties

In the serum of 2 healthy volunteers who received a single dose of 2.0 g of an ethanolic dry extract of Tormentil (3.5–4.5:1; ethanol 60%) and 16 colitis patients who received 3.0 g daily, neither tannins nor their metabolites could be detected for a period of up to 8 or 6 hours, respectively [Huber 2007].

Preclinical safety data

Acute toxicity
No acute toxicity was observed in mice during the 72 hours after oral (300 mg/kg) and i.p. (200 mg/kg) administration of a tormentil extract (4:1; acetone 75 %) [Lund 1985].

Mice treated with a high dose of tormentic acid (600 mg/kg i.p.) did not show any visible signs of acute toxicity during 7 days of observation. Tormentic acid caused a slight sedation

in the animals after 30 and 90 min, with a small decrease in awareness and motor activity. No changes were observed in CNS excitation, posture, motor coordination and autonomic response [Ivorra 1988].

Intraperitoneal injection of more than 10 mg/kg agrimoniin to mice led to stretching and writhing reactions within 5 min. Average LD_{50} (male/female) was 101.4/102.7 mg/kg (i.p.), 31.2/35.4 mg/kg (i.v.) and > 1000 mg/kg (p.o.). After p.o. administration the animals developed only a slight depressed state for about 2 hours [Miyamoto 1987].

Clinical safety data

In a study in 16 patients with active ulcerative colitis who received a tormentil dry extract (3.5–4.5:1; ethanol 60 %) at increasing doses of 1.2, 1.8, 2.4 and 3.0 g/d for 3 weeks each, mild upper abdominal discomfort was experienced by 6 patients (38 %), but did not require discontinuation of the medication. The extract appeared safe up to 3.0 g/d [Huber 2007].

In a randomized, double-blind, placebo-controlled trial in 40 children aged between 3 months and 7 years suffering from rotavirus diarrhoea, no clinical side effects were detected in the treatment group (20 children) receiving tormentil extract (1:10, ethanol 40 %) [Subbotina 2003].

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The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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Monographs

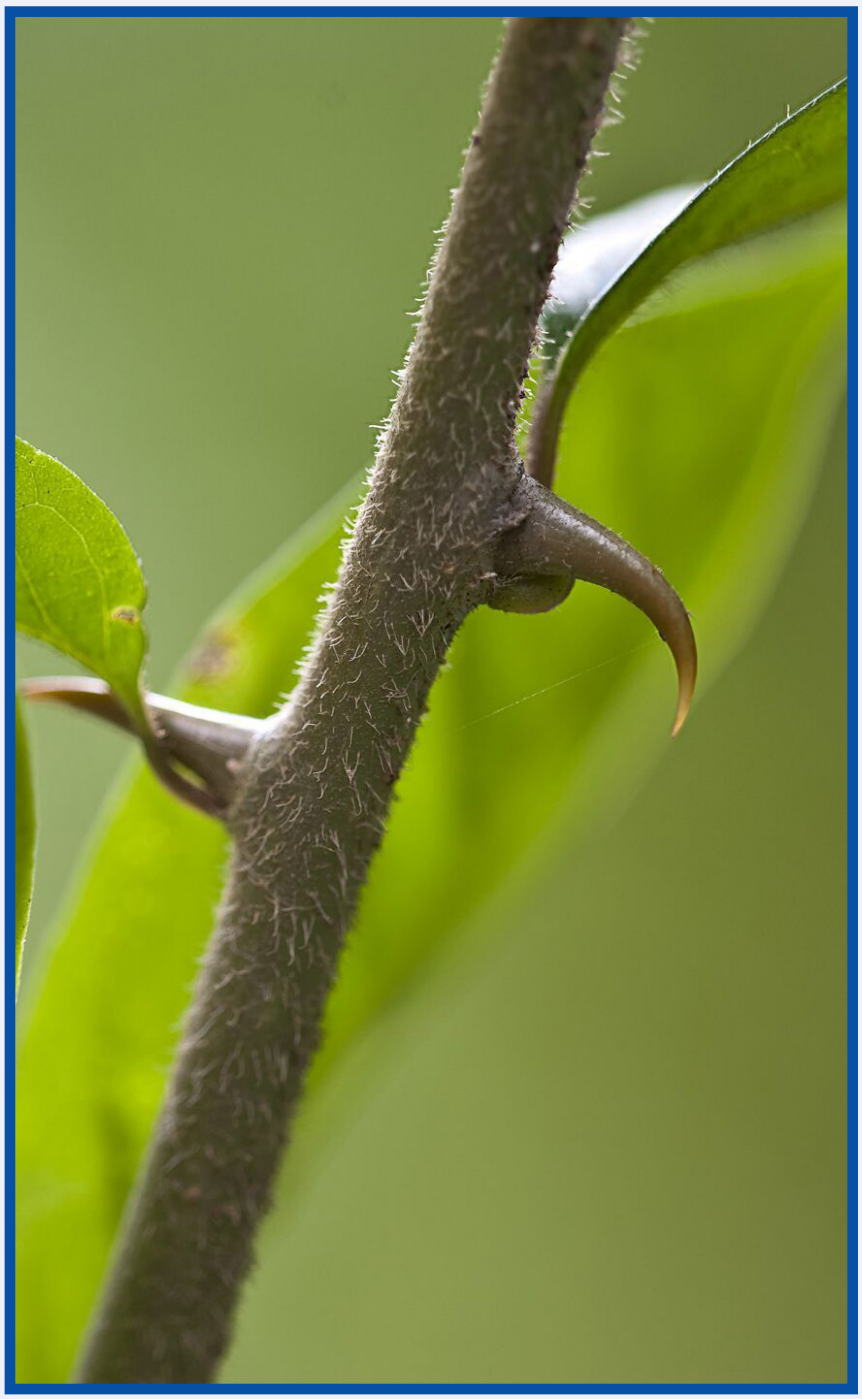
Online Series

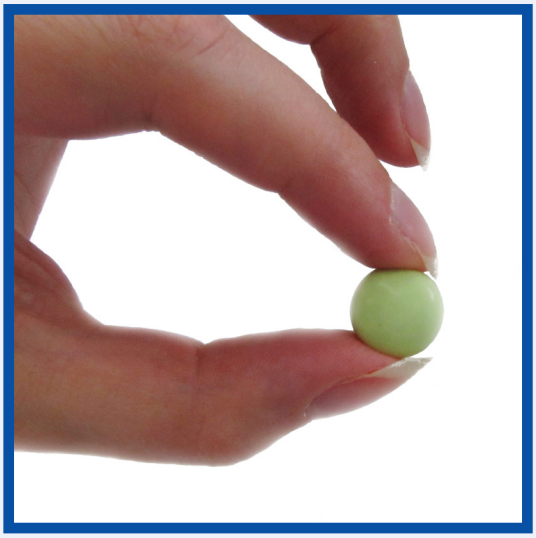
The Scientific Foundation for Herbal Medicinal Products

Uncariae tomentosae cortex

Cat's Claw Bark

2018





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MONOGRAPHS

***The* Scientific Foundation for**
Herbal Medicinal Products

UNCARIAE TOMENTOSAE CORTEX
Cat's Claw Bark

2018

E/S/C/O/P
European Scientific Cooperative
On Phytotherapy

ESCOP Monographs were first published in loose-leaf form progressively
from 1996 to 1999 as Fascicules 1-6, each of 10 monographs

Second Edition, completely revised and expanded

Second Edition, Supplement 2009

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
CCl_4	carbon tetrachloride
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase

INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

UNCARIAE TOMENTOSAE CORTEX 2018

Cat's Claw Bark

DEFINITION

Cat's claw bark consists of the inner stem bark of *Uncaria tomentosa* (Willd. ex Roem. et Schult.) DC. It contains not less than 0.7 per cent of oxindole alkaloids expressed as uncarine E ($C_{21}H_{24}N_2O_4$, M_r 368.4) and calculated with reference to the dried drug.

A draft monograph for the European Pharmacopoeia has been published [Pharmeuropa 2012].

CONSTITUENTS

Characteristic constituents are oxindole alkaloids (0.15-2.6%), which are either pentacyclic (0.1-2.6%) or tetracyclic (0.0-1.0%) derivatives. Pentacyclic oxindole alkaloids consist of isopteropodine (uncarine E), pteropodine (uncarine C), isomitraphylline, mitraphylline, speciophylline (uncarine D) and uncarine F, while tetracyclic oxindole alkaloids are rhynchophylline, isorhynchophylline, corynoxeine and isocorynoxeine [Condori Peñaloza 2015; Bertol 2012; Pilarski 2010; Reis 2008; Kitajima 2002 and 2001; Ganzera 2001; Laus 1997].

Other constituents are indole alkaloids (akuammigine, tetrahydroalstonine, hirsutine, hirsuteine, dihydrocorynantheine), monoterpenoid glucoindole alkaloids (carboxystrictosidine derivatives), the β-carboline alkaloids such as harman [Hemingway 1974; Laus 1997; Kitajima 2002 and 2001], quinovic acid glycosides (0.05-0.8%) [Condori Peñaloza 2015; Cerri 1988, Aquino 1989, Pavei 2012], ursolic and oleanolic acid-type glycosides [Aquino 1990, Kitajima 2000, Kitajima 2004], sterols (campesterol, sigmasterol, sitosterol) [Senatore 1989], polyphenols and procyanidines (such as chlorogenic acid and further quinic acid esters, rutin, epicatechin and cinchonaines) (0.07-0.3%) [Navarro 2017; Condori Peñaloza 2015; Pavei 2010, Sheng 2005, Wirth 1997] and 7-deoxyloganic acid [Muhammed 2001].

CLINICAL PARTICULARS

Therapeutic indications
Adjuvant for the treatment of mild inflammatory conditions [The ABC Clinical guide to herbs]. Efficacy in this indication is plausible on the basis of literature and traditional use.

Posology and method of administration

Dosage
Adults: Decoction (20 g/l): daily dose 60 -100 ml [Jones 1995; The ABC Clinical guide to herbs 2003; Roth 2002].

Method of administration
For oral administration.

Duration of administration
No restriction.

Contra-indications
Should not be used by organ transplant patients [The ABC Clinical guide to herbs 2003].

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
It should be noted that a cat's claw extract has been shown to inhibit cytochrome P450 3A4 *in vitro* [Budzinski 2000, Foster 2003].

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A case study concerning the interaction between cat´s claw bark and protease inhibitors in an HIV-positive patient with cirrhosis associated with hepatitis C infection was reported. Serum trough concentrations (C_{min}) of atazanavir, ritonavir and saquinavir were conspicuously high (1.22, 6.13 and 3.4 µg/mL respectively) compared to recommended C_{min} values of 0.8, 0.25 and 2.1 µg/mL. Self-medication with cat´s claw bark was found to be the cause as the C_{min} levels normalized within 15 days after cat´s claw withdrawal [Galera 2008].

In a further case study, worsening of Parkinson's disease motor symptoms was reported after oral intake of cat´s claw bark. A 38 year old man was being treated with levodopa and pergolide and motor symptoms were well controlled. A few days after the first intake of a cat´s claw infusion, tremor and hypokinesia markedly increased. Following the withdrawal of cat´s claw bark intake after three weeks, he remained markedly hypokinetic for 1 more week. Motor symptoms then progressively improved to reach the basal state [Consentino 2008].

Cat´s claw bark (capsules containing 0.5% pentacyclic oxindole alkaloids) enhanced the action of diazepam (2 mg/kg) on spontaneous motor activity when administered orally at 3.5 and 7.14 mg/kg b.w. to mice. The sedative effects of diazepam were partially suppressed by the 7.14 mg/kg dose, whereas a stronger effect of diazepam on the exploratory ability and movement was observed when administered together with cat´s claw bark at 3.5 mg/kg ($p<0.001$). Muscle relaxant activity of the antidepressant drug was influenced to a small extent at both doses [Quilez 2012].

Ingestion of 1 mL of an aqueous cat´s claw bark extract (32 mg/mL) for 7 days affected the biodistribution of the radiobiocomplex sodium pertechnetate in rats (n=5) (3.7 MBq, 0.3 mL injected via the ocular plexus) compared to a control group (n=5) which received 0.9% NaCl solution. A significant ($p<0.05$) alteration in sodium pertechnetate uptake from 0.57 to 0.39% radioactivity (ATI)/g was observed in the heart, from 0.07 to 0.19% ATI/g in the pancreas and from 0.07 to 0.18%ATI/g in muscle. No significant differences were observed for the uptake in other organs such as brain, liver, duodenum, kidney, spleen, lung, stomach, blood, bone, thyroid and testis [Moreno 2007].

A single patient with systemic lupus erythematosus experienced acute renal failure associated with ingestion of 4 capsules of cat´s claw bark per day. Acute allergic interstitial nephritis was diagnosed, creatinine levels and proteinuria were increased compared to urine analysis before the intake and returned to normal upon discontinuation of intake. The patient had taken the cat's claw together with numerous oral medications including prednisone, atenolol, metolazone, furosemide and nifedipine [Hilepo 1997].

Pregnancy and lactation
No data available.

In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Anti-inflammatory activity
A decoction (20 g/L) significantly inhibited lipopolysaccharide-induced (1 µg/mL) production of macrophagal cytokine TNF-α (65-85% inhibition, $p<0.01$, IC_{50} 1.2 ng/mL) [Sandoval 2000].

In a further study, a similar extract (50 g/L) showed an IC_{50} value of 14.1 ng/ml in the same model. Alkaloid free HPLC-fractions (0.1 and 100 ng/mL) exhibited effects similar to the full extract (20-40% inhibition at 0.1 ng/mL, 65-80% at 100 ng/mL) and they also inhibited NO release [Sandoval 2002].

A decoction of cat´s claw bark (20 g/L, 0.56 - 0.70% pentacyclic oxindole alkaloids) stimulated IL-1 and IL-6 production by alveolar rat macrophages in a dose-dependent manner in the range from 0.025 - 0.1 mg/mL (IL-1: maximum 10 fold compared to control, IL-6: 7.5 fold). Enhanced IL-1 and IL-6 levels in lipopolysaccharide-stimulated macrophages (IL-1: maximum 5.2 fold compared to control, IL-6 > 2 fold) were also shown [Lemaire 1999].

A decoction at 100 µg/mL attenuated peroxynitrite-induced apoptosis in epithelial cells and macrophages ($p<0.05$), inhibited LPS-induced NO formation by 60% ($p<0.05$), iNOS gene expression and cell death, and markedly inhibited the activation of NF-κB [Sandoval 1998].

The same extract at 10 µg/mL significantly reduced PEG_2 production in murine macrophages after LPS (0.05 µg/mL) stimulation ($p<0.05$), whereas no effect was observed without the LPS stimulus [Piscoya 2001].

A purified aqueous extract (<0.05% alkaloids) reduced the 6-hydroxydopamine induced increase in iNOS expression, NF-κB and TNF-α in a human dopaminergic neuron neuroblastoma cell line ($p<0.05$) [Shi 2013].

An 80% ethanol extract (8:1; 5.61% penta- and tetracyclic oxindole alkaloids) impaired NF-κB DNA binding at a concentration of 500 µg/mL using Jurkat T cells and TNF-α as stimulus, whereas an aqueous extract (0.26% oxindole alkaloids) only slightly prevented NF-κB DNA binding at the same concentration. The extracts at 50 µg/mL inhibited COX-1 (7.8% and 32.7% respectively) and COX-2 (21.7% and 12.2% respectively) [Aguilar 2002].

Treatment of THP-1 monocyte like cells with an ethanolic extract inhibited the MAP kinase signalling pathway and altered cytokine expression. LPS-dependent expression of TNF-α was inhibited by 45-95% at 10-320 µg/mL ($p<0.05$). In contrast, IL-1β expression was augmented by 1.2-1.4 fold at 40-160 µg/mL, but was completely blocked at 320 µg/mL. Treatment of the cells with the extracts alone augmented IL-1β expression by 20 fold, but did not affect the expression of TNF-α In LPS stimulated cells treatment with the extract blocked ERK1/2 and MEK1/2 phosphorylation in a dose dependent manner, but did not influence the protein expression. Treatment with the extract inhibited LPS-dependent activation of specific NF-κB and activator protein family components. When NF-κB subunits nuclear localization signals were blocked, TNF-α production was diminished, whereas IL-1β expression remained unchanged [Allen-Hall 2007, 2010].

The procyanidine cinchonain 1b (42.5 µM/mL) inhibited

5-lipoxygenase by 100%, while (-)-epicatechin and cinchonain 1a were not active [Wirth 1997].

Quinic acid inhibited the NF-κB activity induced by activating Jurkat T cells with PMA and ionomycin, without effecting cell proliferation. The compound inhibited LPS-induced breakdown of IκBα. The antioxidant pyrolidine dithiocarbamate as positive control inhibited the breakdown and, in contrast to quinic acid, also affected the phosphorylation and resynthesis of IκBα [Akesson 2005].

Effects on immune functions
A dry extract (3% total alkaloids, no further details) stimulated macrophage phagocytosis up to 4.7 fold ($p<0.01$) at 0.13-1.3 mg/mL, whereas no effect was observed on the synthesis of IL-12 or IFN-γ or on the activity of natural killer cells. LPS and IL-12 in the presence of IL-2 were used as positive controls [Groom 2007].

Treatment of Dengue-virus infected human monocytes with a 50% ethanolic extract (2.9% alkaloids) or a non-alkaloidal fraction of the extract (0.1-100 µg/mL) did not impair the virus-induced increases in TNF-α, IFN-α, IL-10 and IL-6 production. However, the cytokine levels of TNF-α and IFN-α were significantly reduced by treatment with an alkaloid enriched fraction at 100 µg/mL ($p<0.05$); resulting cytokine levels were similar to those after dexamethasone treatment at 0.05 mM. IL-6 was not inhibited by the treatment. IL-10 was not significantly altered by cat´s claw bark, although a strong tendency for IL-10 inhibition was observed after treatment with the alkaloid fraction (572±219 pg/mL vs. 244±60 pg/mL after treatment) [Reis 2008].

A modified aqueous extract (alkaloids <0.01%, quinovic acid glycosides not detected, total phenol content 42%, arabinogalactan-protein content 24%) significantly increased NO (by 50% at 160 µg/mL, $p<0.05$), IL-6 (by 73% at 80 µg/mL, $p<0.05$) and TNF-α (by 230% at 80 µg/mL, $p<0.05$) production compared to the negative control whereas IL-1β levels were reduced (at 5-160 µg/mL, $p<0.05$) in mouse peritoneal macrophages [Lenzi 2013].

An alkaloid mixture (containing penta- and tetracyclic oxindole alkaloids, 0.9 g/100 g dried plant material) showed an enhanced effect on granulocyte phagocytosis in two in vitro tests (chemiluminescence assay, granulocyte test modified after Brandt), down to a concentration of 10^{-3} mg/mL. The most pronounced activity was found for isopteropodine (13-60%, 10^{-2}–10^{-6} mg/mL). Pteropodine, isomitraphylline and isorhynchophylline showed half of the activity, while rhynchophylline and mitraphylline were inactive [Wagner 1985a, Wagner 1985b].

Addition of pentacyclic oxindole alkaloids (1 µM, 7 days; 4% speciophylline, 6% uncarine F, 2% mitraphylline, 3% isomitraphylline, 28% pteropodine and 57% isopteropodine) to supernatants of human endothelial cells (EA.hy926) induced the proliferation of resting or low activated human B and T lymphocytes. Proliferation of normal human T- and B-lymphoblasts as well as of Raji and Jurkat lymphoblastoid cell lines was inhibited (viability not impaired) and the myeloid cell line U-937 was not affected. No effect on proliferation was found when alkaloids alone, or together with a supernatant of untreated endothelial cells, were added to the lymphocytes. Thus, proliferation was not influenced directly by the alkaloids. No activity was found for tetracyclic derivatives (67% rhynchophylline and 33% isorhynchophylline) on proliferation itself, but there was a dose-dependent inhibitory effect of pentacyclic oxindoles on Raji and Jurkat cells [Wurm 1998].

Oxindole alkaloid mixtures (50-500 µg/mL) dose dependently decreased neopterin formation and tryptophan degradation induced by IFN-γ in stimulated or non-stimulated human peripheral blood mononuclear cells; pentacyclic derivatives ($p<0.01$ at 150-500 µg/mL) were less active than tetracyclic ones ($p<0.01$ at 50-500 µg/mL) [Winkler 2004].

Antioxidant activity
Inhibition of lipid peroxidation in rat liver homogenates was determined with methanolic extracts of cat's claw root and stem bark (dichloromethane pre-extracted; root: IC_{50} 259 µg/mL, stem bark: IC_{50} 56 µg/mL, catechin as control: IC_{50} 1.0 µg/mL). Aqueous extracts of roots were inactive and aqueous extracts of stem bark were only active in high doses (100 and 1000 µg/mL). Methanolic extracts also inhibited a free radical-mediated damage of the sugar moieties of DNA (root IC_{50} 82 µg/mL; stem bark IC_{50} 16 µg/mL; catechin: IC_{50} 5.0 µg/mL) [Desmarchelier 1997].

In another assay, a decoction (50 g/L; 0.9% oxindole alkaloids, 2.4% flavonols) showed inhibition of lipid peroxidation in mouse brain homogenates (IC_{50} 148 µg/mL). Inhibition of DPPH and ABTS radical production was observed in a concentration dependent way (DPPH: IC_{50} 21 µg/mL, ABTS: IC_{50} 7.7 µg/mL). The extract also provided protection of deoxyribose against hydroxyl radicals in a dose-dependent manner [Sandoval 2002].

A similarly prepared decoction (20 g/L) at a dose of 10 µg/mL prevented the loss in cell viability (RAW 264.7) induced by 0.3 µM DPPH ($p<0.01$) [Sandoval 2000].

A purified aqueous extract (<0.05% alkaloids, 8-10% carboxyalkylesters) scavenged DPPH and ABTS radicals in a dose-dependent manner (IC_{50} 32.2 µg/mL and 20.1 µg/mL respectively). At 25 µg/mL, hydroxyl anions were scavenged by 55.7%, superoxide anions by 21.0% and singlet oxygen radicals by 14.2%. Quinic acid as constituent was inactive at 25 µg/mL, and the scavenging rate on hydroxyl radicals was only about 15% at 5 mg/mL. The extract also inhibited 6-hydroxydopamine induced increase of reactive oxygen species (ROS), reduction of mitochondrial membrane potential, loss of cell viability as well as increase in lipid peroxidation (by 28.9%) in a human dopaminergic neuron neuroblastoma cell line ($p<0.01$) [Shi 2013].

Pteropodine scavenged DPPH radicals by 98% at 250 µg/mL [Paniagua-Perez 2009].

Antiviral activity
Treatment of Dengue-virus infected human monocytes with a 50% ethanolic extract (2.9% alkaloids) at 10 mg/mL significantly decreased viral antigen detection ($p<0.05$), an alkaloid fraction showed an effective inhibitory activity at 1 µg/mL ($p<0.05$), whereas an alkaloid-free fraction was inactive. Cell rates were similar to those after dexamethasone treatment at 0.05 mM [Reis 2008].

Six quinovic acid glycosides were tested for their antiviral activity against two RNA viruses. All compounds inhibited vesicular stomatitis virus infections in a chicken embryo related (CER) cell line at dose levels close to cytotoxic doses (MIC_{50} 20-71 µg/mL, LD_{50} 80-150 µg/mL). Five of the tested compounds were inactive against rhinovirus 1B infections in HeLA cells, only one reduced the cytopathogenic effect by 50% at a dose of 30 µg/mL (maximal non-toxic concentration for HeLA cells: 60 µg/mL) [Aquino 1989].

A concentrated, freeze-dried 40% hydroethanolic extract (183.8 µg/mg total polyphenols, 15.4 µg/mg alkaloids and 67.8 µg/mg

quinovic acid glycosides) inhibited HSV-1 replication in Vero cells, when added to cells simultaneously (IC_{50}<15.75 µg/mL) but not after virus infection. No virucidal effect was observed when virus suspensions were pre-incubated with the extract. The extract did not prevent penetration of HSV into cells but showed a strong inhibition of its attachment. An alkaloid (62.7 µg/mg) and a quinovic acid glycoside (217.8 µg/mg) fraction were inactive [Caon 2014].

Antiproliferative and cytotoxic effects
An aqueous extract dose-dependently reduced [^{3}H]-oestradiol-specific receptor binding in cytosol from oestrogen-dependent tumours (ductal carcinoma). Pre-treatment of the cytosol with cat's claw bark or tamoxifen as control (20 µg each) reduced specific binding by 47.2% and 69.3% respectively [Salazar 1998].

Extracts of cat's claw stem bark were tested for their inhibitory effects on proliferation in a human breast cell line (MCF7). The methanolic extract (33:1) was more effective than an aqueous extract (50:1) (IC_{50}-values 38 and 270 µg/ml respectively). At the maximum concentration (1 mg/mL) both extracts were toxic [Riva 2001].

In another study, a purified aqueous extract (< 0.05% alkaloids, 8-10% carboxy alkyl esters) inhibited proliferation of human leukaemic cells (HL60) and lymphoma cells (Raji), with IC_{50}-values of 71 µg/mL and 84 µg/mL respectively. Reduced inhibitory activity was seen in the leukaemic cell lines K562 (IC_{50} 200 µg/mL) [Sheng 1998, Akesson 2003a].

The antitumor activity of the same extract was also demonstrated in the clonogenic assay, a dose-dependent response being observed on the colony survival (IC_{50} 83 µg/mL, HL60). In the MTT assay, IC_{50} values of 100 µg/mL (HL60) and 267 µg/mL (K562) were calculated. The suppressive effect on tumour cell growth appeared to be mediated via induction of apoptosis, as demonstrated by characteristic morphological changes, internucleosomal DNA fragmentation after agarose gel-electrophoresis and DNA fragmentation quantification. The extract induced a dose-dependent and delayed type of apoptosis (48 hours after exposure), whereas no significant increase of necrosis was observed [Sheng 1998].

The extract also inhibited proliferation of normal mitogen-activated mouse B- and T-lymphocytes; the effect was not caused by toxicity or induction of apoptosis. Furthermore, no interference with IL-2 production or receptor signalling was observed [Akesson 2003a].

An ethanolic extract (14:1) dose dependently inhibited TPA induced Epstein-Barr virus early antigen activation in Raji cells [Kapadia 2002].

Of various tested cat´s claw bark extracts, an ethanolic extract (1 g/10 mL, 37°C, 3.5% pentacyclic oxindole alkaloids) was shown to be the most active one against Lewis lung carcinoma (IC_{50} = 25.06µg/ml), cervical carcinoma (IC_{50} = 35.69µg/ml) and colon adenocarcinoma (IC_{50} = 49.06µg/ml), whereas an alkaloid enriched fraction (51.7% pentacyclic oxindole alkaloids) was especially effective in inhibiting proliferation of cervical carcinoma (IC_{50} = 23.57µg/ml), breast carcinoma (IC_{50} = 29.86µg/ml) and lung carcinoma cell lines (IC_{50} = 40.03µg/ml). An aqueous dry extract (1 g/10 mL, 37°C, 0.43% pentacyclic oxindole alkaloids) showed the lowest activity against all cell lines [Pilarski 2010].

The aqueous extract as well as the alkaloid rich fraction were also studied for their effects on the Wnt-signaling pathway, a central regulator of development and tissue homeostasis. The alkaloid rich fraction inhibited the Wnt-signaling activity in a human cervical carcinoma cell line (HeLa, IC_{50} = 38 µg/mL), and in the human colorectal cancer cell lines HCT116 (IC_{50}=60 µg/mL) and SW480 (IC_{50}=29 µg/mL). The aqueous extract was less active (IC_{50}= 278 µg/mL, 150 µg/mL and 190 µg/mL respectively). Considering the ratio of a Tcf-reporter construct (TOP-luciferase) and a mutated reporter construct (FOP-luciferase), it was evident that the effects were only specific in HeLa and HCT116 cells at concentrations of >300 µg/mL, whereas the alkaloid-rich fraction displayed an inhibitory effect at much lower concentrations. No effect was observed on beta-Catenin levels, but expression of endogenous Wnt target gene cMyc was reduced by both extracts. Wnt-inhibition IC_{50} values were much lower than 50% growth inhibition; it is suggested that the decrease in Wnt-signaling is not caused by reduced cell proliferation. The alkaloid rich fraction was clearly less toxic to non-cancer cells and to cells without activated Wnt-signaling than to cancer cells with aberrantly activated Wnt-signaling activity [Gurrola-Diaz 2011].

Hexane, ethyl acetate, butanol and methanol cat´s claw bark extracts inhibited viability of the human promyelotic leukemic cell line HL-60 by 50% at 13.0 µg/mL, 18.0 µg/mL, 13.5 µg/mL and 50.3 µg/mL respectively. The ethyl acetate extract was further investigated; at 100 µg/mL it clearly induced morphological changes and chromosomal condensation in HL-60 cells. The observed apoptotic effect was dose dependent with 74.7% apoptotic cells at 100 µg/mL. Cells treated with the extract underwent rapid loss of mitochondrial transmembrane potential, stimulation of phosphatidylserine flip-flop, release of mitochondrial cytochrome c into cytosol, induction of caspase-3 and -9 activity and induced cleavage of DNA fragmentation factors. Apoptosis was accompanied by up-regulation of Bax, down-regulation of Bcl-XL and cleavage of Mcl-1 [Cheng 2007].

Pentacyclic oxindole alkaloids, with the exception of mitraphylline, inhibited proliferation of acute lymphoblastic leukaemia T cells in a dose-dependent manner (MTT-assay and clonogenic assay). The effect of the most potent alkaloids uncarine F and pteropodine correlated with the induction of apoptosis. After 48 h, the two alkaloids increased apoptotic cells by 57% at 100 µmol/L ($p<0.01$). Apoptosis of alkaloid treated cells was not impaired by Bcl-2 overexpression, and no activation of caspase-9 was observed. The same dose did not increase apoptosis in G0/1-arrested cells; however, at 200 µmol/L the effect was comparable to a dose of 100 µmol/L in dividing cells [Bacher 2005].

In another study, mitraphylline had a stronger antiproliferative effect on a glioma (IC_{50}=20 µM) and a neuroblastoma cell line (IC_{50}=12.3 µM) than cyclophosphamide (IC_{50}=50.85 µM and 8.05 µM respectively) or vincristine (IC_{50}=30.43 µM and 10.05 µM respectively) as positive controls [Garcia Prado 2007].

The alkaloid also inhibited the proliferation of Ewing´s sarcoma and a breast cancer cell line by 50% at 17.15 and 11.80 µM respectively, cyclophosphamide at 67.60 µM and 38.01 µM, and vincristine at 39.16 µM and 44.66 µM respectively [Garcia Gimenez 2010].

Isopteropodine and pteropodine at 100 µM inhibited proliferation in human medullary thyroid carcinoma and breast carcinoma cells by 57.1%, in a phaeochromocytoma cell line by 36% and in a melanoma cell line by 13%. A methanolic extract at 100-200 µM did not show activity. In the medullary thyroid carcinoma cells a decrease in mitochondrial dehydrogenase activity and an increase in caspase-3 and -7 as well as in poly(ADP-ribose) polymerase fraction was observed, whereas bcl-2 overexpression remained constant [Rinner 2009].

Cytotoxicity of (iso)pteropodine, (iso)mitraphylline, uncarine D, rhynchophylline, 7-deoxyloganic acid, as well as β-sitosteryl glucoside was tested in several human cancer cell lines using doxorubicin (DOX) as control. Uncarine D showed weak cytotoxic activity against malignant melanoma (IC_{50} = 30 µg/mL; DOX < 1.1), epidermoid carcinoma (IC_{50} = 35 µg/ml; DOX 1.7), ductal carcinoma (IC_{50} = 34 µg/mL; DOX 1.7) and ovarian carcinoma (IC_{50} = 34 µg/mL; DOX 2.0), whereas pteropodine was only cytotoxic in ovarian carcinoma (IC_{50} = 37 µg/mL), and the other compounds were inactive [Muhammad 2001].

Isopteropodine and pteropodine showed moderate cytotoxicity against several mammalian cell lines with IC_{50} values from 17 to 51 µg/mL. Both compounds exhibited weak DNA-damaging activity against the yeast strains RS321 and RS322, which lack one of the three known DNA repair pathways. No effect was detected against the corresponding wild-type strain [Lee 1999].

A quinovic acid glycoside enriched fraction decreased the growth and viability of bladder cancer cell lines T24 (IC_{50} 78.4 µg/mL after 72h) and RT4 (IC_{50} 99.4 µg/mL after 72h). In T24 cells the fraction induced apoptosis by activating caspase-3 and NF-κB, but it did not induce cell cycle arrest or alter phosphorylated and total protein levels of PTEN and ERK [Dietrich 2014].

Antimutagenic activity
Sequentially prepared extracts of cat's claw bark (light petroleum, chloroform, chloroform-methanol 9:1, methanol and water) and some chromatographic fractions of the chloroform-methanol extract exerted a protective action against photomutagenesis induced by 8-methoxy-psoralen plus UVA (15 min of irradiation) in *S. typhimurium* TA 102 at a dose of 100 µg/mL. The methanolic extract showed the most pronounced activity (59%). β-Carotene was used as a positive control (68%, same dose) [Rizzi 2003].

Skin protective activity
Incubation of organogenic human skin cultures for 24 h with a purified aqueous extract (5 mg/mL) after exposure to 100 mJ/cm^2 of UVB reduced the number of UV-induced necrotic cells by 95% ($p<0.001$). When cells were incubated for 6 h with the same dose and then irradiated by UVB (0-100 mJ/cm^2), no effect was observed on the level of thymine dimers as UVB induced DNA damage. However, after re-treatment with the extract for 24 h, a reduction of thymine dimer producing cells of 26% was observed at 75 mJ/cm^2 and 63% at 100 mJ/cm^2 compared to the cells before the 24 h incubation period ($p<0.001$) [Mammone 2006].

Influence on cytochrome P450
A non-specified extract of cat's claw was found to be an *in vitro* inhibitor of human cytochrome P450 3A4 (IC_{50} at concentrations of less than 1% of the extract; fluorometric microtiter plate assay) [Budzinski 2000].

In a further study, a methanolic extract (0.13% pentacyclic oxindole alkaloids; aliquot of 25 mg/mL) inhibited the activities of cytochrome P450 2C9, 2C19, 2D6 and 3A4 (11.4, 5.2, 13.4 and 56.8% inhibition respectively). Determination of the interaction on marker substrates by the extract and the protease inhibitor nelfanavir showed an additive effect of the two drugs with only little evidence of synergism [Foster 2003].

Effect on muscarinic and 5-HT2 receptors
The effects of isopteropodine and pteropodine on the function of Ca^{2+}-activated Cl^- currents evoked by stimulation of G protein-coupled muscarinic M_1 acetylcholine and 5-HT_2 receptors in Xenopus oocytes injected with rat cortex total RNA were studied. The alkaloids at 1-30 µM failed to induce membrane current, but markedly enhanced current responses evoked by acetylcholine and serotonin. Both compounds produced 2.7-3.3 fold increases in the acetylcholine response with EC_{50} values of 9.52 and 9.92 µM and 2.4-2.5-fold increases in the serotonin response with EC_{50} values of 13.5 and 14.5 µM respectively. Mitraphylline failed to modulate current responses. No effects were observed on currents mediated by glutamate receptors or N-methyl-D-aspartate, kainic acid or glycine [Kang 2002].

Effect on human erythrocytes
Incubation of a human erythrocyte suspension with an ethanolic dry extract (8:1, 20% polyphenols, 34.8 mg alkaloids per g extract) at 100-500 µg/mL, and incubation with an aqueous dry extract (9:1, 10% polyphenols, 4.3 mg alkaloids per g extract) at 250-500 µg/mL, resulted in a slight, but statistically significant ($p<0.001$) decrease in haemoglobin outflow. Haemolysis, formation of met-Hb at 5-500 µg/mL or haemoglobin denaturation at 250 µg/mL were not observed for either extract within 24 h of incubation. The extracts at 250 µg/mL did not cause a statistically significant increase in ROS levels or in lipid peroxidation after 1 h of incubation. Both extracts dose dependently depleted the extent of 2,4-dichlorophenol (250 µg/mL) provoked haemoglobin oxidation and lipid peroxidation, and decreased the level of ROS and haemolysis. The effects were statistically significant ($p<0.001$) at 100 µg/mL for the ethanolic and at 250 µg/mL for the aqueous extract. No protective effect was observed against catechol-induced (100 µg/mL) haemolysis or oxidation [Bors 2011].

Incubation of erythrocytes for 1 h and 5 h with the same extracts at 250 µg/mL resulted in reduced changes in catalase activity induced by 2,4-dichlorophenoxyacetic acid and 2,4-dichlorophenol ($p<0.001$). Changes induced by catechol were significantly reduced after 1h of incubation ($p<0.001$), but not after 5h. An effect on catalase activity was not observed without the inducing agents [Bukowska 2012].

Slight disturbances in size and shape of erythrocytes were observed by flow cytometry and phase contrast microscopy after incubation with 100 µg/mL ethanolic extract and with 250 µg/mL aqueous extract ($p<0.001$). At 250 µg/mL, externalization of phosphatidylserine on the erythrocyte surfaces was noticed [Bors 2012].

In vivo studies

Effects on immune functions
Enhancement of phagocytosis in mice, determined by carbon-clearance test (after Biozzi), was more pronounced after i.p. administration of an alkaloid-rich aqueous macerate (10 mg/kg b.w., 24 h before the test; 2.3 fold compared to control) than after administration of an alkaloid-free macerate (1.7 fold compared to control). The alkaloid mixture itself showed significant activity only after adding a tannin mixture (2.2 fold compared to control) [Wagner 1985b].

A small but significant ($p<0.05$) elevation in the white blood cell count of rats was found after treatment with a purified aqueous extract (40 and 80 mg/kg b.w. daily for 8 weeks or 160 mg/kg b.w. for 4 weeks by gavage) when compared to controls [Sheng 2000a].

In a further study, the effect of the same extract on doxorubicin-induced leucopoenia was evaluated in rats (2 mg/kg b.w., i.p. 3 times at 24 h intervals). After the last treatment the animals were daily gavaged with the extract for 16 consecutive days (40 and 80 mg/kg b.w.). As a positive control, a granulocyte colony stimulator was administered (s.c., 5 and 10 µg/kg b.w. for 10 days). The white blood cells recovered significantly ($p<0.05$) faster in treated than untreated groups (cat's claw group:

recovery in 4 days at a dose of 80 mg/kg and in 9 days at 40 mg/kg; granulocyte colony stimulator: within 24 h). However, the recovery by cat's claw bark was a more natural process, with all fractions of white blood cells being increased proportionally, whereas the control mainly elevated the neutrophil cells. These results were confirmed by microscopic examination of the blood smears [Sheng 2000b]. A comparable effect was observed when a quinic acid ester enriched fraction was administered at 200 mg/kg [Sheng 2005].

A dose-dependent increase in spleen cell numbers was observed when a purified aqueous extract was supplied to mice in drinking water at daily doses of approximately 125, 250 or 500 mg/kg b.w.. This increase reached a plateau within 3 weeks. In contrast to the total number of spleen cells, the proportions of B cells, T cells, NK cells, granulocytes and memory lymphocytes remained unchanged compared to control after 63 days. The total number of thymocytes and body or spleen weight were not influenced by the treatment. The effect on spleen cells was reversible as cell density returned to normal levels within four weeks after interruption of application. Furthermore, no significant effects on precursor cell maturation or on the output rate of mature T cells from thymus were observed. The extract significantly ($p<0.05$) prolonged lymphocyte survival in lymphoid tissues without increasing the proliferation rate [Akesson 2003b].

In a consecutive study, the effect of the same extract (500 mg/kg b.w.) was compared to that of quinic acid, a component of the extract (125, 250 or 500 mg/kg b.w.). In both groups the number of spleen cells increased significantly within 21 days ($p<0.02$). In the extract-treated group no increase in white blood cells, blood lymphocytes or erythrocytes was observed. In contrast, mice treated with increasing concentrations of quinic acid had increasing numbers of white blood cells and lymphocytes ($p<0.002$). However, the body weight of those animals (n=10) was significantly reduced ($p<0.01$) [Akesson 2005].

Treatment of rats with a purified hot water extract (daily dose of 40 and 80 mg/kg b.w. for 8 weeks) dose-dependently enhanced PHA stimulated lymphocyte proliferation in splenocytes ($p<0.05$ and $p<0.01$ respectively) [Sheng 2000a].

Treatment of BALB/c male mice once a day with 125, 500 or 1250 mg/kg b.w. of a 50% hydroalcoholic extract (2.9% oxindole alkaloids) for 28 days (no further treatment) resulted in a significant increase in the relative number of lymphocytes ($p<0.05$) associated with a decrease in granulocytes ($p<0.01$). The increase of mature CD^{4+} T-lymphocytes was more pronounced in the groups treated with 125 or 500 mg/kg (27.8% and 32.3% respectively, compared to 21.3% in the control group; $p<0.05$). Whereas a reduced percentage of CD^{4+} T-lymphocytes and a higher percentage of $CD45RA^{+}$ B-lymphocytes was observed in the group treated with the highest dose (80% compared to 51.7% in the group treated with 500 mg/kg b.w. and 63.5% in the control group). This increase in B-lymphocytes was associated with an enlargement of the splenic marginal zone (relative organ weight 0.37 vs 0.30 in the control, $p<0.05$) and an increase in the cellularity of thymic medulla [Domingues 2011a].

The same extract was tested in experimental immune-mediated diabetes in mice. Diabetes was induced by five i.p. injections of streptozotocin (MLDS) at 5 mg/kg b.w. on 5 consecutive days. Treatment of mice (7 groups, n=8) with the extract by gavage at 10-400 mg/kg b.w. for 21 days resulted in a significant reduction of diabetes incidence and glycaemic levels ($p<0.05$), the highest dose completely abrogated diabetes incidence at day 12 ($p<0.001$). The morphometric analysis of insulitis revealed a clear protective effect, animals treated with the extract at 400 mg/kg b.w. presented a higher number of intact islets ($p<0.001$), a significant inhibition of destructive insulitis ($p<0.05$), and protection against the loss of insulin-secreting β-cells. Application of MLDS resulted in a reduced number of CD^{4+} cells which was normalized by the treatment at 100 or 400 mg/kg b.w. ($p<0.05$), CD^{8+}cell counts were not significantly influenced. Furthermore, the treatment resulted in a TH2 cytokine polarization with a significant increase in IL-4 production and in $FoxP3^{+}$ trag regulatory T-cells [Domingues 2011b].

In another study, mice receiving filgrastim at 3 and 9 µg i.p. and oral doses of 5 and 15 mg of a 70% hydroalcoholic extract for four days (n=6 per group) showed significant recovery ($p<0.005$) of the neutrophil counts compared to the control after ifosfamide-induced neutropenia. The effect of the cat´s claw bark treatment was calculated as 85.2 % compared to that of filgrastim as a positive control. No differences were observed in levels of non-protein thiols or in the activities of antioxidant enzyme catalases or superoxide dismutase among all groups [Farias 2011].

Cat´s claw protected mice against a lethal dose of *Listeria monocytogenes*. Mice were treated orally with an extract (1% alkaloids, dissolved in water, no further details) at 10-200 mg/kg b.w. for 7 consecutive days. The animals were infected with a lethal dose of *L. monocytogenes* 3 h after the end of treatment and observed for 30 days. All untreated mice died within 5 days, whereas 15% of animals treated with the extract at 50 mg/kg and 35% of animals treated at ≥100 mg/kg survived the infection. The treatment also prevented the myelosuppression and splenomegaly caused by sublethal infections due to significantly increased numbers of granulocyte-macrophage progenitors in the bone marrow ($p<0.001$). A dose of 100 mg/kg stimulated progenitor production within 24 h, and the effect was sustained for up to 3 days at least. In contrast, the increase of progenitors in the spleen observed at 48 and 72 h after infection was fully reversed ($p<0.001$); in non-infected mice no effect on spleen progenitors was observed. The same dose also upregulated the production of IL-1, IL-6 and colony-stimulating factors. No significant changes were observed in white blood cell counts. A significant increase of neutrophils ($p<0.05$) and reduction of lymphocytes and monocytes ($p<0.01$) were observed at 48 h and 72 h after bacterial challenge [Eberlin 2005].

A hydroalcoholic dry extract (10% yield) was found to be immunostimulatory in a study involving mice infected with formalin-inactivated whole *Sendai* virus (500 µg measured as total protein in 200 µL phosphate-buffer saline pH 7.2). One group (A) was treated intragastrically with the virus suspension alone (n=6), another (B) with virus and 0.56 mg extract (n=6), a third group (C) with the virus and 5.6 mg extract (n=6) seven times on alternate days. A fourth group (D, n=6) was immunized with the virus (5 µg s.c.) twice, at day 0 and 12. The saliva IgA antibody response was significantly higher in group C than in groups A, B and D at days 2, 7 and 14 after the last immunization, IgA titres were significantly increased between days 2 and 14 only in group C ($p<0.05$). Significant differences were also observed in serum IgG titres in groups B-D at day 7 with a maximum response and a significant decrease between day 7 and 14 after the last immunization. The response of group A remained reduced; the highest response was observed for group D. The serum haemagglutinin antibody response was increased in groups B, C and D within 2 to 14 days after the last immunization ($p<0.05$), being most pronounced in group D [Bizanov 2005].

In a study assessing lymphocyte production in 6 groups of mice (n=5 each), four of the groups received pteropodine (100-600 mg/kg b.w. i.p.), one group received distilled water (0.4 ml) as control and the final group received α-interferon (0.01 µL/kg

b.w.). Lymphocyte production was significantly induced after 24 h from 44.5% in the control group to 58.5-68.4% in the pteropodine groups ($p<0.05$) and compared to 50.3% after α-interferon administration. After 96 h lymphocyte counts in the control group were 47.4%, and significantly ($p<0.05$) higher in the pteropodine groups 56.0-78.8%, and the α-interferon group (56.0%) [Paniagua-Perez 2009].

Antiinflammatory activity
An ethanolic extract (80%, 8:1; 5.61% penta- and tetracyclic oxindole alkaloids) reduced mouse paw oedema at a concentration of 50 mg/kg b.w., equivalent to 7 mg/kg b.w. indomethacin (application via gastric probe). The same effect was observed for 200 mg/kg b.w. of an aqueous extract (0.26% oxindole alkaloids) [Aguilar 2002].

A decoction (20 g/L, diluted 1:4, provided in the drinking water ad libidum) markedly attenuated indomethacin-induced enteritis in mice. Significant reduction of myeloperoxidase activity ($p<0.05$), morphological damage and liver metallothionein protein concentration was shown (control: 30.5 µg/g tissue, indomethacin treated animals: 216.6 µg/g tissue, indomethacin and cat's claw bark: 69.6 µg/g tissue) [Sandoval 1998].

In a further study, a protective effect of an aqueous extract against indomethacin-induced gastritis in rats was observed. Micropulverized drug (5 mg/mL) was given as a decoction in the drinking water for 3 days prior to oral administration of 20 mg/kg b.w. indomethacin. The degree of mucosal injury, apoptosis in stomach epithelial cells and TNFα mRNA induction in the mucosa was significantly reduced ($p<0.01$) [Sandoval 2002].

The effect of a decoction (20g/L) on ozone-induced lung inflammation in mice was evaluated by assessing total protein levels of bronchoalveolar lavage fluid (BALF), cytology, lung histopathology and morphometry. To characterize ozone-induced acute pneumonitis, 40 mice were randomly assigned to 4 groups. One group was exposed to room air only, the other three groups were exposed to 2 ppm ozone for 4 h and killed 0, 4 and 8 h after the exposure. Total protein in BALF increased with increased time after the exposure, after 8 h total protein was significantly higher ($p<0.05$) than in the control group or in mice killed immediately after the exposure. Ozone inhalation was also characterized by a high number of infiltrating neutrophils within the bronchiole´s lumen and wall immediately after exposure; no neutrophils in cytology smears were found in the control group. Histologically, the bronchiolar epithelium showed necrosis and sloughing of bronchiolar epithelial cells with minimal oedema. Oral administration of the decoction in the drinking water (50 or 100%) for an 8 day period to 24 mice did not influence the total protein level in BALF if the animals were killed immediately after the exposure compared to a control group (n=12). In the treated groups sacrificed after 8h the protein levels were markedly decreased (in the 100% treatment group by 50% compared to control, $p< 0.05$). Furthermore, a lower degree of necrosis, a higher number of intact epithelial cell nuclei in bronchial wall and a decreased number of infiltrating neutrophils were observed [Cisneros 2005].

In another study, the effect of a decoction (20g/L) on LPS-induced acute lung injury in mice was evaluated by assessing inflammatory cell concentration of bronchoalveolar lavage fluid (BALF) and histology. Intranasal instillation of LPS (100 µg/kg b.w.) induced a significant increase in polymorph nuclear neutrophils and macrophages in BALF, which was reduced to basal values in animals (n=8) treated with a single dose of dexamethasone (2.5 mg/kg b.w.) or in mice pre-treated with cat´s claw bark decoction for 7 (n=8) or 90 (n=8) days (administered in water ad libitum) ($p<0.05$) but not in animals pre-treated for 15 or 30 days. No significant changes were observed without LPS instillation. Both cat´s claw and dexamethasone treatments reduced LPS-induced lung oedema, exudation and lung injury histology. Toxic effects were not observed in any of the cat´s claw treated groups; water and food consumption, body and organ weight, kidney, liver and lung pathology were not affected [Roque 2009].

In another study, root bark extracts were tested (petroleum ether, chloroform, chloroform-methanol 9:1, methanol and water) in rats (p.o.). Only the chloroform-methanol extract (50 mg/kg b.w.) and the aqueous extract (84 mg/kg b.w.) reduced carrageen-induced paw oedema, by 69.2 and 41.2% respectively. Compounds isolated from the chloroform-methanol extract by bioactivity-guided fractionation (quinovic acid glycosides, ursolic and oleanolic acid, 5α-carboxystrictosidine) showed only weak activity (at max. 33% at a dose of 20 mg/kg b.w.) compared to the most effective fraction (46.8%, 4.2 mg/kg b.w.) [Aquino 1991].

A quinovic acid glycoside fraction of cat´s claw bark (100 mg/kg b.w., i.p.) significantly ($p<0.001$) reduced IL-8 levels in the bladder of mice with cyclophosphamide-induced haemorrhagic cystitis. It prevented the increased expression of purinergic P2X7R receptors ($p<0.05$) and reduced the massive migration of neutrophils measured as a decrease in myeloid peroxidase activity ($p<0.05$) in bladder tissue [Dietrich 2015].

Mitraphylline impaired the release of TNF-α by 50% when administered to mice orally at 30 mg/kg b.w. for 3 days before LPS stimulation. It also inhibited the release of IL-1α and -1β by nearly 70%. The production of the pleiotropic cytokine IL-4 by activated T-cells, mast cells or basophils was reduced by 40%, whereas IL-17 production was reduced by 50%. No impact on levels of other cytokines was observed [Rojas-Duran 2012].

Renoprotective effects
A study evaluating the renoprotective effect of cat's claw involved 4 groups of rats (n=7 each); 2 control groups: one submitted to laparotomy (sham control), one submitted to 45 minutes of renal clamping causing renal ischaemia; and two treatment groups, administered an aqueous dry extract (20 mg, p.o., 1 x day) for 5 days prior to either the laparotomy or renal clamping. In the untreated ischaemic animals creatinine clearance was significantly reduced (0.17 vs. 0.54 ml/min) and the excretion of urinary peroxides was increased (11 vs. 3 nmol/g) compared to both treated groups and the sham control ($p<0.05$). Excretion of malondialdehyde was increased in the ischaemic group compared to the sham control (281 vs. 98 nmol/g; $p<0.05$) and decreased by the treatment of laparotomized rats (108 nmol/g) or ischaemic rats (151 nmol/g) ($p<0.05$) [Vattimo 2011].

Antiproliferative and cytotoxic effects
An aqueous dry extract (1 g /100 mL, 37°C, 0.43% pentacyclic oxindole alkaloids) inhibited tumour growth in mice with Lewis lung carcinoma when administered i.p. at 0.5 mg/day (n=6) and 5 mg/day (n=5) on 21 consecutive days ($p=0.0009$). The number of leukocytes in blood samples collected on the last day of treatment was significantly decreased ($p=0.00002$) in the group (n=6) treated with 0.05 mg/day. No differences were found in any of the groups concerning erythrocytes, platelets or haemoglobin. No antitumour activity was observed when animals were treated with an ethanolic dry extract (3.6% pentacyclic oxindole alkaloids) or an alkaloid enriched fraction (51.7% pentacyclic oxindole alkaloids) at 0.05 and 0.5 mg/day. Interestingly, the aqueous extract was the least active preparation when tested in vitro in cell lines (Lewis lung carcinoma: IC_{50}=461.26 µg/mL), whereas alkaloid-rich preparations were the most active (IC_{50}=25.06 µg/mL) [Pilarski 2010].

In a further study, a 70% hydroalcoholic dry extract (5.03% alkaloids) reduced the growth of murine carcinosarcoma in rats when administered by gavage at 10, 50 or 100 mg/kg b.w. for 14 days (starting at day 1 after tumour inoculation) by 46, 58 and 64% respectively ($p<0.05$). The treatment did not reverse the increase of blood levels of lactate dehydrogenase and gamma-glutamyltranferase, although all doses were effective in reducing urea plasma levels ($p<001$). Without treatment the tumour induced significant changes in all hepatic enzymes related to oxidative stress; catalase was reduced by 79%, superoxide dismutase increased by 252% and glutathione-S-transferase decreased by 59% compared to the baseline group. Treatment with the extract normalized the activities of these enzymes, in the case of superoxide dismutase only the highest dose (100 mg/kg b.w.) was effective. In the tumour tissue, catalase and superoxide dismutase activity were decreased by the treatment in all groups ($p<0.05$). No significant differences were found in activity of glutathione-S-transferase, reduced glutathione levels and lipid peroxidation [Dreifuss 2010].

Antimutagenic activity
Repair of DNA strand breaks 3 h after 12 Gy whole body irradiation was significantly ($p<0.05$) improved by daily oral treatment of rats with a purified aqueous extract (0, 40 and 80 mg/kg b.w.) for 8 weeks [Sheng 2000a].

In a consecutive study, DNA strand breaks were induced by 3 x 2 mg/kg b.w. doxorubicin (DX) applied i.p. at 24 h intervals. On day 10 after the first injection, spleen single cell suspensions were prepared and DNA single strand breaks were evaluated. Rats supplemented daily with 80 mg/kg b.w. of the extract (by gavage, starting 24 h after the last DX injection) showed a significant increase of DNA repair ($p<0.05$), no difference was found for the group treated with 40 mg/kg [Sheng 2000b].

The frequency of sister-chromatid exchanges was significantly lower (3.10-4.53; $p\leq0.05$) in mice administered with both i.p. pteropodine at 100-600 mg/kg b.w. and DX (10 mg/kg b.w.) compared to DX alone (14.06). Micronucleated polychromatic erythrocytes were significantly ($p\leq0.05$) reduced after 24h, from 19.1 in the DX group to 2.1-7.6 in the pteropodine groups) and after 96h, from 22.8 in the DX group to 3.0-6.0 in the pteropodine groups [Paniagua-Perez 2009].

Antinociceptive activity
Pre-treatment of mice with an alkaloid fraction of cat´s claw bark at 10, 30 and 100 mg/kg b.w. (i.p.) caused a significant and dose dependent reduction in the number of abdominal writhing responses by approximately 54, 72 and 97% when compared to vehicle-treated control ($p<0.01$). In the capsaicin test, a statistically significant reduction in the response to the noxious stimulus by 11, 66 and 94% ($p<0.01$) was observed in the pre-treated groups. Formalin induced responses were reduced by 8-94% ($p<0.05$ and 0.01) at doses of 10 - 300 mg/kg b.w. in the early phase (0-5 min after formalin injection) and 28-100% after 15-30 min. The antinociceptive action in the formalin test was significantly ($p<0.01$) reversed by i.p. pre-treatment of animals with ketanserin, but not by naltroxenone, atropine, prazosin, yohimbin, L-arginine or reserpine indicating the involvement of interactions with 5-HT_2 receptors. In the tail flick test, i.p. treatment of the animals with 300 mg/kg b.w. caused a significant increase in latency by 192% ($p<0.01$ after 120 min) and in the hot plate test by 311% ($p<0.01$). The motor response of animals was not affected [Jürgensen 2005].

A quinovic acid glycoside fraction of cat´s claw bark significantly inhibited nociceptive responses ($p<0.05$) in mice with cyclophosphamide induced haemorrhagic cystitis 4 h after i.p. administration (20-100 mg/kg b.w.), but not in healthy animals [Dietrich 2015].

Effects on CNS
An alkaloid fraction of cat´s claw bark (10-20 mg/kg b.w., i.p.), single alkaloids (10-40 mg/kg b.w., i.p.) as well as the muscarinic receptor agonist oxotremorine (0.01 mg/kg b.w., i.p.) significantly ($p<0.05$) attenuated the deficit in passive avoidance response in mice induced by scopolamine (3 mg/kg b.w., i.p.). Locomotor activity was not impaired by scopolamine or the alkaloids. Isopteropodine (20 mg/kg b.w., i.p.) also blocked the impairment of passive avoidance performance by the nicotinic receptor antagonist mecamylamine (15 mg/kg b.w., i.p.) and the N-methyl-D-aspartate receptor antagonist (±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, 7.5 mg/kg b.w., i.p.), but did not affect the deficit caused by diazepam (2 mg/kg b.w., i.p.). Rhynchophylline significantly reduced the mecamylamine-induced deficit but failed to attenuate effects of CPP and diazepam ($p<0.05$) [Mohamed 2000].

Effect on metabolic activity of blood granulocytes
An aqueous extract (1 g/10 mL, 0.43% alkaloids) administered to mice p.o. in 7 daily doses of 200 µg significantly stimulated granulocyte activity by 100% compared to the control (n=10 in each group, $p<0.001$) whereas an ethanolic extract (1g/10 mL, 3.48% alkaloids) showed a slightly inhibitory effect on O_2-production and leukocyte counts [Nowakowska 2010].

Effect on endometriosis
Endometriosis was induced in twenty-four female rats by auto-transplantation techniques. Ten animals were treated with 32 mg/day of a cat´s claw bark extract (32 mg/mL) by gavage and 12 animals with 0.9% saline solution (1mL/100g b.w.) for 14 consecutive days. The initial average volumes of the autotransplants did not differ between the groups ($p=0.18$), but the final average volumes were significantly different ($p=0.001$). There was an increase (from 38.1 to 75.7 mm^3, $p=0.01$) between the initial and final average volumes in the control group. Treatment with cat´s claw bark caused a marked reduction in the growth over time (from 45.5 to 27.3 mm^3, $p=0.009$). Histologically, in the treatment group, six rats had a well-preserved epithelial layer (60%), three (30%) mildly preserved epithelium and one (10%) poorly preserved epithelium. The epithelial layer occasionally presented sporadic epithelial cells. The control group presented seven cases (58.3%) of well-preserved epithelial cells and five cases (41.7%) of mildly preserved epithelial cells [Neto 2011].

Pharmacological studies in humans
Considering the comparable contribution of constituents in cat´s claw bark and root as well as the traditional use of both parts, studies are included in which preparations of the root or root bark were utilized:

Increase of lymphocytes in humans
Thirteen otherwise untreated HIV-patients received 20 mg/day of a hydrochloric acid root extract (10:1, 12 mg pentacyclic oxindole alkaloids per g). Results of blood tests were obtained at the beginning and after 2.2-5.0 months of treatment. Low leukocyte numbers (<4000 per µL, 2 of 13 patients) were raised and high leukocyte numbers (>9000 per µL, 2 of 13 patients) were lowered. The relative and absolute lymphocyte count increased significantly in all test persons (mean value: from 24.0% to 33.7%, $p=0.002$) [Keplinger 1999].

Persistent response to pneumococcal vaccine
A randomized study was carried out in male volunteers (age 40-60, n=23) to investigate the response to a polyvalent pneumococcal polysaccharide vaccine. One group (n=11) was treated with a daily dose of 2 x 350 mg of a purified aqueous extract for two months, the second group (n=12) did not receive any treatment. On day 30 of the study vaccination was performed

and the level of natural immunization was characterized (control group: 23.5%, treatment group: 33.9%, $p<0.05$). On day 60 of the study the cat´s claw bark treatment was stopped. In both groups immunization was achieved, with immunization levels of 72.8% for the control and 52.3% for the treatment group ($p<0.05$). Five months after vaccination, pneumococcal immunity was stable in the cat´s claw group, whereas the control group experienced a clear decrease ($p<0.01$). Blood analysis during the treatment showed that most of the haematological parameters were unaffected, only the lymphocyte/neutrophil ratio was increased [Lamm 2001].

Antimutagenic activity
Enhancement of DNA repair by cat's claw bark was investigated in twelve volunteers in a randomized study. One group received 250 mg per day of a purified aqueous extract for 8 weeks, the second 350 mg and the third group was the control. DNA repair after induction of DNA damage by a standard dose of hydrogen peroxide was measured in freshly prepared peripheral blood lymphocytes 3 times before and 3 times after administration. No differences were found concerning resistance to DNA damage. However, a statistically significant increase in DNA repair was observed (12-15%, $p<0.05$) after treatment. Furthermore, a tendency towards increased PHA induced lymphocyte proliferation was found in both verum groups [Sheng 2001].

Two 35 year old healthy donors, a smoker (approximately 20 cigarettes a day for 15 years) and a non-smoker, were treated for 15 days with a decoction of cat's claw bark (about 6.5 g/day). The urine was tested in the Ames-test, with and without activation by β-glucuronidase. The non-smoker's urine did not show any mutagenic activity before, during and after the treatment. The smoker's urine, however, had mutagenic activity before treatment, which was markedly decreased by the treatment (effect persisted 8 days after treatment) [Rizzi 1993].

Clinical studies

Effect on side effects of chemotherapy
The effectiveness of cat´s claw bark in reducing adverse effects of chemotherapy, particularly neutropenia and leukopenia, was studied in 40 breast cancer patients in a randomized clinical trial. All patients had undergone complete breast cancer resection (invasive ductal carcinoma stage II) and were going to begin adjuvant chemotherapy with a doxorubicin-based scheme for six cycles of 21 days. One group (designated as A, n=20) received the 6 cycles of chemotherapy, the second group (designated as B, n=20) received additionally cat´s claw bark (3x100 mg of a 70% hydroethanolic dried extract, 2.57% pentacyclic oxindole alkaloids, no tetracyclic derivatives). Twenty healthy women participated as the control group. At day zero no significant differences in haematological parameters were observed between the three groups.

A significant reduction in the leukocyte and neutrophil counts was observed in group A during the treatment (leukocytes from 6653 ± 1158 to 3247 ± 1117 cells/mm^3, $p<0.05$; neutrophils from 3588 ± 1081 to 1083 ± 368 cells/mm^3, $p<0.05$). Whereas the values of group B (leukocytes from 6800 ± 1458 to 5469 ± 1626 cells/mm^3, neutrophils from 3496 ± 1108 to 4016 ± 1545 cells/mm^3) remained close to the healthy control values at day 0 (leukocytes 6800 ± 1458 cells/mm3, neutrophils 3510 ± 1077 cells/mm3).

The lymphocyte number was reduced in both groups of cancer patients compared to the control ($p<0.05$). Monocyte counts (control group 487.6 cells/mm3) were not significantly changed by the treatment in group A (from 541 ± 161 to 500.9 ± 226) but were increased in group B (from 515.3 ±169 to 817.0 ± 444.6 cells/mm^3, $p<0.05$).

No significant differences between the patient groups were observed concerning immune response and antioxidant defence. The results of the Comet assay suggested a protective effect of cat´s claw bark on DNA during the treatment. At day 0 no differences were observed between the patient groups. At the end of the sixth cycle, however, the Comet assay index was significantly lower in group B ($p<0.05$) compared to group A [Araujo 2012].

In a randomized interventional trial, the effectiveness of cat´s claw bark in minimizing the side effects of chemotherapy and improving the antioxidant status of colorectal cancer patients was studied. Patients (n=43) who had undergone complete resection of their cancer (stage IIB, III or IV) and due to start chemotherapy with 5-fluorouracil/leucovorin and oxaliplatin (FOLFOX4, 6 cycles of 15 days) were randomly divided into 2 groups. The verum group (A, n=20) was treated with chemotherapy and cat´s claw bark (3x100 mg of a 70% hydroethanolic dried extract, 2.57% pentacyclic oxindole alkaloids, no tetracyclic derivatives), group B (n=23) received only chemotherapy. Administration of cat´s claw bark was suspended on chemotherapy treatment days due to possible interactions. No significant differences were observed in haematological parameters (haemoglobin, MCH, MCV, white blood cells and platelets count) between the two groups. Cat´s claw supplementation also did not change oxidative stress values or activity of antioxidant enzymes (catalase and superoxide dismutase), DNA damage at the level of eukaryotic cells and did not affect immunological parameters [Farias 2012].

In a prospective, single-centre, open-label phase II study, the potential of cat´s claw root bark for symptomatic management of terminal cancer patients was assessed. Patients (n=51) with various solid tumours, without any further therapeutic options, were treated with a dry extract of cat´s claw root bark (3x100 mg, 5.0% total alkaloids) for 8 weeks. The average overall quality of life ($p=0.0411$) and social functioning ($p=0.0341$) improved as assessed by the European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire (version 3). Improvement in fatigue ($p=0.0496$) was assessed by the Chalder Fatigue Questionnaire. Other symptoms such as insomnia, pain, lack of appetite and body weight remained unchanged. No changes were observed in laboratory test results (complete blood count, kidney function, ALT, AST, ALP, GGT, bilirubin) and inflammatory parameters (C-reactive protein, erythrocyte sedimentation rate, α-1-acid glycoprotein) and cytokines (IL-1, IL-6 and TNF-α). No tumour response was detected according to the Response Evaluation Criteria in solid tumours but the disease stabilized for more than 8 months in four participants. The medication was well tolerated by most patients, three gastrointestinal adverse events were classified as being more severe, two of which may be attributed to the treatment [Carvalho Lopes de Paula 2015].

Effect on mild Alzheimer´s disease
In a randomized, placebo controlled pilot study, the effect of a proprietary extract of cat´s claw bark or root (fraction of a methanol extract, no further details) in mild Alzheimer´s disease was investigated. Patients (n=20) were treated with the extract at a dose of 350 mg three times daily for a year, while 20 patients received a placebo. No significant differences were observed between the groups concerning mini-mental state examination and Alzheimer disease assessment scale. The cerebrospinal fluid β-amyloid level, as well as the outcome of MRI measurements (hippocampal, temporal and total brain volume), were not significantly different at 3 and 12 months [Quinn 2004].

Effect on denture stomatitis
Forty-eight patients with denture stomatitis type II, all denture wearers for at least 1 year and in good general health, were

randomly assigned into 3 groups to receive either 2% miconazole gel (positive control, n=15), 1.5% hydroxyethyl cellulose gel (placebo, n=16) or 2% cat´s claw bark gel (no further details, experimental group, n=17). All patients received their treatment on admission (day 0) and were told to apply the gel (2.5 mL = 1 teaspoon) to the base of the denture after meals, 3 times daily for 1 week. Denture stomatitis level as well as fungal load (*Candida* sp.) of the mucosa and denture were recorded on days 0, 7 (after 1 week of treatment) and 14 (1 week after end of treatment). Severity of stomatitis and fungal load were reduced at day 7 and 14 in all groups ($p<0.05$), but with no significant differences between the groups [Tay 2014].

Effect on active rheumatoid arthritis
Forty patients suffering from rheumatoid arthritis and undergoing sulfasalazine or hydroxychloroquine treatment were enrolled in a randomized 2 phase study for 52 weeks. During the first phase (24 weeks, double-blind, placebo-controlled) patients (n=21) were treated with cat's claw (3 x 20 mg of an aqueous acid dried root extract, 1.47% pentacyclic oxindole alkaloids, no tetracyclic derivatives) or placebo (n=19). Comparison of the groups after the first phase showed that patients in the verum group had a greater reduction in the number of painful joints than those of the placebo group (by 53.2 vs. 24.1%, p=0.044). A reduction of tender joints (p=0.001), Ritchie Index (p=0.002) and of the duration of morning stiffness (p=0.002) was observed compared to baseline values, whereas in the placebo patients, the number of tender joints, Ritchie Index and duration of morning stiffness were not significantly reduced. No changes were observed for the number of swollen joints, patient assessment of disease activity and pain. The laboratory variables were unchanged except an increased rheumatoid factor (RF) level in the placebo group (p=0.041) (other parameters: CRP, antinuclear antibodies, complete blood count, hepatic and renal variables).

In the second phase of the study (28 weeks) all patients received the extract. In the former verum group, a further reduction of symptoms was found in the second phase. In patients who received the extract only in phase two, there was a reduction in the number of painful joints (p=0.003), number of swollen joints (p=0.007) and Ritchie Index (p=0.004) compared to the end of phase 1. None of the further observed changes reached statistical significance [Mur 2002].

Pharmacokinetic properties
The alkaloid mitraphylline was stable in simulated gastric fluid (pH 1.2), but unstable in simulated intestinal fluid (pH 6.8; 13.6% degradation after 2 hours). It was subjected to efflux-mediated P-glycoprotein in both Caro-2 and MDR-MDCK monolayers which might limit its intestinal absorption when administered orally. It was metabolized by human liver microsomes with half-lives of 50 min (no details on metabolites). Plasma protein binding was determined by equilibrium dialysis (<90%) [Manda 2014].

Preclinical safety data

Mutagenic effects
No mutagenic effects of different extracts (light petroleum, chloroform, chloroform-methanol 9:1, methanol and water) and chromatographic fractions of cat's claw bark were found in the Ames-test, with and without activation by β-glucuronidase (<100 µg/plate) [Rizzi 1993].

No genotoxic effects of a cat´s claw aqueous extract (plant part not specified) were observed at 0 - 1.9 mg/mL (calculated as dry plant material / mL water) using the somatic mutation and recombination test in wings of *Drosophila melanogaster* [Romero-Jimenez 2005].

Cytotoxic effects
Aqueous extracts (500 mg/mL) did not show any toxic effects in Chinese hamster ovary cells and the bacteria *Photobacterium phosphoreum* in the neutral red assay, tetrazolium assay and microtex test as well as on total protein content (concentrations 10-100 mg/mL) [Santa 1997].

No effect on cell viability was observed after administration of 25-200 mg/mL of an aqueous cat´s claw extract (20g/L, diluted 1:4) in epithelial cells (HT29) and macrophages (RAW 264.7) [Sandoval 1998].

Acute and chronic toxic effects
The LD_{50} of a water/ethanol extract (4:1, 4% alkaloids; no further information; 0.63-5 g/kg b.w.) was found to be > 5 g/kg b.w., the maximum tolerable dose was 2.5-5 g/kg b.w., as sedation and diarrhoea were noticed [Sheng 2000a].

Administration of an aqueous root extract to mice (16.6:1, 3.5% pentacyclic oxindole alkaloids) resulted in lethargy and piloerection. Two of ten mice died within 4 hours of treatment. Autopsy revealed gastro-intestinal haemorrhage and pallor of the liver and spleen. Recovery of survivors was complete within five days after treatment. The mice showed normal body weight gains and normal autopsy findings. The acute LD_{50} of the aqueous extract in mice was greater than 16 g/kg b.w. [unpublished data, cited in Keplinger 1999].

A daily dose of 1 g/kg b.w. (aqueous hydrochloric acid root extract, 10:1, 0.75% oxindole alkaloids) administered p.o. for 28 days caused a slight increase in percentage of lymphocytes and decrease in neutrophil granulocytes. Furthermore, an increase of relative weight of the kidneys in rats was observed. The histology of kidneys was normal. No other findings and no mortalities were observed during the study [unpublished data, cited in Keplinger 1999].

No toxic signs or symptoms, and no differences in food consumption and body weight, were observed in rats after administration of up to 80 mg/kg b.w. of a purified aqueous extract (daily single dose, gavage) for 8 weeks or a dose of 160 mg/kg b.w. for 4 weeks. No differences in organ weight (heart, liver, spleen, and kidney) and no pathological changes were noticed in either group. When a single dose of a purified aqueous extract (up to 8 g/kg b.w.) or capsules containing bark powder (up to 2 g/kg b.w.) was administered to rats (n=5) by gavage, no toxic symptoms or deaths occurred within 2 weeks. Another aqueous cat´s claw bark extract (4:1; 160 mg/kg b.w., as daily single dose for 4 weeks) caused significant reduction in body weight ($p<0.05$) and food consumption ($p<0.01$), but relative organ weights were unchanged [Sheng 2000a].

After treatment of BALB/c male mice once a day with 125, 500 or 1250 mg/kg b.w. of a 50% hydroalcoholic extract (2.9% oxindole alkaloids) for 28 days (no further treatment), no treatment-related mortality and no abnormalities with respect to their coat, eye colour, touch response, strength, salivation, body weight gain and water or food consumption were observed. The bone marrow, lymph nodes, kidneys and liver did not exhibit any significant histological alterations [Domingues 2011a].

Clinical safety data
In a human volunteer study (n=4), no negative effects were observed after treatment with 350 mg/day (5 mg/kg b.w.) of a purified aqueous extract for 6 weeks on haematological analysis, body weight and work attendance. No toxic symptoms were observed [Sheng 2000a].

No negative side effects were observed during and after the

treatment of 23 male volunteers with 250 mg or 350 mg of the same extract for 2 months, or of 12 volunteers for 8 weeks in another study, according to medical examination, clinical chemistry and blood cell analysis [Sheng 2001, Lamm 2001].

Cat´s claw bark related toxic side effects were not observed in colorectal cancer patients (n=20), who had undergone complete resection of their cancer (stage IIB, III or IV) after treatment with chemotherapy with 5-fluorouracil/leucovorin and oxaliplatin (6 cycles of 15 days) and cat´s claw bark (3x100 mg of a 70% hydroethanolic dried extract, 2.57% pentacyclic oxindole alkaloids, no tetracyclic derivatives) within 12 weeks (clinical symptoms, serum clinical chemistry, whole blood analysis, leukocyte differential counts) [Farias 2012].

In a study involving 21 patients with rheumatoid arthritis undergoing sulfasalazine or hydrochloroquine therapy and treatment with cat's claw (3 x 20 mg of an aqueous acid-extracted dry root extract, 73.5% pentacyclic oxindole alkaloids, no tetracyclic derivatives), 12 adverse events occurred. One patient from the verum group withdrew from the study due to gastritis and one from the placebo group (n=19, 12 adverse effects) due to diarrhoea. In a second phase of the study all patients (n=40) were treated with the plant extract. Seven further adverse events were seen, but none could be clearly attributed to the drug intake. No major side effects were observed [Mur 2002].

In another study, patients (n=51) with various solid tumours without further therapeutic options were treated with a dry extract of cat´s claw root bark (3x100 mg, 5.0% total alkaloids). A number of mild adverse events were observed; in general the medication was well tolerated. Three gastrointestinal adverse events were classified as being more severe, two of which may be attributed to the treatment [Carvalho Lopes de Paula 2015].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Supplement 2009
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003

LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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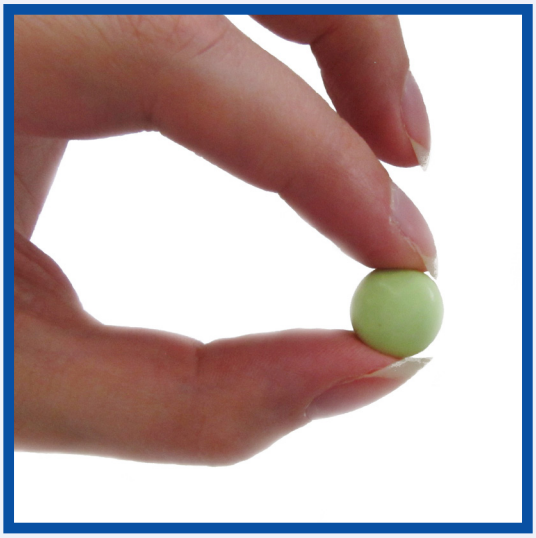
The Scientific Foundation for Herbal Medicinal Products

Urticae folium/herba

Nettle Leaf/Herb

2018



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MONOGRAPHS

***The* Scientific Foundation for**
Herbal Medicinal Products

URTICAE FOLIUM/HERBA
Nettle Leaf/Herb

2018

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Plant illustrated on the cover: *Urtica dioica*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
CCl_4	carbon tetrachloride
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase

INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

URTICAE FOLIUM/HERBA 2018

Nettle Leaf/Herb

DEFINITION

Nettle Leaf/herb consists of the whole or cut dried leaves of *Urtica dioica* L., *Urtica urens* L., or a mixture of the 2 species. It contains minimum 0.3 % for the sum of caffeoylmalic acid and chlorogenic acid expressed as chlorogenic acid (dried drug).

The material complies with the monograph of the European Pharmacopoeia [Nettle leaf].

CONSTITUENTS

Caffeic acid esters, principally caffeoylmalic acid in *Urtica dioica* (up to 1.6%) but none in *Urtica urens*; chlorogenic acid (up to 0.5%) and small amounts of neochlorogenic acid and free caffeic acid in both species.
Flavonoids, principally kaempferol, isorhamnetin, quercetin and their 3-rutinosides and 3-glucosides. Anthocyanins, such as peonidin 3-*O*-rutinoside, peonidin 3-*O*-(6''-*O*-p-coumaroyl glucoside), rosinidin 3-*O*-rutinoside. 13-hydroxyoctadecatrienoic acid, diastereoisomeric 3-hydroxy-α-ionol glucosides, scopoletin, sitosterol and its 3-glucoside, glycoprotein, free amino acids (30 mg/kg).
Minerals (ca. 18% expressed as ash) including potassium (1.8-2.0%) and silicon (0.9-1.8%). The potassium-sodium ratio has been determined as 63:1 in the unprocessed drug.
Stinging hairs on the leaves contain acetylcholine, histamine, 5-hydroxytryptamine (serotonin) and small amounts of leukotrienes.
[Collier 1956; Piekos 1976; Lutomski 1983; Chaurasia 1987; Ellnain-Wojtaszek 1988; Czarnetzki 1990; Bauer 1997; Budzianowski 1991; Lapke 1993; Neugebauer 1995; Schomakers 1995; Szentmihalyi 1998; Schulze-Tanzil 2002].

CLINICAL PARTICULARS

Therapeutic indications

Adjuvant in the symptomatic treatment of arthritis, arthroses and/or rheumatic conditions [Ramm 1995,1996,1997; Hansen 1996; Enderlein 1997; Wolf 1998; Randall 2000].

Nettle leaf or herb is also used as to enhance renal elimination of water in inflammatory complaints of the lower urinary tract [Schilcher 1988,1992; Jaspersen-Schib 1989; Czygan 2002].

Posology and method of administration

Dosage

Internal use

Adults: Hydroalcoholic extracts corresponding to 8-12 g of nettle leaf daily, divided into 2-3 doses [Ramm 1995,1996,1997; Hansen 1996; Wolf 1998]; 3-5 g of the drug as an infusion up to three times daily [Urtica BHP 1983; Van Hellemont 1988 Jaspersen-Schib 1989; Schilcher 1992]; tincture 1:5 (25% ethanol) 2-6 ml three times daily [Urtica BHP 1983]; 15 mL of fresh juice up to three times daily [Kirchhoff 1983].

External use

Adults: Fresh nettle leaf applied to the skin in the area of pain for 30 seconds once daily [Randall 2000].

Method of administration

For oral or topical administration.

Duration of use

No restriction.

Contraindications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicinal products and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
Gastrointestinal upset or allergic response in a few individuals after oral use [Ramm 1995,1997; Hansen 1996; Enderlein 1997; Wolf 1998].

Overdose
No case of overdose reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Anti-inflammatory activity
A hydroethanolic extract (6.4-8:1) and its main phenolic constituent, caffeoylmalic acid, were tested for inhibitory potential on the biosynthesis of arachidonic acid metabolites by rat leukaemic basophilic granulocytes (RBL-1 cells). The extract (0.1 mg/mL) and the isolated acid (1 mg/mL) showed partial inhibitory effects of 20.8% and 68.2% respectively on the 5-lipoxygenase-derived synthesis of leukotriene B_4; caffeoylmalic acid exhibited concentration-dependent activity with an IC_{50} of 85 µg/mL. Both the extract and the acid showed strong concentration-dependent inhibition of the synthesis of cyclooxygenase-derived prostaglandins (IC_{50} of 92 µg/mL for the extract and 38 µg/mL for the acid) [Obertreis 1996].

The same extract significantly and dose-dependently reduced LPS-stimulated release of two proinflammatory cytokines, TNF-α and IL-1β, in human whole blood from 6 healthy volunteers. After 24 hours, TNF-α concentration was reduced by 50.8% and IL-1β concentration by 99.7% using the highest tested extract concentration of 5 mg/mL ($p<0.001$); after 65 hours the inhibition was 38.9% and 99.9% respectively ($p<0.001$). The extract and LPS stimulated the release of IL-6 in human blood when used separately, but showed no additive effect when used simultaneously; IL-6 acts antagonistically to IL-1β, decreasing IL-1β-induced PGE_2 synthesis by fibroblasts and synovial cells. Selected constituents of nettle leaf (caffeoylmalic acid, chlorogenic acid, caffeic acid, quercetin and rutin), tested in the same way did not influence the release of TNF-α, IL-1β or IL-6 at concentrations up to 5×10^{-5} mol/litre [Obertreis 1996].

Th1 and Th2 cells (T helper cells) have cytokine patterns which regulate cell-mediated and humoral immune responses. Th1 cells produce IL-2 and IFN-γ, proinflammatory cytokines that induce a cascade of inflammatory responses. Th2 cells produce IL-4, IL-5 and IL-10. The cytokine patterns of these Th effector cells are antagonistic and cross-regulated; thus agents that promote Th1 cytokine expression inhibit Th2 cytokine production and vice versa. The water-soluble fraction from a nettle leaf extract (6.4-8.1:1) significantly inhibited phytohaemagglutinin (PHA)-stimulated production of Th1-specific IL-2 ($p<0.01$) and IFN-γ ($p<0.02$) in human peripheral blood mononuclear cells (PBMC) in a dose-dependent manner, by 50 ± 32% and 77 ± 14% respectively at the highest concentration tested (equivalent to 400 µg/mLexpressed as the total extract). The dose-dependent inhibiting effect on IL-2 and IFN-γ expression was also detected by reverse transcriptase-polymerase chain reaction in PHA-stimulated PBMC. In contrast, the extract fraction enhanced the secretion of Th2-specific IL-4 by PHA-stimulated PBMC, but dose-dependently decreased IL-10 secretion [Klingelhoefer 1999].

Incubation of HeLa cells with various concentrations of an extract (6.4-8.1:1) before stimulation with TNF demonstrated that the extract potently and dose-dependently inhibited the formation of an NF-κB DNA complex and inhibited NF-κB reporter gene activity; in both cases even stronger inhibition was observed with a water-soluble fraction of the extract. Pretreatment with the water-soluble fraction also inhibited NF-κB activation in stimulated Jurkat T, L929 fibrocarcinoma and MonoMac6 cells. Further experiments with stimulated HeLa and Jurkat T cells suggested that the water-soluble fraction of the extract inhibits NF-κB activation not by modification of DNA binding but by preventing the degradation of its inhibitory subunit IκB-α [Riehemann 1999].

In an *ex vivo/in vitro* study, 2 x 670 mg of an extract (6.4-8.1:1) was administered daily for 21 days to 18 healthy volunteers from whom blood samples were taken at 0, 7 and 21 days. Testing of the whole blood samples revealed, in comparison with day 0 values, significant reductions in LPS-stimulated release of two cytokines, TNF-α and IL-1β: at day 7 and day 21, release of TNF-α had decreased by 14.6% ($p<0.01$) and 24.0% ($p<0.001$), and IL-1β by 19.2% ($p<0.01$) and 39.3% ($p<0.001$), respectively. When a water-soluble fraction from the same batch of nettle leaf extract, at various concentrations, was incubated for 24 hours with 0-, 7- and 21-day whole blood samples from the volunteers, concentration-dependent (and time-dependent in the sense of 7- and 21-day samples from the volunteers) inhibition of LPS-induced release of the same cytokines was demonstrated; at the highest concentration of extract fraction (160 µg/mL) incubated with 21-day blood samples, release of TNF-α decreased by 79.5% ($p<0.001$) and IL-1β by 99.2% ($p<0.001$) compared to day 0 values of blood untreated with the extract fraction [Teucher 1996].

An extract, prepared as 0.25 mg/mL of a lyophilized aqueous extract in water, produced 93% inhibition of PAF-induced exocytosis of elastase from human neutrophils. The same extract (0.2 mg/mL) showed no activity in a test for inhibition of the biosynthesis of prostaglandins [Tunon 1995].

A dry 95%-isopropanolic extract (19-33:1) and 13-hydroxy-octadecatrienoic acid (a constituent present in the extract) at 10 µg/mL significantly suppressed IL-1β-induced expression of matrix MMP proteins on cultured human chondrocytes: relative expression of MMP-1, MMP-3 and MMP-9 decreased by 63-96% ($p = 0.0014$ to 0.0057) with the extract and 60-88% ($p = 0.0078$ to 0.054) with 13-hydroxyoctadecatrienoic acid [Schulze-Tanzil 2002].

A dry 95%-isopropanolic extract (19-33:1) exhibited an immunosuppressant effect in preventing the maturation of cultured human myeloid dendritic cells (without affecting their viability), leading to reduced induction of primary T cell responses [Broer 2002].

Other effects
Aqueous extracts (not further specified) produced slight contraction followed by relaxation in isolated uterine smooth muscle from the non-pregnant mouse. Application of the extracts to uterine muscle from the pregnant mouse produced a diametrically opposed effect, increase of muscular tone and contractions of considerable amplitude [Broncano 1987].

Inhbitory effects of various extracts (petroleum ether, ethyl acetate, methanol and water) and flavonoid aglycone and flavonoid glycoside fractions (1 mg/mL) from *Urtica dioica* leaves on rat platelet aggregation induced by thrombin 0.5 U/mL were analysed. Only the ethyl acetate extract (60-80%), flavonoid aglycones (86.5%) and flavonoid glycosides (82.8%) were significantly ($p< 0.001$) active [El Haouari 2006].

The results from perfusion of glucose-containing Islets of Langerhans by an aqueous extract (10 g/200 mL) showed an increase in insulin secretion both in 2.8 and 16.7 mM solutions [Farzami 2003].

The antiproliferative activity of an aqueous extract of *Urtica dioica* at 0, 0.375, 0.75, 1.5, 2 and 3 mg/mL concentrations on MCF-7 cell line was tested after 24, 48 and 72 hrs. After 72 hrs a dose-dependent manner antiproliferative activity was observed (IC_{50}: 2 mg/mL). There was a significant difference in cell viability between 0.375 mg/mL and 0.75 mg/mL ($p< 0.05$) [Fattahi 2013].

The antioxidant activity of an aqueous extract of *Urtica dioica* was evaluated by MTT and showed significant activity ($p< 0.05$) at 3-12 mg/mL concentration [Fattahi 2013].

A hydroethanolic dry extract (not further specified) showed histamine receptor antagonist activity with an IC_{50} of 251 (±13) µg/mL and histamine receptor negative agonist activity with an IC_{50} of 193 (±71) µg/mL. It also showed inhibition of mast cell tryptase, COX-1, COX-2 and haematopoietic prostaglandin D2 synthase with IC_{50} values of 172 (±28), 160 (±47), 275 (±9) and 295 (±13) µg/mL respectively [Roschek 2009].

Ex vivo experiments
An ethanolic extract (5 g/100 mL) of *Urtica dioica* caused significant inhibition (25 µL, 50 µL ($p< 0.01$) and 100 µL ($p< 0.02$)) of adenosine deaminase activity in prostate tissue from patients with prostate cancer (Gleason scores 4-7) [Durak 2004].

In vivo experiments

Antidiabetic Activity
Normal and streptozotocin diabetic rats were injected i.p. with a fraction obtained from an aqueous extract of *Urtica dioica*. A significant ($p< 0.05$) rise in the level of serum insulin was observed after 60 min. Glucose level showed a decrease, initiated at 60 min and 120 min [Farzami 2003].

A hydroalcoholic extract (not further specified) of *Urtica dioica* was administered to Wistar rats at 100 mg/kg/day i.p. for five days and then hyperglycaemia was induced with streptozotocin. After five weeks, blood glucose concentrations were significantly ($p<0.05$) lower in the treatment group compared to an untreated diabetic group (303.6±100.6 and 454.7±34.5 respectively). The percentage of β-cells was significantly ($p<0.05$) higher in the treatment group compared to the untreated diabetic group (1.9 and 22.9 % respectively) [Golalipour 2007].

Hepatoprotective Activity
The effects of 14 days treatment with a hydroethanolic extract (80% ethanol; 50 and 100 mg/kg b.w. p.o.) of *Urtica dioica* were investigated in the liver of Swiss albino mice (8-9 weeks old). Significant increases were observed for the activities of cytochrome b5 (cyt b5), NADH-cytochrome b5 reductase (cytb5R; $p< 0.001$ at doses of 50 mg/kg and 100 mg/kg), glutathione S-transferase and DT-diaphorase ($p< 0.005$ at 50 mg/kg, $p< 0.05$ at 100 mg/kg), and glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase ($p< 0.001$ at doses of 50 mg/kg and 100 mg/kg). Both treatment groups showed significantly lower activity of cytochrome P450, lactate dehydrogenase ($p< 0.001$), NADPH-cytochrome P450 reductase ($p< 0.005$, $p< 0.001$), total sulfhdryl groups ($p< 0.05$, $p< 0.005$) and protein bound sulfhydryl groups (100 mg/kg $p< 0.001$) [Özen 2003].

A study investigated the effects of 14 days treatment with a hydroethanolic extract (80% ethanol; 50 and 100 mg/kg b.w. p.o.) of *Urtica dioica* on hepatic aniline 4-hydroxylase (A4H), NADH and NADPH-NADH in Swiss albino mice (8-10 weeks old). Hepatic aniline 4-hydroxylase (A4H) cofactor requirements were significant at both doses for cofactors NADH ($p< 0.05$), NADPH-NADH at 50 mg/kg ($p< 0.01$) and at 100 mg/kg ($p< 0.005$), and not significant for NADPH at 50 mg/kg but significant at 100 mg/kg ($p< 0.01$). Also A4H activity on Mg^{2+} in mice was found to be significant at doses of 50 mg/kg ($p< 0.05$) and 100 mg/kg ($p< 0.005$), and on Ca^{2+} at 50 mg/kg ($p< 0.01$), but not significant at 100 mg/kg [Özen 2009].

Diuretic effects
The effects of continuous intravenous perfusion into anaesthetized rats for 1.25 hours of solutions (in isotonic 0.9% saline) of a dry aqueous extract at two dose levels, 4 mg/kg/hour or 24 mg/kg/hour, were compared with the effect of furosemide (as a control diuretic), similarly perfused at 2 mg/kg/hour. Compared to control periods (perfusion of saline only), the extract caused dose-dependent increases in diuresis (urine volume) of 11% and 84% (both $p<0.001$) and in natriuresis of 28% and 143% (both $p<0.001$) respectively, while furosemide increased diuresis by 85% and natriuresis by 155% (both $p<0.001$) [Tahri 2000].

No effect on diuresis or ion excretion could be demonstrated in rats after oral administration of an aqueous extract at a dose of 1 g/kg b.w. [Lasheras 1986].

Furthermore, no significant diuretic effect was observed during 2 hours after oral administration to rats of an unspecified ethanolic extract at 1 g/kg b.w., whereas urinary excretion increased significantly after intraperitoneal administration of 500 mg/kg [Tita 1993].

Although the potassium-sodium ratio of dried nettle leaf was determined as 63:1, the ratio in a decoction (2 g of dried leaf boiled in 200 ml of deionized water) was found to be much higher at 448:1 [Szentmihalyi 1998].

Spontaneous motility
An infusion and aqueous extract (3:1) produced dose-dependent reductions in spontaneous motility and body temperature in rats and mice when administered i.p. at doses of 1.739 and 3.748 g/kg b.w. for the infusion and 303 and 606 mg/kg for the extract [Broncano 1987]. An aqueous extract at a dose of 750 mg/kg led to a significant reduction in spontaneous activity in mice during the first 16 hours after administration [Lasheras 1986].

Hypotensive effects
In the perfusion experiments described above under *Diuretic effects*, the diuretic and natriuretic effects were accompanied by a dose-dependent hypotensive effect. Compared to control periods (perfusion of isotonic 0.9% saline only), perfusion of a dry aqueous extract (in isotonic saline) reduced arterial blood

pressure by 15% at 4 mg/kg/hour and 38% at 24 mg/kg/hour (both $p<0.001$); while furosemide at 2 mg/kg/hour reduced arterial blood pressure by 28% ($p<0.001$). The hypotensive effect was reversible within about 1 hour of recovery after the lower dose of extract or furosemide, but was persistent after the higher dose of extract, indicating a possible toxic effect at that dose level [Tahri 2000].

Nettle herb produced a rapid but only transient decrease of 31.7% on the blood pressure of anaesthetized rats after i.v. administration of an aqueous extract at a dose of 25 mg/kg b.w. [Lasheras 1986]. In cats, an aqueous extract (3.3:1) administered by cannula at a dose of 26.6 mg/kg b.w. produced a marked hypotensive effect and bradycardia, which was not compensated by subsequent administration of adrenaline [Broncano 1983].

Hyperglycaemic activity
Both an 80% ethanolic extract and an aqueous decoction, evaporated to dryness, resolubilized and administered to mice at the equivalent of 25 g drug/kg b.w. 2 hours prior to glucose load, produced hyperglycaemic effects in an oral glucose tolerance test [Neef 1995].

Analgesic activity
After administration of an aqueous extract at a dose of 1200 mg/kg, mice showed much greater resistance to thermal stimulation in the hot plate test at 55°C, taking 190% longer time to react than control animals [Lasheras 1986].

An ethanolic extract (not further specified) reduced the writhing response to phenylquinone in rats after oral (1 g/kg) and intraperitoneal (500 mg/kg) treatment but demonstrated no analgesic activity in the hot plate test [Tita 1993].

An *U. urens* extract (80% ethanol) showed significant anti-nociceptive activity in chemically induced mouse pain models; in the writhing test (96.5% inhibition at 250 mg/kg i.p., $p<0.01$), the formalin test (62.8% inhibition at 500 mg/kg p.o., $p<0.05$) and significant ($p<0.01$) antiinflammatory activity in the carrageenan-induced rat hind paw oedema test (41.5% inhibition at 300 mg/kg) [Marassini 2010].

Local anaesthetic activity
Local application to the rat tail of 0.05 mL of an aqueous extract (100 mg lyophilized extract per mL), in the same region as subsequent application of heat in the tail flick test, produced a local anaesthetic effect comparable to that of lignocaine [Lasheras 1986].

Clinical studies

Adjuvant treatment of arthritis, arthroses and/or rheumatic conditions
Five open, multicentric, post-marketing surveillance studies have been carried out on patients with arthritic or rheumatic complaints using a preparation containing a dry hydroethanolic extract (6.4-8:1) at a daily dosage of 2 x 670 mg (corresponding to 9.648 g of dried leaf per day). In each study a proportion of the patients also continued other therapies, primarily non-steroidal anti-inflammatory drugs (NSAIDs), while others received only the nettle leaf extract. Assessments were carried out through patient questionnaires and consultations with physicians. Overall, 80-95% of patients rated the efficacy of the extract, and 93-95% its tolerability, as good or very good [Ramm 1995,1996,1997; Hansen 1996; Wolf 1998]:

- 152 patients with rheumatic pains, of whom about 60% had degenerative disorders of joints, were treated in a 3-week study. Pain symptoms assessed by a visual analogue scale (VAS) improved in 70% of patients by at least one third: pain at rest by 50%, movement pain by 51%. In patients receiving only nettle leaf extract (n = 12) pain decreased by 43% [Ramm 1995].

- 219 patients, mainly with degenerative or inflammatory joint disorders, were treated in another 3-week study. Pain symptoms (VAS) improved by at least one-third in 70% of patients; in patients with pain of degenerative origin by about 50%. Patients taking only nettle leaf extract found it as effective as the extract + NSAIDs [Hansen 1996].

- 223 patients with arthritis (over 50% gonarthritis, 34% coxarthritis) were treated in a 6-week study. Pain intensity (VAS) decreased by 56% and objective assessment of all findings on joints (reddening, overheating, swelling, pressure pain and joint discharge) improved by 60-65% on average. A reduction in complaint symptoms of at least 33% from initial values was experienced by 75% of the patients and this was reflected in patients' responses to a Quality of Life questionnaire. Out of the 130 patients initially taking concomitant NSAIDs, 106 (81%) reduced or discontinued their NSAID dosage [Ramm 1997].

- 8955 patients suffering pain and impairment of mobility due to osteoarthritis or rheumatoid arthritis were treated in a 3-week study. The total symptom scores (1-5 point scales for pain at rest, exercise pain and restricted mobility) of 96% of patients decreased by 45% on average and no clinically relevant differences in efficacy were evident between the group taking only the extract compared to those continuing with NSAIDs or other therapy. 64% of those patients who initially continued NSAID treatment reduced (38%) or discontinued (26%) their NSAID dosage later in the study [Ramm 1996].

- 819 patients with gonarthritis were treated in a 12-month study. Symptoms such as pain, joint stiffness and impaired joint function, assessed by a quantitative gonarthritis-specific questionnaire, decreased by 61% on average. The frequency of each of 5 symptoms (swelling, pressure pain, reddening, joint discharge and overheating) decreased significantly ($p<0.001$) [Wolf 1998].

In an open, randomized 14-day study, patients with acute arthritis received daily either 2 × 100 mg of diclofenac (n = 17) or 50 mg of diclofenac and 50 g of a prepacked stewed nettle leaf purée (water content 95.5%, caffeoylmalic acid content 20 mg) (n = 19). Both groups also received the gastroprotective misoprostol (a prostaglandin analogue). The main criterion of the study was relative improvement in elevated serum levels of C-reactive protein, which decreased by about 70% in both groups. Assessments (verbal rating score 0-4) of physical impairment, subjective pain and pressure pain by patients, and stiffness by physicians, showed improvements in the range 52-77%, with no significant differences between the two groups [Enderlein 1997].

An exploratory study was carried out on the alleviation of pain by external application of fresh nettle leaves, which causes urtication. From analysis of recorded, semi-structured interviews between a doctor and 18 people who had tried this self-treatment for joint or muscle pains, 15 out of 18 claimed that nettle treatment worked on every application; 17 out of 18 reported pain relief after the first course of treatment and had found no other treatment as effective as nettle leaf. Onset of pain relief occurred in less than 24 hours in 11 out of 18 patients. The stinging sensation was reported by 14 out of 18 as 'not painful, with a not unpleasant warmth' and, other than

3 cases of localised numbness for 6-24 hours and a few rashes, no side effects were reported [Randall 1999].

Subsequently a randomized, double-blind, crossover study was carried out involving 27 patients with persistent osteoarthritic pain at the base of the thumb or index finger, none of whom had previously used nettle leaf as a treatment. Existing analgesic or anti-inflammatory treatments were continued during the study. A fresh leaf from pot-grown, non-flowering *Urtica dioica* or *Lamium album* (white deadnettle, as a placebo of similar appearance), handled through plastic, was applied to the affected area once daily for 30 seconds in a standard manner. One week of treatment was followed by a wash-out period of 5 weeks before one week of the alternative treatment. Compared to placebo, significantly greater reductions in scores were observed with nettle leaf on a visual analogue scale for pain ($p = 0.026$) and the Stanford health assessment questionnaire for disability ($p = 0.0027$). No serious side affects were reported and the localized rash and itching associated with nettle leaf was acceptable to 23 of the 27 patients [Randall 2000].

Diuretic effects
In an open 2-week study, 32 patients suffering from myocardial or chronic venous insufficiency were treated daily with 3 × 15 mL of nettle herb pressed juice. A significant increase in the daily volume of urine was observed throughout the treatment, the volume on day 2 being 9.2% higher ($p<0.0005$) than the baseline amount in patients with myocardial insufficiency and 23.9% higher ($p<0.05$) in those with chronic venous insufficiency. Minor decreases in body weights (about 1%) and systolic blood pressure were also observed. Serum parameters were unaffected [Kirchhoff 1983].

Antidiabetic effect
In a randomized, double-blind, placebo-controlled clinical trial, the effects of a dried hydroethanolic *Urtica dioica* extract (ethanol 70%, yield 20.24%) were examined in type 2 diabetic patients. Patients continued with their same dose of conventional oral antihyperglycaemic drugs during the trial. They received one capsule every 8 hours for 3 months, containing either 500 mg of the extract (n=46) or placebo (n=46). Effects on the blood levels of fasting glucose, postprandial glucose, glycosylated haemoglobin (HbA1c), creatinine and liver enzymes SGOT and SGPT, as well as on systolic and diastolic blood pressure were evaluated. Compared with placebo, the extract lowered the blood levels of fasting glucose, 2 hours postprandial glucose and HbA1c significantly ($p<0.001$, $p= 0.009$, and $p= 0.006$ respectively) without any significant effects on the other parameters ($p>0.05$) [Kianbakht 2013].

Antioxidant Effect
A randomized, double-blind, placebo-controlled trial involved 50 patients with type 2 diabetes, administered with either 100 mg/kg b.w. of a hydroethanolic extract (45% ethanol; 2.7 g dry matter per 1L extract) or placebo for 8 weeks. In blood samples collected after 8 weeks, *in vitro* Total Antioxidant Capacity (TAC) and Superoxide Dismutase (SOD) were significantly ($p<0.05$) increased in the intervention group compared to the control group [Namazi 2012].

Pharmacokinetic properties
No data available.

Preclinical safety data
The intraperitoneal LD_{50} of an aqueous extract of *Urtica dioica* herb in mice has been determined as 3.625 g/kg b.w. [Lasheras 1986].

An ethanolic extract of *Urtica dioica* herb showed low toxicity in both rats and mice after oral and intraperitoneal administration at the equivalent of up to 2 g of dried drug per kg b.w. [Tita 1993].

Clinical safety data
No serious adverse effects were reported from 5 clinical studies in which a total of 10,368 patients took 2 x 670 mg of a dry hydroethanolic extract (6.4-8:1), corresponding to about 9.7 g of dried leaf, daily for periods varying from 3 weeks to 12 months; the incidence of minor adverse effects (mainly gastrointestinal upsets or allergic reactions) was 1.2-2.7% [Ramm 1995-1997; Hansen 1996; Wolf 1998]. In a study where 19 patients received 50 g of a stewed nettle leaf purée daily for 14 days, 3 patients reported meteorism [Enderlein 1997].

A case of gynaecomastia in a man and a case of galactorrhoea in a woman were reported after consumption of 2-3 cups nettle tea daily for one month. However, the causal relationship was unclear [Şahin 2007].

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E/S/C/O/P
MONOGRAPHS

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Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
ARCTII RADIX	Burdock Root	Online Series, 2016
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BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
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ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
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HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003

LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Second Edition, 2003
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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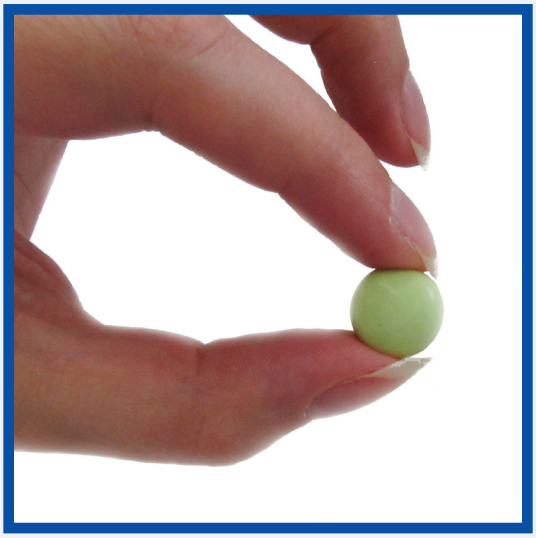
Online Series

The Scientific Foundation for Herbal Medicinal Products

Urticae radix

Nettle Root

2015



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MONOGRAPHS

***The* Scientific Foundation for**
Herbal Medicinal Products

URTICAE RADIX
Nettle Root

2015

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ESCOP Monographs were first published in loose-leaf form progressively
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Urticae radix - Nettle Root

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

Edited by Simon Mills and Roberta Hutchins
Cover photographs by Simon Mills (*Urtica dioica*) and Martin Willoughby
Cover and text design by Martin Willoughby
Typeset in Optima by Roberta Hutchins

Plant illustrated on the cover: *Urtica dioica*

FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
Verso
Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
Back cover

Information on the member organizations and people involved in ESCOP's activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

URTICAE RADIX

Nettle Root

2015

DEFINITION

Nettle root consists of the whole, or fragmented, underground parts of *Urtica dioica* L., *Urtica urens* L., their hybrids or mixtures of these.

The material complies with the European Pharmacopoeia [Nettle Root].

CONSTITUENTS

Approx. 0.1% agglutinin (UDA), a lectin which can be separated into 11 isolectins and which contains 86 amino acid residues [Peumans 1984; Van Damme 1988; Broekaert 1989; Willer 1991, 1992; Balzarini 1992; Beintema 1992; Lerner 1992, Wagner 1994a; Frank 1998; Ganzéra 2005]; a mixture of polysaccharides, basically 2 glucans, 2 rhamnogalacturonans and 1 arabinogalactan [Willer 1992; Wagner 1992, 1994b; Frank 1998]; scopoletin, β-sitosterol, β-sitosterol glucoside and other sterols and sterol glucosides [Schilcher 1986; Chaurasia 1986a, 1987a,b; Frank 1998]; phenylpropanes (homovanillyl alcohol and its glucoside) as well as lignans such as (+)-neo-olivil and its derivatives, (–)-secoisolariciresinol, (–)-isolariciresinol and dehydrodiconiferyl alcohol, and lignan glucosides [Chaurasia 1986b, 1987a; Kraus 1990a,b; Ganßer 1995a,b; Schöttner 1997a,b; Orčić 2015]; ceramides [Kraus 1991a]; hydroxy fatty acids including (10*E*,12*Z*)-9-hydroxy-10,12-octadecadienoic acid [Kraus 1991b] and the isomeric 9,10,13-trihydroxy-11-octadecenoic and 9,12,13-trihydroxy-10-octadecenoic acids [Ganßer 1995a,b]; monoterpene diols and their glucosides [Kraus 1991c].

CLINICAL PARTICULARS

Therapeutic indications
Symptomatic treatment of micturition disorders (dysuria, pollakisuria, nocturia, urine retention) in benign prostatic hyperplasia (BPH) [Djulepa 1982; Tosch 1983; Schilcher 1988a,b, 1989, 1992; Stahl 1984; Vontobel 1985; Vahlensieck 1986, 1996; Vandierendounck 1986; Dathe 1987; Maar 1987; Bauer 1988; Feiber 1988; Friesen 1988; Goetz 1989; Jaspersen-Schib 1989; Belaiche 1991; Fischer 1992; Kaldewey 1995; Brom 1996; Engelmann 1996; Bracher 1997; Veit 1998; Chrubasik 2007; Pagano 2014] at stages I and II as defined by Alken [Alken 1973] or stages II and III as defined by Vahlensieck [Vahlensieck1996].

Posology and method of administration

Dosage

Daily dose: 4-6 g of the drug as an infusion [Schilcher 1992; Jaspersen-Schib 1989]; 300-600 mg of dried native extract (7-14:1, 20% V/V methanol) [Djulepa 1982; Tosch 1983; Stahl 1984; Vontobel 1985; Vandierendounck 1986; Dathe1987; Maar 1987; Bauer 1988; Feiber 1988; Friesen 1988; Fischer 1992; Brom 1996; Bracher 1997; Veit 1998; Pagano 2014] or 378-756 mg of dried native extract (12-16:1, 70% V/V ethanol) [Kaldewey 1995]; 4.5-7.5 mL of fluid extract (1:1, 45% ethanol) [Goetz 1989] or 15 mL of fluid extract (1:5, 40% ethanol) [Belaiche 1991]; comparable extracts at equivalent dosages.

Method of administration
For oral administration.

Duration of administration
No restriction.

Contra-indications
None known.

Special warnings and special precautions for use
All cases of difficulty in micturition require clarification by a physician and regular medical checks in order to rule out the need for other treatment, e.g.

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Stages of Benign Prostatic Hyperplasia as defined by Alken	Stages of Benign Prostatic Hyperplasia as defined by Vahlensieck
Stage I Dysuria, pollakisuria, possibly nocturia, reduction in projection of the urine stream, no residual urine (stage of compensation of the bladder musculature).	**Stage I** No micturition problems More or less marked BPH Urine stream: greater than 15 mL/s maximal flow No residual urine No bladder trabeculation
Stage II Same symptomatic as under I, except with residual urine (incipient decompensation of the bladder musculature).	**Stage II** Intermittent micturition problems (frequency, calibre of urine stream) More or less marked BPH Urine stream: between 10 and 15 mL/s maximal flow No or low (≤ 50 mL) residual urine No or incipient bladder trabeculation
Stage III Complete stoppage or overflow of the bladder (decompensation of the bladder musculature).	**Stage III** Permanent micturition problems (frequency, calibre of urine stream) More or less marked BPH Urine stream: less than 10 mL/s maximal flow Residual urine more than 50 mL Trabeculated bladder
	Stage IV Permanent micturition problems (frequency, calibre of urine stream) More or less marked BPH Urine stream: less than 10 mL/s maximal flow Residual urine more than 100 mL Overflowing bladder Stoppage of the upper urinary tract

surgical intervention. Consultation with a physician is particularly necessary in cases of blood in the urine or acute urine retention.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
Not applicable.

Effects on ability to drive and use machines
None known.

Undesirable effects
Gastrointestinal complaints [Djulepa 1982; Stahl 1984; Vontobel 1985; Kaldewey 1995; Engelmann 1996; Pagano 2014]. Rare cases of allergic skin reactions [Tosch 1983, Pagano 2014].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments
Significant suppression (average 67%) of SHBG binding capacity by a 10% solution of a 20% methanolic extract of nettle root has been demonstrated. It appears that the binding of 5α-dihydrotestosterone to proteins can be influenced by the extract [Schmidt 1983]. An aqueous extract of nettle root dose-dependently inhibited the binding of SHBG to solubilized receptors from human prostatic tissue; in the same experiment neither a 70% ethanolic extract of nettle root nor *Urtica dioica* agglutinin were effective [Hryb 1995].

The lignan secoisolariciresinol and a mixture of isomeric (11*E*)-9,10,13-trihydroxy-11-octadecenoic and (10*E*)-9,12,13-trihydroxy-10-octadecenoic acids isolated from nettle root reduced the binding activity of human SHBG; methylation of the mixed hydroxy acids increased their activity by about 10-fold [Ganßer 1995a].

(10*E*,12*Z*)-9-hydroxy-10,12-octadecadienoic acid isolated from an aqueous-methanolic extract of nettle root inhibited aromatase activity [Kraus1991b; Bartsch 1992]. On the other hand, aromatase inhibition by five other compounds isolated from a methanolic extract of nettle root was only weak (less than 1% compared to 4-hydroxy-androst-4-ene-3,17-dione) [Ganßer 1995b].

A polysaccharide fraction from an aqueous extract of nettle root showed activity in the lymphocyte transformation test. Isolated polysaccharides produced significant, concentration-dependent reduction of haemolysis (95% reduction at 1 mg/mL) in classical and alternative complement tests. From these results anti-inflammatory and immunomodulating activities were deduced [Wagner 1989; Willer 1990,1992]. Lectin fractions from *Urtica dioica* agglutinin stimulated the proliferation of human lymphocytes in the lymphocyte transformation test [Wagner 1989; Willer 1992].

Organic solvent extracts of nettle root inhibited Na^+, K^+-ATPase activity of human BPH tissue cells by 28-82% at 0.1 mg/mL. Steroidal compounds from nettle root, such as stigmast-4-en-3-one, stigmasterol and campesterol, inhibited the enzyme activity by 23.0-67.0% at concentrations from 10^{-3} to 10^{-6} M. These results suggest that some hydrophobic constituents such as steroids inhibit the membrane Na^+, K^+-ATPase activity of the prostate, which may subsequently suppress prostate-cell metabolism and growth [Hirano 1994].

A lectin fraction from *Urtica dioica* agglutinin inhibited by 53% the binding of epidermal growth factor (EGF) to EGF receptors in cells cultured from human prostatic tissue [Willer 1992]. The growth of cells cultured from human BPH tissue was significantly inhibited by 5 different fractions from a 20% methanolic extract of nettle root [Enderle-Schmidt 1988]. *Urtica dioica* agglutinin at 500 ng/mL to 100 µg/mL dose-dependently inhibited the binding of ^{125}I-labelled EGF to its receptor on human A431 epidermoid cancer cells [Wagner 1994].

Lignans present in polar extracts of nettle root, (+)-neo-olivil, (–)-secoisolariciresinol, dehydrodiconiferyl alcohol, isolariciresinol and (–)-3,4-divanillyltetrahydrofuran, as well as the main intestinal metabolites of plant lignans in humans (enterodiol, enterolactone and enterofuran), showed binding affinity to human SHBG. Outstandingly high affinity was exhibited by (–)-3,4-divanillyltetrahydrofuran, which is present only in traces in nettle root [Schöttner 1997b].

A 20% methanolic extract of nettle root significantly ($p<0.05$), and time- and concentration-dependently, inhibited the proliferation of human prostatic epithelial LNCaP (**l**ymph **n**ode **ca**rcinoma of the **p**rostate) cells; maximum growth reduction of 30% was obtained on day 5 at a concentration of 10^{-6} mg/mL. In contrast, the extract had no antiproliferative effect on human prostatic stromal cells. Comparable inhibition ($p<0.05$) of proliferation of human epithelial LNCaP cells by a polysaccharide-rich fraction (POLY M) of the same extract was observed over a 7-day period. Again, the inhibition was time- and concentration-dependent, with maximum suppression of 50% on day 6 at concentrations of 10^{-9} and 10^{-11} mg/mL. No cytotoxic effects of the extract or the fraction POLY-M on cell proliferation were observed [Lichius 1999a; Konrad 2000].

Nettle root extract inhibited cell proliferation in cultures of prostate tissue taken from BPH patients [Rausch 1992].

It has been shown that *Urtica dioica* agglutinin binds to the cell membrane of prostatic adenoma cells from BPH patients [Sinowatz 1994] and can inhibit the action of growth factors involved in the regulation of prostate growth [Wagner 1992].

An ethanolic and a petroleum ether extract inhibited 5α-reductase with an IC_{50} of 120 µg and 190 µg, respectively, compared to 1.06 µg by finasteride [Nahata 2012].

In vivo experiments

Ten dogs suffering from BPH, treated daily for 100 days with 90 mg/kg b.w. of a nettle root extract (3.5-7:1, 20% V/V methanol), showed an average decrease of 30% in prostate volume [Daube 1988]. The same extract did not inhibit testosterone and dihydrotestosterone stimulated growth of the prostate in castrated rats [Rhodes 1993].

A crude aqueous fraction from nettle root containing 4 different polysaccharides, administered orally at 40 mg/kg, significantly inhibited carrageenan-induced rat paw oedema by 36.8% after 5 hours ($p<0.01$) and 63.6% after 22 hours ($p<0.005$). This anti-inflammatory activity was comparable after 5 hours, and markedly superior after 22 hours, to that exhibited by indometacin at 10 mg/kg (42.1% and 27.3% inhibition respectively) [Wagner 1989, 1992, 1994c].

In a BPH model involving implantation of mouse fetal urogenital sinus tissue into the ventral prostate glands of adult male mice, a 20% methanolic extract of nettle root administered orally for 28 days reduced experimentally-induced prostate growth by 51.3%, compared to 26.5% by an aqueous extract [Lichius 1997]. In the same model, a polysaccharide-rich fraction (POLY-M) from the 20% methanolic extract reduced prostate growth by 33.8% whereas *Urtica dioica* agglutinin increased prostate growth by 20.1% and secoisolariciresinol by 37.2% [Lichius 1999b].

An ethanolic and a petroleum ether extract were administered orally to rats with testosterone-induced increased prostatic weight. At 50 mg/kg b.w. the nettle extracts reduced this weight increase by 70% and 73% respectively. At 10 mg/kg b.w the respective reductions were close to 30%, compared to 50% for β-Sitosterol at this level. The positive control finasteride at 1 mg/kg b.w reduced the weight increase by 80%. In the negative control group, administered only subcutaneous testosterone, urine output was reduced by 77% after 4 weeks. Treatment with the extracts reduced the urinary obstruction to 2 and 5%, β-Sitosterol to 11% and finasteride to 1% (at the above mentioned concentrations) [Nahata 2012].

Pharmacological studies in humans

31 men aged between 58 and 82 years with BPH at stages I to II were treated daily for 20 weeks with 1200 mg of a dried nettle root extract preparation (3.5-7:1; 20% V/V methanol). From fine needle aspiration biopsies of the prostate at 4-weekly intervals, morphologically significant changes in prostatic adenoma cells were detected that may relate to competitive inhibition of SHBG binding capacity by the extract [Ziegler 1982].

Prostatic cells taken by needle biopsy from 33 BPH patients treated with nettle root extract for about 6 months were investigated by fluorescence microscopy. Compared with normal prostatic cells, a decrease in homogenous granules was detected in hyperplastic cells from the BPH patients, indicating that biological activity in these cells had decreased [Ziegler 1983]. The presence of nettle root constituents or their metabolites in prostate tissue obtained (through prostatectomy) from BPH patients treated with nettle root extract was demonstrated by fluorescence microscopy. The granular fluorescence was not observed in prostate tissue from patients not treated with nettle root extract, but could be simulated to some extent by *in vitro* incubation of this tissue with nettle root extract [Dunzendorfer 1984].

Morphological examination of prostate tissue obtained by needle biopsy from BPH patients before and 6 months after therapy with nettle root extract confirmed ultrastructural changes in the smooth muscle cells and epithelial cells of the prostate [Oberholzer 1987].

Clinical studies

Clinical studies performed with dried native extract of nettle root (7-14:1, 20% V/V methanol) in a preparation containing an equal amount of diluent. As in the published reference papers where mg amounts are stated, ***the following dosages relate to the extract preparation, of which only 50% was native extract (7-14:1).***

A review of 34 clinical trials including 40,000 patients concluded that there is evidence of efficacy for the methanolic extract in

the improvement of BPH related complaints [Chrubasik 2007].

In a randomized, double-blind, placebo-controlled study with 40 BPH II patients (1200 mg of extract preparation per day, n = 20; placebo, n = 20), statistically significant ($p<0.05$) decreases in micturition frequency and SHBG levels were observed in the verum group after 6 months [Fischer 1992].

Significant improvements of 14% in average urinary flow rate and 40-53% in residual urine volume were observed in BPH patients (n = 32) who received 600 mg of extract preparation daily for 4-6 weeks in a randomized, placebo-controlled (n = 35), double-blind study [Dathe 1987].

Fifty BPH stage I-II patients enrolled in a double-blind, controlled study were treated daily for 9 weeks with 600 mg of extract preparation (n = 25) or placebo (n = 25). A significant increase of 44% in micturition volume ($p<0.05$) and a highly significant decrease in serum levels of SHBG ($p = 0.0005$) were observed. The latter effect was probably due to the SHBG binding capacity of the extract [Vontobel 1985].

In an open, multicentre study with 5492 patients receiving 600-1200 mg of extract preparation per day for 3-4 months, significant improvements in nycturia and daytime micturition frequency were observed [Tosch 1983]. A 50% decrease in nycturia was reported in an open, multicentre study with 4051 BPH patients who received 1200 mg of extract preparation per day for 10 weeks [29]. In another open, multicentre study, with 4480 BPH patients receiving 600-1200 mg of extract preparation per day for 20 weeks, significant improvements ($p<0.01$) in urinary flow and residual urine volume were observed [Friesen 1988].

111 BPH patients with nycturia received 1200 mg of extract preparation per day for 10 weeks in an open study. Nocturnal micturition frequency decreased in 55% of cases [Vandierendounck 1986]. 37 out of 39 BPH I-III patients experienced improvements in urinary flow, residual urine, nycturia and pollakisuria after a 6-month treatment with 600-1200 mg of extract preparation per day [Maar 1987]. In another open study, residual urine decreased in 67 out of 89 BPH patients receiving 600 mg of extract preparation per day for 3-24 months [Djulepa 1982].

Significant decreases ($p<0.05$) in prostate volume and residual urine volume as well as in serum SHBG, oestradiol and oestrone levels were observed in an open study with 253 BPH patients who received 1200 mg of extract preparation per day for 12 weeks [Bauer 1988]. Decreases in prostate volume in 54% of cases and residual urine in 75% of cases were observed in an open study with 26 BPH patients who received 1200 mg of extract preparation per day for 4-24 weeks [Feiber 1988].

A one-year double-blind, randomized, placebo-controlled, multicentre study involving 124 patients (mean age 64 ±0.6) treated with 459 mg nettle root dry extract once a day demonstrated a significant ($p=0.0233$) difference in decrease of the International Prostate Symptom Score (IPSS) in favour of the verum group, by 5.7 points (from 18.7 ±0.3 to 13.0 ±0.5) compared to 4.7 points (from 18.5 ±0.3 to 13.8 ±0.5) in the placebo group (122 patients; mean age 63). The secondary objective variables showed positive but non-significant results in favour of the verum group: maximal urinary flow was increased by 3mL/s while residual urinary volume was lowered by 5 mL, compared to 2.9 mL/s and 4 mL respectively for placebo. Analysis showed better results for quality of life improvement in the verum group (65%) than in the placebo group (62%) ($p=0.69$) [Schneider 2004].

Clinical studies performed with other nettle root preparations
In a double-blind, multicentre study, 41 BPH patients were treated daily for 3 months with either 2 x 3 mL of an aqueous extract preparation equivalent to 4.68 g of a fluid extract (1:1, 16% ethanol) (n = 20) or placebo (n = 21). A decrease in residual urinary volume of 19.2 mL in the verum group compared to 10.7 mL in the placebo group, and an increase in maximal urinary flow of 7.1 mL/s in the verum group compared to 4.4 mL/s in the placebo group, were observed. A significantly greater improvement ($p = 0.002$) in the IPSS was also reported in the verum group [Engelmann 1996].

Daily treatment for 60 days with 90-150 drops of a fluid extract (1:1, 45% ethanol; Ph. Fr.) led to a 66% decrease in residual urine in an open study with 10 BPH patients [Goetz 1989].

In an open study with 67 BPH patients, a reduction in nocturnal micturition frequency was observed after 6 months of daily treatment with 3 x 5 mL of a fluid extract (1:5, 40% ethanol) [Belaiche 1991].

In an open multicentre study involving 1319 patients with BPH and/or prostatitis, daily treatment for 6 months with 378-756 mg of a native extract of nettle root (12-16:1, 70% V/V ethanol) led to substantial improvements in dysuria, nycturia, pollakisuria, urinary flow and residual urine volume. 79.9% of the patients reported an improvement in their quality of life [Kaldewey 1995].

In a double blind, randomized, placebo-controlled, partial crossover study, 620 patients with lower urinary tract symptoms (LUTS) secondary to BPH, aged between 55 and 72 (mean age 63), were treated for 6 months with a preparation of *Urtica dioica* fluid extract (120 mg per dose; not further specified) (n = 305) or placebo (n = 315) three times daily with meals. Unblinding was carried out after completion of the six month trial, and for an 18 month follow-up period patients were allowed to continue with *Urtica dioica* treatment or to crossover if they had previously been on placebo. Patients with a follow up >16 months after participation in the trial were included in the analysis. After the initial 6 months, verum treatment demonstrated a significantly greater ($p<0.001$) reduction in residual urinary volume than placebo, from 73 mL ± 32.6 to 36 mL ± 25.5 in the verum group compared with 74 mL ± 29.6 to 71 mL ± 24.4 in the placebo group, and a significantly greater increase in maximal urinary flow ($p<0.05$) for verum (8.2 mL/s) compared to placebo (3.4 mL/s). Crossing over to *Urtica dioica* resulted in a reduction in urinary retention to a volume of 38 mL ± 25.5 and an increase of the maximal urinary flow by 7.4 mL/s. The size of the prostate was measured by transrectal ultrasonography (TRUS) and showed a significant reduction ($p<0.001$) of the prostate volume in the verum group from 40.1 cc ± 6.8 to 36.3cc ± 4.2, this increased again to 39.5 cc ± 6 in those patients who discontinued the medication during the follow up, while the volume stayed decreased in those patients who continued the medication during follow-up. There was also a significantly greater improvement to the IPSS in the verum group compared to placebo ($p<0.002$) [Safarinejad 2005].

In an open, prospective phase IV multicentre trial, 100 patients with LUTS received four capsules each containing 240 mg of a dry extract (5.4-6.6:1; ethanol 20% V/V) daily for 24 weeks. Treatment with the preparation resulted in a significant ($p<0.0001$) reduction in the IPSS from 19.5 ± 4.8 to 13 ± 5, a significant ($p<0.0001$) increase in the maximal urinary flow rate from 9.7 mL/s ± 2.2 to 13.2 mL/s ± 3.8 and a non-significant reduction of the residual urinary volume by 24.8 mL (±150). Digital measurement (n=48) showed no reduction in prostate volume after 24 weeks [Klein-Bischoff 2007].

Pharmacokinetic properties
After oral administration of 20 mg of purified *Urtica dioica* agglutinin (UDA) to patients and healthy volunteers, 30-50% was excreted unchanged in the faeces. The concentration in urine was in the 1-10 ng/mL range and the total amount of UDA in urine was less than 1% of the administered dose. These data confirmed the extreme stability of UDA in the digestive tract and its partial uptake and renal clearance [Samtleben 1996].

Preclinical safety data
No data available.

Clinical safety data
Over 16,000 patients were treated with nettle root extracts in clinical studies [Djulepa 1982; Tosch 1983; Stahl 1984; Vontobel 1985; Vandierendounck 1986; Dathe 1987; Maar 1987; Bauer 1988; Feiber 1988; Friesen 1988; Goetz 1989; Belaiche 1991; Fischer 1992; Kaldewey 1995; Engelmann 1996] and have taken daily doses of up to 756 mg of hydroalcoholic dry native extract for periods of up to 6 months or, in a few cases, 300 mg of dry native extract for 24 months. The incidence of adverse events was generally under 5%. No serious adverse effects have been reported, the majority of complaints being mild gastrointestinal upsets. In a large open study involving 1319 patients, the incidence of adverse events probably or possibly related to treatment with nettle root extract was 1.0% [Kaldewey 1995].

According to a review, the majority of 40,000 patients participating in clinical trials were treated with an extract prepared with 20% methanol. As adverse effects were only reported as occurring in 2% of all cases, and these were of minor concern, treatment with this particular extract was suggested to be safe [Chrubasik 2007].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
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AGRIMONIAE HERBA	Agrimony	Supplement 2009
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ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
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Although publication in the form of books was convenient in the past, ESCOP recognizes that online publication now offers a number of advantages, not least in facilitating rapid publication of individual monographs as soon as all stages of preparation have been completed. Commencing from 2011, therefore, new and revised monographs will be published online only.

The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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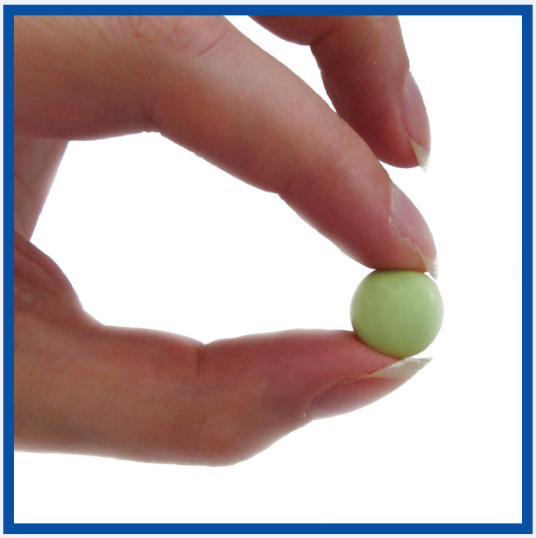
Bearberry Leaf

2012



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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

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Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

UVAE URSI FOLIUM

2012

Bearberry Leaf

DEFINITION

Bearberry leaf consists of the whole or cut dried leaf of *Arctostaphylos uva-ursi* (L.) Spreng. It contains not less than 7.0% of anhydrous arbutin ($C_{12}H_{16}O_7$; M_r 272.3), calculated with reference to the dried drug.

The material complies with the monograph of the European Pharmacopoeia [Bearberry Leaf].

CONSTITUENTS

The main characteristic constituent of bearberry leaf is arbutin (5 to 15%) accompanied by variable amounts of methylarbutin (up to 4%) and by small amounts of the free aglycones hydroquinone (less than 0.3%) and methylhydroquinone [Sticher 1979; Linnenbrink 1984;Veit 1992; Blaschek 2007]. Other constituents include gallic acid, galloylarbutin and up to 20% of gallotannins [Hegnauer 1989]; flavonoids, especially glycosides of quercetin, kaempferol and myricetin [Hegnauer 1989; Blaschek 2007]; and 0.4 to 0.75% triterpenes, mainly ursolic acid and uvaol [Hegnauer 1989; Blaschek 2007].

CLINICAL PARTICULARS

Therapeutic indications

Uncomplicated infections of the lower urinary tract [Frohne 1970; Kedzia 1975; Bradley 1992; Wichtl 1999; Fintelmann 2009; Blaschek 2009; Schilcher 2010] such as cystitis, when antibiotic treatment is not considered essential [Coe 1989; Furrer 1994; Weber 1995].

Posology and method of administration

Dosage

Adults: Cold water macerates of 9 to 12 g dried leaf corresponding to 400-800 mg of arbutin per day, divided into 2-4 doses; equivalent preparations [Blaschek 2009; Schilcher 2010].

Not recommended for children below 12 years [Blaschek 2007; Schilcher 2010].

Method of administration
For oral administration. Patients should be advised to consume plenty of liquid during the treatment [Fintelmann 2009].

Duration of administration
Treatment should be continued until complete disappearance of symptoms (up to a maximum of 1 week) [Schilcher 2010].

Contraindications
Pregnancy, lactation, children below 12 years of age [Schilcher 2010].

Special warnings and special precautions for use
If symptoms worsen after 48 hours medical advice should be sought.

Interaction with other medicaments and other forms of interaction
None reported in clinical studies.

Pregnancy and lactation
Bearberry leaf should not be used during pregnancy or lactation [Mills 2005].

Effects on ability to drive and use machines
None known.

Undesirable effects
Nausea and vomiting may occur due to stomach irritation from the high tannin

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content of bearberry leaf [Blaschek 2009].

Overdose
No reports are available on overdose of bearberry leaf.

PHARMACOLOGICAL PROPERTIES

The ability of bearberry leaf to resolve uncomplicated infections of the lower urinary tract is based on its antibacterial and anti-inflammatory properties. Although the exact mechanisms are not fully elucidated the pharmacodynamic and pharmacokinetic data suggest that most of the effect is mediated by arbutin [Jahodar 1985a; Moskalenko 1986; Holopainen 1988; Rios 1987; Paper 1993].

Pharmacodynamic properties

In vitro experiments

Antimicrobial activity
The antimicrobial activity of a 70% ethanolic bearberry leaf extract (1:5) was tested using the disc assay. The extract demonstrated maximal antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Mycobacterium smegmatis* [Moskalenko 1986].

The antimicrobial activity of an 80% ethanolic whole plant extract was tested against *Escherichia coli*, *Proteus vulgaris*, *Streptococcus faecalis*, *Enterobacter aerogenes* and compared to streptomycin. The greatest activities were found against *E. coli* (approx. 1/100 of the activity of streptomycin) and *P. vulgaris* (approx. 1/300) [Holopainen 1988].

A 95% ethanolic bearberry leaf extract showed antibacterial activity against *Staphylococcus aureus* (MIC 2 g/L) [Rios 1987].

The effect of arbutin on different bacteria was related to their extracellular ß-glucosidase activity. The highest ß-glucosidase activity was found in *Streptococcus faecalis* (100%), *Proteus vulgaris* (100%), *Klebsiellae* (95%) and *Enterobacteriae* (72%), and the lowest in *E. coli* (11.6%). A 90 to 100% bactericidal effect was achieved for the species *Klebsiella pneumoniae* and *S. faecalis* at 4 µg/ml and for *Enterobacter cloacae* and *P. vulgaris* at 5.6µg/ml [Jahodář 1985a].

Older experiments revealed strong antibacterial properties of urine samples obtained from healthy volunteers after consumption of arbutin or bearberry tea. This effect was only observed with urine adjusted to pH 8, whilst urine of pH 6 was ineffective [Frohne 1970; Kedzia 1975].

Urine samples of volunteers, after ingestion of a 60% ethanolic extract (corresponding to 420 mg arbutin), were incubated with *E. coli* which was able to deconjugate the hydroquinone (HQ) glucuronide and sulphate to free HQ [Siegers 1997]. Further investigations demonstrated that the majority of the free HQ was found inside the bacteria, this way exerting the antibacterial effect [Siegers 2003]. Accordingly, the pH of urine neither modifies HQ formation nor the antibacterial effect of HQ derivatives [Garcia 2010].

Anti-inflammatory activity
Preincubation of rat peritoneal mast cells with a methanolic bearberry leaf extract at 20, 100 and 500 µg/ml reduced histamine release to 88%, 65.1% and 23.6%, respectively [Kubo 1990].

The ability of a bearberry leaf extract to prevent LPS-induced up-regulation of NO production was examined on N-11 murine microglia and in RAW 264.7 murine macrophages. The extract at concentrations between 5 and 50 µg/ml, corresponding to 3 to 30 nM arbutin, significantly reduced LPS-induced NO production with an IC_{50} value of around 20 µg/mL [Shanmugam 2008].

Arbutin was shown to decrease the neutrophil oxidative burst dose-dependently, starting at concentrations of 1µM. A more detailed study on isolated human neutrophils showed that arbutin potently decreased the external oxidant concentration without reduction of the oxidative burst arising inside neutrophils [Jančinová 2007].

Anti-adherence activity
An aqueous extract of bearberry leaf enhanced the surface hydrophobicity of 40 *E. coli* and 20 *Acinetobacter baumannii* strains and increased the cell aggregation capacity [Türi 1997].

An aqueous extract decreased the surface hydrophobicity of *Helicobacter pylori* and enhanced the cell aggregation capacity in the salt aggregation test [Annuk 1999].

Other activities
A bearberry leaf extract (not further specified; 10 µg/ml) showed antioxidant activity in U937 cells and strongly protected cells against DNA damage induced by hydrogen peroxide and *t*-butanol [Carpenter 2006].

Aqueous and methanolic extracts of five commercial bearberry leaf preparations demonstrated *in vitro* inhibition of cytochrome P450 isoenzymes [Chauhan 2007].

In vivo experiments

Anti-inflammatory activity
A 50% methanolic bearberry leaf extract was administered *p.o.* to mice at doses of 20, 100 and 500 mg/kg b.w. immediately before and 16 hours after picryl chloride application. The extract did not show a relevant inhibitory effect on ear swelling when compared to prednisolone. However, the effect of prednisolone was enhanced by combination with bearberry leaf. In the same experiment, arbutin had a significant effect only at an oral dose of 50 mg/kg in combination with prednisolone ($p<0.01$) [Kubo 1990]. Oral administration of 20, 100 or 500 mg/kg of the bearberry leaf extract reduced acetic acid-induced capillary vascular permeability in mice by 10.4, 21.7 and 27.9% respectively. A significant effect ($p<0.05$) was observed only for the highest dose [Kubo 1990]. The extract significantly reduced carrageenin-induced paw oedema in Wistar rats at an oral dose of 100 and 500 mg/kg, the effect being significant for the highest dose ($p<0.05$) [Kubo 1990)].

Oral administration of arbutin at 50 mg/kg/day for 28 days was shown to decrease plasma levels of the pro-inflammatory cytokine IL-6 in a rat model of adjuvant arthritis. The control plasma level of interleukin-6 was 29.4±2.6 pg/ml and was increased on days 14 and 21 of arthritis to 119.9±14.3 and 101.3±11.3 pg/ml, respectively. In the group treated with arbutin, the respective IL-6 concentrations were 72.8±12.0 and 35.5±8.8 pg/ml. In addition, arbutin was found to inhibit neutrophil activity in the experimental arthritis model [Jančinová 2007].

Diuretic activity
An aqueous extract of bearberry leaf was administered *i.p.* to 10 male rats as a single dose of 50 mg/kg b.w.; a control group of 10 rats received hypotonic saline solution and another group of 10 rats received hydrochlorothiazide at 10 mg/kg. The urine volume from rats treated with the extract was higher than from the controls and was comparable to the volume after

hydrochlorothiazide [Beaux 1999].

Female Wistar rats received a bearberry leaf extract (prepared from 3 g of the drug/L) ad libitum instead of tap water, with a diet fortified with calcium (8 g/kg b.w.) for 12 days. After that period, two 24-hour urine samples were collected. Excretion of calcium, citrate, phosphate, creatinine, pH and diuresis were evaluated in control and treated rats. No relevant differences between control and treated rats were observed in any of the parameters [Grases 1994].

Pharmacokinetic properties

Arbutin, the main active constituent of bearberry leaf, is rapidly absorbed after oral administration of the pure substance or bearberry leaf tea or bearberry leaf extract preparations via a Na^+/glucose carrier [Paper 1993; Schindler 2002; Quintus 2005; Blaut 2006].

In vitro and human studies have demonstrated that arbutin does not undergo hydrolysis in the stomach and reaches the small intestine intact. The contribution of bacteria to arbutin hydrolysis in the small intestine is very small [Paper 1993; Blaut 2006].

In rats, arbutin is eliminated unchanged, while in humans arbutin is eliminated in the form of conjugated metabolites. Urinary excretion of free HQ (less than 0.6% of the administered dose) and arbutin metabolites (HQ-glucuronide and HQ-sulphate) occurs within a few hours, with maximum excretion during the first 6 hours; within 24 hours approximately 70-100% of the administered dose is excreted [Frohne 1970; Kedzia 1975; Paper 1993; Siegers 1997; Schindler 2002; Quintus 2005; Blaut 2006].

Older *in vitro* experiments suggest the uptake of arbutin and a lack of free HQ in biopsy homogenates from the small intestine of rat, hamster and chicken as well as in human small intestine of healthy volunteers [Garcia et al. 2010].

An *in vivo* study with Fischer 344 rats investigated the tissue distribution of ^{14}C-hydroquinone (^{14}C-HQ; 25 or 50 mg/kg b.w.) following single or repeated oral administration. After a single dose, HQ was rapidly absorbed, metabolized and eliminated as conjugated HQ. Free HQ eliminated in the urine represented less than 1% of the total administered dose. The mean radioactivity recovered in the combined tissues and carcasses ranged from 0.51% to 0.96% of the dose. Livers and kidneys contained the highest concentrations of ^{14}C-HQ (0.24% and 0.04%, respectively). In a repeated dose study, 8 rats were treated daily for 14 days with unlabeled HQ (25 mg/kg) and with ^{14}C-HQ on day 15. Blood concentration increased to a plateau in the first 30 min and declined to the limits of quantification by 24 h after dose administration. A mean of 87-94% of the dose was recovered in the urine and an additional 1-3% of the dose was recovered in the faeces [English 2005].

The elimination of arbutin (20, 100 and 400 mg/kg) was investigated in female Wistar rats. Up to 16 hours after oral administration nearly 82% of the arbutin was eliminated unchanged, even after changing urine pH; and excretion was complete after 30 hours. Free HQ as a possible degradation product was not detected [Jahodář 1985b].

In a study on the metabolism of arbutin following oral intake of bearberry leaf, twelve healthy volunteers received 3 x 2 coated tablets containing a 60% ethanolic extract of bearberry leaf (corresponding to 420 mg arbutin). Only 0.6% of the arbutin was excreted as free HQ, whereas 70% (294 mg) was detected as HQ-conjugates to glucuronic and sulphuric acid in the urine. In 6 volunteers no free HQ was detected [Siegers 1997].

In a preliminary pharmacokinetic study in 3 volunteers, the time-dependent renal excretion of arbutin metabolites HQ-glucuronide, free HQ and HQ-sulphate was investigated after ingestion of an aqueous bearberry leaf extract containing 150 mg of arbutin. More than 50% of the arbutin was excreted within 4 hours, mainly in the form of the metabolites HQ-glucuronide and HQ-sulphate; and more than 75% of the total administered arbutin was excreted within 24 hours. The elimination of free HQ was negligible in 2 out of 3 volunteers, and only in the third reached 5.6% of the total administered arbutin dose [Quintus 2005].

A cross-over study in 6 healthy volunteers investigated the bioavailability of an aqueous preparation of bearberry leaf compared to that of enteric-coated tablets containing the same herbal substance. The mean arbutin content in one enteric-coated tablet corresponding to 250 mg bearberry leaf was 50 mg. The aqueous extract was prepared to contain the same amount of arbutin. The participants received 2 tablets or 500 mg of the aqueous preparation. Five urine samples were collected within 24 hours. After a washout phase of 24 hours the volunteers received a single dose of the other preparation. The mean total amount of arbutin detected after 24 hours in urine of subjects treated with the aqueous preparation was 86.6 ± 16.3 mg. In urine from subjects treated with coated tablets, the total amount of arbutin was 82.8 ± 16.1 mg. Therefore, a very high percentage of arbutin was recovered in the urine for both herbal preparations after 24 hours. No free HQ was detected and differences in the pH of the urines (6.5 to 8) had no influence on the amount of free HQ. After administration of the aqueous extract, most of the arbutin was eliminated within 3 hours (53% of the total arbutin), whereas with the coated tablets maximum arbutin elimination was found after 3 to 6 hours (31% of the total arbutin) [Paper 1993].

An open, randomized, crossover study with 16 healthy volunteers compared the bioavailability of a single oral dose of bearberry leaf dry extract as film-coated tablets or as a herbal tea. Participants received either 2 tablets containing 472.5 mg of bearberry leaf dry extract (corresponding to 105 mg arbutin each) or 100 ml of an aqueous herbal preparation containing 945.0 mg of bearberry leaf dry extract (corresponding to 210 mg arbutin). Urine was collected 90 min prior to the experiment and during the periods 0 to 4, 4 to 8, 8 to 12, 12 to 24 and 24 to 36 hours after drug administration. The three major metabolites identified in urine were free HQ, HQ-glucuronide and HQ-sulphate. After administration of the film-coated tablets 66.7 % of the arbutin was excreted in the urine and after the aqueous herbal preparation 64.8%. The major metabolite HQ-glucuronide accounted for 67.3% and 70.3% after coated tablet or herbal tea administration, respectively. Both metabolites were rapidly excreted mainly during the first 4 hours for both preparations. No further excretion of arbutin metabolites was detected in urine more than 24 hours after extract administration. The mean amounts of free HQ and each HQ metabolite in urine did not differ significantly between the two preparations. The relative availability of arbutin from the tablets, in relation to the aqueous herbal preparation, was 103.3% based on the relative urinary excretion of the arbutin metabolites (total HQ, free HQ, HQ-glucuronide and HQ-sulphate) [Schindler 2002].

Preclinical safety data

Single dose toxicity
No data have been reported for bearberry leaf. No toxic effects were observed in mice treated with arbutin at 50-200 mg/kg b.w., *p.o.* or *i.p.* [NTP 2006].

The oral LD_{50} value of hydroquinone was greater than 375

mg/kg in male and female Sprague-Dawley rats [Topping 2007], 400 mg/kg in mice, 550 mg/kg in guinea pigs, 300 mg/kg in pigeons, 70 mg/kg in cats and 200 mg/kg in dogs [Woodard 1949]. The high sensitivity of cats is related to lower glucuronide conjugation activity in the liver and intestinal tract. Administration in the food decreased the rate and extent of HQ absorption, thus increasing oral LD_{50} values up to 1050 mg/kg in rats [DeCaprio 1999].

LD_{50} values for HQ by parenteral administration have been reported as 115-160 mg/kg in the rat and 190 mg/kg in the mouse [DeCaprio 1999].

Dermal application of 200 mg /kg of HQ to rabbits under occlusive wrap for 24 hours did not result in neurobehavioural effects or mortality [Topping 2007].

Repeated dose toxicity
No data have been reported for bearberry leaf.

Arbutin administered intraperitoneally to mice, at a dose of 8 g/kg b.w. for a period of 2 weeks, did not induce toxic effects [NTP 2006].

Subchronic exposure of male and female Sprague-Dawley rats to free HQ (200, 64, 20 mg/kg b.w./day) revealed that doses up to 64 mg/kg HQ resulted in acute behavioural effects including tremors and reduced activity. Tremors occurred within 1 hour of dosing and resolved at hour 6. Brain weights were not altered by HQ administration, but mean terminal body weight was reduced by approximately 7% after doses of 200 mg/kg in males. No treatment-related morphological or histological changes of organs were observed. Neuropathologic examination of the CNS and PNS did not reveal any morphologic lesions associated with HQ administration or due to repetitive CNS stimulation by HQ. No nephrotoxic effects were observed in rats given 200 mg/kg [Topping 2007].

Two toxicity studies with free HQ were conducted in F344 rats and B6C3F mice for 2 and 13 weeks and the animals were treated 5 days/week. Mice received 0, 31, 63, 125, 250 or 500 mg HQ/kg b.w. in corn oil by gavage for 14 days. Rats received 0, 63, 125, 250, 500 or 1000 mg HQ/kg b.w. on the same schedule. No chronic toxicity was observed at doses below LD_{50} for rats and mice, which range from 200 to 500 mg/kg (NTP 1989). In mice, 4/5 males and 5/5 females at the dose of 500 mg/kg and 3/5 males at 250 mg/kg died before the end of the study. Tremors followed by convulsions were seen at doses of 250 and 500 mg/kg. All rats receiving a dose of 1000 mg/kg as well as 1/5 male rats and 4/5 female rats at the 500 mg/kg dose died before the end of the study. Clinical signs included tremors lasting up to 30 min after each dosing of 500 and 1000 mg/kg [NTP 1989; Kari 1992].

In a 13-week study, mice and rats received 0, 25, 50, 100, 200 or 400 mg free HQ/kg b.w. administered in corn oil by gavage. Doses below LD_{50} did not show any signs of toxicity. Free HQ at 400 mg/kg caused death in all rats and 3/10 female rats had already died before the end of the study at 200 mg/kg. Tremors and convulsions were observed in most rats receiving 400 mg/kg and in several female rats receiving 200 mg/kg. Inflammation and/or epithelial hyperplasia of the fore-stomach were seen in 4/10 male rats and 1/10 female rats receiving 200 mg/kg. Toxic nephropathy, characterized by tubular cell degeneration in the renal cortex, was seen in 7/10 male and 6/10 female rats receiving 200 mg/kg and in 1/10 females receiving 100 mg/kg. [NTP 1989; Kari 1992].

In a long-term repeated-dose toxicity study, mice were given 0, 50 or 100 mg HQ/kg b.w. for up to 2 years. Rats received 0, 25 or 50 mg HQ/kg b.w. on the same schedule. At the highest dose, the mean body weights in mice and rats were approximately 5 to 14% lower than those of controls. No significant differences in survival were observed between treated and control animals. In male rats receiving 50 mg/kg HQ a significantly higher severity of nephropathy ($p<0.01$) and compound-related lesions in the liver including anisokaryosis, syncytial alteration and basophilic foci were observed [Kari 1992]. Mononuclear cell leukaemia in female rats occurred with an increased incidence in the dosed groups (vehicle control, 9/55; low dose, 15/55; high dose, 22/55) [NTP 1989].

Earlier studies showed that in the rat kidney the outer stripe of the outer medulla, particularly in renal proximal tubules, were mainly affected by the toxicity of HQ. In re-evaluations [Hard 1997; Whysner 1995] these results were interpreted as an exacerbation of a spontaneous, age-related disease occurring primarily in male rats (chronic progressive nephropathy), with no known counterpart in humans [IARC 1999; McGregor 2007; NTP, 2009].

Reproductive toxicity
No data have been reported for bearberry leaf.

A study in 240 Sprague-Dawley rats evaluated the effects of arbutin on the reproductive performance of the parent rats as well as on the development and reproductive performance of the offspring. Arbutin was administered s.c. at doses of 25, 100 or 400 mg/kg b.w. daily to male rats before mating and to female rats during pregnancy and lactation. All pregnant parent rats delivered with normality. No effect on reproduction of male and female rats or the development of the offspring was observed at doses up to 100 mg/kg. No treatment-related change in the reproductive performance of the F1 rats was observed. Foetal toxicity occurred at doses of 400 mg/kg[Itabashi 1988].

Genotoxicity and Mutagenicity
Bearberry leaf has been shown to be non-mutagenic in the Ames test using *Salmonella typhimurium* strains TA98 and TA100, and in the *Bacillus subtilis* rec-assay [Morimoto 1982]. In the cytochalasin block micronucleus test a 70% ethanolic bearberry leaf extract (0.025 - 0.2 mg/ml) did not show clastogenic activity as it did not affect the yield of micronuclei either in irradiated or unirradiated human blood lymphocytes [Jokic 2003].

An *in vitro* study investigated the mutagenic capacity of arbutin (0.1-10 µM) in mammalian cells after deglycosylation to free HQ by human intestinal bacteria. Mutagenicity was detected by the determination of mutant frequency in Chinese hamster V79 cells.

Four of nine representative human intestinal species investigated, namely *Eubacterium ramulus*, *Enterococcus casseliflavus*, *Bacteroides distasonis* and *Bifidobacterium adolescentis*, deglycosylated arbutin at rates of 21.08, 16.62, 8.43 and 3.59 nmol x min^{-1}/mg protein, respectively. In contrast, homogenates from small intestinal mucosa and cytosolic fractions from colon mucosa deglycosylated arbutin at substantially lower rates: 0.50 and 0.09 nmol x min^{-1}/mg protein, respectively. Arbutin, unlike HQ, did not induce gene mutations in Chinese hamster V79 cells in the absence of an activating system. However, in the presence of cytosolic fractions from *E. ramulus* or *B. distasonis*, arbutin (1mM) was strongly mutagenic. Cytosolic fraction from *Escherichia coli*, showing no arbutin glycosidase activity, was not able to activate arbutin in this model system [Blaut 2006]. Hydroquinone was found to be mildly myeloclastogenic in the micronucleus test in SPF mice after oral administration of

a toxic dose (200 mg/kg b.w.) [Gad-el-Karim 1985].

Urine samples from 12 volunteers after oral intake of a preparation corresponding to 420 mg arbutin/day did not show mutagenic potential *in vitro* in the Ames test with typical bacterial strains. Neither was any mutagenic potential found in vivo in the micronucleus assay in mice [Siegers 1997].

Carcinogenicity
No data have been reported for bearberry leaf.

Clinical safety data

An observational case report assumed that ingestion of an unspecified bearberry leaf preparation for three years at an unspecified dose caused bilateral bull's-eye maculopathy in a 56-year-old woman [Wang 2004].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Supplement 2009
ALOE CAPENSIS	Cape Aloes	Second Edition, 2003
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Second Edition, 2003
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
BALLOTAE NIGRAE HERBA	Black Horehound	Supplement 2009
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BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Second Edition, 2003
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Supplement 2009
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Second Edition, 2003
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Second Edition, 2003
FUMARIAE HERBA	Fumitory	Supplement 2009
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Supplement 2009
GRINDELIAE HERBA	Grindelia	Supplement 2009
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013
HYPERICI HERBA	St. John's Wort	Second Edition, 2003
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
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MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
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MILLEFOLII HERBA	Yarrow	Supplement 2009
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PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
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PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Second Edition, 2003
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Second Edition, 2003
RATANHIAE RADIX	Rhatany Root	Supplement 2009
RHAMNI PURSHIANI CORTEX	Cascara	Second Edition, 2003
RHEI RADIX	Rhubarb	Second Edition, 2003
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Second Edition, 2003
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Second Edition, 2003
SALICIS CORTEX	Willow Bark	Second Edition, 2003
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Second Edition, 2003
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VIOLAE HERBA CUM FLORE	Wild Pansy	Supplement 2009
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Supplement 2009
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

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The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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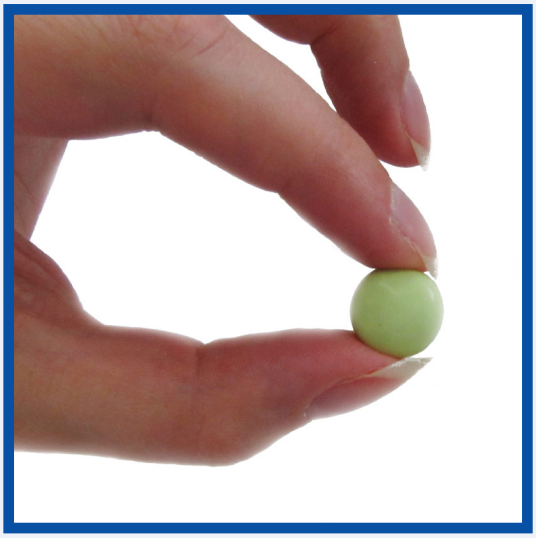
Vaccinii macrocarpi fructus

Cranberry

2020



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2020

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Plant illustrated on the cover: *Vaccinium macrocarpon*

FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

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Information on the member organizations and people involved in the production of ESCOP monographs and other activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
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ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

VACCINII MACROCARPI FRUCTUS 2020

Cranberry

DEFINITIONS

Cranberry
Cranberry consists of the ripe, fresh or dried fruits of *Vaccinium macrocarpon* Aiton.

Cranberry Liquid Preparation
Cranberry liquid preparation is a bright red juice derived from the fruits of *Vaccinium macrocarpon* Aiton or *Vaccinium oxycoccus* L. It contains no added substances.

The material complies with the monograph of the United States Pharmacopeia [Cranberry liquid preparation].

CONSTITUENTS

Cranberry
The main characteristic constituents of cranberry are A-type proanthocyanidins [Foo 2000a,b] and lower amounts of B-type proanthocyanidins [Feliciano 2013]; quinic, malic and citric acids (2.6-3.6%) [Jensen 2002, Coppola 1986]; benzoic acid (up to 0.5%), hydroxybenzoic acids and hydroxycinnamic acids, mainly in bound forms [Marwan 1982, Chen 2001, Zuo 2002, Murphy 2003, He 2006]; anthocyanins, especially peonidin 3-arabinoside and 3-galactoside [Hong 1986, Ohnishi 2006, Neto 2007, Kylli 2011]; flavonol glycosides, mainly of quercetin and myricetin [Yan 2002, Vvedenskaya 2004, Neto 2007]; iridoids (monotropein and 6,7-dihydromonotropein) [Jensen 2002]; ursolic acid and derivatives [Schmandke 2004, He 2006]; fructose, xyloglucans and other sugars [Hong 1986, Hotchkiss 2015] and volatile compounds [Zhu 2001].

Cranberry Liquid Preparation
The content requirements of the United States Pharmacopeia include: not less than 2.4% of dextrose, 0.7% of fructose, 0.9% of quinic acid, 0.9% of citric acid and 0.7% of malic acid; a quinic acid to malic acid ratio of not less than 1.0; and not more than 0.05% each of sorbitol and sucrose [Cranberry liquid preparation].

CLINICAL PARTICULARS

Therapeutic indications
Prevention of urinary tract infections (UTIs) [Ferrara 2009, Takahashi 2013, Foxman 2015, Maki 2016].

Posology and method of administration

Dosage

Adults: 240-750 mL per day of a cranberry liquid preparation containing 25-100% of cranberry juice, divided into 2 or 3 portions; 200-500 mg of cranberry dry extract or juice concentrate twice daily [Avorn 1994, Walker 1997, Stothers 2002, Hess 2008, McMurdo 2009, Maki 2016, Vostolova 2015]; other equivalent preparations [Foxman 2015].

Children 2-18 years of age: 2-15 mL of cranberry juice per kg body weight [Foda 1995, Afshar 2012, Salo 2012]; other equivalent preparations [Fernández-Puentes 2015]

Note: The liquid and solid cranberry preparations that have been used in clinical studies are mainly commercial products, variously and often inadequately described as cranberry juice, cranberry juice cocktail, cranberry concentrated juice, cranberry concentrate, cranberry extract, cranberry concentrated extract etc. The heterogeneity of products and dosages used make it difficult to define a minimum or optimal dosage and composition for effective cranberry preparations [Jepson 2008, Nowack 2008, Guay 2009].

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Method of administration
For oral administration.

Duration of administration
No restriction. If symptoms persist or worsen, medical advice should be sought.

Contra-indications
None known.

Special warnings and special precautions for use
Increased medical supervision and international normalized ratio (INR) monitoring should be considered for any patient on warfarin and taking high amounts of cranberry juice regularly [UK Medicines and Healthcare Products Regulatory Agency 2004, Ansell 2009].
Patients with a history of calcium oxalate nephrolithiasis should seek medical advice before intake of cranberry.

Interaction with other medicaments and other forms of interaction
A pharmacokinetic interaction between cranberry juice and warfarin is unlikely, especially with moderate cranberry consumption [Greenblatt 2006a,b, Li 2006, Lilja 2007, Ansell 2008, Zikria 2010].

Pregnancy and lactation
Except under medical supervision, cranberry should not be taken during pregnancy or lactation in amounts greatly exceeding those found in foods [Barnes 2007].

Effects on ability to drive and use machines
None known.

Undesirable effects
None of clinical relevance reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Anti-adherence activity
Cranberry juice inhibited by more than 75% the adherence to uroepithelial cells of more than 60% of isolates of *Escherichia coli* from clinical samples (out of 77 isolates tested) [Sobota 1984].

Inhibitory effects of cranberry juice on the adherence to eukaryotic cells of various *E. coli* strains were investigated in several models. The inhibitory activity against type 1 fimbriated *E. coli* was ascribed to the fructose content of cranberry juice. Inhibitory activity against P-fimbriated bacteria was non-dialyzable, suggesting that high molecular weight constituents are involved [Zafriri 1989].

Electron micrographic evidence from P-fimbriae-producing strains of *E. coli* grown in media with a cranberry juice content of 25% suggested that the juice acts on the cell wall, preventing the proper attachment of fimbrial subunits, or as a genetic control preventing the expression of normal fimbrial subunits, or both [Ahuja 1998].

Atomic force microscopy was used to investigate bacterial surface characteristics and adhesion forces between a silicon nitride probe surface and two strains of *E. coli*, one P-fimbriated and the other non-fimbriate, after exposure to dilutions of a cranberry juice neutralized to pH 7. The results demonstrated molecular-level changes on the surfaces of P-fimbriated *E. coli* upon exposure to neutralized cranberry juice [Liu 2006].

Presence of 10% juice or the equivalent amount of a proanthocyanidin (PAC)-enriched fraction during cultivation of *E. coli* resulted in the downregulation of flagellar basal body rod and motor proteins as shown in gene expression analysis. In SEM images a decrease in the visible P-fimbriae was observed [Johnson 2008].

P-fimbriated *E. coli* can form strong bonds with the Gal-Gal disaccharide receptor on uroepithelial cells, while non-fimbriated *E. coli* interactions are only non-specific. In the P-fimbriated *E. coli*-uroepithelial cell system, Gibbs free energy of adhesion values increased as a function of increasing cranberry juice concentration up to the point where adhesion became unfavourable. The results suggested that cranberry juice may disrupt bacterial ligand-uroepithelial cell receptor binding, thus inhibiting bacterial attachment to the host tissue [Liu 2008].

Atomic force microscopy resulted in a significant decrease in adhesion forces to a model surface when *E. coli* (non-fimbriated HB101 or P-fimbriated HB101pDC1) was cultivated under addition of either light cranberry juice cocktail (L-CJC) or PACs ($p<0.001$). Exposure of *E. coli* HB101pDC1 to increasing concentrations of L-CJC or PACs decreased bacterial attachment to human ureteral epithelial cells (CRL 9520 VA) ($p<0.001$) [Pinzon-Arango 2009].

P-fimbriated *E. coli* HB101pDC1 or non-fimbriated *E. coli* HB101 were attached to atomic force microscopy tips, and the adhesion forces between *E. coli* and uroepithelial cells in solutions containing 0, 2.5, 5, 10 and 27% cranberry juice were measured. The adhesion forces between *E. coli* HB101pDC1 and uroepithelial cells decreased dose-dependently [Liu 2010].

Adherence of *P. aeruginosa* PAO1 to uroepithelial cells decreased significantly ($p<0.001$) in the presence of cranberry juice as compared to control. Biofilm formation on sterile catheters was significantly ($p<0.001$) reduced with substantially less exopolysaccharides in the biofilms. With the juice, lower levels of alginate, cell surface hydrophobicity, pyochelin, haemolysin and phospholipase C were also observed in the culture supernatant of the biofilm cells [Harjai 2014].

In tests of cranberry juice extract and fractions thereof, surface adhesion of the antibiotic-resistant pathogenic *E. coli* B78 and the non-pathogenic strain *E. coli* HB101 to silicon nitride tips was determined by atomic force microscopy (AFM). The extract and three flavonoid-rich fractions did not change the adhesion of *E. coli* HB101. In contrast, a significant ($p<0.001$) decrease in the adhesion forces of strain B78 was shown, as compared to control [Gupta 2016].

Freeze-dried cranberry powder (9 mg PAC/g) reduced the mean adherence of a P-fimbriated uropathogenic *E. coli* isolate to vaginal epithelial cells from 18.6 to 1.8 bacteria per cell ($p<0.001$). At 50 µg/mL a purified PAC extract from fresh cranberry reduced mean adherence of this strain to primary cultured bladder epithelial cells from 6.9 to 1.6 bacteria per cell ($p<0.001$) in a linear and dose-dependent manner [Gupta 2007].

In uropathogenic *E. coli* strain CFT073 exposed to dried cranberry powder (CP; 5, 10, and 20 mg/mL) or PACs (0.1 mg/mL), expression of the flagellin gene involved in flagella formation, and motility was inhibited. Transmission electron

microscopy imaging revealed fewer flagella in bacteria grown with CP or PACs. Swimming and swarming motilities were hindered significantly ($p< 0.001$ for CP at 10, 15, 20 mg/mL and for CP at 5, 10, 15, 20 mg/mL, as well as PACs at 0.1 mg/mL, respectively) [Hidalgo 2011].

An extract enriched in PACs at concentrations of 5, 25, 75 and 137 mg PACs/mL demonstrated a dose-dependent inhibition of adherence of *E. coli* to human urinary bladder carcinoma T24 cells by 17%, 52%, 76 and 89% respectively [Risco-Rodríguez 2015]. Urine samples from rats taking this preparation as powder for oral suspension or as tablets (118 mg PACs/animal) showed an inhibition of *E. coli* adherence to bladder epithelial cells of 83% and 52% respectively. The lower dose of 59 mg PACs/animal also resulted in an inhibition of adherence (40% and 29% respectively) [Risco 2010].

In an adhesion/invasion assay a dry PAC extract significantly ($p<0.01$) reduced invasion of canine kidney cells by pathogenic *E. coli* CFT073 and O157:H7, *Enterococcus faecalis* 29212, and *Pseudomonas aeruginosa* 10145. Adhesion of Gram-positive *E. faecalis* decreased upon treatment, whereas adhesion of the Gram-negative strains slightly increased [Tukenkji 2010].

Two dry extracts, standardized for PACs, at concentrations of 5 -180 µg/mL significantly ($p<0.05$) inhibited adhesion of an *E. coli* strain carrying PAOGII fimbriae to uroepithelial cells. Another extract showed significant inhibition ($p<0.05$) only from 20 µg/mL upwards [Ermel 2012].

Adherence to uroepithelial cells of *E. coli* strains 8 and 269 was concentration-dependently reduced by an extract (not further specified). After exposure to 0.75×MIC of the extract (38 and 55 mg/mL respectively), the percentage of adherent bacteria was 18.4% and 22.9% for *E. coli* strains 8 and 269 respectively, as compared to control [Wojnicz 2012].

An extract significantly ($p<0.05$) inhibited the adhesion to polystyrene surfaces of *Candida albicans* ATCC 3153 at concentrations of 10, 20 and 40 mg/mL and of *C. glabrata* IHEM9556 at the two higher concentrations. Pretreatment of surfaces with the extract induced an anti-adhesion activity mainly against the different strains of *C. glabrata* and an anti-biofilm activity against various strains of *C. albicans* [Girardot 2014].

In contrast, a dose-dependent increase in bacterial adhesion of two *E. coli* strains to T24 bladder cells was observed with a cranberry extract with 21% PACs from 10 to 100 µg/mL ($p<0.05$ and 0.01 respectively). Confocal laser scanning microscopy and scanning electron microscopy proved that the extract led to the formation of bacterial clusters on the outer plasma membrane of the host cells without subsequent internalization. This activity was not observed with a PAC-depleted extract. The latter extract showed significant inhibition of adhesion of type 1 fimbriae dominated and mannose-sensitive strain NU14 ($p<0.01$ at 50 and 100 µg/mL) but had no inhibitory activity against P- and F1C-fimbriae dominated strain 2980. Quantitative gene expression analysis indicated that PAC-containing as well as PAC-depleted extracts increased the *fimH* expression in NU14. For strain 2980 the PAC-containing extract led to upregulation of P- and F1C-fimbriae, whereas the PAC-depleted extract had no influence on gene expression [Rafsanjany 2015].

An extract (not further specified) from concentrated cranberry juice (0.5 and 1 mg/mL) significantly ($p<0.05$) reduced biofilm formation of a mixture of fourteen strains of uropathogenic *E. coli* [Margetis 2015].

Pre-incubation of fimbriae expressing *E. coli* with a spray dried extract (41% PACs) inhibited adhesion to villi from pig jejunal mucosa by 75.3 and 95.8% at 20 and 100 µg respectively in strain $F4^+$, and by 100% at both concentrations in strain $F18^+$. Binding of isolated F4 and F18 fimbriae to pig intestinal epithelium was reduced by pretreatment of the fimbriae with 2 and 20 mg/mL of the extract [Coddens 2017].

A significant ($p<0.05$) inhibition of *Vibrio cholera* biofilm formation during the development/maturation stage by reducing the biofilm matrix production and secretion was observed after addition of a water-soluble cranberry extract (4.0% PACs) to the culture medium at 2 mg/mL. Downregulation of the expression of several genes responsible for the production of the bacterial polysaccharide and matrix proteins was observed [Pederson 2018].

A cranberry dry extract (DER 25:1, PACs>2.7%) significantly decreased adhesion of uropathogenic *E. coli* strains UTI89 to human T24 bladder cells and CFT073 to A498 kidney cells in a concentration-dependent manner from 250 to 2500 µg/mL ($p<0.05$ to 0.001). Urine samples of male volunteers (n=8) after consumption of 900 mg/day of the extract for 7 days inhibited adhesion of strain UTI89 to T24 cells, whereas the urine of female volunteers (n=8) remained without effect. Protein analysis of the urine samples indicated increased amounts of Tamm-Horsfall protein (syn. uromodulin) in the active samples [Scharf 2019].

PACs, consisting predominantly of epicatechin oligomers (degree of polymerization [DP] 3-5), inhibited the adherence of uropathogenic P-fimbriated *E. coli* to cellular surfaces containing α-Gal(1→4)β-Gal receptor sequences similar to those on epithelial cells of the urinary tract [Foo 2000a,b].

A non-dialyzable, high-MW fraction from cranberry juice (MW > 12000-15000) significantly ($p<0.01$) inhibited sialic acid-specific adhesion of several strains of *Helicobacter pylori* to immobilized human gastric mucus and to human gastric HT-29 cells at a concentration of 100 µg/mL [Burger 2000]. The IC_{50} of the fraction was below 100 µg/mL for adhesion of some clinical isolates of *H. pylori* to both human gastric mucus and cultured gastric epithelial cells [Burger 2002].

Confluent monolayers of gastric cell lines were exposed to suspensions from 83 different isolates of *H. pylori* in the presence and absence of a non-dialyzable, high-MW fraction from cranberry juice. Adhesion of two thirds of the isolates to gastric cells was inhibited by the fraction at 0.2 mg/mL. Only 13 isolates (16%) were resistant to both the fraction and metronidazole, and 30 (36%) were resistant to the fraction alone. There was no cross-resistance [Shmuely 2004].

High molecular weight PACs (100 µg/mL) were examined for their potential to reduce the initial adhesion of uropathogenic *E. coli* CFT073 and *E. faecalis* 29212 to two model materials (PVC and PTFE). The experiments demonstrated a decreased attachment of both bacteria to PVC and PTFE when either bacteria, the model materials or both bacteria and model materials were pretreated with PACs. The most potent reduction was seen with *E. faecalis* and coated material surfaces (90% inhibition with PVC; 88% inhibition with PTFE) [Eydelnant and Tufenkji 2008].

Immobilized oral bacterial fructosyltransferase (FTF, involved in the pathogenesis of dental diseases) was treated with non-dialyzable, high-MW material (NDM; MW 15000) from concentrated cranberry juice at concentrations between 0 µg/mL and 200 µg/mL, with or without the addition of salivary amylase. NDM attached firmly to immobilized FTF in a dose-dependent manner, and the attachment was reduced in the presence of

salivary amylase (affinity constant 10^6 M^{-1} and 0.2 x 10^6 M^{-1}, respectively). At 200 µg/mL, NDM with sucrose inhibited the activity of immobilized FTF by 63% within minutes as compared with sucrose alone [Feldman 2010].

PACs (100 µg/mL) completely suppressed swarming motility of *Pseudomonas aeruginosa* ($p<0.05$), while a weaker decrease was also observed after treatment with cranberry powder, natural cranberry juice, diet cranberry juice cocktail containing artificial sweeteners, and cranberry tea. Twitching and swimming motilities were reduced to a lesser extent by the PACs [O'May 2011].

A highly purified A-type PAC inhibited adherence of sensitive and multi-drug resistant strains of *E. coli* to vaginal and bladder epithelial cells dose-dependently up to 70% with 50 µg/mL ($p<0.001$) [Gupta 2012].

A-type PACs were significantly more effective ($p<0.05$) in increasing agglutination of extra-intestinal pathogenic *E. coli* 5011 as compared to B-type PACs. The reduction of invasion of gut epithelial Caco-2 cells by *E. coli* was also significantly ($p<0.05$) more pronounced with A-type PACs [Feliciano 2013].

Adhesion of different strains of uropathogenic *E. coli* and *Proteus mirabilis* to HT1376 human bladder carcinoma cells was dose-dependently reduced by A-type PACs (15, 25, 50, 75 and 100 µg/mL) by up to 75%, as compared to controls. A reduction in motility of two *P. mirabilis* strains was observed [Nicolosi 2014].

Addition of PACs at 100 µg/mL to agar plates reduced the swarming motility of *P. aeruginosa* and inhibited biofilm formation by 40.9% ($p<0.05$) and 55.7% ($p<0.01$) at 1 and 10 µg/mL respectively. Proteomic analysis of PAC-treated *P. aeruginosa* revealed upregulation of 12 proteins related to iron siderophores or cation transporters and 5 proteins putatively involved in amino acid synthesis. Two proteins related to ATP synthesis, a likely cytochrome C protein (PA2482) and several proteins involved in DNA and RNA synthesis were downregulated, and several citric acid cycle proteins were reduced. Murine macrophages (J774A.1) and human embryonic kidney cells (HEK293T/17) pretreated with 10 µg/mL PACs were significantly ($p<0.05$) prevented from *P. aeruginosa*-mediated lysis as compared to untreated control [Ulrey 2014].

A xyloglucan fraction inhibited the adhesion of uropathogenic *E. coli* CFT073 and UTI89 strains to T24 human bladder epithelial cells in a dose-dependent manner with half-maximal inhibitory concentrations of 0.82 and 0.66 mg/mL respectively. A non-dose-dependent response was shown for adhesion of enterohaemorrhagic E. coli O157:H7 to HT29 human colonic epithelial cells [Hotchkiss 2015].

A phenolic-free carbohydrate fraction from cranberry fruit, containing predominantly oligosaccharides with xyloglucan and arabinan residues, reduced biofilm production of the uropathogenic *E.coli* strain CFT073 and the non-pathogenic strain MG1655b by 50% at 1.25 mg/mL, but did not inhibit bacterial growth [Sun 2015].

Application of a combination of cranberry PACs (DP4 and DP9) with myricetin reduced the insoluble exopolysaccharide (EPS) content (>80% reduction vs. vehicle-control; $p<0.001$) produced by *Streptococcus mutans*-derived glucosyltransferases, which is essential for the initiation of cariogenic biofilms. The 3D architecture of biofilms showed a defective EPS-matrix and failure to develop microcolonies on saliva-coated hydroxyapatite surface. The mechanical stability of the biofilms was significantly ($p<0.001$) weakened [Kim 2015].

A cranberry juice powder dissolved in water to a 25% concentration significantly ($p<0.01$) reduced the adherence of various oral *Streptococcus* strains to saliva-coated hydroxyapatite beads. A non-dialyzable, high-MW fraction from cranberry juice (MW > 14000) at a concentration of 100 µg/mL significantly ($p<0.01$) inhibited biofilm formation by *Streptococcus mutans*, *S. criceti*, *S. oralis* and *S. mitis* on saliva-coated hydroxyapatite beads. At 500 µg/mL biofilm formation by *S. sobrinus* and *S. sanguinis* was also significantly ($p<0.05$) inhibited [Yamanaka 2004].

Adhesion of three different strains of *E.coli* to human buccal cells was significantly ($p<0.001$) reduced up to 80% by a dry extract at a concentration containing 180 µg/mL PACs [Margetis 2015].

At a concentration of 0.6-2.5 mg/mL a non-dialyzable, high-MW fraction from cranberry juice dissociated co-aggregates formed by a wide range of oral bacteria. Gram-negative bacteria were more sensitive than Gram-positive bacteria [Weiss 2002].

At 0.066-1.33 mg/mL a non-dialyzable, high-MW fraction (MW >15000) from cranberry juice significantly inhibited the activity of two components of dental biofilm, glucosyltransferase and fructosyltransferase ($p<0.05$); it also inhibited the adhesion of *Streptococcus sabrinus* to hydroxyapatite beads ($p<0.05$) [Steinberg 2004]. In the same concentration range the non-dialyzable fraction promoted desorption of *Streptococci* from dental biofilm in the presence and absence of extracellular glucans and fructans ($p<0.05$), although the effect was more pronounced in the absence of these polysaccharides [Steinberg 2005].

A-type PAC oligomers of defined DP influenced 3D biofilm architecture and *Streptococcus mutans*-transcriptome responses. Accumulation of biofilms formed in a saliva-coated hydroxyapatite biofilm model was significantly ($p<0.0001$) impaired by application of PACs, and genes involved in bacterial adhesion, acid stress tolerance and glycolysis were affected. Genes (*rmpC*, *mepA*, *sdcBB* and *gbpC*) associated with sucrose-dependent bacterial binding were repressed. PACs of DP 4 and DP 8 to 13 were most effective in disrupting bacterial adhesion to glucan-coated apatite surface (>85% inhibition vs. vehicle-control; $p<0.0001$) and gene expression [Feng 2013]. In a similar study, topical application of a purified PAC fraction resulted in significantly ($p<0.05$) reduced biomass and total amount of extracellular insoluble polysaccharides of *S. mutans* biofilms as compared to vehicle control [Koo 2010].

A-type PACs significantly ($p<0.05$) prevented biofilm formation of *C. albicans* in a dose-dependent manner (23% reduction at 6.25 µg/mL to 80% reduction at 100 µg/mL). At 25 and 50 µg/mL, adherence to oral epithelial cells was reduced by 42% and 90% respectively, with complete inhibition observed at 100 µg/mL. A similar effect was observed in adherence to saliva-coated acrylic resin discs [Feldman 2012].

Co-aggregation of dental plaque forming bacteria was tested with a combination of either *Streptococcus sanguinis* (Ss) with *Fusobacterium nucleatum* (Fn) or *Porphyromonas gingivalis* (Pg) with Fn. The crude non-dialyzable fraction from a juice concentrate (NDM) and its phenol-rich fraction prepared with 75% acetone (NDMac) were effective in inhibiting co-aggregation of Ss and Fn (MIC = 470 µg/mL) and of Fn and Pg (MIC = 940 µg/mL). Biofilm formation by three bacterial strains, *Streptococcus mutans*, *Streptococcus epidermidis* and a methicillin-resistant strain of *Staphylococcus aureus*, was significantly ($p<0.001$) reduced by NDMac at concentrations of 80 µg/mL and by NDM at 320 µg mL [Neto 2017].

Anti-inflammatory activity
A phenol-rich extract from fresh cranberries at 1, 10 and 50 µg/mL decreased COX-2 expression ($p<0.05$) and suppressed degradation of IκBα ($p<0.05$) in unstimulated HT-29 human colon adenocarcinoma cells cultivated in serum-free medium. In phorbol 12-myristate 13-acetate (PMA)-stimulated cells, increased COX-2 expression and IκBα degradation were reduced by pretreatment with the extract at 10 µg/mL ($p<0.05$) [Narayansingh 2009].

A defatted 90% methanolic extract from fresh fruits inhibited COX-2 activity in human T lymphocytes with an IC_{50} of 12.8 µg/mL and the NF-κβ transcriptional activation with an IC_{50} of 19.4 µg/mL. The release of IL-1β, IL-6, IL-8 and TNF-α from LPS-stimulated human peripheral blood mononuclear cells was significantly ($p<0.001$) inhibited at a concentration of 50 µg/mL. iNOS activity in murine RAW 264.7 cells remained unchanged [Huang 2009].

In J774 macrophages, LPS-induced NO production was inhibited significantly ($p<0.01$) by concentrations of 30 µg/mL and 100 µg/mL of an extract enriched in hydroxybenzoic and hydroxycinnamic acids, anthocyanins and flavonols. Significant effects on iNOS or COX-2 expression were not detected. In human THP-1 macrophages the extract (100 µg/mL) inhibited LPS-induced IL-6, IL-1β and TNF-α production [Kylli 2011].

IL-8 secretion from MKN-45 stomach cancer cells, induced by 25 isolated strains of *Helicobacter pylori*, was significantly ($p<0.001$) inhibited by an extract (5.4% total polyphenols, 11.2% total organic acids) at a concentration of 1 mg/mL [Matsushima 2013].

An extract (not further specified), and a polyphenol-rich fraction thereof, reduced LPS-elicited induction of IL-1β in mouse Raw 264.7 macrophages in a dose-dependent manner [Grace 2014].

In LPS-induced BV-2 microglia, an extract enriched in anthocyanins (20 µg/mL) significantly lowered levels of NO species by 36.5% ($p<0.0001$) and of ROS by 9.2% ($p<0.01$) [Ma 2018].

Pre-incubation of Caco-2/15 cells with three fractions from freeze-dried cranberries (250 µg/mL; low-, medium- and high molecular mass phenolics respectively) prevented iron/ascorbate-mediated lipid peroxidation and counteracted LPS-mediated inflammation significantly ($p<0.01$ to $p<0.001$). Increased pro-inflammatory cytokines (TNF-α and IL-6), COX-2 and prostaglandin E2 as well as elevated NFκB activation were decreased. Nrf2 downregulation was enhanced. Oxidative stress-dependent mitochondrial dysfunctions (rise in ATP production, upregulation of Bcl-2, decline of protein expression of cytochrome c and apoptotic-inducing factor) as well as changes in the protein expression of mitochondrial transcription factors (mtTFA, mtTFB1, mtTFB2) were restored by the high molecular phenolic fraction [Denis 2015].

At 10-50 µg/mL the non-dialyzable fraction (MW > 14000) from cranberry juice potently inhibited pro-inflammatory cytokine (IL-1β, IL-6, TNF-α) and chemokine (IL-8, RANTES) responses of a macrophage cell line triggered by LPS from the five major periodontopathogens ($p<0.05$) [Bodet 2006a]. The same fraction dose-dependently inhibited the proteolytic activities of several bacteria known to contribute to periodontal tissue destruction ($p<0.05$) [Bodet 2006b].

The effects of A-type PACs (25, 50, and 100 µg/mL) on MMP production in LPS-stimulated macrophages were investigated. The increased secretion of MMP-7, MMP-8 and MMP-13 was significantly reduced at all concentrations ($p<0.05$). The LPS-induced production of MMP-3 was only decreased at 100 µg/mL ($p<0.05$), and a synergistic effect was observed at 25 µg/mL. Elevated MMP-1 and MMP-9 secretion was diminished at 50 and 100 µg/mL ($p<0.05$). Pretreatment of the macrophages with the PACs significantly ($p<0.05$) reduced LPS-induced NF-κB p65 activation. The LPS-enhanced levels of phosphorylated JNK2 (Thr183/Tyr185), AKT (Ser473), AKT (Thr308), and MEK1 (Ser217/Ser221) were reduced with 50 µg/mL PACs. MMP-1 mediated type I collagen degradation and MMP-9-mediated gelatine degradation were inhibited significantly ($p<0.05$) at all concentrations [La 2009a].

Pretreatment of oral epithelial cells with A-type PACs (25, 50, and 100 µg/mL) prior to stimulation with *C. albicans* resulted in a significant decrease ($p<0.05$) of the elevated secretion of IL-6 and IL-8 in a dose-dependent manner. Pretreatment of the cells with 50 µg/mL of the fraction significantly ($p<0.05$) decreased *C. albicans*-induced activity of NF-κB p65 to the level of non-stimulated cells. Elevated levels of phosphorylated kinases AKT (Ser473), ERK1/2 (Thr202/Tyr204) and MEK1 (Ser217/Ser221) were significantly ($p<0.05$) reduced upon pretreatment at 50 µg/mL [Feldman 2012].

In human gingival epithelial cells and normal human gingival fibroblasts IL-17 stimulated the production of IL-6 ($p<0.005$) and IL-8 ($p<0.03$). Non-toxic levels of non-dialyzable high molecular material from juice inhibited constitutive IL-6 and IL-8 production by epithelial cells ($p<0.01$) and fibroblasts ($p<0.03$) as well as IL-17-stimulated cytokine production [Tipton 2013b].

Aggressive periodontitis (AgP) gingival fibroblasts were incubated with high molecular weight non-dialyzable material rich in PACs (NDM) from cranberry juice or NDM+LPS from *Fusobacterium nucleatum* or *Porphyromonas gingivalis*. NDM (50 µg/mL) inhibited LPS-stimulated NF-κB p65 ($p<0.003$) and constitutive or LPS-stimulated matrix metalloprotease (MMP)-3 ($p<0.02$). Constitutive or LPS-stimulated IL-6 production in AgP fibroblasts ($p<0.0001$) was increased by NDM, but inhibited in normal human gingival fibroblasts ($p<0.01$) [Tipton 2013a].

In human gingival epithelial cells IL-1β-stimulated IL-6 production was significantly decreased after incubation with NDM (25 µg/mL; $p<0.001$) or inhibitors of NF-κB or AP-1. The increased activation of NF-κB or AP-1 after IL-1β was reduced by NDM ($p<0.0001$). NDM did not significantly affect IL-1β-stimulated levels of phosphorylated intermediates in the NF-κB pathway (IκBa) or the AP-1 pathway (c-Jun, ERK1/2) [Tipton 2014].

Human temporomandibular joint synovial fibroblasts from joints with and without degenerative osteoarthritis were incubated with IL-1β (0.001–1 nM) and NDM (25–250 µg/mL). Increases of IL-6, IL-8 and VEGF production were dose-dependently reduced by NDM (25, 50, 100 and 250 µg/mL). NDM decreased IL-1β-elevated nuclear levels of NF-κB and AP-1 ($p<0.04$) [Tipton 2016].

Antimicrobial activity
Liquid extracts (50 µl of 3g of homogenated berries or berry cake in 10 mL acidified ethanol) showed activity against six Gram-positive and four Gram-negative bacteria, with *Bacillus cereus* being the most sensitive [Viskelis 2009].

Three cranberry extracts (enriched in water-soluble phenolic compounds (E1), apolar phenolic compounds (E2) or anthocyanins (E3)) were investigated against seven bacterial strains. The strongest effects were observed for E1 with MICs between 12.6 and 50.4 µg phenolics/well [Côté 2011].

Six different microorganisms (*Candida glabrata*, *C. lusitaniae*, *C. krusei*, *C. tropicalis*, *C. albicans* and *Cryptococcus neoformans*) were significantly ($p<0.001$) inhibited by an extract enriched in phenolics and four PAC-rich subfractions with MIC_{50} values between 0.5 and 63 µg/mL [Patel 2011].

Addition of 10% cranberry juice or of a PAC fraction (10, 50 or 100µg/mL) to the cultivation broth strongly increased the doubling time of *E.coli*. Altered expression of genes associated with iron transport and binding, the transcripts for essential metabolic enzymes, ATP synthesis, and fumarate hydratase was shown. The changed expression of genes associated with iron transport was attributed to the strong iron-chelating capacity of PACs. Addition of exogenous iron to the growth media partially reversed the inhibitory effect on bacterial growth [Lin 2011].

Three extracts standardized for PACs reduced the growth of *Staphylococcus spp.*, but not of *E. coli*, with MICs in the range 0.02–5 mg/mL. The extracts also inhibited biofilm production in several clinically relevant strains of *Staphylococcus* (*S. epidermidis*, *S. aureus*, methicillin-resistant *S. aureus*, *S. saprophyticus*) and *E. coli*, but did not eradicate the established biofilm [LaPlante 2012].

Treatment of *S. aureus* with a water-soluble extract (containing at least 88% carbohydrates and 2 to 3.8% total phenolic compounds) or an ethanolic extract from cranberry press-cake revealed a transcriptional signature typical of peptidoglycan-acting antibiotics (upregulation of genes *vraR/S*, *murZ*, *lytM*, *pbp2*, *sgtB*, *fmt*). The effect of the ethanolic extract on peptidoglycans was confirmed by the marked inhibition of incorporation of D-[^{3}H]alanine. The combination of β-lactams and this extract in checkerboard assays revealed a synergistic activity against *S. aureus* including strain MRSA COL. For this strain a 512-fold decrease of the MIC of amoxicillin in the presence of the ethanolic extract at 128 µg/mL was observed [Diarra 2013].

Growth of several pathogenic *E. coli* strains was significantly inhibited by an extract with defined PAC content at 50 to 1000 µg/mL ($p<0.04$ to 0.0001) [Margetis 2015].

A reduction in cell viability of approximately 40% was observed for monocyte-derived macrophages or oral epithelial cells treated with cell wall of *Peptostreptococcus micros*. Pretreatment of both cell lines with NDM dose-dependently reduced the toxic effect to approximately 15% ($p<0.05$) [La 2009b].

Polymeric PACs (mean DP 35) from lyophilized cranberries showed strong activity against *S. aureus* VTT E-70045 [Kylli 2011].

A potent and dose-dependent effect of PACs on the cytoskeleton of HeLA cells was demonstrated. Actin pedestal formation and localized actin polymerization required for infection with two strains of enteropathogenic *E. coli* and *S. typhimurium* were clearly diminished. Invasion of HeLa cells by *S. typhimurium* was reduced in a concentration-dependent manner. PACs had no effect on bacterial growth, nor the production of bacterial effector proteins of the type III secretion system [Harmidy 2011].

Different fractions from cranberry powder (sugars plus organic acids, monomeric phenolics, anthocyanins plus PACs, anthocyanins or PACs alone) were found to inhibit *E. coli* O157:H7, *Listeria monocytogenes* and *Lactobacillus rhamnosus* to various degrees, with *L. monocytogenes* being the most susceptible [Lacombe 2013].

Antiviral effects

Monolayers of monkey kidney epithelial-like MA-104 cells were inoculated with 0.2 ml cranberry juice before or after treatment with Simian rotavirus SA-11 and bovine reovirus type 3. Pretreatment of the monolayers at juice concentrations from 16% to 30% reduced reovirus infectivity titers by >90% and of rotavirus to a similar extent. Transmission electron microscopy did not detect virus penetration or egress among pretreated monolayers. Detection of rotavirus RNA was markedly reduced after pretreatment. Posttreatment was less effective in the reduction of viral infectivity titers than pretreatment [Lipson 2010].

The influence of cranberry juice and a PAC fraction on the infectivity of murine norovirus (MNV-1), feline calicivirus (FCV-F9), MS2(ssRNA) bacteriophage and phiX-174(ssDNA) bacteriophage was studied. Viruses (~7 log^{10} or ~5 log10 PFU/mL) were mixed with equal volumes of the juice or 0.30, 0.60, and 1.20 mg/mL final PAC concentration and viral infectivity was evaluated with plaque assays. At low viral titres, FCV-F9 was undetectable after all treatments. All other tested titres were reduced by the treatment [Su 2010].

Pretreatment of Vero cells with an extract (>360 mg/g PACs) resulted in a concentration-dependent inhibition of *Herpes simplex* virus 1 and 2 (IC_{50} against HSV-1 and HSV-2 replication 14.2 ± 0.5 mg/mL and 9.6 ± 0.2 mg/mL respectively). Two PAC-A containing fractions of the extract were shown to be responsible for the activity. Gene expression studies showed that the attachment of HS viral particles to the target cells was impaired at an early step in a concentration-dependent manner. The extract and the PAC fractions led to a loss of infectivity of the HSV particles by targeting the viral envelope glycoproteins gD and gB [Terlizzi 2016].

An extract (366 mg/g total PACs) inhibited influenza A and B virus replication in MDCK cells in a concentration-dependent manner with an IC_{50} value of 4.5 µg/mL for both viruses. The extract prevented the attachment and entry of the viruses and exerted virucidal activity. The extract and PAC-A2 directly interacted with the ectodomain of viral haemagglutinin (HA) glycoprotein [Luganini 2018].

Neuraminidase activity (NA) of influenza A and B strains was inhibited by NDM (MW 12,000-15,000). The most potent effect was observed against two H1N1 strains (IC_{50} 187 and 197 µg/mL respectively). The NA inhibition in two influenza B strains (IC_{50} 441 and 578 µg/mL respectively) and of bacterial neuraminidase of Streptococcus pneumoniae (IC_{50}, 594 µg/mL) was much less [Oiknine-Djian 2012].

Anticancer activity

An extract from cranberry fruit and fractions from it were tested for their ability to induce quinine reductase, an enzyme that can inactivate certain carcinogens, and to inhibit the induction of ornithine decarboxylase, an enzyme involved in tumour proliferation. The ethyl acetate fraction induced quinine reductase, doubling its activity at a concentration of 3.7 µg of tannic acid equivalent (TAE) per 150 µl of medium. The IC_{50} values (as TAE per 150 µl of medium) for inhibition of ornithine decarboxylase were 6.0 µg for the PAC fraction, 7.0 µg for the extract and 55.0 µg for the ethyl acetate fraction [Bomser 1996].

A fraction from a methanolic extract inhibited the growth of two human tumour cell lines with IC_{50} values in the range 16-125 µg/mL [Yan 2002].

The IC_{50} of a proanthocyanidin-rich fraction from an extract was 5.67 µg/mL in an ornithine decarboxylase assay, indicating a significant activity against the promotion stage of chemically-induced carcinogenesis [Kandil 2002].

Fractions of an extract enriched with sugars, organic acids, total polyphenols, PACs or anthocyanins were tested for antiproliferative effects against human cancer cell lines at concentrations corresponding to their content in the extract. The extract and all fractions except the sugar fraction showed significant antiproliferative activity compared to controls ($p \leq 0.01$), the total polyphenol fraction being the most active against all cancer cell lines [Seeram 2004].

Pretreatment with a proanthocyanidin-rich fraction inhibited viability and proliferation of SEG-1 human oesophageal adenocarcinoma cells in a time- and dose-dependent manner. At 50 µg/mL, acid-induced cell proliferation was also significantly reduced, apoptosis was induced and a cell cycle arrest at the G1 checkpoint occurred (all $p<0.05$) [Kresty 2008].

Gene expression analysis of NCI-H460 human lung cancer cells after treatment with 50 µg/mL of an A-type PAC (DP 4-5) fraction showed upregulation of numerous processes linked to cell death and downregulation of cell cycle linked processes. The treatment also resulted in significant ($p<0.05$) and rapid induction of apoptosis [Kresty 2011].

A PAC fraction from frozen fruits (PAC-1), consisting of mainly A-type PACs with DP 2 to 11, exhibited cytotoxicity against platinum-resistant human ovarian SKOV-3 cancer cells (IC_{50} = 79 µg/mL) by inducing classic apoptotic changes, but was non-cytotoxic to lung fibroblasts. Synergistic effects with paraplatin were observed: pretreatment of SKOV-3 cells with the fraction (106 µg/mL) led to a distinct reduction of the paraplatin IC_{50} value, and co-treatment revealed reduced cell proliferation at lower concentrations than individual treatment with the fraction or with paraplatin [Singh 2009]. Purification of this fraction resulted in fraction PAC-1A, which selectively reduced the viability of various human neuroblastoma cell lines and showed synergistic effects with cyclophosphamide [Singh 2012].

The proanthocyanidin-rich isolate PAC-1 inhibited viability and proliferation of chemotherapy-resistant SKOV-3 ovarian cancer cells by causing cell cycle arrest in the G2/M phase and increasing the generation of intercellular ROS. Apoptosis was induced through activation of caspases 3, 7 and 8, blocking of the activation of the pro-survival factor AKT and inactivation/ cleavage of DNA repair factor PARP. PAC-1 also blocked vascular endothelial growth factor (VEGF)-stimulated receptor phosphorylation in HUVEC cells [Kim 2012].

In human oesophageal adenocarcinoma cells, a PAC fraction (DP 4-5) at 50 and 100 µg/mL induced caspase-independent cell death mainly via autophagy and low levels of apoptosis in acid-sensitive JHAD1 and OE33 cells, but resulted in cellular necrosis in acid-resistant OE19 cells ($p<0.05$ as compared to vehicle treated cells). Cell death involved PI3K/ AKT/mTOR inactivation, pro-apoptotic protein induction (BAX, BAK1, deamidated BCL-xL, Cytochrome C, PARP), modulation of MAPKs (P-P38/P-JNK) and G2-M cell cycle arrest [Kresty 2015].

An extract from frozen fruits with sugars removed showed significant ($p<0.05$) and dose-dependent cytotoxicity against DU145 human prostate cancer cells at 10, 25 and 50 µg/mL with an IC of 46% at 50 µg/mL. At this concentration the extract led to an arrest in the G1 phase of the cell cycle associated with significant ($p<0.05$) decreases in the expression of CDK4, cyclin A, cyclin B1, cyclin D1 and cyclin E and increased expression of p27 [Deziel 2012].

A sugar-free extract and two fractions (rich in flavonoids and PACs respectively) reduced viability of human DU145 prostate adenocarcinoma cells in a dose- dependent manner with IC50 values < 50 µg/mL. Cell death occurred due to apoptosis as shown by increased caspase-8 and -9 activity [MacLean 2011].

A PAC-enriched fraction from frozen fruits decreased viability of DU145 prostate cancer cells by 30% at a concentration of 25 µg/mL. The fraction inhibited MMP 2 and 9 activity via an increase in the expression of TIMP-2 and a decrease in the expression of EMMPRIN. The expression of PI-3 kinase and AKT proteins and the translocation of the NF-kB p65 protein to the nucleus and c-fos protein levels were decreased. The phosphorylation of p38 and ERK1/2 as well as the c-jun protein levels increased [Deziel 2010].

In a similar experiment with a flavonoid fraction (25 µg/mL), viability of DU145 cells, as well as MMP-2 and MMP-9 activity, was decreased due to increased TIMP-2 expression. Urokinase plasminogen activator (uPA) activity was reduced through effects on specific temporal MMP regulators and uPA regulators and by affecting the phosphorylation and/or expression of specific MAP kinase, PI-3 kinase, NF-kB and AP-1 pathway associated proteins [MacPhee 2014].

In DU145 cells, a fraction containing A-type PACs (DP 2-12) at 25 µg/mL caused cell cycle arrest in the G2/M-phase, decreased the expression of cyclin A, cyclin B1, $p16^{INK4a}$, p21, and pRBp107 protein levels and increased protein expression levels of cyclin E, cyclin D1, CDK2, CDK4 and p27 [Kim 2014].

Antioxidant activity

Cranberry juice with a polyphenolic content corresponding to 1548 mg gallic acid equivalents/L inhibited cupric ion-dependent low density lipoprotein (LDL) oxidation at a dilution of 0.1% ($p<0.001$) [Wilson 1998].

Ethyl acetate fractions from cranberry containing mainly flavonol glycosides and anthocyanins showed 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity with EC_{50} values of 33-40 µg/mL. Cyanidin 3-galactoside exhibited the highest antioxidant activity (EC_{50}: 1.45 µM) in LDL + VLDL oxidation assays, comparable to tocopherol [Yan 2002].

Moderate free radical-scavenging activities and inhibition of lipid peroxidation were observed for fractions from cranberries and cranberry pomace [Caillet 2011, 2012].

Fractions enriched in free, esterified or bound phenolics prepared from different cranberry genotypes showed antioxidant activity in several assays (ORAC, Trolox equivalent antioxidant activity, TBARS, ferric reducing capacity and others) [Abeywickrama 2016].

Effects on enzymes involved in drug metabolism

In human liver microsomes, with the use of mycophenolic acid as a probe, UDP-glucuronosyltransferase (UGT) 1A9 was weakly inhibited by a juice (>40% total PACs) with an IC_{50} of 260 µg/mL [Mohamed and Frye 2011].

A methanolic extract was tested for CYP2C8 inhibition in human liver microsomes with amodiaquine and pioglitazone as probes. The formation of the metabolites N-desethylamodiaquine and hydroxypioglitazone was reduced with IC_{50} values of 24.7 and 24 µg/mL respectively [Albassam 2015].

Fractions from a methanolic extract of freeze-dried juice were tested for their inhibitory potential on recombinant CYP3A4 and CYP3A5 enzymes with the probe midazolam. At 10 µg/mL, the inhibition towards rCYP3A4 varied from 10% (butanol-soluble) to 50% (hexane-soluble) and the extent of inhibition toward rCYP3A5 from 0% (chloroform-soluble) to

23% (hexane-soluble). The hexane-soluble fraction differed significantly ($p<0.05$) from all other fractions. At 50 µg/mL, the hexane- and chloroform-soluble fractions reduced rCYP3A4 activity by at least 75%. Towards rCYP3A5 the inhibition by all fractions was much less with the hexane-soluble fraction resulting in the strongest effect (62% inhibition) [Ngo 2009].

Maslinic and corosolic acid, isolated from a methanolic extract in an activity-guided approach, showed the strongest inhibition of enteric CYP3A activity for the substrate midazolam. The IC_{50} values were 7.4 and 8.8 µM for maslinic and corosolic acid respectively, using human intestinal microsomes and 2.8 and 4.3 µM respectively, using recombinant CYP3A4 as the enzyme source [Kim 2011a].

Other effects
Cranberry juice at a concentration of 1% dilated phenylephrine-induced contractions in rat aortic rings ($p<0.002$). The vasodilation was shown to be dependent on endothelial cell nitric oxide formation [Maher 2000].

Human retinal pigment epithelium cells (ARPE-19) were irradiated with blue light to mimic age-related macular degeneration. Treatment with an ethyl acetate extract from a concentrated juice at 12.5, 25 or 50 µg/mL resulted in a significant ($p<0.05$) increase of proliferation. Similar effects were seen after treatment with the non-condensed and the condensed tannin fraction of the ethyl acetate extract [Chang 2017].

An extract, its ethyl acetate fraction, water fraction and three subfractions significantly ($p<0.05$) inhibited formation of glycated haemoglobin in human blood. Most of the fractions reduced glucose-mediated glycation of human albumin with EC_{50} values from 21-52 µg/mL, better than aminoguanidine (EC_{50} 64 µg/mL) as positive control [Liu 2011]

Formation of fructose-induced glycation end-products was reduced by an anthocyanin-enriched fraction (100 µg/mL) by 74.5%. A significant ($p<0.0001$) inhibition of Aβ-fibrillation was observed in two models. H_2O_2-induced cytotoxicity in BV-2 microglia decreased by 18.7% ($p<0.001$) [Ma 2018].

In differentiated 3T3-L1 adipocytes, treatment with an aqueous extract (25-100 µg/mL) decreased lipid accumulation and generation of reactive oxygen species. Adipogenesis and lipogenesis were suppressed via downregulation of the expression of *PPARγ* and other genes. The extract significantly increased the expression of adiponectin ($p<0.001$ at all concentrations) and decreased leptin expression ($p<0.001$ at 100 µg/mL) [Kowalska 2017].

Pretreatment of neuroblastoma SH-SY5Y cells with lyophilized cranberry juice (25, 50 and 100 µg/mL) for 24 h significantly increased cell viability after exposure to hydrogen peroxide (100 µM) ($p<0.05$ to 0.01). The juice increased mitochondrial activity, reduced intracellular ROS production and lipid peroxidation due to hydrogen peroxide and upregulated the activity of catalase ($p<0.05$) and superoxide dismutase ($p<0.01$) [Casedas 2018].

An anthocyanin-fraction increased Akt phosphorylation in a dose-dependent manner in HUVECs. Cell migration and capillary-like tube formation were significantly ($p<0.01$) enhanced at a concentration of 0.2 mg/mL. The effects were abolished by pretreatment with wortmannin, a PI3 kinase inhibitor [Tulio 2012].

A fraction enriched in A-type PACs (10, 25 and 50 µg/mL) significantly decreased the formation of differentiated osteoclasts in a dose-dependent manner ($p<0.05$, 0.01 and 0.01, respectively) via an inhibition of cell maturation of pre-osteoclastic cells exposed to RANKL and M-CSF. The secretion of IL-8, involved in osteoclastogenesis, was significantly increased, whereas MMP-2 and -9, related to the resorptive activity of osteoclasts, were reduced ($p<0.01$ for all experiments). A significant ($p<0.05$) reduction of released helical peptides from the bone matrix was observed upon treatment of osteoclasts [Tanabe 2011].

Complexes of oligomeric PACs (DP 2-11) with bovine serum albumin significantly ($p<0.05$) reduced COX-2 and iNOS expression in LPS-stimulated RAW 264.7 macrophages in a concentration-dependent manner. The effects of the same concentrations of PACs alone were less pronounced. PACs complexed with hen egg-white lysozyme modulated antigen uptake, processing and presentation in murine peritoneal macrophages as shown by an increase of IL-2 expression in co-cultures with a T-cell hybridoma line [Carballo 2017].

In vivo experiments

Anti-adherence activity
Urine collected from mice receiving a cranberry cocktail (not further specified) for 14 days in place of normal drinking water, inhibited the adherence of *E. coli* to uroepithelial cells by approximately 80% ($p<0.01$) [Sobota 1984]. After administration of cranberry juice (25% or 100%; 3 x 1 mL/d for 3 days) to Sprague-Dawley rats, urine was collected. Treatment of uropathogenic *E.coli* with the urine significantly reduced the adhesion to human urinary bladder carcinoma T24 epithelial cells ($p=0.0181$ for 25% juice and $p=0.0045$ for 100%). Haemagglutination of a fimbriated *E.coli* strain decreased by 40.9% ($p=0.0038$) and 39.8% ($p<0.021$), biofilm formation by 53.9% ($p=0.0006$) and 72.9% ($p=0.0001$), respectively [Chen 2013].

Dogs with recurrent history of UTIs received orally either a cranberry extract (not further specified; 1 g/d for dogs < 25 kg and 2 g/d for dogs > 25 kg; n=6) for 6 months or cephalexin (20 mg/kg twice daily; n=6) for fourteen days. None of the dogs developed a UTI during the 6 months. Urine collected from the cranberry group on day 30 significantly ($p<0.05$) reduced bacterial adhesion of different E. coli strains to MDCK cells as compared to urine samples obtained before extract administration. The day 60 urine showed significantly ($p<0.05$) better effects than days 0 and 30 urine [Chou 2016].

Urine samples from Sprague-Dawley rats collected 2, 4, 8 and 24 hours after a single oral dose of an extract (100 mg/kg containing 15% total PACs) were studied for inhibition of adhesion of P-fimbriated E. coli to HAT-29 cells. The 8 h sample showed the highest activity ($p<0.05$). A 47% reduction in *E. coli* binding was still evident with the sample collected after 24 h [Peron 2017a].

In piglets on standard chow or chow supplemented with 1g/kg or 10 g/kg cranberry extract (41% PACs) for three days before and eight days after challenge with $F18^+$ *E. coli*, no differences in faecal excretion of *E.coli* were observed. In a second approach with 1% extract in the chow and 0.1% in the drinking water, a decrease of *E. coli* in the faeces was observed with significant overall reduction ($p<0.0001$). Score and duration of diarrhoea were also significantly reduced ($p<0.05$ at days 3 to 7 for score and $p=0.038$ for duration). Total serum antibody and IgM levels were significantly lower ($p<0.001$ and 0.01 respectively) [Coddens 2017].

Protective effects on intestinal mucosa and kidney
In male IRC mice, dysfunction of the mucosal immune system

and a suppression of the mucosal barrier function was induced by elemental enteral nutrition (EEN). Mice received 8 mg, 50 mg or 100 mg PACs/kg b.w. in addition to EEN for five days. Body weights were significantly ($p<0.05$) lower in all EEN groups as compared to the group on standard chow. Decreased intestinal tissue IL-4 and IL-13 levels after EEN were significantly ($p<0.05$) improved by 100 mg PACs and by 8, 50 and 100 mg PACs, respectively. IL-1β, IL-6 and TNF-α remained unchanged by EEN or EEN+PACs. Goblet cell numbers and luminal mucin 2, reduced with EEN, were attenuated ($p<0.05$) with 50 and 100 mg PACs [Pierre 2013].

In a similar experiment with only one PAC group (100 mg/kg), decreased phosphorylated STAT-6, polymeric immunoglobulin receptor and small intestine luminal IgA concentrations after EEN were significantly ($p<0.05$) improved with the extract [Pierre 2014].

Cranberry juice (15%; 1 mL/d) was administered orally to female LACA mice for 14 days. On day 15 the animals were infected by introduction of *Pseudomonas aeruginosa* PAO1 into the bladder. Significantly ($p<0.001$) lower renal colonization with bacteria was observed in the cranberry group as compared to untreated controls 5 days after infection. Inflammation with infiltration of polymorphonuclear leukocytes into the interstitium, vascular permeability and destruction of glomeruli and tubules in renal tissue was reduced by the treatment as well as the expression of renal pathology markers, MDA and RNI ($p<0.01$) [Harjai 2014].

Effects on life span
The life span of *Caenorhabditis elegans* wild-type N2 worms was increased by an extract (not further specified; 2 mg/mL) from 12 to 15.9 days. Life span extension was induced via the insulin/IGF signalling pathway and the nuclear localisation of the gene *DAF-16* through osmotic stress resistant-1 and its downstream effector UNC-43. Concentrations of > 6 mg/mL reduced the mean life span. In different stress models, higher resistance of worms (pretreated with 2 mg/mL of the extract for two generations) to heat shock and to osmotic stress was observed [Guha 2013].

Feeding of *C. elegans* with a uropathogenic *E. coli* strain reduced lethal time 50% (LT50) from 279.6 hours (feeding on non-virulent *E. coli* OP50) to 97.6 hours. Pretreatment of uropathogenic *E. coli* with urine from rats which had received cranberry juice (25% or 100%; 3 x 1 mL per day for 3 days) resulted in an improvement of the reduced LT_{50} to 117.4 hours (25% juice) and 158.1% (100% juice) [Chen 2013].

Addition of 2 mg/mL of a water-soluble cranberry extract (standardized to 4.0% PACs) to the growth medium significantly extended the life span of *C. elegans* CL2006 worms (expressing β-amyloid (Aβ) peptides) by 16.2% from 14.8 to 17.2 days ($p=0.006$), and delayed paralysis due to Aβ toxicity via heat shock transcription factor (HSF)-1. The total amount of Aβ species in the cranberry-treated worms was around 20% less than in controls ($p<0.05$). The extract also significantly ($p<0.05$) enhanced the solubility of proteins in aged worms [Guo 2016].

Life span in three life stages (healthy span 3–30 days, transition span 31–60 days and senescence span 61 days to the death of all flies) of adult *Drosophila* flies was extended by addition of 2% of a PAC-enriched extract to their diet. Life span extension was associated with reduced phosphorylation of ERK, slightly increased phosphorylation of AKT and a reduced level of 4-hydroxynonenal protein adducts [Sun 2014].

Addition of 5 and 20 mg/mL of an extract enriched in anthocyanins to the diet of male fruit flies increased the mean life span from 48 to 51 ($p<0.05$) and 53 ($p<0.01$) days respectively. The higher dose significantly delayed the age-dependent decline in climbing activity compared with control ($p<0.01$). The effects were accompanied by upregulation of the ageing-related genes SOD1 and downregulation of MTH, InR, TOR and PEPCK [Wang 2015].

In male ICR mice ageing was accelerated by 80 mg/kg/d galactose i.p. for 8 weeks. Oral treatment with a cranberry concentrate (15, 30 and 60 mg/kg/d PACs) resulted in a reduction of elevated hepatic and brain TBARS and serum and brain MAO levels. Hepatic CAT activity was significantly decreased by all doses ($p<0.05$). The lowest dose also significantly lowered the plasma 8-isoprostane level [Jiao 2017].

Anti-inflammatory effects
Male Sprague-Dawley rats received an atherogenic diet supplemented with 5 and 10% freeze-dried cranberry powder for 6 weeks. After challenge with LPS (0.5 mg/kg i.p. 15 hours prior to sacrifice) significantly ($p<0.05$) lower mean serum levels of CRP and IL-1β and higher serum IL-10 and NO levels were observed in the cranberry groups [Kim 2011b].

Colitis was induced in mice with dextran sulphate sodium (DSS) for 7 days in weeks 3 and 6 of feeding with either normal chow, chow containing 0.1% or 1% cranberry extract (not further specified) or 1.5% whole dried cranberry powder (DC). Animals on the high extract dose and on DC showed a later onset of colitis and a lower disease activity index in both weeks on DSS. DSS-induced shortening of the colon and increased colonic myeloperoxidase were significantly improved by DC ($p<0.05$). DC treatment resulted in reduced structural damage in colonic mucosa and less infiltration of inflammatory cells when compared to DSS alone. All cranberry treatments significantly reduced serum levels of TNF-α compared to DSS control, while DC also significantly reduced serum IL-1β (both $p<0.05$) [Xiao 2015].

Addition of 0.8% of a polyphenol-rich cranberry fraction (80.9% total phenolics; approx. 0.1% dimeric PACs and 0.7% anthocyanins) to the diet of high fat (HF)-fed obese C57BL/6J mice for 10 weeks resulted in a significant decrease in plasma ALT (31%, $p<0.01$) and in histological severity of non-alcoholic fatty liver disease (33% reduction in area of involvement, $p<0.01$; 29% decrease in lipid droplet size, $p<0.05$) compared to only HF-fed control mice. Hepatic protein levels of TNF-α and C-C chemokine ligand 2 were reduced by 28% ($p<0.01$) and 19% ($p<0.05$) respectively. The extract also decreased hepatic mRNA levels of toll-like receptor 4 (63%) and NF-κB (24%), as well as a number of downstream target genes and markers of macrophage infiltration [Glisan 2016].

Mice received a methanolic extract at doses of 50 and 200 mg/kg p.o. for 10 days. The extract significantly ($p<0.05$) reduced carrageenan-induced paw oedema by 42.9% and 36.9% after 5 hours respectively. Increased lipid peroxidation and influx of neutrophils, as measured by myeloperoxidase activity, in inflamed paws were decreased by both doses of the extract, significantly ($p<0.05$) only at the high dose [Nardi 2016].

A hydroethanolic extract (50, 100, and 200mg/kg) or vehicle was administered orally to mice at 1, 25 and 49 hours after induction of acute pancreatitis. The extract decreased L-arginine-induced abdominal hyperalgesia (from 48 to 72 h) and pancreatic oedema, pancreatic and pulmonary neutrophil infiltration, as well as levels of TNF-α, IL-1β and IL-6 after 72 h. Hyperamylasaemia and hyperlipasaemia were also reduced in comparison to the vehicle-treated group. Moreover, lipoperoxidation, carbonyl radicals, non-protein sulfhydryl

groups, and activity of catalase and superoxide dismutase, but not glutathione peroxidase, were restored by the extract [Santana 2018].

Experimental periodontitis in female BALB-c mice induced by mixed infection with *Porphyromonas gingivalis* and *Fusobacterium nucleatum* resulted in distinct alveolar bone loss after 42 days, which was significantly less ($p<0.05$) following pre-treatment of the two strains with NDM (MG > 12.000) from cranberry juice (4 mg/mL) and addition of the same dose of NDM to the drinking water of the mice [Polak 2013].

Antitumour effects
Chemopreventive effects of cranberry juice concentrate were evaluated in a model of bladder cancer in female Fischer-344 rats given N-butyl-N-(4-hydroxybutyl)-nitrosamine (150 mg, 2 x per week for 8 weeks). Cranberry juice concentrate (1.0 or 0.5 mL/rat/d), or water only, was administered by gavage for up to 6 months, starting one week after the final dose of the carcinogen. A dose-dependent effect of cranberry treatment was observed, with a reduced number of bladder tumours (-38%) in the 1.0 mL cranberry group compared to the water only control. The extract neither affected body weight gain nor caused other signs of toxicity [Prasain 2008].

Colon tumours were induced in male Fischer-344 rats by injection of azoxymethane at the age of 7 and 8 weeks. From week 4 the animals received either standard rodent feed, standard feed supplemented with 5 or 10% cranberries, or 2.5% and 5% cranberry juice for 42 weeks. A significant ($p<0.05$) reduction in the number of tumours, tumour size and the ratio of tumours/tumour bearing rat was observed in all cranberry groups as compared to control with the cranberries showing better effects than the juice. Activities of SOD and CAT increased in all cranberry groups [Sunkara 2009].

BALB/c nu/nu mice were inoculated s.c. with 5×10^6 human gastric SGC-7901 cancer cells pretreated with 0, 5, 10, 20 or 40 mg/mL of a dry cranberry extract (80% acetone). After 28 days, mean tumour diameter and weight of xenografts in the 10 and 20 mg cranberry groups were significantly lower than those in the control group ($p<0.05$ and 0.001 respectively). Tumour volume was also reduced ($p<0.05$ after 20 mg/mL). Mice did not develop any tumours with 40 mg/mL [Liu 2009].

An acidic ethanolic extract (yield 12.5%) was administered orally to male CD-1 mice at 200, 400 and 800 mg/kg b.w. daily for one week, concurrently with 200 mg/kg/day p.o. benzo[a] pyrene (B[a]P). Other groups received either no treatment, B[a]P 200 mg/kg alone or the cranberry extract alone at 800 mg/kg. B[a]P-induced increased frequency of micronucleated normochromatic erythrocytes was significantly ($p<0.05$) and dose-dependently reduced by all doses of the extract. The decrease in the index of polychromatic/normochromatic erythrocytes caused by B[a]P was significantly ($p<0.05$) improved by the highest extract dose [Madrigal-Santillan 2012].

The effects of freeze-dried cranberry powder were investigated against colitis-associated colon tumorigenesis induced by azoxymethane/dextran sulphate sodium in mice. The animals received a basal diet or the same diet containing 1.5% cranberries for 20 weeks. The supplementation resulted in a significant reduction of tumour incidence, multiplicity, burden, and average tumour size (all $p<0.05$) compared to the group on the basal diet. Gene and protein expression levels of pro-inflammatory cytokines IL-1β, IL-6, and TNF-α were significantly decreased (all $p<0.05$). Multiple signalling pathways/proteins related to inflammation, cell proliferation, apoptosis, angiogenesis and metastasis in the colon, such as COX-2, HO-1, NQO1, p-PI3K, PI3K, p-Akt, Akt, p21, p27, cyclin D1, CDK 4, p-Rb, EGFR, p53, cleaved caspase-3, cleaved PARP, VEGF, MMP-2, and MMP-9 were improved [Wu 2018].

An NDM fraction (MG 12,000–30,000) from cranberry juice inhibited the growth of tumours after i.p. inoculation of Balb/C female mice with Rev-2-T-6 lymphoma cells. The animals received 160 mg/kg i.p. the day after inoculation and 80 mg/kg/d for a further 2 weeks. A second group received half of these doses in the same regimen. Sixty days after inoculation 4 out of 5 mice without NDM developed tumours. In both NDM groups tumours were observed in none of the animals due to an increase in the immune response toward Rev-2-T-6 cells [Hochman 2008].

Oral administration to mice of a PAC fraction (DP 4-5, 250 µg/mouse, 6 days a week for 19 days) inhibited human oesophageal adenocarcinoma OE19 tumour growth by 67.5% via modulation of AKT/mTOR/MAPK signalling and induction of the autophagic form of LC3B [Kresty 2015].

Effects on lipids and glucose metabolism
Male Sprague-Dawley rats received either an atherogenic diet (HFC) or atherogenic diet supplemented with 2% or 5% cranberry powder for 6 weeks. Serum total cholesterol and LDL-cholesterol levels were not significantly different with the addition of cranberry powder. However, serum HDL-cholesterol level was significantly increased and serum protein carbonyl and TBARS levels significantly decreased in the 5% cranberry group compared with the HFC control (all $p<0.05$). Plasma FRAP value tended to be increased by cranberry powder [Kim 2008].

In Golden Syrian hamsters fed with a high-fat, high-cholesterol diet, addition of 2% cranberry concentrate powder (2-3.8% total phenolics, 0.3-1% total anthocyanins) to the diet for 20 weeks resulted in significantly reduced ($p<0.05$) liver weight to body weight ratio, heart rate, blood pressure and aortic esterified cholesterol concentrations. HDL- to VLDL- and HDL to LDL-cholesterol ratios remained unchanged [Kalgaonkar 2010].

The effect of a polyphenol-rich cranberry powder (CP) on metabolic parameters associated with metabolic syndrome was investigated in growing rats fed a high fructose diet (HFD, containing 58% fructose) or the HFD diet containing an additional 3.3, 6.6 or 33 g CP/kg for eight weeks. Fasting plasma glucose and triglycerides were reduced by the addition of CP to HFD. The AUC following an oral glucose tolerance test was 35–50% higher in animals fed the HFD vs. a starch-based control diet, and was decreased to control levels by the low or medium CP diet. CP tended to lower fasting plasma insulin. Homeostatic models of insulin resistance and β-cell function were lowest in animals fed low or medium CP diets ($p<0.05$). Rats on the control diet had a slightly higher food intake, final body weight and abdominal fat compared to animals on the other diets. Kidney weight was higher in the HFD group while CP decreased kidney weight to normal levels [Khanal 2010a].

A further study investigated the effect of cranberry pomace on selected metabolic parameters (increased fasting plasma insulin, cholesterol, and triacylglycerols (TAG), post-prandial plasma TAG homeostatic assessment models of insulin resistance and β-cell function) associated with high fructose feeding (58% by weight) in growing Sprague–Dawley rats. Addition of 1.5 or 3% extruded or un-extruded pomace to the high fructose diet for 8 weeks effectively ameliorated fasting plasma insulin, cholesterol, TAG level and insulin resistance. The best effects were observed with 3% extruded pomace [Khanal 2010b].

Male Sprague-Dawley rats received an atherogenic diet alone or supplemented with 5 or 10% freeze-dried cranberry powder for 6 weeks and were subsequently challenged with LPS (0.5 mg/kg i.p.) 15 hours prior to sacrifice. Mean serum HDL-cholesterol was significantly ($p<0.05$) higher in rats receiving 5% cranberry powder as compared to the atherogenic diet alone [Kim 2011b].

Male Fischer 344 rats were fed normal rodent chow with or without 2% cranberry powder from 6 to 22 months of age. Both groups displayed an age-related decline in basal plasma insulin concentrations, which was significantly ($p<0.05$) delayed by cranberry with a portal insulin concentration 7.6-fold higher at the end of the study, coupled with improved β-cell function. Supplementation led to increased β-cell glucose responsiveness during an oral glucose tolerance test, but insulin resistance values were similar in both groups. Total β-cell mass and expression of pancreatic and duodenal homeobox 1 and insulin within islets were enhanced, and insulin release from insulin-producing β-cells was increased [Zhu 2011].

Dietary supplementation of normal and obese C57BL/6 male mice with 2% of an ethanolic extract from cranberries (enriched in flavonoids) in addition to low-fat or high-fat diet resulted in an amelioration of insulin resistance and plasma lipid profile in obese mice, and a reduction of visceral fat mass. The adiponectin-AMPK pathway was identified as the main mediator of the improvement. In contrast, the reduced plasma atherogenic cholesterol observed in normal mice with cranberry supplementation was correlated to a downregulation of the hepatic cholesterol synthesis pathway [Shabrova 2011].

Daily treatment of ovariectomised rats with cranberry juice at 7 mg/kg for 8 weeks returned circulating levels of TC, triglycerides, HDL, non-HDL, and non-HDL/HDL cholesterol ratio close to normal values ($p<0.01$ to 0.001). Juice consumption improved endothelium-dependent relaxation in the aorta of the rats ($p<0.001$) by restoring phosphorylated eNOS levels, reversing the up-regulated levels of the renin–angiotensin system and normalising the elevated NAD(P)H oxidase expression and oxidative stress [Yung 2013].

Obese diabetic C57BL/KsJ-*db/db* mice received either a normal diet (control) or an atherogenic diet with or without the addition of 5 or 10% cranberry powder (120mg anthocyanins/100g; 2.6 g PACs/100g) for 6 weeks. Consumption of the atherogenic diet resulted in elevated serum total cholesterol and atherogenic index (AI), and reduced HDL-cholesterol. With 10% cranberry powder, mean HDL-cholesterol was increased close to control levels, whereas the AI remained higher than in the control rats. LPS-induced elevated serum insulin was lowered by more than 60% with both amounts of cranberry powder ($p<0.05$), and mean serum glucose level was reduced by approximately 19% with the lower amount ($p>0.05$). Mean activity of liver cytosolic glutathione peroxidase was significantly increased by LPS but found to have comparable values to without LPS in the 10% cranberry group ($p<0.05$). A reduction of approximately 89% in serum protein carbonyl was also observed at the higher amount ($p>0.05$) [Kim 2013].

The effects of consuming a diet enriched with 2% of a cranberry extract (88.8 mg PACs and 2.5 mg anthocyanins/g) for 28 days on the antioxidant status of non-obese mice and mice with monosodium glutamate–induced obesity were examined. Only in obese animals did the extract reduce the MDA content in erythrocytes and increase the activity of NAD(P)H:quinone oxidoreductase in the liver, which was accompanied by an increase in the corresponding mRNA levels. Catalase activity was raised in erythrocytes and the small intestine (all $p<0.01$) [Bousova 2015].

The effects of a cranberry extract (200 mg/kg p.o. for 8 weeks) were compared in C57Bl/6J male mice receiving either normal chow or a high fat-high sucrose (HFHS) diet to induce obesity. The extract significantly reduced HFHS-induced weight gain and visceral obesity ($p<0.05$) and decreased liver weight and triglyceride accumulation in association with blunted hepatic oxidative stress and inflammation. Insulin sensitivity improved, as revealed by improved insulin tolerance, lower homeostasis model assessment of insulin resistance and decreased glucose-induced hyperinsulinaemia during an oral glucose tolerance test. The extract lowered intestinal triglyceride content and alleviated intestinal inflammation and oxidative stress. The treatment markedly increased the proportion of the mucin-degrading bacterium *Akkermansia* in the gut microbiota [Anhe 2015].

In another study, the animals received the different diets for 3 weeks, then 8 weeks of treatment with the same extract as above. The extract did not reverse weight gain or fat mass accumulation in either diet group, but did fully reverse hepatic steatosis in the obese group, which was linked to upregulation of genes involved in lipid catabolism (e.g. PPARα, PPARγ; $p<0.001$) and downregulation of pro-inflammatory genes (e.g. COX2, NF-κB; $p<0.001$; TNFα $p<0.05$) in the liver. The extract also improved glucose tolerance and normalized insulin sensitivity in obese mice. The gut microbiota of obese mice significantly ($p<0.001$) changed in the cranberry group [Anhe 2017].

Male Wistar rats (120 days old) were fed either a standard diet (SD) or a high fat diet to induce obesity for 30 days. Both obese and normal rats subsequently received by gavage either 200 mg/kg cranberry extract/d (4 mg total polyphenols) or water as a control for a further 30 days. At the end of the study, body weights in the high fat + cranberry group were significantly lower ($p<0.05$) compared to high fat controls, and comparable to rats on the SD or cranberry-supplemented SD. Supplementation of the high fat diet with cranberry also resulted in less visceral fat, corticosterone, liver glucocorticoid sensitivity, cholesterol and triglycerides, and microsteatosis. High fat diet-increased lipid peroxidation in plasma and tissues, as well as higher protein carbonylation in liver and adipose tissue, was improved by the cranberry supplementation [Peixoto 2018].

Wistar rats received either a high fat diet (HFD) or a high fat diet supplemented with 3% unextruded cranberry pomace (UCP; with 32 mg/100 g anthocyanins and 59 mg/100 g flavonols) or 3% extruded cranberry pomace (ECP; no anthocyanins and 7.5 mg/100 g flavonols) for 8 weeks to study the effects on faecal fat excretion, liver index, lipid and carbohydrate metabolism, and the inhibition of oxidative stress due to HFD. Both supplements significantly improved the plasma antioxidant capacity and decreased lipid peroxidation ($p<0.05$). The addition of UCP significantly ($p<0.05$) increased faecal lipid excretion and decreased serum triglycerides [Bajerska 2018].

Hamsters were randomly assigned to either a high-cholesterol control diet (HCD), an HCD diet supplemented with 1% cranberry anthocyanins (CL) or an HCD diet supplemented with 2% cranberry anthocyanins (CH), for six weeks. Plasma total cholesterol and aorta atherosclerotic plaque decreased dose-dependently with increasing amounts of cranberry anthocyanins, whereas the excretion of both neutral and acidic sterols increased as compared to HCD ($p<0.05$ for CH). mRNA levels of intestinal genes of transporters, enzymes and proteins involved in cholesterol absorption and metabolism remained unchanged [Wang 2018].

Cranberry powder (4% PACs) at concentrations of 0.016% and 0.08% dose-dependently reduced fat accumulation in Caenorhabditis elegans ($p<0.05$) by 43% and 74%, respectively,

without affecting food intake, locomotor activities or body size. In mutant worms, the effects on different genes involved in lipogenesis were established in detail [Sun 2016].

Antimicrobial activity
Over 5 days eighty mice were infected on three occasions with *H. pylori* and two weeks later randomly allocated to four groups for various oral treatments: 0.5 mL of a cranberry juice daily for 30 days; triple therapy (amoxicillin 50 mg/kg, bismuth subnitrate 6.15 mg/kg and metronidazole 22.5 mg/kg, daily for 14 days), a combination of cranberry juice and triple therapy; or no treatment (control). The "clearance rate" for *H. pylori* was assessed after 24 hours, the eradication rate 4 weeks after treatment. The clearance rate was 80% in the cranberry group, 100% in the triple therapy group and 90% in the combination group compared to the control group. The eradication rate in the cranberry group was only 20% compared to 80% in the triple therapy group and 80% in the combination group.
In a prevention study, mice were treated with 0.5 mL of cranberry juice daily for 30 days and in the last 5 days (days 26-30) divided into four groups. One group was challenged orally with *H. pylori* on days 26, 28 and 30 (when the mice were not given juice), a second group with *H. pylori* suspended in cranberry juice, a third group with *H. pylori* 6 hours after receiving juice, and a control group with *H. pylori* but no cranberry juice. None of the mice were protected from infection and the numbers of colonizing bacteria were not reduced [Xiao 2003].

In a mouse model of mastitis, *S. aureus*-infected mammary glands were treated with either amoxicillin (0.005 mg/gland), an ethanolic extract of cranberry pomace (20 mg/gland) or a combination of both (0.005+20 mg/gland); the combination significantly ($p<0.05$) reduced bacterial counts in infected glands [Diarra 2013].

In BALB-c mice, intranasal instillation with a highly infective oropharyngeal *E. coli* strain resulted in 59.2% mortality after 48 hours. Mortality caused by the same strain pre-cultured with a dry extract (180 mg/mL PACs) was significantly ($p<0.01$) lower at 18.5%, and deaths occurred only during the first 24 hours due to increased bacterial clearance accompanied by a decreased inflammatory immune response and pulmonary neutrophil recruitment. Profuse neutrophil infiltration and severe alveolitis was observed in lungs challenged with non-exposed *E. coli*, whereas lung injury was significantly ($p<0.05$) attenuated in mice challenged with PAC-exposed *E. coli* [Margetis 2015].

Effects on drug metabolising enzymes
Male Wistar rats were treated orally with a standardized extract (containing 1.6 mg of anthocyanidins and 36 mg of PACs) in two dosage schemes (long-term: 14 days, 0.5 mg PACs/kg/d; short-term: 1 day, 1.5 mg PACs/kg). Long-term administration showed significantly ($p<0.05$) increased activities of hepatic CYP3A and CYP1A1 (by 38% and 34% respectively). Both treatments significantly ($p<0.05$) enhanced hepatic carbonyl reductase 1, UDP-glucuronosyl transferase and glutathione-S-transferase activities. No change in the activity of these enzymes was observed in the small intestine [Bartikova 2014].

Other effects
Male Wistar rats received a 90% methanolic extract from lyophilized cranberries (100 mg/kg p.o. for 10 days) and were challenged with doxorubicin (DOX; 15 mg/kg i.p. on day 7). DOX-induced bradycardia, ST segment depression and prolongation of ST and QT intervals in ECG were completely restored by the extract ($p<0.05$). The extract significantly inhibited DOX-provoked elevated myeloperoxidase activity, GSH depletion as well as accumulation of GSSG, MDA and protein carbonyls in cardiac tissues ($p<0.05$). The DOX-induced decreases of cardiac activities of SOD, glutathione peroxidase (GSH-Px) and glutathione reductase (GR) were significantly ($p<0.05$) improved. Treatment with the extract significantly ($p<0.05$) reduced the DOX-induced rise of serum LDH, creatine phosphokinase, creatine kinase-MB and troponin I level. Histopathological changes in cardiac tissues were also improved [Elberry 2010].

Unfit mares completed three graded exercise tests (GXTs) in a cross-over design after receiving either water (2 L) or cranberry extract (30 g in 2 L of water) 1 hour prior to testing with 7 days wash-out. Blood samples were taken prior to dosing (pre-exercise), at the end of each step of the GXT, at the end of the exercise and at 2, 5 and 30 min, 1, 2, 4 and 24 h post-GXT. No effect of treatment was observed on maximal oxygen uptake, run-time to fatigue, core temperature, total blood protein or haematocrit. The cranberry group had significantly ($p<0.05$) lower TNF-α mRNA expression than control [Liburt 2010].

A water-soluble cranberry extract (4.0% PACs) at 2 mg/mL enhanced innate immunity of *C. elegans* against *Vibrio cholerae* infection as shown by an elongation of survival time. Supplementation only slightly reduced the intestinal colonization of *V. cholerae*, but upregulated the expression of innate immune genes, such as clec-46, clec-71, fmo-2, pqn-5 and C23G10.1. In *C. elegans* mutants p38 MAPK signalling, insulin/IGF-1 signalling and HSF-1 were shown to be involved in the host immune response to the extract [Dinh 2014].

Male albino Wistar rats were intoxicated with CCl_4 (1.6 g/kg b.w. s.c., twice a week for 30 days). During this period one group was treated intragastrically with 7 mg/kg of a cranberry extract enriched in phenolics. The extract prevented changes in liver mitochondrial ultrastructure. The number of damaged mitochondria/100 mitochondria was 10.9 in untreated controls, 17.7 in intoxicated controls and 13.8 in the extract group ($p<0.05$ as compared to CCl_4 group) [Lapshina 2015].

Sprague-Dawley rats infected in the mouth with *Streptococcus mutans* consumed 56% sucrose with their feed and 5% sucrose with the drinking water. Topical treatment with a PAC fraction twice daily for 5 weeks led to the incidence and severity of smooth-surface caries being significantly reduced in the PAC group as compared with vehicle control, and 40-45% less severe smooth-surface lesions of dentin were observed (both $p<0.05$). The influence on the incidence and severity of sulcal-surface caries (at the dentin exposed level) was less [Koo 2010].

In a mouse model, oral administration of bovine reovirus type 3 resulted in systemic haemorrhage, diarrhoea and dehydration. Deaths occurred 3 to 4 days after inoculation. Co-inoculation of the virus with a 40% concentration cranberry juice cocktail (0.2 ml by gavage) revealed normal mucosa in the colon, intact goblet cells and dense faeces on histological analsysis [Lipson 2010].

Experimental periodontitis in female BALB-c mice induced by mixed infection with *Porphyromonas gingivalis* and *Fusobacterium nucleatum* resulted in distinct alveolar bone loss after 42 days, which was significantly ($p<0.05$) less after pretreatment of the two strains with NDM (MG > 12.000) from cranberry juice added to the drinking water [Polak 2013].

Pharmacological studies in humans

Anti-adherence activity
Following consumption of 140 mL of cranberry juice, urine samples from 15 out of 22 healthy volunteers showed significant ($p<0.05$) inhibition of adherence of a clinical isolate of *E. coli* to uroepithelial cells [Sobota 1984].

Isolates of *E. coli*, *Proteus* and *Pseudomonas* from patients with urinary tract infections (UTIs), were tested for adherence to urinary epithelial cells by four different techniques with a cranberry juice cocktail (CJC): pre-incubation of the bacteria with CJC; pre-incubation of the urinary epithelial cells with CJC; pre-incubation of the bacteria with urine collected from healthy individuals 2 hours after drinking 340 mL of CJC; and use of urinary epithelial cells collected from healthy individuals 2 hours after drinking CJC. Significant ($p<0.05$) anti-adherence activity of cranberry was demonstrated by each technique [Schmidt 1988].

In an open, controlled study 39 uropathogenic P-fimbriated *E. coli* isolates obtained from women with UTIs were incubated with urine collected from healthy women over a 12-hour period before and after consumption of 240 mL of a CJC. Urine collected after CJC consumption prevented adhesion of 80% of the 39 *E. coli* isolates and 79% of the 24 antibiotic-resistant isolates, while pre-consumption urine failed to prevent adhesion of any of the isolates. Anti-adherence activity was evident in the urine within 2 hours and for up to 10 hours after CJC consumption [Howell 2002].

In a double-blind, placebo-controlled, cross-over study 20 healthy volunteers were randomized to receive on separate occasions, with a wash-out period of at least 6 days, a single dose of 250 mL or 750 mL of a cranberry drink (containing 27% of cranberry juice concentrate) or a placebo drink. Compared to urine samples from the placebo group, urine collected the morning after consumption of the cranberry drink significantly and dose-dependently inhibited adherence of six uropathogenic *E. coli* strains from patients with UTIs to a human urinary epithelial bladder cell line [Di Martino 2006].

Eight volunteers consumed a preparation (36 or 108 mg cranberry) or placebo in a double-blind, randomised, cross-over trial with a wash-out period of at least 6 days between each regimen. Twelve hours after consumption, the first morning urine was collected, and four different *E. coli* strains were cultured in the samples. A significant ($p <0.001$) dose-dependent decrease in bacterial adherence of all strains to human T24 epithelial cells was seen after both doses of cranberry as compared to placebo [Lavigne 2008].

Urine of healthy women who had ingested cranberry extract (not further specified) significantly ($p<0.0001$) reduced the bacterial adhesiveness of two strains of *E. coli* (ATCC 25922, ATCC 35218) to HT1376 human bladder carcinoma cells (-50.9%). No changes were observed with placebo [Tempera 2010].

In a multi-centre, randomized, double-blind, placebo-controlled, cross-over study, 32 women consumed cranberry powder standardized to 18, 36 or 72 mg PACs or placebo. Urine samples collected from 1 to 6 hours as well as at 24 hours after consumption were tested for anti-adhesion activity in a mannose-resistant haemagglutination assay and with uropathogenic *E. coli* on human T24 epithelial cells. Significant ($p<0.001$) anti-adhesion activity was observed in the cranberry groups as compared to placebo. The inhibition dose-dependently increased with PAC equivalents and was prolonged until 24 h with 72 mg of PAC [Howell 2010].

Four male volunteers took 300 mg of a cranberry dry extract (50% ethanol soluble) twice daily for 7 days, with urine samples collected prior to and on days 3 and 7 of consumption. A significant reduction of the adhesion of a uropathogenic *E. coli* strain to T24 bladder cells was observed ($p<0.01$ at both time points) [Rafsanjany 2015].

After consumption of a single dose of cranberry juice cocktail (470 mL containing 0.04% PACs) or placebo by 12 healthy participants, in a cross-over study with a washout period of 1 week, urine was collected at 0, 0 to 3, 3 to 6, 6 to 9, 9 to 12, and 24 hours after consumption. The juice significantly ($p<0.05$) increased the anti-adhesion activity of uroepithelial cells against P-fimbriated *E. coli* compared with placebo [Mathison 2014].

In a similar randomized, double-blind, placebo-controlled, cross-over set-up, 10 volunteers ingested single doses of 450 mL of cranberry beverages or placebo. In a second experiment, 60 participants took two doses of the beverages, in the evening and the following morning. Urine samples were taken at baseline and 0–3, 3–6, 6–9 and 24 hours, and from 0 to 6 hours post-intervention, respectively. The mean anti-adhesion activity, as determined by the suppression of agglutination of mannose-resistant human red blood cells, following incubation with uropathogenic P-fimbriated *E. coli* was significantly ($p<0.05$) higher in cranberry groups as compared to placebo [Kaspar 2015].

In a prospective study, 72 patients with recurrent UTIs were randomized to take either cranberry capsules (2 x 60 mg PACs/d; n=36) or Lactobacillus capsules (2 x 400 million bacteria/d) as 'placebo' for 12 weeks. Compared to 'placebo', cranberry significantly reduced the urinary mean bacterial adhesion score (0.28 vs. 2.14; $p<0.001$) and biofilm formation ($p<0.01$) [Singh 2016].

After ingestion of 360 mg of extract (42.6% A-type PACs and 14.6% B-type PACs) and 200 mg of quercetin by healthy volunteers, urine samples were collected after 2 ,4, 6, 8 and 24 h. Changes in antiadhesive properties of uropathogenic E. coli to HAT-29 epithelial cells after treatment with the urine samples were strongest ($p<0.001$) in urine samples taken at 6–8 h. Metabolomic analysis revealed valeric acid- and valerolactone-derivatives in these samples [Peron 2017b].

Antimicrobial activity of urine

In a crossover study, 8 healthy volunteers received three successive regimens with a minimum 6 day wash-out period. Volunteers received with their evening meal either 3 capsules of cranberry PACS (36 mg each), 3 placebo capsules or 1 cranberry and 2 placebo capsules. Urine samples were taken 12 hours later (first urine of the day). A *C. elegans* model was used to evaluate the influence of the urine following cranberry intake on the ability of 4 different uropathogenic *E. coli* strains to kill the worms. Urine collected after intake of 108 mg cranberry PACs resulted in reduced mortality of the worms feeding on 3 of the *E. coli* strains. The effects were significant in 2 strains with type 1 pili and P-fimbriae ($p<0.001$) and another strain with type 1 pili but no P-fimbriae ($p=0.02$) as compared to the placebo-treated *E. coli* strains [Lavigne 2008].

A similar assay was used to evaluate the influence of urine following intake of cranberry powder standardized to 18, 36 and 72 mg PACs on the virulence of a uropathogenic *E. coli* strain. Pre-treatment of the bacteria with the urine of patients who took the cranberry preparations reduced the ability to kill worms as compared to untreated *E. coli*. The effect was most pronounced with 72 mg of PACs [Howell 2010].

Effects on urinary stone risk factors

Calcium oxalate is the most common type of urinary stone. In dietary excess, oxalate can be a causative factor in nephrolithiasis; cranberry juice contains moderately high levels. Urine samples collected from 5 healthy volunteers before and after taking the recommended dosage of cranberry concentrate tablets for one

week were analysed for oxalate and other components. Average oxalate levels in the urine were significantly ($p<0.01$) increased by 43.4%, from 29.0 to 41.2 mg/day over the 1-week period. While urinary pH dropped from 5.82 to 5.54 (not significant) and urinary calcium increased by 12.8% (not significant). On the other hand, there were significant increases in urinary levels of the stone formation inhibitors magnesium (+ 47.3%, $p<0.02$) and potassium (+ 67.5%, $p<0.006$) [Terris 2001].

In two other cross-over studies [Keßler 2002, Gettman 2005], involving daily intake of cranberry juice preparations, urinary excretion of oxalate also increased. On the other hand, in a further cross-over study, involving 20 healthy men, daily consumption of 500 mL of cranberry juice significantly ($p<0.001$) reduced urinary excretion of oxalate [McHarg 2003]. This contradiction reflects the complexity of the processes demonstrated by Terris, and other effects that may minimise the risk of urinary stone formation [Gettman 2005].

In a cross-over study on the effect of fruit juices on risk factors associated with kidney stone formation, 12 healthy male subjects adhered to a standard diet (including beverages) for 5 days during each of four study phases. On the fifth day in each phase they consumed 330 mL of juice (cranberry or plum or blackcurrant) diluted to 750 mL with mineral water or, as the control, 750 mL of mineral water. Compared to the control, cranberry juice significantly lowered the urinary pH from 6.35 to 6.18 ($p\leq0.05$) and increased the excretion of oxalic acid ($p\leq0.05$). Uric acid excretion was unchanged, but the "relative supersaturation value" for uric acid increased from 0.67 to 0.99 ($p\leq0.05$), which could increase the risk of uric acid stone formation [Keßler 2002].

In a further cross-over study, 20 healthy male students without previous history of kidney stones were randomized to receive daily for 2 weeks either 500 mL of cranberry juice (oxalate 86 mg/L, ascorbic acid 300 mg/L) diluted with 1500 mL water, or 2000 mL water only as control, and after a 2-week wash-out the alternative treatment. Analysis of urine samples collected at baseline and on day 14 of each phase revealed that cranberry juice significantly reduced the excretion of oxalate ($p<0.001$) and phosphate ($p<0.014$), and increased citrate excretion ($p = 0.001$) as compared to the control [McHarg 2003].

Healthy subjects (n=12) and calcium oxalate stone formers (n=12) were randomized in a cross-over study to receive a controlled metabolic diet and either 1 litre cranberry juice cocktail (CJC, 27% cranberry juice) or 1 litre deionized water daily for 7 days and, after a 3-week wash-out, the alternative treatment. Blood samples and 24-hour urine collections on the last 2 days of each phase were analysed for stone risk factors and serum chemistry. Since no significant differences were evident between normal subjects and stone formers in response to the CJC the groups were combined. Cranberry juice significantly increased urinary calcium (from 154 to 177 mg/day, $p = 0.0008$) and urinary oxalate (from 26.4 to 29.2 mg/day, $p = 0.04$), while urinary uric acid decreased (from 544 to 442 mg/day, $p<0.0001$), and urinary pH dropped from 5.97 to 5.67 ($p = 0.0005$). Although cranberry juice had a mixed effect, the authors concluded that overall it probably does not substantially affect the risk of stone formation [Gettman 2005].

Antioxidant and anti-inflammatory activity
The effect of cranberry consumption on plasma antioxidant capacity was evaluated in 9 healthy female volunteers. After an overnight fast they consumed with wash-out periods of one week, 500 mL of cranberry juice or 500 mL of a 9% sucrose solution. Blood samples were collected 5 minutes before and 0.5, 1, 2 and 4 hours after intake of the beverage. Consumption of the juice, resulted in a significant increase in plasma antioxidant capacity ($p<0.001$), mainly attributable to an increase in plasma vitamin C concentration ($p<0.001$) rather than a modest increase in total phenolics ($p<0.05$ after 1 hour) [Pedersen 2000].

In 20 healthy female volunteers, consumption of 750 mL/day of cranberry juice for 2 weeks did not alter blood or cellular antioxidant status or biomarkers of lipid status pertinent to heart disease as compared to placebo or baseline. The juice had no effect on basal or induced oxidative DNA damage [Duthie 2006].

After a single dose (240 mL) of high fructose corn syrup (HFCS) or the syrup supplemented with cranberry juice, plasma antioxidant capacity, glucose, triglycerides and ascorbate were measured 6 times over 7 hours in 10 normal volunteers. The decrease in plasma antioxidant capacity after HFCS at all time points was improved with cranberry juice [Vinson 2008].

In a randomized, double-blind, placebo-controlled, cross-over trial, 12 participants consumed a single dose of cranberry juice cocktail or placebo with a washout period of 1 week. Blood was collected at 0, 2, 4, 8, and 24 hours after consumption. The juice significantly ($p<0.05$) increased GSH concentrations and SOD activity compared with placebo at 24 hours. No significant differences were observed in GSSG, GSH:GSSG ratio, CRP, NO production and oxidative damage of DNA. IL-4 was significantly ($p<0.05$) reduced after 4 hours whereas other cytokines remained unchanged [Mathison 2014].

In a double-blind, randomized, placebo-controlled study, 16 male members of a rowing team received two capsules per day for 6 weeks of either cranberry extract (n=9) or placebo (n=7). Each cranberry capsule contained 360 mg of cranberry extract (10% PACs; equivalent to 4320 mg cranberry fruit). The participants performed a 2000 m rowing test at the beginning and at the end of the supplementation. Blood samples were taken prior to each exercise test, one minute after the test, and after a 24-h recovery period. The levels of hepcidin, IL-6, TNF-alpha, ferritin, iron, soluble transferrin receptor and myoglobin were determined, along with total iron-binding capacity, unbound iron-binding capacity and total antioxidant capacity (TAC). At the end of the study period, athletes in the cranberry group had significantly higher resting, post-exercise and post-recovery levels of TAC than controls [Skarpanska-Stejnborn 2017].

Overweight or obese volunteers (n=78; BMI 27–35) ingested 450 mL of a low calorie, high polyphenol cranberry extract beverage or placebo daily for 8 weeks in a randomized, double-blind, placebo-controlled trial. Blood and urine samples were collected at baseline and after 8 weeks. Compared to placebo, a single cranberry dose at baseline lowered endothelin-1 and elevated NO, the GSH:GSSG ratio and interferon-γ ($p<0.05$). After 8 weeks of cranberry intervention, fasting C-reactive protein was significantly ($p<0.05$) lower as compared to placebo. No effects on any other inflammatory biomarkers were seen [Chew 2019].

Investigations on possible interactions
A number of cases of suspected interaction between cranberry consumption and the anticoagulant warfarin have been reported since 1999 [UK Medicines and Healthcare Products Regulatory Agency 2003, 2004, Suvarna 2003, Zikria 2010, Hamann 2011, Haber 2012]. From assessment of the data available in several reports none of the cases was considered to plausibly demonstrate that loss of anticoagulant control was caused by cranberry juice [Bussey 2006, Greenblatt 2006a,b]. Additionally, in several case reports excessive amounts of juice or sauce were ingested (0.4-2 L/d for 1 week to 1 month) [Zikria 2010, Haber 2012].

In a study investigating the effect of cranberry juice on CYP2C9, the NSAID flurbiprofen was used as a surrogate for S-warfarin, since both drugs are metabolized almost exclusively by CYP2C9. In human volunteers, clearance of a single 100 mg dose of flurbiprofen was not significantly changed by 230 mL of cranberry juice administered the night before and 30 minutes before the flurbiprofen [Greenblatt 2006b].

In an open, randomized, 3-way cross-over study, 12 healthy male volunteers received single oral 200 mg doses of cyclosporine (as surrogate for R-warfarin), with either 240 mL of cranberry juice or water, under fasting conditions and multiple whole blood samples collected up to 36 hours. The juice had no significant effect on the overall disposition of cyclosporine, the ratios of least squares means for AUC_t, AUC_{inf}, and C_{max} for cyclosporine were 95.0%, 93.4%, and 95.2% respectively [Grenier 2006].

In another study, 10 healthy volunteers took 3 × 200 mL of cranberry juice or water daily for 10 days. On day 5 they ingested simultaneously 10 mg of racemic warfarin, 1 mg of tizanidine and 0.5 mg of midazolam as probe drugs for CYP2C9, CYP1A2 and CYP3A4 enzymes respectively. The juice did not change the anticoagulant effect of warfarin nor inhibit the activities of CYP2C9, CYP1A2 or CYP3A4 [Lilja 2007].

The effect of cranberry juice on prothrombin time, as assessed by the international normalized ratio (INR), was investigated in a randomized, double-blind study. Seven patients with atrial fibrillation, on a stable dose of warfarin for 3 months, were randomized to consume 250 mL of cranberry juice daily for 7 days then, after a wash-out period of 7 days, placebo for 7 days, or vice versa. No significant interaction between cranberry juice and warfarin was evident; the INR did not change significantly from baseline and differences between the cranberry and placebo groups were not significant [Li 2006].

In a randomized, double-blind study, patients on warfarin with a history of stable anticoagulation were given 230 mL/d of cranberry juice (n = 14) or placebo (n = 16) for 2 weeks. Daily intake of cranberry juice by patients on warfarin therapy appeared to have no significant effect on the INR compared to placebo [Ansell 2008].

A randomized, open-label, cross-over study demonstrated that although cranberry did not alter warfarin *pharmacokinetics* or plasma protein binding it could alter the *pharmacodynamics* of warfarin in certain individuals, depending on genetic factors. Variability in warfarin response has been attributed by some authors to genetic variability of *CYP2C9** (cytochrome P450 2C9) and *VKORC1* (vitamin K epoxide reductase subunit 1) genes. The study involved healthy male subjects of known, varying genotype with respect to *CYP2C9* and *VKORC1*. From 16 subjects randomized to one of three treatments, 12 completed the study (4 per treatment arm). The volunteers received a single high dose of 25 mg of racemic warfarin orally either alone or after 2 weeks of pretreatment with cranberry juice concentrate in capsules (2 × 500 mg three times daily, corresponding to 57 g fresh fruit per day) or garlic tablets. Administration of the cranberry or garlic preparations continued for a further 7 days when the last blood sample was drawn. Following a wash-out period of 2 weeks, the subjects crossed over to the other treatments.

Cranberry increased the INR AUC by 30%. The mean INR_{max} attained after warfarin and cranberry was 2.8 compared to 2.6 after warfarin alone [Mohammed Abdul 2008].

In an open, cross-over study with a wash-out period of at least 2 weeks, 8 healthy volunteers were given in one phase a single 25 mg diclofenac tablet with 180 mL of water and in the other phase pretreated for 5 days with 180 mL cranberry juice drink (containing 27% cranberry) twice a day and on day 6 administered a 25 mg diclofenac tablet and a further 180 mL cranberry juice. No significant differences were observed in t_{max}, C_{max} or AUC0-∞ of diclofenac following cranberry consumption [Ushijima 2009].

Possible interactions between amoxicillin or cefaclor and consumption of cranberry juice were investigated in 18 healthy women receiving a single oral dose of amoxicillin (500 mg and 2 g) with or without 240 mL of cranberry juice cocktail in a cross-over design. Another test was conducted with 500 mg cefaclor with or without 360 mL cranberry juice cocktail. Concurrent use of cranberry juice had no significant effects on the extent of oral absorption or renal clearance of amoxicillin and cefaclor. However, T_{max} of amoxicillin was significantly higher with the juice (0.75 hours with 500 mg and 1 hours with 2 g; $p<0.01$). For cefaclor a significant decrease in C_{max} ($p< 0.01$) and a longer T_{max} ($p< 0.05$), but no difference in the mean AUC with concomitant use of cranberry juice was observed [Li 2009].

Healthy volunteers (n=16) received every 15 minutes 3 x 240 mL of a concentrated juice or water and 5 mg midazolam with the third portion. Ten blood samples were taken during the 12 hours after the last dose. As compared to water, the concentrate significantly increased the geometric mean AUC0-∞ of midazolam by 30% ($p<0.001$), decreased the geometric mean AUC0-∞ ratio of 1´-hydroxymidazolam/midazolam by 40% ($p<0.001$) and had no effect on geometric mean terminal half-life. This indicated inhibition of enteric, but not hepatic CYP3A-mediated first-pass metabolism of midazolam [Ngo 2009].

In a similar study, 16 volunteers followed the same administration scheme of cranberry concentrate, but took 10 mg warfarin and 10 mg vitamin K concomitantly with the last dose of concentrate. Relative to water, the juice decreased the geometric mean S-to-R-warfarin Cmax ratio significantly ($p<0.05$) by 11%. No significant differences were detected between water and the concentrate with respect to the geometric mean AUC0–∞ ratio and terminal half-life of S/R-warfarin [Ngo 2010].

In a randomized, double-blind, placebo-controlled trial, 30 patients on stable warfarin anticoagulation consumed 240 mL cranberry juice cocktail (n=14) or 240 mL placebo beverage (n=16) daily for 2 weeks. INR values and plasma levels of R- and S-warfarin were measured during the intervention and a 1-week follow-up period. The groups did not differ significantly in mean plasma R- and S-warfarin concentrations. Four patients in each group developed minimally elevated INR (3.38-4.52) during the treatment period. Mean INR differed significantly ($p<0.02$) only on treatment day 12 [Ansell 2009].

Ten patients on stable warfarin therapy received 240 mL of cranberry juice twice daily for 7 days. No differences in the mean prothrombin time between baseline and at days 2, 6 and 8 were observed [Mellen 2010].

Other effects

A randomized, double-blind, placebo-controlled intervention in 45 healthy volunteers compared a low calorie cranberry beverage (CB; 450 mL/d; n = 22) with a placebo beverage (PB; n = 23) for 10 weeks on health outcomes related to cold and influenza symptoms. A significant ($p<0.001$) increase in the proliferation index of γδ-T cells *ex vivo* was observed in the CB group as compared to placebo. The total incidence of colds and

* As a convention, these designations are italicised when referring to genes (CYP2C9 is the gene that encodes the enzyme CYP2C9).

influenza did not differ between the groups, but the proportion of the total number of symptoms was significantly (p = 0.031) lower in the CB group. Levels of cytokines (IL-1α, IL-1β, IL-13, MIP-1β, TNF-α) in peripheral blood mononuclear cell did not differ between the groups, but IFN-γ was significantly higher in the CB group [Nantz 2013].

In a randomized, double-blind, controlled, cross-over trial, flow-mediated dilation (FMD), blood pressure, pulse wave velocity and augmentation index were investigated in ten healthy males at baseline, 1, 2, 4, 6 and 8 h post-consumption of cranberry juices containing 409, 787, 1238, 1534, and 1910 mg of total cranberry (poly)phenols (TCP), or a control drink. Dose-dependent increases in FMD were observed at all time-points with a peak at 4 h and maximal effects with 1238 mg TCP. No significant changes were seen in blood pressure, pulse wave velocity and augmentation [Rodriguez-Mateos 2016].

Urine and faecal samples were collected from 10 healthy individuals before and at the end of intake of 42 g/d of sweetened dried cranberries for 2 weeks. In the urinary proteome, 22 proteins changed significantly (p<0.05) from pre- to post-samples. Of 25 proteins found in all subjects, the vesicular integral membrane protein, the phosphoinositide 3-kinase interacting protein 1 and uromodulin significantly decreased (p=0.001). Changes in the faecal microbiome were a decrease in the *Firmicutes:Bacteroidetes* ratio in 7 of 10 volunteers, an increase in *Akkermansia* bacteria, increases in commensal bacteria and decreases or the absence of bacteria associated with negative health effects [Bekiares 2018].

Overweight or obese volunteers (n=78; BMI 27–35) underwent 3 oral glucose tolerance challenges (prior to intervention, after a single dose of verum or placebo in week 0 and in week 8) during a randomized, double-blind, placebo-controlled trial. The participants ingested 450 mL/d of a low calorie, high polyphenol cranberry extract beverage or placebo for 8 weeks. Blood and urine samples were collected at baseline, after 8 weeks and during the challenges. The cranberry beverage significantly (p<0.05) reduced serum insulin and increased HDL cholesterol compared to placebo [Chew 2019].

High-molecular-weight NDM (MW <12000) from cranberry juice concentrate in a mouthwash (3 mg/mL) was evaluated by colonization of 40 children with *Streptococcus mutans*. The children rinsed their mouth one minute daily for 30 days with 5 mL of the preparation or a placebo solution. The mean plaque and salivary scores after 30 days were significantly decreased with the preparation (p<0.0001 for both scores as compared to baseline; p<0.008 and 0.007, respectively, as compared to placebo) [Gupta 2015].

Epidemiological data
An evaluation of US data for adults (n=10334) showed significantly (p<0.015) lower levels of C-reactive protein for consumers of cranberry juice cocktails (average consumption 404 mL/2 days) and trends towards lower weight and waist circumferences, levels of BMI, fasting glucose, insulin, total cholesterol and triglyceride values [Duffey and Sutherland 2015].

Clinical studies

See Table 1 for a brief summary of clinical studies.

Prevention of bacteriuria and symptomatic urinary tract infections (UTIs)

In a randomized, double-blind, placebo-controlled study, 153 volunteers (women; mean age 78.5 years) were randomized to 300 mL of a commercial cranberry beverage or a placebo drink daily for 6 months. Urine samples were tested at baseline and at monthly intervals for 6 months for bacteriuria and white blood cells (an indication of pyuria). For subjects in the cranberry group, the odds of bacteriuria ($\geq 10^5$ organisms/mL) with pyuria were only 42% of the odds in the placebo group (p = 0.004) and, for those with bacteriuria-pyuria in the previous month, the odds of remaining so were only 27% of the odds in the placebo group (p = 0.006) [Avorn 1994].

In a randomized, double-blind, placebo-controlled cross-over study, women (28-44 years) with a history of recurring UTIs and with a symptomatic UTI treated with antibiotics on commencement were randomly assigned to one of two groups. Treatment began with either 400 mg of a cranberry extract or placebo daily for 3 months, followed by the alternative treatment for a further 3 months. Out of 19 subjects, 10 completed the study (4 taking cranberry first, 6 placebo first) and among these 10 a total of 21 UTIs were recorded, only 2 being for non-*E. coli* bacteria. Cranberry was significantly (p<0.005) more effective than placebo in reducing the occurrence of UTIs; only 6 occurred during the 3 months of cranberry treatment compared to 15 during 3 months on placebo [Walker 1997].

To compare cranberry extract with low-dose trimethoprim for prevention of recurrent UTIs, women aged 45-93 years with two or more antibiotic-treated UTIs in the previous 12 months were randomized to 500 mg cranberry extract (not further specified; n = 69) or 100 mg of trimethoprim (n = 68) daily for 6 months. Antibiotic-treated UTIs were experienced by 39 of the 137 participants, with no significant difference between the numbers in each group (25 in the cranberry group, 14 in the trimethoprim group) or the median times to first recurrence (84.5 days in the cranberry group, 91 days in the trimethoprim group [McMurdo 2009].

A double-blind, non-inferiority trial compared 12-months of prophylactic treatment with 480 mg trimethoprim-sulfamethoxazole (TMP-SMX) or 2 x 500 mg cranberry extract daily in premenopausal women with recurrent UTIs (n=221). After 12 months, the mean number of patients and the proportion of patients with at least 1 symptomatic UTI was higher in the cranberry group than in the TMP-SMX group (4.0 vs. 1.8; p=0.02) and 78.2% vs. 71.1%, respectively. Median time to the first UTI was 4 months for the cranberry group and 8 months for the TMP-SMX group. After 1 month, in the cranberry group, 23.7% of faecal and 28.1% of asymptomatic bacteriuria *E.coli* isolates were TMP-SMX resistant, whereas in the TMP-SMX group, 86.3% of faecal and 90.5% of asymptomatic bacteriuria *E. coli* isolates were TMP-SMX resistant [Beerepoot 2011].

A controlled, double-blind trial was conducted in children with recurrent UTIs, 85 were aged 1 month to 1 year and 107 were aged from 1 to 13 years. As prophylaxis, the children received 0.2 mL/kg of a syrup containing 3% cranberry extract (n=117) or 0.2 mL/kg of a syrup containing 8 mg/mL trimethoprim (n=75) for one year. The cumulative rate of UTIs in infants treated with trimethoprim was 28% as compared to 35% with cranberry. In children over 1 year the rate was 35% with trimethoprim and 26% with cranberry [Fernández-Puentes 2015, Uberos 2015].

To evaluate the effectiveness of cranberry in the prophylaxis of UTIs, 150 women (21-72 years) were randomized to one of three treatments for a year (n = 50/group): cranberry juice + placebo tablets, placebo juice + cranberry tablets (not further specified), or placebo juice + placebo tablets. Tablets were taken twice daily, 250 mL of juice three times daily. All the subjects had experienced at least two UTIs during the previous year. The number of patients experiencing at least one symptomatic UTI

per year decreased significantly in both the cranberry juice and cranberry tablet groups (both $p<0.05$), to 10 patients (20%) and 9 patients (18%) respectively, compared to 16 patients (32%) in the placebo group [Stothers 2002].

In a randomized, double-blind, placebo-controlled study 376 elderly, hospitalized patients (mean age 81 years) were administered 300 mL of cranberry juice daily (n = 187) or placebo (n = 189) and followed up for 35 days or until hospital discharge. A total of 21 out of 376 participants (5.6%) developed a symptomatic UTI, 7/187 in the cranberry juice group and 14/189 in the placebo group. As a secondary outcome, there were significantly ($p = 0.027$) fewer infections with *E. coli* in the cranberry group [McMurdo 2005].

The effects of cranberry on asymptomatic bacteriuria in pregnancy were assessed in a double-blind, placebo-controlled study in 188 women at less than 16 weeks of gestation. The women were randomized to a 240 mL cranberry juice cocktail (CJC; 27% cranberry juice) three times daily (group A), CJC at breakfast then placebo at lunch and dinner (group B), or placebo three times daily (group C). Of the 188 women randomized, 73 withdrew (mainly due to gastrointestinal upset). In all there were 27 UTIs in 18 of the women, of which 6 were in 4 of the group A women, 10 in 7 of the group B women and 11 in 7 of the group C women. There were reductions in the frequency of asymptomatic bacteriuria and all UTIs, by 57% and 41% respectively, in women who took multiple daily doses of CJC when compared to placebo only [Wing 2008].

In a randomized, double-blind, placebo-controlled trial, 319 women with acute UTI received 240 mL of a low-calorie cocktail containing 27% cranberry juice (112 mg PACs per dose) or placebo twice daily for 6 months. Participants were followed up until a second UTI or for 6 months. An overall recurrence rate of 16.9% (20% for verum and 14% for placebo) with no significant difference between the groups was found [Barbosa-Cesnik 2011].

In a randomized, double-blind, placebo-controlled trial, children (n=255; mean age 4 years) treated for UTI were randomized to either cranberry juice (n=129) or placebo (n=134) for 6 months. Twenty children (16%) in the cranberry group and 28 (22%) in the placebo group had at least 1 recurrence of UTI, with no differences in timing between the first recurrences. Episodes of UTI were 27 and 47 in the cranberry and placebo groups, respectively, and the UTI incidence density per person-year at risk was 0.16 episodes lower in the cranberry group ($p=0.035$). The children in the cranberry group had significantly ($p<0.001$) fewer days on antibiotics [Salo 2012].

Children (n=40; 5 to 18 years; at least two non-febrile UTIs in the year before) were randomized to either 2 mL/kg/day juice containing 37% PACs (verum) or the same amount without PACs (placebo) for one year in a double-blind study. In the verum group, 5 UTIs occurred in 5 patients as compared to 15 UTIs in 8 patients in the placebo group. In the 13 and 14 patients who completed the study, respectively, an average incidence of UTIs of 0.4/patient after verum and 1.5/patient after placebo ($p=0.045$) was observed [Afshar 2012].

A randomized, double-blind, placebo-controlled study involved 213 patients suffering from multiple UTI relapses. Patients consumed either 125 mL cranberry juice (40 mg PACs/dose; n=107) or a placebo beverage (n=106) once daily for 24 weeks. Only in the subgroup of females above 50 years was a significantly ($p = 0.0425$) lower rate of relapse of UTIs between groups observed [Takahashi 2013].

In a prospective, randomized, double-blind, placebo-controlled study, 171 multiple sclerosis outpatients with urinary disorders (pollakiuria, urgency, dysuria and/or urinary incontinence) received either cranberry powder for oral solution (18 mg PACs) twice daily or placebo for 1 year. No differences in the time to the first symptomatic UTI across the year, the 1-year UTI rate and the number of UTIs in each patient were observed between the groups [Gallien 2014].

Long-term care facility residents (n=928, mean age 84) with different UTI risk at baseline received cranberry capsules (500 mg preparation; 9 mg PACs) twice daily for 12 months or placebo in a double-blind, randomized, multicentre trial. In participants with high UTI risk at baseline (n = 516), the incidence of UTI was significantly ($p=0.04$) lower with verum (n=253) than with placebo (n=263). No difference in UTI incidence between cranberry (n=205) and placebo (n=207) was found in participants with low UTI risk (n = 412) [Caljouw 2014].

Patients undergoing eligible gynaecological surgery (n=160) were enrolled in a randomized, double-blind, placebo-controlled trial on the efficacy of cranberry in preventing UTIs after surgery with catheter placement. The participants received 2 cranberry capsules (each equivalent to 236 mL juice) or placebo twice daily for 6 weeks after surgery. The occurrence of UTIs was significantly ($p<0.008$) lower in the cranberry group compared with placebo [Foxman 2015].

Women with a history of UTIs (n=182) were administered for 6 months either 2 x 250 mg/d cranberry powder capsules (0.56% PACs; n=89) or placebo capsules (n = 93) in a randomized, double-blind, placebo-controlled trial. The number of UTIs during the observation period was significantly lower in the cranberry group (10.84%) compared to placebo (25.81%) and the time to the first UTI significantly longer (both $p= 0.04$) [Vostalova 2015].

A randomized, double-blind, placebo-controlled trial compared the effects of cranberry in 55 uncircumcised boys and 12 circumcised boys with histories of uncomplicated UTI. The uncircumcised boys were divided into group 1 (n = 28) taking 120 mL/d cranberry juice and group 2 (n = 27) taking placebo juice for 6 months. The circumcised boys in group 3 (n=12) also received placebo juice. At the end of treatment, the incidences of bacteriuria, mainly caused by *E. coli*, were 25%, 37% and 33.3% in groups 1, 2 and 3, respectively, significant ($p<0.05$) between group 1 and both other groups [Wan 2016].

The effects of cranberry capsules (36 mg PACs) on bacteriuria with pyuria were studied in a randomized, double-blind, placebo-controlled trial in elderly women (n=185; over 65 years). Patients with or without bacteriuria with pyuria at baseline took 2 capsules/day (equivalent to appr. 600 mL of cranberry juice) or placebo for 1 year. No significant difference in the presence of bacteriuria with pyuria between the treatment group vs. control (29.1% vs. 29.0%) or in the number of symptomatic UTIs (10 in the treatment group vs. 12 in the control group) or other secondary outcomes was observed [Juthani-Mehta 2016].

In a randomized, double-blind, placebo-controlled, multicentre trial, women with a history of a recent UTI were assigned to the intake of 240 mL of a cranberry beverage (119 mg PACs; n = 185) or a placebo beverage (n = 188) daily for 24 weeks. In the cranberry group 39 UTI episodes were observed as compared to 67 episodes in the placebo group ($p=0.016$). Clinical UTI with pyuria was significantly ($p=0.037$) reduced with verum. The time to UTI with culture positivity did not differ significantly between groups [Maki 2016].

In a randomized, double-blind, placebo-controlled trial, 124 men aged over 45 years, with PSA levels <2.5 ng/mL and an IPSS ≥8 received 250 or 500 mg dry cranberry powder or placebo for 6 months. At the end of the trial both cranberry groups showed a dose-dependent effect with significant lowering of the IPSS (–3.1 and –4.1 in the 250- and 500 mg groups, p=0.05 and p<0.001, respectively) compared to placebo (–1.5). Significant differences in the maximum and average urinary flow rate, post-void residual volume and bladder voided volume were observed in the 500 mg group compared to baseline (p<0.018, 0.040, 0.027 and 0.014, respectively) [Vidlar 2016].

In a randomized, placebo-controlled, double-blind study, elderly women with a hip fracture (n=227) were treated with 2 capsules daily of either placebo or cranberry (each containing 550 mg cranberry powder; 4.19 mg PACs), from admission until 5 days postoperatively. No differences were observed between the groups in the propor-tion of patients with hospital-acquired postoperative positive urine cultures [Gunnarsson 2017].

Patients with recurrent UTIs (n=72) were randomized in a prospective study to take cranberry capsules (2 x 60 mg PACs/d; n=36) or *Lactobacillus* capsules (2 x 400 millions bacteria/d) for 12 weeks. A significant (p<0.001) decrease in recurrent UTIs was observed after the cranberry preparation (33%) as compared to the *Lactobacillus* (89%). Mean burning micturition score (0.19 vs. 1.47), microscopic pyuria score (0.36 vs.2.0) and bacterial growth in urine were also significantly reduced (all p< 0.001) [Singh 2016].

One month after discharge following ileal conduit diversion, patients either started to take cranberry extract (n=20; 2 x 400 mg with 1.8% PACs daily) for 3 months, or received training about avoidance of UTIs (n=20), or were left without any intervention (n=20). No significant differences in the groups were observed in UTI symptoms such as increased body temperature, leukocytosis and increased CRP levels. Flank pain score was significantly (p<0.05) lower in the experiment groups (cranberry and training) at weeks 13 to 16. Only one patient in the cranberry group (5%) and 8 patients (40%) in the control group developed urinary tract infections [Temiz 2018].

TABLE 1 CLINICAL STUDIES WITH CRANBERRY PREPARATIONS

First Author Year	Study design	Type of patient No. of patients (test/control)	Daily medication Duration of treatment	Outcome for cranberry group (in comparison with placebo or ref. drug group)
Prevention of bacteriuria and symptomatic urinary tract infections (UTIs)				
Fernández-Puentes 2015	R / D-B comparison with ref. drug	Children 1 month-1 year: 85 Children 1-13 years: 107	0.2 mL/kg syrup with 3% Cranberry extract (n=117) 0.2 mL/kg syrup with 8 mg/mL Trimethoprim (n=75)	In children under 1 year cranberry inferior to trimethoprim. In children over 1 year cranberry not inferior to trimethoprim.
Beerepoot 2011	R / D-B comparison with ref. drug	Women, 23-46 years, with recurrent UTIs: 221 (110/111)	2 x 500 mg Cranberry extract 480 mg Trimethoprim-sulfamethoxazole 12 months	Cranberry extract less effective at prevention of UTIs, but did lead to less resistant E. coli isolates.
McMurdo 2009	R / D-B comparison with ref. drug	Women, 45-93 years, with history of UTIs: 137 (69/68)	500 mg Cranberry extract 100 mg Trimethoprim 6 months	No significant difference between cranberry and trimethoprim in incidence of UTIs or time to recurrence.
Gunnarsson 2017	R / D-B / P-C	Elderly women (mean 83 years) with hip fractures: 144 (75/69)	2 x 550 mg Cranberry powder with 4.19 mg PACs Placebo capsules From admission to 5 days postoperatively	No significant difference in postoperative positive urine cultures.
Maki 2016	R / D-B / P-C	Women (mean 41 years) with recurrent UTIs: 373 (185/188)	240 mL Cranberry drink 240 mL Placebo drink 24 weeks	Significantly less UTIs (p=0.016) and clinical UTIs with pyuria (p=0.037).
Vidlar 2016	R / D-B / P-C	Men (mean 53 years) with PSA levels <2.5 ng/mL and IPSS ≥8: 124 (43/38/41)	250 mg dry Cranberry powder 500 mg dry Cranberry powder Placebo 6 months	Significantly lower IPSS vs. placebo (250 mg p=0.05; 500 mg p<0.001). Significant differences in maximum and average urinary flow rate, post-void residual volume, bladder voided volume (500 mg vs. baseline; p<0.018, 0.040, 0.027 and 0.014, respectively).
Juthani-Mehta 2016	R / D-B / P-C	Elderly women (mean 86.4 years); 185 (92/93)	2 Cranberry capsules with 36 mg PACs 2 Placebo capsules 1 year	No significant differences in bacteriuria with pyuria as well as secondary outcomes.
Wan 2016	R / D-B / P-C	Uncircumcised (55) and circumcised (12) boys, 6 to 18 years, with histories of uncomplicated UTIs: 67 (28/27/12)	120 mL Cranberry juice 120 mL Placebo juice 120 mL Placebo juice 6 months	Significantly lower incidence of bacteriuria in cranberry group as compared to both placebo groups (p<0.05).

Vostolova 2015	R / D-B / P-C	Women (mean 37 years) with recurrent UTIs: 182 (89/93)	2 x 250 mg Cranberry dry fruit capsules with 0.56% PACs 2 x Placebo capsules 6 months	No. of UTI episodes significantly lower and time to the first UTI significantly longer (both p=0.04).
Foxman 2015	R / D-B / P-C	Women, 23-88 years, with eligible gynaecological surgery: 160 (80/80)	2 x 2 Cranberry capsules 2 x 2 placebo capsules 6 weeks	Significantly lower incidence of UTIs) in cranberry group (p=0.008).
Caljouw 2014	R / D-B / P-C	Elderly persons (mean 84 years): 928 High UTI risk: 516 (253/263) Low UTI risk: 412 (205/207)	2 x 500 mg Cranberry capsules with 9 mg PACs 2 x Placebo capsules 12 months	Significantly lower incidence of UTIs in high risk group (p=0.04). No difference in low risk group.
Gallien 2014	R / D-B / P-C	Patients with MS (mean 49 years): 171 (82/89)	2 x Cranberry powder for oral solution 2 x Placebo 1 year	No significant difference between the groups in time to first UTI, no. of UTIs/year and per patient.
Takahashi 2013	R / D-B / P-C	Patients, 20-79 years, with recurrent UTIs: 213 (107/106)	125 mL Cranberry juice (40 mg PACs) 125 mL Placebo beverage 24 weeks	Significantly lower relapse rate of UTIs in the subgroup of females over 50 years (p=0.0425).
Afshar 2012	R / D-B / P-C	Children, 5-18 years, with at least 2 UTIs the year before: 40 (20/20)	2 mL/kg Cranberry juice with or without 37% PACs 1 year	Significantly lower incidence of UTIs (65%; p=0.045) in cranberry group.
Salo 2012	R / D-B / P-C	Children (mean 4 years) with UTIs: 255 (126/129)	5 mL/kg Cranberry juice Placebo 6 months	Less recurrent UTI episodes and significantly less antimicrobial days (p<0.001).
Barbosa-Cesnik 2011	R / D-B / P-C	Women (mean 21.2 years) with acute UTIs: 319 (155/164)	2 × 240 mL Cranberry drink 2 × 240 mL Placebo drink 6 months	No significant difference in recurrence rate of UTIs.
Wing 2008	R / D-B / P-C	Healthy pregnant women at less than 16 weeks gestation:188 (58/63/67)	2 × 240 mL Cranberry drink 2 × 240 mL Placebo drink or 1 of each; until delivery	Reduced frequency of asymptomatic bacteriuria (57%) and all UTIs (41%).
McMurdo 2005	R / D-B / P-C	Older patients, no history of UTI: 376 (187/189)	2 × 150 mL Cranberry juice 2 × 150 mL Placebo drink 15-16 days	Fewer symptomatic UTIs in cranberry group, but not significant (p=0.122).
Stothers 2002	R / D-B / P-C	Women, 21-72 years, with recurrent UTIs: 150 (50/50/50)	3 × 250 mL Cranberry juice 2 Cranberry tablets Placebo; 12 months	Significantly lower Incidence of UTIs in cranberry groups (both p<0.05).
Walker 1997	R / D-B / P-C cross-over	Women, 28-44 years, with recurring UTIs: 10	400 mg Cranberry extract Placebo; 3 months each	Cranberry significantly more effective in reducing occurrence of UTIs (p<0.005).
Avorn 1994	R / D-B / P-C	Elderly women, (mean 78.5 years); 153 (72/81)	300 mL Cranberry cocktail Placebo drink; 6 months	Odds ratio of bacteriuria with pyuria only 42% relative to placebo group (p<0.004).
Temiz 2018	Randomized controlled	Patients after ileal conduit diversion: 20/20/20	Cranberry capsules (2 x 9 mg PACs) Training on avoidance of UTIs No intervention	No differences in temperature, leukocyto-sis, CRP levels; significantly (p<0.05) lower flank pain score with cranberry at weeks 13 to 16; UTIs: 5% in the cranberry group, 40% in control group
Singh 2016	Randomized controlled	Patients, 15-76 years, with recurrent UTIs:72 (36/36)	Cranberry capsules (2 x 60 mg PACs) Lactobacillus capsules (2 x 400 million bacteria) 12 weeks	Significant decrease in recurrent UTI, mean burning micturation score, microscopic pyuria score and bacterial growth in urine (all p< 0.001).
Sengupta 2011	Randomized open controlled	Women, 18-40 years, with recurrent UTIs: 60 (21/23/16)	500 mg Cranberry powder (14.5 mg/g PACs) 1000 mg Cranberry powder (14.5 mg/g PACs) No intervention; 90 days	Significant reduction of E. coli in urine in both cranberry groups (p=0.0151 low dose; p<0.0001 high dose, compared to baseline).

Vidlar 2010	Randomized open controlled	Men (mean 63 years) at risk of prostate disease with UTIs: 42 (21/21)	3 x 500 mg Cranberry powder No intervention 180 days	Significant improvement in IPSS, quality of life, urination parameters compared to baseline (all $p<0.05$). Lower total PSA levels ($p<0.05$) compared to control.
Ferrara 2009	Randomized open controlled	Girls, 3-14 years, with recurring UTIs: 84 (27/28/29)	50 mL Cranberry drink 100 mL Lactobacillus drink No intervention; 6 months	Recurrence of UTIs lower in cranberry group ($p=0.05$).
Kontiokari 2001	Randomized open controlled	Women, (mean 29-32 years) with UTIs caused by E. coli:150 (50/50/50)	50 mL Cranberry drink 100 mL Lactobacillus drink No intervention; 6 months	Recurrence of UTIs lower in cranberry group (6 months, $p=0.023$; 12 months, $p=0.048$).
Haverkorn 1994	Randomized cross-over	Elderly persons (mean 81 years): 17	2 × 15 mL Cranberry juice Same volume of water 4 weeks each	Lower incidence of bacteriuria in cranberry phase than in water-only phase ($p=0.004$).
Barnoiu 2015	Open controlled	Patients undergoing ureteral double J catheter insertion: 62 (31/31)	120 mg Cranberry extract No intervention in routine prophylactic therapy	Significantly lower UTI rates in cranberry group (12.9 vs. 38.7%; $p<0.04$).
Ledda 2017	Open controlled	Adolescents,12-18 years, with recurring UTIs: 36 (19/17)	1 x 120 mg Cranberry extract capsule with 36 mg PACs + life style change Life style change only 2 months	Significantly lower no. of UTIs ($p=0.0001$).
Ledda 2016	Open controlled	Men (mean 67 years) with recurrent UTIs: 44 (23/21)	1 x 120 mg Cranberry extract capsule with 36 mg PACs + life style change Life style change only 2 months	Significantly lower no. of UTIs ($p=0.0062$).
Ledda 2015	Open controlled	Patients (mean 39 years) with recurrent UTIs: 44 (22/22)	1 x 120 mg Cranberry extract capsule with 36 mg PACs + life style change Life style change only 2 months	Significantly lower UTI rates and shorter duration of UTIs in cranberry group.
Kirchhoff 2001	Open controlled	Patients in two hospital geriatric units:338 (187/151)	2 × 175 mL Cranberry juice 2 × 175 mL Mixed (non-cranberry) juice; 4 weeks mean	No significant influence on incidence of UTIs.
Burleigh 2013	Open uncontrolled	Women, 18-64 years, with history of UTIs: 20	42 g sweetened dried cranberries	Significant decrease of mean UTI rate/6 months ($p=0.004$) and of geometric mean of CFU/mL of isolated E. coli ($p=0.040$) pre- and post-consumption.
Sánchez Ballester 2013	Open uncontrolled	Women (mean 35.2 years) with recurrent symptomatic postcoital urinary tract infections: 20	Cranberry extract (118 mg PACs/day) 6 months	Significant reduction of UTIs ($p<0.0001$) and positive urine cultures ($p<0.001$). Significant improvement of quality of life ($p=0.0002$).
Bailey 2007	Open uncontrolled	Women, 25-70 years, ≥ 6 UTIs in past year: 12	2 × 200 mg Cranberry extract 12 weeks	No UTIs in 12 weeks; none in 8 participants after 2 years.
Papas 1966	Open uncontrolled	Adults with acute UTI: 60	450 mL Cranberry juice 21 days	Positive clinical response in 53% of patients; improvement in further 20%.
Pagonas 2012	Retrospective	Kidney transplant recipients (mean 54.5 years): 82 (39/25/18/30)	2 x 50 mL Cranberry juice 3 x 500 mg Methionine Both agents No prophylaxis 1 year before and 1 year after initiation of treatment	Significant reduction in no. of UTIs ($p<0.001$), symptoms ($p<0.008$) and pyuria/nitrituria ($p<0.02$) with cranberry as compared to pretreatment period.

Prevention of bacteriuria and symptomatic UTIs: patients with neurogenic bladder (NB) SCI = Spinal cord injury				
Hess 2008	R / D-B / P-C cross-over	Men, 28-79 years, with NB secondary to SCI: 57	500 mg Cranberry extract Placebo 6 months each treatment	Frequency of UTIs reduced by 70% ($p<0.05$ for both incidence and number of patients with UTIs).
Lee 2007	R / D-B / P-C	Patients, 16-82 years, with NB secondary to SCI: 305 (78/75/75/77)	2 × 800 mg Cranberry 2 × 1 g Methenamine hippurate 800 mg Cranberry + 1 g MH Placebo; Up to occurrence of UTI or 6 months	No significant reduction in incidence of symptomatic UTIs.
Waites 2004	R / D-B / P-C	Adults, 20-73 years, with NB secondary to SCI: 48 (26/22)	2 g conc. Cranberry juice Placebo; 6 months	No reduction in incidence of bacteriuria or UTIs.
Linsen-meyer 2004	R / D-B / P-C cross-over	Adults with NB secondary to SCI: 21	3 × 400 mg Cranberry Placebo; 4 weeks each	No significant effect on bacteriuria or frequency of symptomatic UTI.
Schlager 1999	D-B / P-C cross-over	Paediatric patients, 2-18 years, with NB: 15 (9/6, 6/9)	Cranberry juice concentrate Placebo drink; 3 months each	No significant effect on bacteriuria or frequency of symptomatic UTI.
Foda 1995	Randomized single-blind cross-over	Paediatric patients, 1.4-18 years, with NB: 21	15 mL/kg Cranberry juice 15 mL/kg water 6 months each	No difference in incidence of UTI between cranberry juice and water phases.
Reid 2001	Open controlled cross-over	Patients with NB secondary to SCI: 15	3 × 250 mL Cranberry juice 3 × 250 mL extra water 1 week each	Cranberry juice significantly reduced bacterial biofilm load in the bladder ($p<0.013$).
Mutlu 2012	Open P-C cross-over	Paediatric patients, 4-18 years, with NB: 20	1 x 1 Cranberry capsule 1 x 1 Placebo capsule 6 months each	Significantly lower UTI infection rate ($p<0.012$) and pyuria $p=0.000$).

Metabolic syndrome				
Shidfar 2012	R / D-B / P-C	Men with Type 2 diabetes (mean 54.8 years): 58 (29/29)	240 mL Cranberry juice 240 mL Placebo juice 12 weeks	Significant decreases in serum glucose and apoB ($p>0.05$ and $p>0.01$). Significant increases in serum apoA-1 and PON-1 activity ($p>0.05$ and $p<0.01$) compared to placebo. All parameters ($p<0.01$) compared to baseline.
Basu 2011	R / D-B / P-C	Women with metabolic syndrome: 31 (15/16)	480 mL Cranberry juice 480 mL Placebo juice 8 weeks	Significantly increased plasma antioxidant capacity and decreased oxidized LDL and malondialdehyde (all $p<0.05$).
Schell 2017	Open controlled	Patients with type 2 diabetes: 25 (12/13)	40 g dried cranberries after high-fat breakfast challenge No cranberries	Significantly lower postprandial increases in glucose, lower serum IL-18 and MDA at 4 h, and higher serum total nitrite at 2 h (all $p<0.05$).
Simão 2013	Open controlled	Patients with metabolic syndrome: 56 (20/36)	700 mL energy-reduced cranberry juice Normal diet	Significant increase in adiponectin ($p=0.01$) and folic acid ($p=0.033$) and decrease in homocysteine ($p<0.001$) in relation to baseline and ($p<0.05$) compared to controls.

Cardiovascular risk factors				
Novotny 2015	R / D-B / P-C	Volunteers (mean 50 years): 56 (29/27)	2 x 240 mL low calorie cranberry juice (173 mg phenolics) 2 x 240 mL Placebo beverage (62 mg phenolics/ serving) 8 weeks	Significantly lower fasting serum triglycerides ($p=0.027$), serum C-reactive protein ($p=0.0054$), diastolic blood pressure ($p= 0.048$) and fasting plasma glucose ($p=0.03$).

Flammer 2013	R / D-B / P-C	Patients (mean 49.5 years) with peripheral endothelial dysfunction and cardiovascular risk factors: 84 (32/37)	2 x 230 mL Cranberry juice cocktail 480 mL Placebo juice 4 months	Endothelial progenitor cells expressing osteocalcin significantly (p<0.019) reduced. No differences in metabolic, inflammatory markers or oxidative stress markers and endothelial function
Ruel 2013	R / D-B / P-C cross-over	Obese men (mean 45 years): 35	500 mL/d low calorie cranberry juice cocktail (400 mg total polyphenols, 20.8 mg anthocyanins) 500 mL/d placebo juice 4 weeks each, with 4 weeks wash-out	No significant changes in augmentation index and cardiometabolic profile.
Dohadwala 2011	R / D-B / P-C cross-over	Patients (mean 62 years) with coronary heart disease: 44	480 mL Cranberry juice (835 mg total polyphenols, 94 mg anthocyanins) 480 mL Placebo beverage 4 weeks each treatment	Significant decrease in carotid-femoral pulse wave velocity from 8.3 to 7.8 m/s (p=0.003).
Ruel 2009	S-B	Obese men (mean 51 years): 30	0, 125, 250 and 500 mL low calorie cranberry juice (74 mg PACs/125 mL) 4 weeks, each	Significant decrease in plasma matrix metalloproteinase-9 (p<0.0005) and plasma NOx concentrations(p<0.05).
Ruel 2008	S-B	Obese men (mean 51 years): 30	0, 125, 250 and 500 mL low calorie cranberry juice (74 mg PACs/125 mL) 4 weeks, each	Significant decreases in plasma OxLDL, intercellular adhesion molecule-1 (both p<0.0001), vascular cell adhesion molecule-1 (p<0.05) and systolic blood pressure (p<0.03).
Ruel 2006	S-B	Obese men (mean 51 years): 30	0, 125, 250 and 500 mL low calorie cranberry juice (74 mg PACs/125 mL) 4 weeks, each	Significant decreases in body weight, BMI, oxidative stress (all p<0.05) and waist circumference (p<0.0001) at the end of the study. Significant increases in plasma HDL-cholesterol after 250 mL (p<0.05) and 500 mL (p<0.001).

Prevention of urinary symptoms in patients under radiation therapy				
Hamilton 2015	R / D-B / P-C	Men with prostate cancer + radiation therapy (mean 68 years): 40 (20/20)	Cranberry capsule with 72 mg PAC Placebo capsule During treatment and for 2 weeks after treatment completion	Decreased incidence of cystitis (p=0.058) and pain/ burning (p=0.045).
Campbell 2003	Randomized controlled	Men with prostate cancer + radiation therapy: 112 (55/57)	3 × 118 mL Cranberry juice 3 × 118 mL Apple juice; 9 weeks	No significant difference in urinary symptoms.
Bonetta 2017	Open controlled	Men with prostate cancer + radiation therapy (mean 70 years): 924 (489/435)	200 mg Cranberry extract 6-7 weeks	Less UTIs and recurrent UTIs, radio¬the¬ra-py-induced dysuria and use of antibiotics and NSAIDs significantly reduced.
Bonetta 2012	Open controlled	Men with prostate cancer + radiation therapy: 370 (184/186)	200 mg Cranberry extract 6-7 weeks	Less UTIs and radiotherapy-induced urinary tract symptoms significantly reduced.

Other studies				
Student 2016	R / D-B / P-C	Men with prostate cancer prior to surgery (mean 65 years): 64 (31/31)	3 x 500 mg Cranberry fruit powder capsules 3 x 500 mg placebo capsules 30 days (mean) prior to surgery	Significant decrease of serum PSA 22.5% (p<0.05).

Zhang 2005	R / D-B / P-C	189 adults infected with Helicobacter pylori: (97/92)	2 × 250 mL Cranberry juice 500 mL Placebo juice; 90 days	Significant suppression of H. pylori infection ($p<0.05$) at days 35 and 90.

R = randomised; D-B = double-blind; P-C = placebo controlled; S-B = single-blind.

In an open, controlled study, 150 women (mean age 29-32 years) with UTIs caused by *E. coli* were randomly assigned to one of three treatments: 50 mL of a cranberry-lingonberry drink (containing 7.5 g cranberry juice concentrate and 1.7 g lingonberry juice concentrate) daily for 6 months (n = 50), 100 mL of a *Lactobacillus* drink five days a week for 12 months (n = 50) or no intervention as control (n = 50), with follow-up of all participants at 12 months. The recurrence of UTIs was significantly lower in the cranberry group than in the control group ($p = 0.014$ at 6 months; $p = 0.052$ at 12 months) [Kontiokari 2001].

In an open, cross-over study, 38 elderly persons (mean age 81 years, completed by n=17) were randomized to either 2 × 15 mL of cranberry juice or water daily for 4 weeks, followed by the alternative regimen for a further 4 weeks. There were significantly ($p=0.004$) fewer occurrences of bacteriuria during the cranberry juice phase than in the water-only phase [Haverkorn 1994].

In a randomized, controlled trial, 84 girls (3 to 14 years) with recurrent UTIs received daily for 6 months either 50 mL cranberry juice concentrate (containing 7.5 g cranberry and 1.7 g lingonberry concentrate; n=28) or 100 mL of a *Lactobacillus* drink (4 x 107 cfu; n=27) or no treatment as a control (n=29). During the observation period, 34 episodes of UTIs occurred: 5 in the cranberry group (18.5%), 11 in the *Lactobacillus* group (42.3%) and 18 in the control group (48.1%), showing a significant ($p<0.05$) reduction in the risk of repeated UTIs in the cranberry group as compared to the two other groups [Ferrara 2009].

Men at risk of prostate disease with UTIs, elevated PSA, negative prostate biopsy and clinically confirmed chronic non-bacterial prostatitis received either 1500 mg/d dried powdered cranberries for 6 months (n=21), or no intervention as control (n=21), in a randomized, controlled, open study. After 180 days, patients in the cranberry group experienced a significant improvement in IPSS, quality of life, urination parameters including voiding parameters (e.g. rate of urine flow, average flow, total volume, post-void residual urine volume) as compared to baseline (all $p<0.05$) and lower total PSA levels ($p<0.05$) as compared to control. There was no influence on blood testosterone or serum CRP levels [Vidlar 2010].

A 90-day randomized, controlled trial in 60 women with a history of recurrent UTIs evaluated the efficacy of cranberry powder containing 14.5 mg/g PACs at two doses, 2 x 250 mg/d (n=21) and 2 x 500 mg/d (n=23) compared to an untreated control group (n=16). At the end of the study, the presence of *E. coli* in urine was significantly reduced in both cranberry groups as compared to baseline ($p=0.0151$ for the low dose and $p<0.0001$ for the high dose), whereas it remained unchanged in the untreated controls. Relief of symptoms was also reported after both cranberry doses. No significant changes were observed in haematological and serum biochemical parameters [Sengupta 2011].

In two geriatric units (mean duration of stay 4 weeks), urine samples from patients with suspected urinary tract infections were cultured and significant growth of bacteria led to antibiotic treatment. Over a 12-month study period, participating patients in one unit received 2 × 175 mL of cranberry juice daily (n = 187) for the length of their stay, while those in the other unit received the same amount of a mixed berry juice (n = 151). Cranberry juice treatment did not significantly influence the incidence of urinary tract infections [Kirchhoff 2001].

In a pilot study involving 44 patients with recurrent UTI, the frequency of UTIs for two months before treatment was compared to that during treatment for two months with120 mg cranberry extract daily (containing 36 mg PACs) plus standard life style and hygiene management advice, or standard management advice only (control). The reduction of UTIs as compared to the pre-treatment period was 73.3% in the cranberry group and 15.4% in the lifestyle change only group ($p=0.012$). The duration of UTIs was also significantly shorter with the extract (2.5 vs. 3.6 days; $p<0.05$) [Ledda 2015]. In a similar study using the same preparation and dosage in 43 elderly men, the number of UTIs in the cranberry group was significantly ($p=0.0001$) reduced from 3.2 before treatment to 0.8 during treatment. No significant change was seen following the life style change only, demonstrating a significant ($p=0.0062$) between group difference [Ledda 2016]. An identical trial was performed in 36 adolescents (12-18 years). The mean number of UTIs during the treatment period in the supplemented group (0.31±0.2) was significantly ($p= 0.0001$) lower compared to both control (2.3±1.3) and to before treatment (1.74±1.1) [Ledda 2017].

A prospective, open trial compared UTI rates following standard prophylactic treatment before ureteral double J catheter insertion either with (n=31) or without (n=31) addition of 120 mg cranberry extract (not further specified). UTI percentage was lower in the cranberry group (12.9% compared to 38.7%; $p=0.04$) compared to the standard prophylactic treatment only [Barnoiu 2015].

In an open pilot study, 12 women (25-70 years) with a history of at least 6 UTIs in the preceding year took 2 × 200 mg of a concentrated cranberry extract (30% total phenols, 25% PACs) daily for 12 weeks. Urinalysis was carried out at baseline and at monthly intervals, and all 12 participants were followed up two years later. During the 12-week study none of the women had a UTI and two years later eight of the women who continued to take cranberry were still free from UTIs [Bailey 2007].

In an open study, cranberry juice was administered to 60 patients with diagnoses of acute UTI. After 3 weeks of treatment with 450 mL/d of cranberry juice, 32 patients (53%) had a positive clinical response (no urogenital complaints, less than 100,000 bacteria per mL urine) and moderate improvement was evident in a further 12 patients (20%), while 16 (27%) showed neither bacteriological improvement nor symptomatic relief [Papas 1966].

In an observational, prospective study, women (n=20) with recurrent symptomatic postcoital UTIs received an extract (118 mg/d PACs) for 6 months as follows: after the 1st sexual intercourse in a week 1 dose daily for 3 days, and after the 2nd and following intercourses in a week 1 dose postcoital. The number of UTIs was significantly ($p<0.0001$) reduced from 2.8 in the 3 months prior to treatment to 0.2 at month 6. The mean quality of life assessed on a VAS was 62.4±19.1 at baseline and increased to 78.2±12.4 at month 6 ($p=0.0002$). All patients had an infection with positive urine culture at baseline, while after

6 months there were only 3 symptomatic infections (p<0.001) [Sánchez Ballester 2013].

Women (n=20; 18-64 years, mean age 37) with a history of recurrent UTIs consumed 42 g/day of sweetened dried cranberries for two weeks. The mean UTI rate per six months pre- and post-consumption decreased significantly (p=0.004) from 2.4 to 1.1. A significant decrease (p=0.040) of *E. coli* isolated from rectal swab eluates pre- and post-consumption was observed [Burleigh 2013].

Women with recurrent UTIs (n=24) consumed 120 mL cranberry juice on average 6 days/week for 8 weeks. At weeks 2, 4 and 8, the Interstitial Cystitis Symptom Index (ICSI) and the Interstitial Cystitis Problem Index (ICPI) were used to evaluate UTI symptoms. Both scores decreased significantly from 2 to 8 weeks (ICSI p<0.0001; ICPI p<0.01) [Bass-Ware 2014].

A retrospective analysis of 82 kidney transplant recipients compared the influence of prophylaxis with cranberry juice (2 x 50 mL/d, n=39) or L-methionine (3 x 500 mg/d, n=25) or both (n=18) on annual recurrent UTIs before and after initiation of prophylaxis, as well as in thirty patients without any prophylactic treatment (control). Overall, prophylactic treatment significantly (p<0.001) decreased the annual UTI incidence by 58.3%, with no change in the control group. Cranberry reduced the annual number of UTI episodes by 63.9% (p<0.001), symptoms such as fever, dysuria, urgency (p<0.008) and pyuria/nitrituria (p<0.02) as compared to the period before the start of prophylaxis. In the L-methionine group, UTI episodes were reduced by 48.7% as well as symptoms and pyuria/nitrituria (all p<0.001). The combination led to a reduction of UTI episodes by 64.5% (p<0.001), of symptoms (p<0.03) and of pyuria/nitrituria (p<0.008). In patients without prophylaxis all parameters remained unchanged [Pagonas 2012].

Prevention of UTI:
Patients with neurogenic bladder
In a randomized, double-blind, placebo-controlled study with a cross-over design, 57 men (28-79 years) with spinal cord injuries and neurogenic bladder were randomized to receive twice daily for 6 months either a 500 mg cranberry extract (not further specified) tablet or placebo, followed by the alternative preparation for a further 6 months, with no wash out period. The primary outcome criterion was the incidence of UTIs; 47 patients completed the study. There were 31 episodes of bacteriuria in the cranberry period and 37 in the placebo period. However, during the cranberry period 6 patients had 7 UTIs, compared to 16 patients and 21 UTIs in the placebo period (p<0.05 for both number of patients and incidence). Cranberry reduced the frequency of UTIs to 0.3 per year compared to 1.0 per year on placebo, with a significant p<0.05) reduction in the likelihood of a UTI and symptoms in every month. Patients with a glomerular filtration rate higher than 75 mL/minute had the greatest benefit [Hess 2008].

A much larger randomized, double-blind, placebo-controlled study examined the efficacy of cranberry (no further details) and methenamine hippurate (MH) in the prevention of symptomatic UTIs in patients (aged 16-82 years, predominantly male) with spinal cord injury (SCI) and neuropathic bladder, but stable bladder management. Randomized into groups, the patients received one of four daily treatments: 2 × 800 mg of cranberry (n = 78), 2 × 1 g of MH (n = 75), 800 mg of cranberry + 1 g of MH (n = 75) or placebo (n = 77). The primary outcome measure was the time from commencement of treatment to the occurrence of a symptomatic UTI, or 6 months if none occurred. No significant benefit in the prevention of UTIs was evident from cranberry or MH treatment [Lee 2007].

In a randomized, double-blind, placebo-controlled study, patients with neurogenic bladder secondary to SCI, and baseline urine cultures showing at least 105 colonies of bacteria/mL, received either 2 g of concentrated cranberry juice (n = 26) or placebo (n = 22) daily for 6 months. Urinalysis and cultures were performed initially and at monthly intervals. No significant differences between groups were observed with respect to bacteriuria and pyuria. In the verum group 10 participants (38.5%) had a total of 16 symptomatic UTI episodes during the 6-month period, while in the placebo group 8 participants (36.4) had 14 symptomatic UTI episodes [Waites 2004].

In a randomized, double-blind, placebo-controlled cross-over study, 37 patients with neurogenic bladder secondary to SCI were randomly assigned to treatment with either 3 × 400 mg cranberry tablets or placebo daily for 4 weeks. After a wash-out period of 1 week the participants took the alternative treatment for a further 4 weeks. The participants were checked weekly and urine analyses were carried out; results on 21 patients were available for evaluation. No favourable effect of the cranberry treatment was evident from urinary bacterial and white blood cell counts or the incidence of UTIs [Linsenmeyer 2004].

A pilot study in 15 patients with SCI demonstrated that drinking 3 glasses of cranberry juice/d significantly reduced the bacterial biofilm load in the bladder (p = 0.013 compared to baseline) due to a reduction in adhesion of Gram-negative (p = 0.054) and Gram-positive (p = 0.022) bacteria to bladder epithelial cells. Intake of the same amount of water did not significantly reduce bacterial adhesion or biofilm presence [Reid 2001].

In a double-blind, placebo-controlled, cross-over study the effect of cranberry juice on the prophylaxis of bacteriuria was determined in children with neurogenic bladder receiving intermittent catheterization. A cranberry juice concentrate (corresponding to 300 mL of cranberry juice) or a placebo drink was given to 15 children daily for 3 months, followed by the alternate treatment for a further 3 months. In this population cranberry juice had no effect on the frequency of bacteriuria [Schlager 1999].

In a randomized, single-blind cross-over study, 40 paediatric patients (1.4-18 years) with neurogenic bladder managed by clean intermittent catheterization were randomized to daily treatment with either a cranberry cocktail (30% cranberry concentrate) or water at 15 mL/kg b.w., divided into 3-4 doses, for 6 months then the alternative treatment for a further 6 months; 21 patients completed the study, of whom 11 received cranberry first. No beneficial effect of cranberry consumption greater than that of water was evident in this study. Fewer UTIs were observed in 9 patients taking cranberry juice and in 9 given water; no difference was noted in 3 patients [Foda 1995].

Children with neurogenic bladder (n=20; mean age 7.25 years) received placebo for 6 months and after a one week break, cranberry extract (not further specified) for a further 6 months in a prospective study. UTI rate and pyuria were significantly (p<0.012 and p=0.000) reduced as compared to placebo [Mutlu 2012].

Urinary symptoms during radiation therapy
During 7 weeks of treatment for prostate cancer by external radiation therapy, 112 men were randomized to daily consumption of either 3 × 118 mL of cranberry juice (n = 55) or the same volume of apple juice (n = 57). No significant difference was observed between the two groups regarding the urinary symptoms in International Prostate Symptom Scores [Campbell 2003].

In a randomized, double-blind, placebo-controlled study, 40 men with prostate cancer undergoing radiation therapy took either cranberry extract (72 mg PACs/day) or placebo throughout their course of radiotherapy and for 2 weeks after treatment completion. The incidence of cystitis was lower (p=0.058) in those taking cranberry (65%) than placebo (90%). While the incidence of pain/burning was significantly (p=0.045) decreased compared to placebo [Hamilton 2015].

Of 370 patients receiving radiotherapy for prostate cancer, 184 were treated with 200 mg of an extract standardized to 30% PACs daily for 6-7 weeks in a non-randomized, open study. In the untreated control group (n=186), 45 UTIs (24.2%) were observed, compared to 16 UTIs (8.7%) in the cranberry group. Urinary symptoms due to radiotherapy were significantly milder in the cranberry group: nycturia (31% vs. 54% increase, p<0.001), urgency (31% vs. 54% increase, p<0.0001), micturition frequency (p<0.0006) and dysuria (p<0.0001) [Bonetta and Di Pierro 2012].

In a similar, randomized, open study with the same medication, 924 patients were enrolled, with 489 receiving the extract and 435 in an untreated control group. Significantly (p=0.0001) less patients experienced UTIs in the cranberry group (53 patients; 10.8%) compared to the control group (107 patients; 24.6%). Recurrent UTIs were seen in 10 control patients (2.3%) and only 4 cranberry patients (0.8%) (p=0.004). Use of antibiotics and NSAIDs was significantly (p<0.001) less with the extract, and a significant (p<0.04-0.001) reduction of dysuria (degrees 0 to 3) was observed as compared to control [Bonetta 2017].

Metabolic syndrome
In a randomized, double-blind, placebo-controlled study, type 2 diabetics on oral glucose-lowering medication (n=30) received 3 x 500 mg cranberry extract or placebo for 12 weeks to examine changes in lipid profiles, oxidized LDL, glycaemic control, components of the metabolic syndrome such as abdominal obesity and raised blood pressure, C-reactive protein and urinary albumin excretion. LDL cholesterol decreased significantly in the cranberry group (p<0.005 compared to baseline and p<0.001 compared to placebo) as well as total cholesterol and the ratio total:HDL cholesterol (p=0.020 and 0.044 respectively, compared to baseline; p<0.001 and p=0.032 respectively, compared to placebo). All other parameters did not change significantly [Lee 2008].

In a randomized, double-blind, placebo-controlled trial, women with metabolic syndrome consumed 480 mL/d cranberry juice (238 mg PACs) or placebo for 8 weeks (n = 15-16/group). The juice increased plasma antioxidant capacity (1.5 ± 0.6 to 2.2 ± 0.4 µmol/L) and decreased oxidized LDL and malondialdehyde (120.4 ± 31.0 to 80.4 ± 34.6 U/L and 3.4 ± 1.1 to 1.7 ± 0.7 µmol/L, respectively), at 8 weeks (all significant (p<0.05) compared to placebo). The intake of juice did not improve blood pressure, glucose and lipid profiles, C-reactive protein and IL- 6 [Basu 2011].

Type 2 diabetic male patients (n=58) were randomized to 240 mL cranberry juice or a placebo drink daily for 12 weeks in a randomized, double-blind trial. With the juice, significant decreases in serum glucose and apoB (p<0.05 and p<0.01, respectively) and significant increases in serum apoA-1 and PON-1 activity (p>0.05 and p<0.01, respectively) were observed at the end of study as compared to placebo as well as compared to baseline (p<0.01 for all) [Shidfar 2012].

Patients (n=56; 42-56 years; BMI 26.3-38.4) with metabolic syndrome consumed a low-calorie cranberry juice (0.7 L/d; 364 mg total phenolics) or placebo for 60 days. The verum group showed a significant increase in adiponectin (p=0.010) and folic acid (p=0.033) and a decrease in homocysteine (p<0.001) in relation to baseline and in comparison with controls (p<0.05). No significant changes in the pro-inflammatory cytokines TNF-α, IL-1 and IL-6 or in C-reactive protein were observed. Oxidative stress measurements resulted in significantly (p<0.05) decreased lipid peroxidation and protein oxidation levels as compared to control [Simao 2013].

Postprandial effects were studied in obese patients with type 2 diabetes (n=25) in a randomized, cross-over trial at fasting and 1, 2 and 4 hours after a high-fat breakfast with or without dried cranberries (40 g). Postprandial serum glucose levels were significantly (p<0.05) lower after cranberries vs. control at 2 and 4 hours. No significant differences between the groups were observed in insulin, insulin resistance, lipid profiles and blood pressure. Among biomarkers of inflammation and oxidation, postprandial serum IL-18 and MDA were significantly lower at 4 h, and serum total nitrite was higher at 2 h in the cranberry group (all p < 0.05). No effects were noted on CRP or IL-6 [Schell 2017].

Cardiovascular risk factors
In a single-blind study, 30 obese men received a low calorie cranberry juice cocktail (CJC) containing 125, 250 and 500 mL cranberry juice (adjusted to 500 mL with placebo cocktail, 74 mg PACs/125 mL). Placebo cocktail (500 ml/d) was consumed during a 4 week run-in period, followed by three 4-week periods with the increasing doses of CJC. Small but significant decreases were observed for body weight, BMI (both p<0.05) and waist circumference (p<0.0001) at the end of the study, as well as significant increases in plasma HDL-cholesterol after 250 mL (p<0.05) and 500 mL (p<0.001). Changes in plasma apo A-I concentration and the reduction in plasma TG were not significant. Plasma total and LDL-cholesterol and circulating apoB levels remained unchanged. Plasma total antioxidant capacity changed significantly: after an increase with 125 mL/d, it decreased with 250 mL and 500 mL (both p<0.05 vs. 125 mL CJC/d). Oxidative stress, as shown by the plasma nitrite/nitrate concentration, was reduced significantly at the end of the intervention (p<0.05 vs. 0 mL CJC/d) [Ruel 2007].

At the end of the study significant decreases in plasma OxLDL cholesterol concentrations, plasma intercellular adhesion molecule-1 (both p<0.0001), vascular cell adhesion molecule-1 (p<0.05) and systolic blood pressure (p<0.03) were observed in the same patients and set-up [Ruel 2008]. The treatment also resulted in a significant decrease in plasma matrix metalloproteinase-9 (p<0.0005) and plasma NOx concentrations (p<0.05) after 12 weeks vs. run-in [Ruel 2009].

In a randomized, placebo-controlled, cross-over study, 44 patients with coronary heart disease received 4 weeks each of 480 mL/d cranberry juice (54% juice, 835 mg total polyphenols, 94 mg anthocyanins) and placebo, with a 2 week wash-out period. Vascular function was measured before and after each beverage. Mean carotid-femoral pulse wave velocity, a measure of central aortic stiffness, decreased after cranberry juice (from 8.3 to 7.8 m/s) in contrast to an increase (8.0 to 8.4 m/s) after placebo (p= 0.003). Brachial artery flow-mediated dilation, digital pulse amplitude tonometry, blood pressure and carotid-radial pulse wave velocity as well as the biochemical profile including TC, LDL-C, HDL-C, triglycerides, glucose, insulin and CRP, did not change significantly [Dohadwala 2011].

The augmentation index (AIx), an index of arterial stiffness, and the cardiometabolic profile of 35 overweight men did not show any significant changes in a randomized, placebo-controlled, double-blind, cross-over study after consumption of 500 mL low

calorie cranberry juice cocktail (400 mg total polyphenols, 20.8 mg anthocyanins) or 500 mL placebo juice/d for 4 weeks. After a 4-week wash-out period the groups were crossed for another 4 weeks. However, a significant (p=0.019) decrease in AIx was noted after cranberry cocktail as compared to baseline [Ruel 2013].

In a randomized, double-blind, placebo-controlled trial, 84 patients with peripheral endothelial dysfunction and cardiovascular risk factors received either 2 x 230 mL/d of a cranberry juice cocktail (1740 µg/mL total phenols, 151 µg/mL total anthocyanins, 2662 µg/mL total PACs) or placebo. The 4-month protocol was completed by 69 patients (cranberry n=32; placebo n=37). No differences between the groups were seen in metabolic, inflammatory or oxidative stress markers and endothelial function. Only endothelial progenitor cells expressing osteocalcin were significantly (p<0.019) reduced by the juice [Flammer 2013].

A double-blind, placebo-controlled, parallel-arm study was conducted in 30 women and 26 men who consumed 240 mL of a low calorie cranberry juice (LCCJ; 173 mg phenolics/serving) or a matched placebo (62 mg phenolics/serving) twice daily for 8 weeks. Fasting serum triglycerides (TGs) were significantly (p=0.027) lower after LCCJ as compared to placebo. Individuals with higher TGs at baseline experienced a better effect. Serum TC, LDL and HDL cholesterol as well as serum apo A-I, apo A-II, and apoB did not differ between the groups. Serum C-reactive protein significantly (p=0.0054) decreased with LCCJ, which also lowered diastolic blood pressure (p= 0.048) and fasting plasma glucose (p=0.03) compared with placebo. The effect on homeostasis model assessment of insulin resistance was more beneficial for participants with high baseline values (p=0.035) [Novotny 2015].

Other effects

In a randomized, double-blind, placebo-controlled study, 189 patients (aged 48.9 ± 11.2 years) with *Helicobacter pylori* infections were randomized to 2 × 250 mL of cranberry juice (n = 97) or matching placebo beverage (n = 92) daily for 90 days. The degree of *H. pylori* infection was determined by a ^{13}C-urea breath test before randomization and on days 35 and 90 of intervention. At day 35, ^{13}C urea breath tests were negative for 14 out of 97 patients in the cranberry group and 5 out of 92 in the placebo group. At day 90 results were negative for 14 out of 97 patients in the cranberry group (of whom 11 had also been negative at day 35) and for 5 out of 92 in the placebo group (of whom 2 had also been negative at day 35). The results were significantly (p<0.05) in favour of cranberry treatment [Zhang 2005].

In a randomized, double-blind placebo-controlled study, 64 patients with prostate cancer received, for a mean of 30 days prior to surgery, either 1500 mg cranberry fruit powder (n=31) or placebo (n=31). Serum PSA significantly (p<0.05) decreased by 22.5% in the cranberry group as compared to baseline, but increased by 0.9% with placebo. A trend to downregulation of urinary beta-microseminoprotein and serum γ-glutamyltranspeptidase, as well as upregulation of IGF-1 was found after cranberry treatment. There were no changes in prostate tissue markers [Student 2016].

Clinical reviews and meta-analysis

From a review of 10 randomized controlled studies [Avorn 1994, Haverkorn 1994, Foda 1995, Walker 1997, Schlager 1999, Kontiokari 2001, Stothers 2002, Waites 2004, Linsenmeyer 2004, McMurdo 2005] involving a total of 1049 participants, it was concluded that cranberry juice may decrease the number of symptomatic UTIs over a 12-month period. Cranberry products were more effective in reducing the incidence of UTIs in women with recurrent UTIs than in elderly men and women or in patients requiring catheterisation [Jepson 2008].

A meta-analysis of pooled results from four studies [Stothers 2002, Kontiokari 2001, Waites 2004, McMurdo 2005] led to the conclusion that cranberry products significantly reduced the incidence of UTIs for up to 12 months in comparison with placebo or control [Jepson 2008].

An updated review and meta-analysis of 24 studies (six cross-over studies, 11 parallel group studies with two arms; five with three arms, and two studies with a factorial design) comprised a total of 4473 participants taking various cranberry products. Compared with placebo, water or no treatment, cranberry products did not significantly reduce the occurrence of symptomatic UTI overall or for any of the subgroups. The effectiveness of cranberry was also not significantly different to antibiotics for women and children. There was no significant difference between gastrointestinal adverse effects from cranberry products compared to placebo/no treatment [Jepson 2012].

Other reviewers also concluded that clinical studies have documented the efficacy of cranberry juice in women with recurrent UTI, with the strongest evidence for sexually active women. However, many of the available studies suffer from limitations, including high numbers of drop-outs and lack of intent-to-treat analysis. The limitation of these studies is the lack of uniformity with regard to the intervention, including the type of cranberry product evaluated (juice, sweetened cocktail, dried juice or extracts in capsules and tablets), the concentration or content of the product, the dosing regimen and duration of intervention [Raz 2004, Nowack 2008, Micali 2014].

Thirteen randomised, controlled trials on the use of cranberry products in the prevention of UTIs, including 1616 subjects, were evaluated qualitatively and 10 of these trials, including a total of 1494 subjects, were further analysed in quantitative synthesis in a systematic review and meta-analysis. The results indicated that cranberry products are associated with a protective effect against UTIs. In subgroup analysis, cranberry products seemed more effective in women with recurrent UTIs, female populations, children, cranberry juice drinkers and subjects using cranberry-containing products more than twice daily [Wang 2012a].

A review of eight clinical trials examined the use of cranberry products for the prevention of UTIs in children and adolescents (aged between 1 month and 18 years). Three studies had been performed in otherwise healthy children and five in paediatric patients with underlying urogenital abnormalities, of which two small trials compared cranberry to antibiotics. In otherwise healthy children, cranberry use was associated with a reduction in the overall number of UTIs and a decrease in the number of antibiotic days per year for UTI treatment. In patients with urogenital abnormalities, results were contradictory. However, cranberry products had similar efficacy as cefaclor or trimethoprim [Durham 2015].

In a meta-analysis of 7 randomized controlled trials in generally healthy, non-pregnant women (n=1498 participants; >18 years) with a history of UTI, comparison of cranberry intervention to placebo or control showed a reduced risk of UTI by 26% with cranberry [Fu 2017].

A compilation of 25 studies involving 4947 participants, in a systematic review with meta-analysis, concluded that intake of cranberry products reduced the incidence of UTIs: patients at some risk for urinary tract infections were more susceptible to the effects of cranberry [Luis 2017].

Four studies in individuals with spinal cord injuries were evaluated in a review which concluded that cranberry supplementation is ineffective for prevention of UTIs in these patients [Navarrete-Opazo 2017].

Pharmacokinetic properties

Pharmacokinetics in vitro
The transport of A-type procyanidin dimers, trimers and tetramers in two fractions from spray-dried cranberry powder through differentiated human intestinal epithelial Caco-2 cell monolayers was studied. After 2 hours, a transport rate of 0.6% for a dimer in one fraction and of 0.4% for trimers and 0.2% for tetramers from another fraction to the basolateral side was observed [Ou 2012].

After incubation of a PAC-enriched extract with human gut microbiota, benzoic acid, 2-phenylacetic acid, 3-phenylpropionic acid, 2-(3-hydroxyphenyl)acetic acid, 2-(4-hydroxyphenyl)acetic acid, 3-(3-hydroxyphenyl)propionic acid and 2-(3,4-dihydroxy-phenyl)acetic acid were identified as metabolites. The mass recovery of the metabolites after 24 hours was equivalent to 20% of the applied procyanidins [Ou 2014].

Incubation of a cranberry extract (9.76 mg/g benzoic acids, 11.1 mg/g hydroxy-cinnamic acids, 2.1 mg/g flavan-3-ols, 0.055 mg/g anthocyanins) with human colonic bacteria resulted in the determination of 27 compounds (phenylacetic, phenylpropionic, benzoic and cinnamic acids, phenols and metabolites of flavan-3-ols, such as phenyl-γ-valerolactones and phenylvaleric acid derivatives). Benzoic acid, 4-hydroxy-5-(3´-hydroxyphenyl)-valeric acid and phenylacetic acid were the most abundant compounds [Sanchez-Patan 2015].

Pharmacokinetics in animals
Sprague-Dawley rats were fed a diet containing 0, 3.3, 6.6 or 33 g/kg of concentrated cranberry powder (1.51 mg/g total anthocyanins, 56.2 mg/g procyanidins, 94.2 mg/g total phenolics, 214 mg/g glucose and 70.6 mg/g fructose) for 50 days. Urine was collected over 24 hours between days 30 and 35 and nineteen phenolic acids were determined. Hippuric, 4-hydroxycinnamic, 3-hydroxyphenylacetic, 4-hydroxyphenylacetic and 3-hydroxyphenylpropionic acid were the major metabolites. For several phenolic acids, the percentage excreted in the conjugated form was generally consistent for the various levels of cranberry in the diet and ranged from 65 to 100% for the individual acids [Prior 2010].

Cranberry concentrate powder (1 g/kg b.w.; 5 days a week for 10 months) was administered orally to Fischer-344 rats. Quercetin, isorhamnetin, myricetin, kaempferol, proanthocyanidin A2, peonidin 3-O- and cyanidin 3-O-galactoside as well as 13 conjugated metabolites of quercetin and isorhamnetin were identified in the urine after treatment. Very low levels of isorhamnetin (0.48 ng/mL) and proanthocyanidin A2 (0.541 ng/mL) were determined in plasma samples. Analysis of amethanolic extraction of urinary bladder tissue showed that the chronic administration of the concentrate resulted in accumulation of quercetin and isorhamnetin in the bladder [Rajbhandari 2011].

Addition of 5% freeze-dried cranberry powder to a fructose-rich diet for Sprague-Dawley rats resulted in significant ($p<0.05$) changes of urinary excretion of 10 phenolic acids after 30 to 40 days: total hippuric acid as the main metabolite increased by >6000 µg/day relative to control, 4-hydroxycinnamic acid and 3-methoxy-4-hydroxyphenylacetic acid each by >180 µg/day [Khanal 2014].

An extract enriched in PACs was administered orally to Sprague-Dawley rats (250 mg/kg b.w. twice daily, at 0 and 12 hours) and urine collected for 24 hours. The urinary metabolome at baseline and after ingestion of the extract showed a clear difference. The urinary levels of hippurate, succinate, lactate and two unknown metabolites increased and metabolites such as α-ketoglutarate and citrate decreased [Liu 2016].

In a metabolomic study, the effects of cranberry supplementation (100 mg/kg/d containing 11 mg/kg PAC-A and 4 mg/kg PAC-B; orally for 35 days) were studied in Sprague-Dawley rats. Microbial PAC metabolites, such as valeric acid and valerolactone derivatives, were shown to be related to cranberry consumption. An increased urinary excretion of glucuronidated metabolites was also observed. The biomarkers of polyphenol intake, catechol sulphate, benzoyl glucuronide and hippuric acid were increased in urine [Peron 2017a].

Pharmacokinetics in humans
Blood samples from a fasted healthy volunteer before, and at 45 and 270 minutes after, ingestion of a very large single dose (1800 mL) of cranberry juice were analysed. No benzoic, phenolic or flavonoid compounds were observed in the plasma before cranberry consumption. Benzoic acid (3 µg/mL) and several hydroxybenzoic acids were detected after 45 and 270 minutes, as well as two hydroxycinnamic acids after 270 minutes, but no free flavonoids [Zhang 2004].

Consumption of cranberry juice (3 x 250 mL/d for 2 weeks; 7 mg/L salicylate) or placebo by healthy women (n=11/group) was associated with a significant increase ($p<0.001$) of salicyluric and salicylic acids in urine at weeks 1 and 2 in the cranberry group compared to placebo. After 2 weeks, a small but significant ($p<0.05$) increase in salicylic acid in plasma was observed as compared to placebo [Duthie 2005].

The excretion of anthocyanins in human urine was investigated in 11 healthy volunteers after consumption of 200 mL of cranberry juice (650 µg total anthocyanins). Maximum urinary levels of anthocyanins were reached between 3 and 6 hours after ingestion. After 24 hours, about 5% of the total anthocyanins had been recovered in the urine, the most abundant compound being peonidin 3-O-galactoside [Ohnishi 2006].

A study in 15 volunteers (mean age: 62 years) with coronary artery disease revealed marked inter-individual differences in plasma anthocyanin pharmacokinetics after consumption of 480 mL cranberry juice (54% juice; 835 mg total polyphenols; 94.5 mg anthocyanins). Maximum anthocyanin concentrations were detected between 1 and 3 h. Anthocyanins showed notable individual differences in C_{max} and AUC_{0-4h}. The pattern of anthocyanin glucosides in plasma and urine reflected their relative concentration in the juice. Plasma concentrations of the individual anthocyanins ranged between 0.56 and 4.64 nmol/L. Total recovery of urinary anthocyanins was 0.79% of the ingested dose [Milbury 2010].

In another study, 11 healthy subjects received 240 mL of a juice, 55 g of a sauce or 40 g dried cranberries, corresponding to doses of 9.1 mg/mL, 11.1 mg/g and 12.4 mg/g phenolics respectively. Blood samples were collected at 0, 0.5, 1, 2, 3 and 4 hours, urine samples before and 6 hours after consumption. Sixteen phenolic, benzoic and cinnamic acids were identified with bound compounds exceeding free phenolics. Plasma levels of most individual compounds declined after reaching a maximum at 0.5 or 1 hours, but for benzoic, trans-cinnamic, vanillic, p-coumaric acids and catechin a second peak was observed. The maximum plasma concentrations of total phenolics were between 0.20 and 1.80 µg/mL, 0.10 and 0.30 µg/mL, and 0.15 and 0.30 µg/mL after consumption of juice, dried fruits and

sauce, respectively. Urinary excretion of phenolic compounds was much higher than in plasma [Wang 2012b].

After overnight fasting, 4 healthy volunteers consumed 0.6 mL/kg of a syrup (1.10% total phenolics, 0.71% PACs, 0.10% anthocyanins). Urine samples were collected before and at 2, 4 and 6 hours after ingestion. More than 30 free polyphenols, phase I and phase II metabolites were tentatively identified such as canthoside A, caffeoyl glucose, dihydroferulic acid, dihydroxybenzoic acid [Iswaldi 2013].

A single-dose pharmacokinetic trial in 10 healthy adults aged over 50 years evaluated the 24 hours absorption and excretion of flavonoids, phenolic acids and PACs from 237 mL of a cranberry juice cocktail (54% juice). Thirteen phenolic acids, 2 flavanols, 4 flavonols, 6 anthocyanins, 4 anthocyanin glucuronides, and 1 PAC were identified. A bimodal distribution of plasma C_{max} was observed for the flavanols, flavonols and 7 phenolic acids, and a trimodal distribution for caffeic acid. The total concentration of phenolics in plasma reached a peak of 34.2 µg/mL between 8 and 10 h, while in urine this peak was 269.8 µg/mg creatinine appearing 2–4 h earlier [McKay 2015].

The clearance of flavonoids was examined in urine samples of 10 healthy women collected at 0, 90, 225, and 360 min after ingestion of 240 mL cranberry juice containing flavonol glycosides. Five flavonol glycosides were identified, with quercetin-3-galactoside the most abundant cranberry flavonol, exhibiting the highest peak urine concentration (C_{max}) of 1315 pg/mg creatinine. Relative to the other flavonoids with T_{max} of 90–151 min, quercetin-3-arabinoside showed delayed clearance with T_{max} of 237 min [Wang 2016].

Ten healthy volunteers on a low polyphenol and anthocyanin-free diet received a single dose of 450 mL of cranberry juice (787 mg total (poly)phenols). Blood samples were collected before and at 1, 2, 4, 6, 8 and 24 hours, and urine for the 24 h after consumption. Sixty phenolic metabolites were identified such as sulphates of pyrogallol, valerolactone, benzoic acids, phenylacetic acids, glucuronides of flavonols, as well as sulfates and glucuronides of cinnamic acids. The most abundant plasma metabolites were hippuric acids, catechols, benzoic acids and phenylacetic acids. Benzoic acids, cinnamic acids and flavonol metabolites appeared in plasma at 1 - 2 hours post-consumption. The 24 h urinary recovery of phenols was 6.2% with mainly hippuric, phenylacetic and benzoic acids [Feliciano 2016].

Sixty (poly)phenol metabolites were detected in the plasma of ten healthy volunteers after consumption of cranberry juices containing 409, 787, 1238, 1534 and 1910 mg of total cranberry (poly)phenols (TCP). Most of them were conjugated and non-conjugated phenolic acids including ferulic and caffeic acid sulphates. Three flavonoid derivatives, namely kaempferol, kaempferol-3-O-β-D-glucuronide and quercetin-3-O-β-D-glucuronide) were also detected [Rodriguez-Mateos 2016].

In a similar study, the volunteers received juice containing 409, 787, 1238, 1534 and 1910 mg total (poly)phenols. Fourteen out of 60 metabolites and total plasma metabolites exhibited a linear dose response ($p<0.05$). Inter-individual variability of the plasma metabolite concentration was broad and dependent on the metabolite. The mean recovery from urine varied between 10.1% for the lowest and 2.1% for the highest dose. Recovery after the lowest dose was significantly ($p<0.001$) different from all other doses [Feliciano 2017].

Preclinical safety data

Acute toxicity
An acute oral LD_{50} of >5 g/kg b.w. was determined in female Sprague-Dawley rats for a whole cranberry powder standardized to 1.5% PACs according to OECD guidelines [Sengupta 2011].

A single dose of an acidic ethanolic extract (yield 12.5%) did not result in any mortalities in male CD-1 mice at concentrations up to 5.6 g/kg b.w. [Madrigal-Santillan 2012].

Subchronic toxicity
Three cranberry products (90% cranberry fruit solids and 1.4% PACs; 90% cranberry fruit solids and 4.75% PACs; 100% cranberry solids and 1.5% PACs) were added to the feed (1.5 g/kg stock feed) of male Wistar rats for 14 weeks. Controls received stock feed only. No significant effects on oxidative stress parameters, catalase, glutathione peroxidase, glutathione reductase, glutathione transferase, SOD, total antioxidant capacity, TBARs, advanced oxidation protein products, total SH-groups or other clinical chemistry markers were observed as compared to control. Haematological parameters, body weight and food consumption were also unaffected by cranberry intake. Only liver glutathione reductase activity and GSH levels were significantly ($p<0.05$) lower in one of the treated groups and plasma alkaline phosphatase in one of the other cranberry groups as compared to placebo. No changes in gross pathology, organ weights, histopathology or genotoxicity were observed. Total cytochrome P450 levels in the livers were unaffected in all groups [Palikova 2010].

Genotoxicity
Addition of three different cranberry products to the feed of male Wistar rats for 14 weeks did not result in any genotoxic effects [Palikova 2010].

Pregnancy
Pregnant and lactating balb/c mice received 44 mg/kg/d of a preparation (220 mg extract corresponding to 5.5 g cranberries) from copulatory plug until 28 days after delivery. Six weeks after delivery, in spleens of progeny from the cranberry group no differences in macroscopy or spleen weight, but lower numbers and larger diameter of lymphatic nodules (both $p<0.0001$) and more CD19+ and CD8+ lymphocytes ($p<0.05$) were observed as compared to control. No macroscopic abnormalities were observed in the thymus and liver or weights of these organs, but an increase in the diameter of glomeruli was seen in the kidneys ($p<0.05$). Serum levels of creatinine and urea remained unchanged, while only concentrations of VEGF and bFGF in offspring were increased after cranberry compared to control [Balan 2017].

Clinical safety data
Cranberry has a good record of safety at normal therapeutic dose levels [Raz 2004, Lynch 2004].
No adverse reactions were reported in numerous studies [Ferrara 2009, Vidlar 2010, Sánchez Ballester 2013, Ledda 2015, Ledda 2016, Wan 2016, Singh 2016].

In two randomized, placebo-controlled studies involving a total of 526 patients, of whom 187 took cranberry preparations for 15 days [McMurdo 2005] and 100 for 12 months [Stothers 2002], the incidence of adverse events was not greater in verum groups than in placebo groups. In a 6-month comparative study

involving 137 women, of whom 69 took a cranberry extract and 68 took trimethoprim, there were fewer withdrawals and fewer gastro-intestinal upsets in the cranberry group (9% and 19% respectively) than in the trimethoprim group (16% and 25%) [McMurdo 2009].

A trial comparing 480 mg trimethoprim-sulfamethoxazole with 2 x 500 mg cranberry extract daily did not show any significant differences between the groups (n=110/111) in adverse events, which included rash, nausea, vomiting, diarrhoea, constipation and vaginal complaints [Beerepoot 2011].

Patients (n=184) being treated with radiotherapy for for prostate cancer, taking 200 mg of an extract standardized to 30% PACs, did not report any adverse effects or allergic reactions, apart from gastric pain in two cases [Bonetta and Di Pierro 2012]. In a similar study, 4 patients (with chronic gastritis) out of 489 complained of gastric pain [Bonetta 2017].

In a randomized, double-blind study in 171 patients with multiple sclerosis, receiving cranberry powder or placebo, no serious adverse events and no differences between the groups were observed. Gastrointestinal disorders were seen in 17.1% of the verum group and 20.5% of the placebo group [Gallien 2014].

In a controlled, double-blind trial in 85 children from 1 month to 1 year of age, and 107 from 1 to 13 years of age, a syrup containing 3% cranberry extract was well tolerated with no adverse effects reported [Fernández-Puentes 2015].

No differences in adverse effects between 2 x 2 cranberry capsules daily for 6 weeks and placebo were observed in a randomized, double-blind, placebo-controlled trial in 160 women after gynaecological surgery. Gastrointestinal upset was the most commonly reported event in both groups [Foxman 2015].

In a trial including 185 elderly patients taking 2 cranberry capsules/day (equivalent to appr. 600 mL cranberry juice) or placebo for 1 year, the 14 protocol-related, non-serious adverse events (e.g. altered mental status, gastrointestinal or oral cavity disturbance, skin and soft tissue event, weight loss) were of similar frequency in both groups [Juthani-Mehta 2016].

In a randomized, double-blind, placebo-controlled trial in 373 women consuming 240 mL cranberry beverage/d or placebo, adverse events including headache, sinusitis and upper respiratory infection occurred in ≤5% in both groups and were not significantly different [Maki 2016].

Pregnancy
In a study involving pregnant women taking cranberry juice or placebo from less than 16 weeks of gestation to delivery, 73 women out of 188 (38.8%) withdrew, 44 due to gastrointestinal upsets [Wing 2008].

In a placebo-controlled study, 72 pregnant women consumed at least 1 × 240 mL of a cranberry juice cocktail (CJC) daily from about 16 weeks of gestation to delivery; 18 women took 2 × 240 mL and 8 took 3 × 240 mL of the CJC. There were no differences between cranberry and placebo groups with regard to obstetric or neonatal outcomes [Wing 2008].

Indirect evidence from a systematic review (including a survey of post-partum women who had taken cranberry preparations during pregnancy) suggested minimal risk during pregnancy when cranberry is consumed in food amounts [Dugoua 2008].

In the Norwegian Mother and Child Cohort Study including more than 100,000 pregnancies from 1999 to 2008, 919 women reported cranberry use during pregnancy (n=121 in the first trimester, n=566 in early pregnancy). No increased risk of congenital malformations, stillbirth/neonatal death, low birth weight, small for gestational age, preterm birth, low Apgar score (<7), neonatal infections or maternal vaginal bleeding in early pregnancy were seen. The only association was between cranberry use in late pregnancy and vaginal bleeding after pregnancy week 17, further sub-analyses of more severe bleeding outcomes did not show a significant risk [Heitmann 2013].

In a study on the compliance with, and tolerability of, cranberry ingestion for prevention of asymptomatic bacteriuria in pregnancy, 33 women participating until delivery received 2 cranberry capsules (containing 32-34 mg PACs; n=14) or placebo capsules (n=19). No antepartum complications, such as foetal malformation, intrauterine foetal growth restriction, oligohydramnios or polyhydramnios were observed. Neonatal outcomes were similar between the two groups [Wing 2015].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Online Series, 2019
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Online Series, 2019
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Online Series, 2019
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI AETHEROLEUM	Caraway Oil	Online Series, 2019
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
CENTAURII HERBA	Centaury	Online Series, 2015
CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHAMOMILLAE ROMANAE FLOS	Roman Chamomile Flower	Online Series, 2019
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
ECHINACEAE ANGUSTIFOLIAE RADIX	Narrow-leaved Coneflower Root	Online Series, 2019
ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EQUISETI HERBA	Equisetum stem	Online Series, 2018
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI AETHEROLEUM	Fennel Oil	Online Series, 2019
FOENICULI FRUCTUS	Fennel Fruit	Online Series, 2019
FRANGULAE CORTEX	Frangula Bark	Online Series, 2017
FUMARIAE HERBA	Fumitory	Online Series, 2018
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
GRAMINIS RHIZOMA	Couch Grass Rhizome	Online Series, 2016
GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
HAMAMELIDIS FOLIUM	Hamamelis Leaf	Online Series, 2012
HARPAGOPHYTI RADIX	Devil's Claw Root	Supplement 2009
HEDERAE HELICIS FOLIUM	Ivy Leaf	Second Edition, 2003
HELICHRYSI FLOS	Sandy Everlastin Flower	Online Series, 2019
HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013

HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Online Series, 2020
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Online Series, 2020
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Online Series, 2020
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
ROSAE PSEUDO-FRUCTUS	Dog Rose Hip	Supplement 2009
ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Online Series, 2018
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
UNCARIAE TOMENTOSAE CORTEX	Cat's Claw Bark	Online Series, 2018
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Online Series, 2018
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Online Series, 2020
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
VITIS VINIFERAE FOLIUM	Red Vine Leaf	Online Series, 2020
ZINGIBERIS RHIZOMA	Ginger	Supplement 2009

E/S/C/O/P Monographs

Online Series

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The second edition of ESCOP Monographs, published as a hardback book in 2003 with a Supplement in 2009, has been widely acclaimed for its authoritative information on the therapeutic uses of herbal medicines. Monographs covering a total of 107 herbal substances include extensive summaries of pharmacological, clinical and toxicological data, and copious references to scientific literature form an important part of each text.

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The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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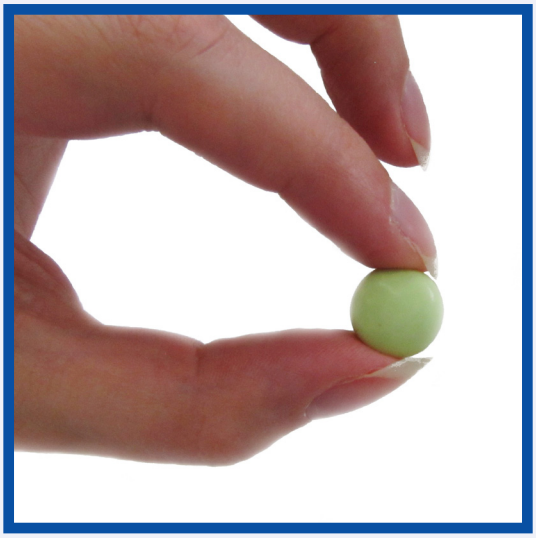
Mullein flower

2014



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VERBASCI FLOS
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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

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Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

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DEFINITION

Mullein flower consists of the dried flower, reduced to the corolla and the androecium of *Verbascum thapsus* L., *V. densiflorum* [syn. *V. thapsiforme* Schrad] and *V. phlomoides* L.

The material complies with the monograph of the European Pharmacopoeia [Mullein flower].

CONSTITUENTS

The characteristic constituents include flavonoids (1.5-4%) such as apigenin, luteolin and quercetin together with their glycosides, tamarixetin glycosides, diosmin, hesperidin, chrysoeriol, eriodictyiol, kaempferol and rutin in *V. phlomoides* (total content 2.4%) [Pápay 1980; Tschesche 1979; Klimek 2010] and in *V. densiflorum* (total content 0.61-0.68%), whereas the flowers of *V. thapsus ssp. thapsus* contain 6-hydroxyluteolin-7-O-glucoside, 3'-methylquercetin and 7,4'-dihydroxyflavone-4'-rhamnoside and a further luteolin triglycoside (verbacoside) [Klimek 2010; Mehrotra 1989; Souleles 1989; Hein 1959].

Iridoid glycosides (0.56% in *V. phlomoides*, 0.13% in *V. densiflorum*) [Grzybek 1996] such as aucubin and catalpol, together with their xylosides and p-coumaroyl esters [Klimek 1996; Osváth 1982; Swiatek 1982].

Phenylethanoid glycosides as verbascoside (acteoside) and forsythoside B (0.74-0.78% in *V. densiflorum*, less than 0.17% in *V. phlomoides*) [Klimek 2010; Grzybek 1996].

Phenolic acids such as caffeic, p-coumaric, ferulic or p-hydroxybenzoic acid and their glycosides [Blohme 2007; Klimek 1996; Osváth 1982]

Triterpene saponins (0.007% isolated from flowers and 0.028% from petals of *V. phlomoides*) as verbascosaponin, the closely related verbascosaponins A and B and desrhamnosylverbascosaponin [Klimek 1996; Schröder 1993a,b; Tschesche 1980]. The presence of saponins has not been confirmed in the flowers of *V. thapsus* and *V. densiflorum*.

Mucilaginous polysaccharides (2-3%) consisting of neutral (arabinogalactan and xyloglucan) and acidic polysaccharides, particularly a highly-branched arabinogalactan with a β-1-6- linked galactose backbone as well as compounds composed of L-arabinose, D-galactose, D-galacturonic acid, D-glucose, D-glucuronic acid, D-mannose , L-rhamnose and D-xylose [Kraus 1987].

Other constituents include phytosterols such as β-sitosterol and stigmasterol, and the bicyclic monoterpene digiprolactone [Hein 1959], carotenoids and xanthophylls [Blohme 2007; Hänsel 2007]. Flowers of *V. phlomoides* contain 2.4% and flowers of *V. densiflorum* 1.6% of fixed oil with palmitic, linolenic and myristic acid as main components [Grzybek 1996].

CLINICAL PARTICULARS

Therapeutic indications

Common cold associated with cough or sore throat as a demulcent and/or a mild expectorant [Bradley 2006; Blohme 2007; Schilcher 2008; Wichtl 2009].

In these indications, the efficacy is plausible on the basis of long-standing use.

Posology and method of administration

Dosage

Adult daily dose: 1-1.5 g of dried drug as an infusion, one to three times daily [Bradley 2006; Blohme 2007; Schilcher 2008; Wichtl 2009].

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Method of administration
For oral administration.

Duration of administration
No restriction. If symptoms persist or worsen, medical advice should be sought.

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with normal medical practice, mullein flower should not be used during pregnancy and lactation.

Effects on ability to drive and use machines
None known.

Undesirable effects
A 33-year old man employed in the medicinal plant industry developed an airborne contact dermatitis, the severity of lesions reduced on vacations and worsened after returning to work. Of 30 herbal drugs tested, a positive reaction was observed after administration of one mixture. Testing of the individual components showed that *Verbascum densiflorum* was responsible for the allergic reaction (positive tests after 48 and 96 h). Patch testing with the plant material in 10 control patients, 5 atopics and 5 non-atopics, was negative [Castro 2006].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

Relatively few pharmacological studies on mullein preparations have been published. Therefore, reports on the pharmacological activities of relevant constituents, notably the iridioid aucubin and the phenylethanoid glycoside verbascoside (acteoside), are also included.

In vitro experiments

Antiviral activity
An aqueous mullein flower dry extract (3.7-4:1) exhibited antiviral activity against fowl plague virus, several influenza A strains, an influenza B strain and *Herpes simplex* Type-1 virus. In the virus-induced cytopathic-effect inhibition assay in Vero cells, the titre of *H. simplex* decreased from 1.7 log units inhibition of virus replication at 50 µg/mL to 2.6 log units at 350 µg/mL after 4 days. The maximum non-toxic concentration (MTC) in Vero cells was determined as 1000 µg/mL after 2 days and 500 µg/mL after 3 days incubation. Plaque reduction of 50% was observed at 190 µg/mL. Virucidal activity was demonstrated by complete inhibition of the virus-induced cytopathic effect in virus-infected Vero cells which had been exposed to the mullein flower extract (300 µg) for 4h [Slagowska 1987].

The antiviral activity against influenza viruses was tested in chorioallantoic membrane cultures, the MTC was 1400 µg/mL in this cell line. The titre of four influenza A strains and one influenza B strain decreased by 1-3 log units, with minimal effective concentration values of 250-1000 µg/mL for influenza A and 1300 µg/mL for the influenza B strain. The strongest inhibition was observed when mullein flower infusion was applied simultaneously or after the virus infection [Zgorniak-Nowosielska 1991].

In another study, combination of an aqueous dry extract (4:1) with amantadine derivatives led to a marked enhancement of the inhibitory effect on the reproduction of an influenza A strain in chick embryo fibroblast cell cultures. The most pronounced enhancement was observed for the combination with amantadine glucuronide [Serkedjieva 2000].

Verbascoside exhibited antiviral activity against respiratory syncytial virus (RSV) in the viral cytopathic effect assay (EC_{50} 0.80 µg/mL) and the plaque-neutralization assay (EC_{50} 9.7 µg/mL) compared to ribavirin as positive control (EC_{50} 1.80 µg/mL and 2.2 µg/mL respectively), but was not active against murine cytomegalovirus and herpes simplex virus type 2 [Kernan 1998].

In another study, verbascoside inhibited vesicular stomatitis virus replication by 53.6% at a concentration of 500 µg/mL in HeLA cells, but did not show any effect against herpes simplex virus type 1 or poliovirus type 1 [Bermejo 2002].

Diosmin and hesperidin inhibited rotavirus infectivity in the viral cytopathic effect assay (IC_{50} 10 µg/mL), quercetin was not active [Bae 2000] .

Antibacterial activity
Mullein flower in olive oil (not further defined) inhibited the growth of *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* in the disc diffusion assay, whereas no effect was observed on *Staphylococcus epidermis* or *Streptococcus pyogenes* [Turker 2002].

Cytotoxic activity
Cytotoxic activity of mullein flower in olive oil (not defined, 600 µl + 150 µL water + 750 µL *Agrobacterium tumefaciens* in PBS) was assessed with the potato disc method. The number of tumors was reduced to 26 (50% inhibition) compared to 53 for water, 60 for 50% methanol (0% inhibition) and 0 for campothecin (100% inhibition) [Turker 2002].

Aqueous mullein flower extracts (5 g in 10 mL water at 37°C or 5 g in 50 mL boiling water and evaporation to 10 mL) had a strong inhibitory effect on the elongation step of protein biosynthesis in isolated rat liver ribosomes. Incorporation of [^{14}C]-leucin into proteins was inhibited by 50% at a concentration of 5% in the incubation mixture. No effect was observed for an ethanolic mullein flower extract (no details available). Fractionation of the aqueous extracts showed that the saponin fraction inhibited elongation factors EF-1 and EF-2, whereas no effect on peptidyltransferase activity was observed. Saponins markedly inactivated ribosomes by 43% (at a dose equivalent to 20 mg mullein flower) probably by inactivation of the binding site of the elongation factors to the ribosomes [Paszkiewicz-Gadek 1990].

Antioxidant and radical-scavenging activity
A 60% ethanolic extract (2g dried flowers in 25 mL, flavonoids 287 mg/L, carotenoids 415 mg/L) exhibited antioxidant activity equivalent to 2.5 mg/mL of ascorbic acid [Mariassyova 2006].

Radical scavenging activity
Forsythoside B and verbascoside showed scavenging activity on DPPH- and on peroxyl radicals comparable to chlorogenic

acid as a positive control, but lower than chlorogenic acid on hydroxyl radicals and in the ferric-reducing assay. Superoxide anions were scavenged to 50% by forsythoside B at 4.2 µg/mL and by verbascoside at 17.5 µg/mL [Deepak 1999; Georgiev 2011]. Verbascoside at 100 µM also significantly ($p<0.05$) reduced superoxide anion formation in LPS and LPS/interferon-γ stimulated human myelomonocytic leukaemia cells [Speranza 2010].

Verbascoside at 50-200 µM inhibited LPS-stimulated NO formation in murine macrophages by 12-59% ($p<0.001$) and by 11-56% ($p<0.001$) in mouse peritoneal exudate macrophages, compared to 64% and 70% respectively by polymixin B (5 µg/mL) as positive control. Expressions of inducible iNOS mRNA, iNOS protein level and iNOS activity in LPS-stimulated cells were not affected. A donor-induced NO reduction by 14-57% ($p<0.001$) was observed at 2-10 µM [Xiong 2000]. In another study, however, verbascoside at 100 µM significantly reduced expression of iNOS mRNA and protein as well as of nuclear NF-κB protein in LPS and LPS / interferon-γ stimulated human myelomonocytic leukaemia cells ($p<0.05$) [Speranza 2010].

Antiinflammatory activity
Aucubin inhibited Ag-induced TNF-α and IL-6 production and expression in RBL-2H3 mast cells in a dose dependent manner with an IC_{50} of 0.10 and 0.19 µg/mL respectively. Maximal inhibition of TNF-α was 73.0% and of IL-6 88.8% [Jeong 2002].

Other activities
A mullein flower infusion (4.6 g/100 mL water) did not affect mucociliary transport velocity in isolated ciliated epithelium from the frog oesophagus 90 s after application at a dose of 200 µl [Müller-Limmroth 1980].

In vivo experiments

Antiinflammatory activity
Aucubin administered orally at 100 mg/kg b.w. inhibited carrageenan-induced rat paw oedema by 33% after 3 hours ($p<0.01$) compared to 44% inhibition by indomethacin (7 mg/kg) as positive control. TPA-induced mouse ear oedema was inhibited by 80% after 4 hours ($p<0.01$) when administered topically at 1 mg/ear (indomethacin: 87% at 0.5 mg/ear). Catalpol did not show significant activity [Recio 1994].

In another study, aucubin administered i.p. at 5 mg/kg b.w. inhibited carrageenan-induced rat paw oedema by 37.9% compared to 49.5% by diclofenac as positive control ($p<0.001$). Both compounds inhibited the inflammatory reaction dose dependently after oral (1.0-10.0 mg/kg) or i.p. (0.5-10.0 mg/kg) administration ($p<0.01$) [Haznagy-Radnai 2012].

Oral administration of verbascoside (200 mg/kg b.w.) inhibited carrageenan-induced hind paw oedema in mice by 19.3-26.9% ($p<0.05$), aucubin (125 mg/kg) by 25.0-33.3% ($p<0.01$), catalpol (19.6 mg/kg) by 11.3-11.5% and indomethacin (10 mg/kg) as positive control by 35.1-45.9% [Kupeli 2007; Akdemir 2011].

In an experimental model of periodontitis in rats verbascoside, administered orally at a dose of 2 mg/kg b.w. daily for 8 consecutive days, significantly decreased several markers of inflammation in gingivomucosal tissue: myeloperoxidase activity ($p<0.001$), malondialdehyde formation ($p<0.01$), NFκB expression ($p<0.01$), iNOS expression ($p<0.01$), nitration of tyrosine residues, activation of the nuclear enzyme poly(ADP-ribose)polymerase ($p<0.01$), expression of apoptosis-inducing proteins Bax and Bcl-2 ($p<0.01$), as well as the degree of gingivomucosal tissue injury [Paola 2011].

In a model of dinitrobenzene sulfonic acid (4 mg per mouse) induced inflammatory bowel disease, the antiinflammatory effect of verbascoside (administered by gavage at 2 mg/kg b.w. every 24 h for 3 days) in mice lacking peroxisome proliferator-activated receptor-α compared to wild-type mice was studied. Verbascoside-mediated antiinflammatory activity was significantly weakened in the animal group without the receptor ($p<0.01$). This was highlighted by the inhibition of myeloperoxidase activity, of intestinal permeability, and of colon injury [Esposito 2010].

Analgesic and antinociceptive acitivity
Verbascoside inhibited acetic acid-induced writhing and tail pressure pain in mice when administered orally at 300 mg/kg b.w. and 100 mg/kg b.w. respectively ($p<0.001$) [Nakamura 1997].

In mice, p-benzoquinone -induced writhings were inhibited by verbascoside by 32.4% ($p<0.01$) at a dose of 200 mg/kg b.w., the same dose of acetylsalicylic acid as positive control inhibited writhings by 58.6% ($p<0.001$); verbascoside did not induce any apparent gastric damage or acute toxicity. The inhibition ratio obtained by administration of aucubin was 35.8% ($p<0.05$) at a dose of 125.1 mg/kg b.w.; after administration of catalpol at a dose of 19.6 mg/kg b.w. writhings were inhibited by 19.4% [Kupeli 2007; Akdemir 2011].

The antihyperalgesic activity of verbascoside was studied in a paw-pressure test in rats. Neuropathic pain was either produced by a chronic constriction injury of the sciatic nerve (CCI) or by an intra-articular injection of sodium monoiodoacetate (MIA). Verbascoside administered i.p. at a dose of 100 mg/kg b.w. significantly reverted hyperalgesia in both groups ($p<0.05$). In the CCI rats, the effect started 15 min after treatment and lasted 45 min, in MIA rats it started after 15 min and lasted 30 min. When administered orally at doses of 300 and 600 mg/kg b.w. the antihyperalgesic effect started 15 min after administration and was still significant after 60 min ($p<0.05$). The pain threshold in animals with non neuropathic pain was not influenced by verbascoside after i.p administration of 50-100 mg/kg b.w. or 300-600 mg/kg b.w. orally [Isacchi 2011].

Wound healing activity
In the linear incision wound model in mice, the wounds were treated topically once a day for nine consecutive days with 0.5 g of a preparation containing 1% verbascoside, vehicle, or a commercial product containing 1% *Centella asiatica* dry extract (positive control). The tensile strength measured on day 10 increased by 25.8% ($p<0.05$) after treatment with the verbascoside containing preparation compared to the vehicle, and 54.5 % ($p<0.001$) after treatment with the positive control. In the circular excision wound model, treatment with verbascoside reduced the wound area by 28.25% ($p<0.05$) at day 10 and 40.52% (0.01) at day 12, compared to the positive control (72.24-100%, $p<0.001$) [Akdemir 2011].

Clinical studies
No clinical studies have been published on preparations of mullein flower.

Pharmacokinetic properties
No data available.

Preclinical safety data
A lyophilized mullein flower infusion (3.7-4:1) did not show toxic effects in Vero cells up to a concentration of 1000 µg/mL at 48 h incubation and up to 500 µg/mL at 72 h incubation. In chick embryo fibroblast cells the maximal non-toxic conentration was 600 µg/mL mullein flower infusion [Slagowska 1987; Zgorniak-Nowosielska 1991].

Verbascoside did not effect gross behaviour or motor coordination of rats in the rota-rod test when administered p.o. at a dose of 1000 mg/kg b.w. [Isacchi 2011]

Genotoxicity of verbascoside
Verbascoside significantly ($p<0.05$) reduced cell viability at 0.01-1 mM and mitotic index at 0.005-0.1 mM in mitogen-stimulated human lymphocytes. It induced a significant and concentration related increase of chromosome aberrations, an increase in the percentage of aberrant cells ($p<0.01$) and in sister chromatid exchanges [Santoro 2008].

Clinical safety data
No data available.

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
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BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
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CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
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CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
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ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
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GENTIANAE RADIX	Gentian Root	Online Series, 2014
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GINSENG RADIX	Ginseng	Second Edition, 2003
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MELILOTI HERBA	Melilot	Second Edition, 2003
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MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Second Edition, 2003
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MILLEFOLII HERBA	Yarrow	Supplement 2009
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MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
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PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
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PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Second Edition, 2003
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
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TARAXACI RADIX	Dandelion Root	Second Edition, 2003
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URTICAE RADIX	Nettle Root	Second Edition, 2003
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The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

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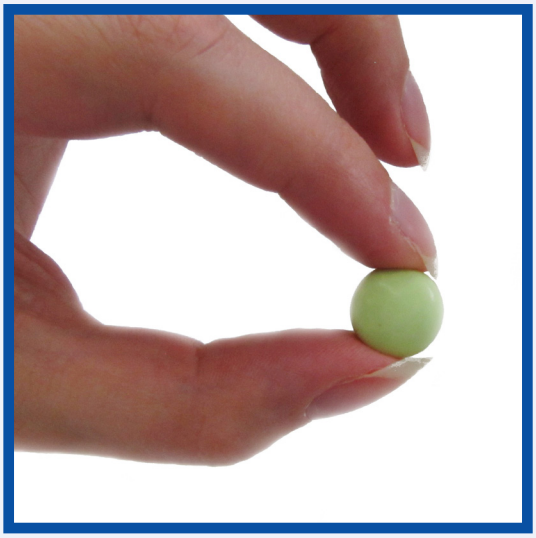

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FOREWORD

It is a great pleasure for me to introduce the online era of ESCOP Monographs. Interest in herbal medicinal products continues to stimulate research on herbal substances and the body of knowledge in this field is steadily growing. ESCOP takes account of this by preparing new monographs and - as the only organisation in the field at the moment - particularly through regular revision of our published monographs. In order to provide readers and authorities with balanced compilations of scientific data as rapidly as possible, ESCOP Monographs will be published online from now on. This contemporary way of publishing adds further momentum to ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to the members of the Scientific Committee, external experts and supervising editors, and to Peter Bradley, the final editor of every monograph published up to March 2011. All have voluntarily contributed their time and scientific expertise to ensure the high standard of the monographs.

Liselotte Krenn
Chair of the Board of ESCOP

PREFACE

Over the 15 years since ESCOP published its first monographs, initially as loose-leaf documents then as two hardback books, ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products. The Second Edition, published in 2003 with a Supplement in 2009, covered a total of 107 herbal substances.

The monograph texts are prepared in the demanding format of the Summary of Product Characteristics (SPC), a standard document required in every application to market a medicinal product for human use within the European Union and ultimately providing information for prescribers and users of individual products.

As a change in style, literature references are now denoted by the name of the first author and year of publication instead of reference numbers; consequently, citations at the end of a monograph are now in alphabetical order. This is intended to give the reader a little more information and perspective when reading the text.

Detailed work in studying the pertinent scientific literature and compiling draft monographs relies to a large extent on the knowledge, skills and dedication of individual project leaders within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are appraised by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved, but it is a time-consuming process.

To accelerate the publication of new and revised monographs ESCOP has therefore decided to publish them as an online series only, commencing in 2011. We trust that rapid online access will prove helpful and convenient to all users of ESCOP Monographs.

As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

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Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency

i.p.	intraperitoneal
IPSS	International Prostate Symptom Score
i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-kB	necrosis factor kappa-B
NO	nitric oxide
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TGF-b	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

VIOLAE HERBA CUM FLORE 2015

Wild Pansy (Flowering Aerial Parts)

DEFINITION

Wild pansy consists of the dried flowering aerial parts of *Viola arvensis* Murray and/or *Viola tricolor* L. It contains not less than 1.5 per cent of flavonoids, expressed as violanthin ($C_{27}H_{30}O_{14}$; M_r 578.5) (dried drug).

The material complies with the monograph of the European Pharmacopoeia [Wild Pansy].

Fresh material may also be used provided that when dried it complies with the monograph of the European Pharmacopoeia.

CONSTITUENTS

The characteristic constituents include

- Flavonoids (up to 2.9%), mainly rutin (up to 1%) and other flavonol-*O*-glycosides together with flavone-*C*-glycosides, principally apigenin di-*C*-glycosides including violanthin (up to 0.8%), violarvensin (up to 0.7%) and vicenin-2, and the luteolin mono-*C*-glucosides isoorientin and orientin [Fraisse 2001, Carnat 1998, Wagner 1972, Glasl 1984, Hänsel 1994, Bradley 2006, Vukics 2008a]. The flowers also contain anthocyanins such as violanin [delphinidin 3-(6-*p*-coumaroyl-rhamnosylglucoside)-5-glucoside] [Saito 1983].
- Phenolic acids (up to 0.5%), mainly salicylic acid and its derivatives methyl salicylate and violutin (methyl salicylate arabinosylglucoside) [Fraisse 2001, Komorowski 1983, Hänsel 1994].
- Carotenoids of the xanthophyll type (up to 0.1%) in the yellow flowers, principally 9-*cis*-violaxanthin (ca. 50%) and all-*trans* violaxanthin (ca. 30%) together with other *cis*- and di-*cis*-violaxanthins. They are mainly present as di-esters, esterified with saturated C_{12}-C_{18} fatty acids and/or b-hydroxy C_{12}-C_{18} fatty acids [Molnar 1980 & 1986, Hansmann 1982].
- Polysaccharides, from ca. 10% [Franz 1969, Zabaznaya 1985] to ca. 25% [Fraisse 2001], consisting mainly of glucose, galactose and arabinose together with xylose and galacturonic acid.
- Cyclotides (about 0.02%): these are macrocyclic peptides containing about 30 amino acids linked in a head-to-tail cyclic backbone constrained by 3 disulfide bonds. The proteins violapeptide I [Schöpke 1993, Göransson 2004] and varvs A-H [Claeson 1998, Göransson 1999] have been isolated from *V. arvensis* while vitri A, varv A and varv E have been isolated from *V. tricolor* [Svangard 2004].

Other constituents include tannins, ascorbic acid, tocopherol and minerals (mainly potassium salts) [Rimkiene 2003, Fraisse 2001, Hänsel 1994, Wichtl 2001].

Saponins were not detected in *V. tricolor* [Schöpke 1993] nor in *V. arvensis* [Schöpke 1993, Fraisse 2001], contrary to an earlier report of about 5% saponins in *V. tricolor* [Tamas 1981].

CLINICAL PARTICULARS

Therapeutic indications

Skin disorders such as eczema, seborrhoea, impetigo and acne, as well as cradlecap and nappy-rash of infants [Bradley 2006, Rimkiene 2003, Hänsel 1994, Wichtl 2001, Van Hellemont 1988, Schilcher 2006].

In these indications, the efficacy is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Internal use
Adults: 1.5-4 g of the drug as an infusion three times daily [Bradley 2006, Hänsel 1994, Wichtl 2001]; fluid extract (1:1, ethanol 25%), 2-4 ml three times daily [Bradley 2006]; dry extract (6:1), 2-4 g daily [Van Hellemont 1988].

Children: proportion of adult daily dose according to age or body weight.

External use
3-4 g of the drug in 150 ml of hot water as a compress or poultice, several times daily [Bradley 2006, Hänsel 1994].

Method of administration
For oral administration and local application.

Duration of administration
No restriction.

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, the product should not be used during pregnancy or lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
None reported.

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Antimicrobial activity
An infusion, a decoction and an ethanolic extract from *V. tricolor* herb exhibited antimicrobial activity against various Gram-positive and Gram-negative bacteria and the fungus *Candida albicans* with MIC and MBC ranging from 0.15 to 5 mg/ml [Witkowska-Banaszczak 2005].

Cytotoxic activity
A 70% ethanolic extract of *V. tricolor* herb and its ethyl acetate, butanol and water fractions were examined for cytotoxic potential on MCF-7 human breast cancer cells, Neuro2a mouse neuroblastoma and normal murine fibroblast cells. The ethyl acetate fraction significantly inhibited cell proliferation in MCF-7 (about 50% ; $p<0.001$ at 200 µg/mL) and in Neuro2a cells (about 25% ; $p<0.01$ at 200 µg/mL). In contrast it was less toxic in normal cells at concentrations up to 400 µg/mL. Further investigation showed induction of apoptosis by increased sub-G1 peak, Bax/Bcl-2 ratio and cleaved caspase-3 level [Sadeghnia 2014].

An aqueous fraction of a dichloromethane : methanol (1:1) extract of *V. tricolor* inhibited proliferation of activated lymphocytes by reducing IL-2 cytokine secretion, as well as reducing production of IFN-g and TNF-a. IL-2 receptor expression and degranulation capacity remained unaffected. Bioassay-guided purification led to 2 active fractions containing cyclotides [Hellinger 2014].

In initial cytotoxicity screening the polypeptide fraction of *V. arvensis* exhibited cytotoxic activity against human embryonic lung tumour cells and T cells. In further screening the fraction exhibited dose-dependent cytotoxic activity against 10 human cancer cell lines with IC_{50} values of 15-45 µg/ml [Lindholm 2002].

The isolated cyclotides varv A and varv F were tested against the same 10 cell lines and were also found to have cytotoxic activity, with IC_{50} values of 2.7-6.4 µM and 2.6-7.4 µM respectively. When varv A was subsequently tested in lymphocytic leukaemia cells from patients and in healthy lymphocytes it exhibited selective toxicity against the leukaemia cells (IC_{50} of 1.34 µM, compared to 12.13 µM in healthy lymphocytes) [Lindholm, Göransson 2002].

Correlation analysis showed that the activity profiles of the polypeptide fraction and the two isolated cyclotides differed significantly from those of antitumour drugs in clinical use, suggesting a new mechanism for cytotoxicity [Lindholm, Gullbo 2002; Lindholm, Göransson 2002].

The cyclotides vitri A, varv A and varv E (isolated from *V. tricolor*) exhibited dose-dependent cytotoxic activity against human lymphoma and myeloma cancer cell lines with IC_{50} values of 0.6 and 1 µM respectively for vitri A, 6 and 3 µM respectively for varv A, and 4 µM in both cell lines for varv E, compared to 0.1 µM in both cell lines for doxorubicin [Svangard 2004].

Other activities
A 50%-methanolic extract from *V. tricolor* was tested for antispasmodic activity using isolated guinea-pig ileum. However, at concentrations ranging from 100 to 800 µg/ml the extract actually exhibited spasmogenic activity [Izzo 1996].

A 70% ethanolic extract of *V. tricolor* flower showed antioxidant activity in the DPPH assay in a dose-dependent manner (IC_{50} 16.0 µg/mL versus 16.6 µg/mL for ascorbic acid) [Piana 2013].

Flavonoid fractions from a methanolic extract and rutin demonstrated antioxidant activity in the Trolox (IC_{50} from 0.12 µg/mL to 17.9 µg/mL) and DPPH assays (IC_{50} from 4.0 µg/mL to 53.0 µg/mL) [Vukics 2008b].

An ethyl acetate fraction from a 70% ethanolic extract of *V. tricolor* herb significantly decreased the diameter of blood vessels on chicken chorioallantoic membrane ($p<0.05$ at 40 µg/egg), while the number of newly formed blood vessels was not suppressed [Sadeghnia 2014].

In vivo experiments

The antinociceptive and anti-inflammatory effect of a gel containing a dried ethanolic (70%) extract of *V. tricolor* flower (at 1%, 3% and 10%) was investigated in adult male Wistar rats with UVB-induced sunburn. The extract (3% in gel) prevented static (100%) and dynamic (49%) mechanical allodynia and

paw oedema (61%). The induced increase in myeloperoxidase activity was inhibited (89%) by the gel (3%). The results were comparable to those obtained with a 1% silver sulfadiazine cream [Piana 2013].

A tincture of *V. tricolor* herb (1:10, ethanol 70%) orally administered to adult male rats (1 mL/100 g) showed a moderate diuretic effect (diuretic index: 1.103; saluretic index Na^+: 1.181; saluretic index K^+: 1.365) [Toiu 2009].

In old experiments wild pansy showed anti-inflammatory activity in animals [Papay 1987] and improvement of induced eczema in rats when the fresh herb was added to their diet for 2 months [Hänsel 1994].

Pharmacological studies in humans
No data available.

Clinical studies
No data available.

Pharmacokinetic properties
No data available.

Preclinical safety data
No data available.

Clinical safety data
No data available.

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Supplement 2009
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Second Edition, 2003
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Second Edition, 2003
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Second Edition, 2003
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
BOLDI FOLIUM	Boldo Leaf	Second Edition, 2003
CALENDULAE FLOS	Calendula Flower	Second Edition, 2003
CAPSICI FRUCTUS	Capsicum	Supplement 2009
CARVI FRUCTUS	Caraway Fruit	Second Edition, 2003
CARYOPHYLLI AETHEROLEUM	Clove Oil	Online Series, 2014
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CENTELLAE ASIATICAE HERBA	Centella	Supplement 2009
CHELIDONII HERBA	Greater Celandine	Second Edition, 2003
CIMICIFUGAE RHIZOMA	Black Cohosh	Online Series, 2011
CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
COLAE SEMEN	Cola	Online Series, 2014
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
CRATAEGI FRUCTUS	Hawthorn Berries	Supplement 2009
CUCURBITAE SEMEN	Pumpkin Seed	Supplement 2009
CURCUMAE LONGAE RHIZOMA	Turmeric	Second Edition, 2003
CURCUMAE XANTHORRHIZAE RHIZOMA	Javanese Turmeric	Supplement 2009
CYNARAE FOLIUM	Artichoke Leaf	Supplement 2009
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ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Supplement 2009
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
EUCALYPTI AETHEROLEUM	Eucalyptus Oil	Second Edition, 2003
FILIPENDULAE ULMARIAE HERBA	Meadowsweet	Online Series, 2015
FOENICULI FRUCTUS	Fennel	Second Edition, 2003
FRANGULAE CORTEX	Frangula Bark	Second Edition, 2003
FUMARIAE HERBA	Fumitory	Supplement 2009
GENTIANAE RADIX	Gentian Root	Online Series, 2014
GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
GINSENG RADIX	Ginseng	Second Edition, 2003
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GRINDELIAE HERBA	Grindelia	Online Series, 2015
HAMAMELIDIS AQUA	Hamamelis Water	Online Series, 2012
HAMAMELIDIS CORTEX	Hamamelis Bark	Online Series, 2012
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MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
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PASSIFLORAE HERBA	Passion Flower	Second Edition, 2003
PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Second Edition, 2003
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Second Edition, 2003
RATANHIAE RADIX	Rhatany Root	Supplement 2009
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RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Second Edition, 2003
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ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Second Edition, 2003
SALICIS CORTEX	Willow Bark	Second Edition, 2003
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
SOLIDAGINIS VIRGAUREAE HERBA	European Golden Rod	Second Edition, 2003
SILYBI MARIANI FRUCTUS	Milk Thistle Fruit	Supplement 2009
SYMPHYTI RADIX	Comfrey Root	Online Series, 2012
TANACETI PARTHENII HERBA	Feverfew	Online Series, 2014
TARAXACI FOLIUM	Dandelion Leaf	Second Edition, 2003
TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
TORMENTILLAE RHIZOMA	Tormentil	Online Series, 2013
TRIGONELLAE FOENUGRAECI SEMEN	Fenugreek	Second Edition, 2003
URTICAE FOLIUM/HERBA	Nettle Leaf/Herb	Second Edition, 2003
URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
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VALERIANAE RADIX	Valerian Root	Supplement 2009
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The second edition of ESCOP Monographs, published as a hardback book in 2003 with a Supplement in 2009, has been widely acclaimed for its authoritative information on the therapeutic uses of herbal medicines. Monographs covering a total of 107 herbal substances include extensive summaries of pharmacological, clinical and toxicological data, and copious references to scientific literature form an important part of each text.

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The European legislative framework for herbal medicines has advanced considerably over the past decade. Directive 2004/24/EC introduced a simplified registration procedure for traditional herbal medicinal products in EU member states and imposed a 2011 deadline for the registration of certain products on the market. The Committee on Herbal Medicinal Products (HMPC), established in 2004 as part of the European Medicines Agency, has made substantial progress in the preparation of Community Herbal Monographs and associated documentation to provide a more harmonized approach to the scientific assessment of herbal medicinal products throughout the European Community

Whether the evaluation of a herbal medicine is based on evidence of clinical efficacy (*well-established use*) or on experience and historical use of that product (*traditional use*) those involved at all levels of the regulatory process need access to detailed, reliable and structured summaries of the available efficacy and safety data. ESCOP monographs meet that requirement and offer an invaluable source of scientific information on herbal medicines to regulators, manufacturers, academics, researchers, health professionals and numerous others.

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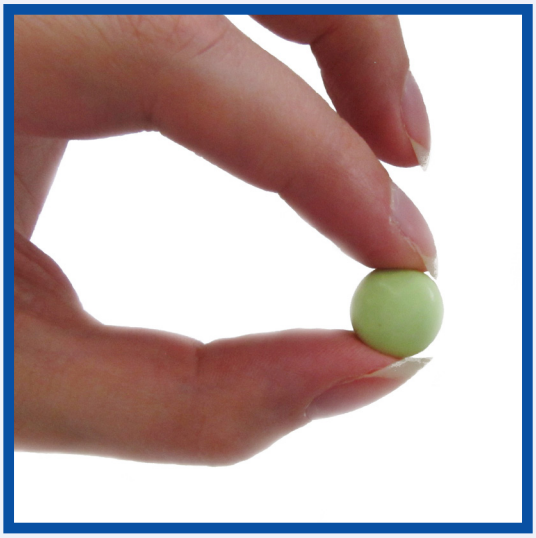
The Scientific Foundation for Herbal Medicinal Products

Vitis viniferae folium

Red Vine Leaf

2020



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Herbal Medicinal Products

VITIS VINIFERAE FOLIUM
Red Vine Leaf

2020

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Second Edition, completely revised and expanded

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Important Note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment may be required. In their efforts to provide information on the efficacy and safety of herbal drugs and herbal preparations, presented as a substantial overview together with summaries of relevant data, the authors of the material herein have consulted comprehensive sources believed to be reliable. However, in view of the possibility of human error by the authors or publisher of the work herein, or changes in medical knowledge, neither the authors nor the publisher, nor any other party involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for results obtained by the use of such information. Readers are advised to check the product information included in the package of each medicinal preparation they intend to use, to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration.

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Plant illustrated on the cover: *Vitis vinifera*

FOREWORD

On behalf of my colleagues in ESCOP it is my great pleasure here to mark the successful online programme for the publication of ESCOP Monographs. Interest in herbal medicinal products and their ingredients continues to stimulate research and the body of knowledge in this field is steadily growing. ESCOP takes full account of this in producing balanced compilations of new evidence, in the form of new ESCOP monographs and by regular revisions of previously published monographs. Our online publication, since 2011, ensures that readers and authorities are updated as rapidly as possible, and builds further on ESCOP's endeavours in the harmonization of European standards for herbal medicinal products.

The Board of ESCOP wishes to express its sincere gratitude to all members of the Scientific Committee, to external experts and to supervising editors. All have contributed their time and scientific expertise voluntarily to ensure the high standard of the monographs.

Dr. Tankred Wegener
Chair of the Board of ESCOP

PREFACE

ESCOP published its first monographs in the 1990's, initially as loose-leaf documents, then as two hardback books in 2003 and 2009. ESCOP Monographs have achieved a reputation for well-researched, comprehensive yet concise summaries of available scientific data pertaining to the efficacy and safety of herbal medicinal products.

The monograph format closely resembles the Summary of Product Characteristics (SPC) template, which is the basis for the information required for authorized/registered medicinal products, e.g. in the package leaflet, as laid down in Directive 2001/83/EC of the European Union.

Detailed work in studying the relevant scientific literature and compiling draft monographs relies on the knowledge, skills and dedication of individual project leaders and their colleagues within ESCOP Scientific Committee, as well as invited experts. After discussion and provisional acceptance by the Committee, draft monographs are reviewed by an eminent Board of Supervising Editors and all comments are taken into account before final editing and approval. In this way a wide degree of consensus is achieved.

We trust that rapid online access is helpful and convenient to all users of ESCOP Monographs. As always, ESCOP is indebted to the many contributors involved in the preparation of monographs, as well as to those who provide administrative assistance and hospitality to keep the enterprise running smoothly; our grateful thanks to them all.

NOTES FOR THE READER

From 2011 new and revised *ESCOP Monographs* are published as an online series only. Earlier monographs are available in two books, *ESCOP Monographs* Second Edition (2003) and the Second Edition *Supplement 2009*, but are not available online for copyright reasons.

After purchase of a single monograph, the specific items to be downloaded are:

Front cover
Title page
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Foreword and Preface
Notes for the Reader
Abbreviations
The monograph text
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Information on the member organizations and people involved in the production of ESCOP monographs and other activities can be found on the website (www.escop.com):

Members of ESCOP
Board of Supervising Editors
ESCOP Scientific Committee
Board of Directors of ESCOP

ABBREVIATIONS used in ESCOP monographs

AA	arachidonic acid
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ALAT or ALT	alanine aminotransferase (= SGPT or GPT)
ALP	alkaline phosphatase
anti-IgE	anti-immunoglobulin E
ASA	acetylsalicylic acid
ASAT or AST	aspartate aminotransferase (= SGOT or GOT)
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BMI	body mass index
BPH	benign prostatic hyperplasia
b.w.	body weight
cAMP	cyclic adenosine monophosphate
CAT	catalase
CCl_4	carbon tetrachloride
CI	confidence interval
C_{max}	maximum concentration of a substance in serum
CNS	central nervous system
CoA	coenzyme A
COX	cyclooxygenase
CSF	colony stimulating factor
CVI	chronic venous insufficiency
CYP	cytochrome P450
d	day
DER	drug-to-extract ratio
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	diphenylpicrylhydrazyl
DSM	Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association)
ECG	electrocardiogram
ED_{50}	effective dose in 50% of cases
EDTA	ethylenediamine tetraacetate
EEG	electroencephalogram
EMA	European Medicines Agency
ENT	ear, nose and throat
ER	oestrogen receptor
ERE	oestrogen-responsive element
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
Gal	galactose
GFR	glomerular filtration rate
GGTP	gamma-glutamyl transpeptidase
GOT	glutamate oxalacetate transaminase (= SGOT)
GPT	glutamate pyruvate transaminase (= SGPT)
GSH	glutathione (reduced)
GSSG	glutathione (oxidised)
HAMA	Hamilton Anxiety Scale
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMPC	Committee on Herbal Medicinal Products (of the EMA)
HPLC	high-performance liquid chromatography
5-HT	5-hydroxytryptamine (= serotonin)
IC_{50}	concentration leading to 50% inhibition
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Tenth Revision
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICSD	International Classification of Sleep Disorders
IFN	interferon
IL	interleukin
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
INR	International Normalized Ratio, a measure of blood coagulation (clotting) tendency
i.p.	intraperitoneal
IPSS	International Prostate Symptom Score

i.v.	intravenous
kD	kiloDalton
KM Index	Kuppermann Menopausal Index
kPa	kiloPascal
LC-MS	liquid chromatography-mass spectrometry
LD_{50}	the dose lethal to 50% of animals tested
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LH	luteinizing hormone
5-LOX	5-lipoxygenase
LPS	lipopolysaccharide
LTB_4	leukotriene B_4
M	molar (concentration)
MAO	monoamine oxidase
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MFC	minimum fungicidal concentration
MIC	minimum inhibitory concentration
Mr	molecular
MRS	Menopause Rating Scale
MRSA	methicillin-resistant *Staphylococcus aureus*
MTD	maximum tolerated dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NBT	nitro blue tetrazolium
NF-κB	nuclear factor kappa-B
NO	nitric oxide
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
n.s.	not significant
NSAID	non-steroidal anti-inflammatory drug
ovx	ovariectomy or ovariectomized
ORAC	oxygen radical absorbance capacity
PA	pyrrolizidine alkaloid
PAF	platelet activating factor
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGE	prostaglandin E
Pgp	P-glycoprotein
PHA	phythaemagglutinin
p.o.	per os
POMS	profile of mood states
PVPP	polyvinylpolypyrrolidone
RANKL	receptor activator of nuclear factor kappa-B ligand
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
s.c.	subcutaneous
SCI	spinal cord injury
SERM	selective oestrogen receptor modulator
SGOT or GOT	serum glutamate oxalacetate transaminase (= ASAT or AST)
SGPT or GPT	serum glutamate pyruvate transaminase (= ALAT or ALT)
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
SSRI	selective serotonin reuptake inhibitor
STAI	state-trait anxiety inventory
$t_{1/2}$	elimination half-life
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TGF-β	transforming growth factor-beta
TNF	tumour necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
URT	upper respiratory tract
URTI	upper respiratory tract infection
UTI	urinary tract infection
VAS	visual analogue scale
VLDL	very low density lipoprotein

VITIS VINIFERAE FOLIUM

2020

Red Vine Leaf

DEFINITION

Red vine leaf consists of the dried leaves from cultivars of *Vitis vinifera* L. known as "teinturiers" and characterized by black grapes and red pulp. It contains not less than 4.0 per cent of total polyphenols and 0.2 per cent of anthocyanins, expressed as cyanidin 3-glucoside ($C_{21}H_{21}O_{11}$; Mr 449).

The material complies with the monograph of the Pharmacopée Française [Vigne rouge – Vitis vinifera].

Note: The leaves turn partially or completely red in autumn. Examples of suitable cultivars are the teinturiers Alicante and Gamay Fréaux [Bruneton 1995; Boucheny 1990].

CONSTITUENTS

The characteristic constituents are phenolic compounds:

- Flavonoids (up to 3.5%), the most abundant being quercetin glycosides (such as quercetin 3-glucuronide, isoquercitrin and hyperoside) and kaempferol 3-glucoside [Ahmed 2015; Andelković 2015; Schneider 2007, 2008; Bombardelli 1995; Boucheny 1990; Diaz 1989].
- Anthocyanins (0.1-1.5%) composed principally of 3-glucosides of peonidin, malvidin and cyanidin [Schneider 2007, 2008; Ezzilli 1997; Boucheny 1990; Laparra 1989; Darné 1988; Wenzel 1987]. The anthocyanin content of the leaves increases during grape maturation then decreases until leaf fall [Ezzilli 1997; Darné 1988].
- Condensed tannins (proanthocyanidins, about 4%), mainly the procyanidin dimers B1 and B2 together with trimers and procyanidin galloylesters [Ahmed 2015; Andelković 2015; Schneider 2007, 2008; Hmamouchi 1997; Boucheny 1990; Bourzeix 1986].
- Other phenolic constituents including flavan-3-ols (free and as galloyl esters), hydroxycinnamic acids (free and esterified with tartaric acid) and other phenolic acids such as p-hydroxybenzoic acid, gallic acid and syringic acid [Ahmed 2015; Andelković 2015; Bombardelli 1995; Bruneton 1995; Boucheny 1990].

CLINICAL PARTICULARS

Therapeutic indications

Internal use

Aqueous dry extract (4-7:1)
Treatment of symptoms of chronic venous insufficiency (CVI) [Butcher 2006; Kalus 2004; Schaefer 2003; Antignani 2001; Kiesewetter 2000] at stages I-II as defined by Widmer and at stages 2-4a [Rabe 2011; Monsieur 2006] as defined by CEAP classification [Eklöff 2004; Meissner 2007].

Stages of chronic venous insufficiency (CVI)
according to Widmer's classification [Antignani 2001, Widmer 1978]

Stage I
Dilated subcutaneous veins, corona phlebectatica paraplantaris, oedema.

Stage II
Trophic lesions (dermatitis, dermatoliposclerosis, white atrophy).

Stage III
Active or healed leg ulcer.

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CEAP classification for chronic venous disorders (CVD) [Eklöf 2004; Porter 1995; Perrin 2005; Meisner 2007]

C0: no visible or palpable signs of venous disease
C1: telangiectasies or reticular veins
C2: varicose veins
C3: oedema
C4a: pigmentation or eczema
C4b: lipodermatosclerosis or atrophie blanche
C5: healed venous ulcer
C6: active venous ulcer
S: symptomatic, including ache, pain, tightness, skin irritation, heaviness, muscle cramps, and other complaints attributable to venous dysfunction
A: asymptomatic

Other preparations
Symptomatic treatment of problems related to varicosis such as painful and heavy legs, as well as haemorrhoids [Fournier 1948; Valnet 1992; Boullard 2001; Van Hellemont 1986]. In these indications, the efficacy is plausible on the basis of human experience and long-standing use.

External use
Topical treatment of varicosis and couperosis [Vanaclocha 2003]. In these indications, the efficacy is plausible on the basis of human experience and long-standing use.

Posology and method of administration

Dosage

Internal use
360-720 mg of red vine leaf aqueous dry extract (4-7:1) daily [Yu 2014; Perrinjaquet-Moccetti 2013; Rabe 2011; Kalus 2004; Kiesewetter 2000].

10 g of dried leaf in 250 mL of water as an infusion, 2-4 cups per day [Elias 1993; Valnet 1992]; 10 mL of fluid extract, once daily [Valnet 1992; Van Hellemont 1986].

External use
As a bath or footbath, a decoction of 60-80 g of dried leaf per litre of water [Vanaclocha 2003].

Method of administration
For oral administration or local application.

Duration of administration
No restriction.

Contra-indications
None known.

Special warnings and special precautions for use
None required.

Interaction with other medicaments and other forms of interaction
None reported.

Pregnancy and lactation
No data available. In accordance with general medical practice, red vine leaf should not be used internally during pregnancy and lactation without medical advice.

Effects on ability to drive and use machines
None known.

Undesirable effects
Mild gastrointestinal complaints have been reported [Kiesewetter 2000].

Overdose
No toxic effects reported.

PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

In vitro experiments

Protection of venous endothelium
An aqueous dry extract (4-6:1, containing 3.3% of quercetin 3-glucuronide and 1.7% of isoquercitrin) and isolated quercetin 3-glucuronide were tested in the presence of platelets and polymorphonuclear granulocytes (PMN) for protective effects on venous endothelial cells (VEC) cultivated on porous filters to form confluent monolayers. The extract strongly inhibited the opening of intercellular clefts in the endothelial barrier caused by release products of the activated platelets and PMN. VEC monolayers pre-incubated with 50 µM quercetin 3-glucuronide for 1 or 7 days were considerably more resistant to the deleterious effects of platelet/PMN release products than VEC layers maintained in the absence of the flavonoid. The extract prevented the increase in hydraulic conductivity provoked by products released from activated blood cells [Nees 2003].

Antiherpetic activity
Various preparations of red vine leaf were tested on *Herpes simplex* virus type I (HSV-1): cryoground powder, an aqueous dry extract (1:5), filtered juice (1 mL corresponding to 3 g of fresh leaf or 600 mg of dried leaf), aqueous dry extracts prepared by maceration at 20°C or 37°C, aqueous dry extract prepared by decoction and a methanolic extract. Antiherpetic activity was determined by measuring inhibition of cytopathogenic effects of HSV-1. Extracts prepared with water at low temperature were the most active ones. Total protection against HSV-1 at a concentration of $10^3 \times ID_{50}$/50 µl (ID = the infectious dose) was obtained with cryoground powder at 50 mg/mL, the aqueous dry extract at 20 mg/ml or pure filtered juice at 0.125 mL/mL [Girre 1990].

Antispasmodic activity
Antispasmodic activity equivalent to that of 1 µg of papaverine was exerted on isolated guinea pig ileum by 0.5-1 mg of a red vine leaf infusion and 10-20 µg of a hydroalcoholic dry extract (50-80% V/V) [Abeles 1962].

Antiparasitic and antimicrobial activity
An aqueous and an ethanolic extract (not further specified), containing 3.99 mg/g and 5.0 mg/g of anthocyanins respectively, demonstrated IC_{50} values of 12.65 mg/mL and 108.9 mg/mL respectively against *Leishmania infantum*. The ethanolic extract destroyed cytoplasmic and nuclear membranes of the parasite and altered the overall shape of the host cell [Mansour 2013].

Methanolic soft extracts (not further specified, containing 2.1 to 2.6 mg/g of flavonoids) alone or in an ointment showed antibacterial activity against *Escherichia coli*, *Proteus mirabilis* and *Bacillus cereus*, but no activity against *Candida albicans* and *Aspergillus brasicalus* [Selka 2013].

Extracts (methanol/acetone/water/acetic acid: 30/42/27.5/0.5: V/V/V/V) showed stronger antimicrobial activity against Gram positive bacteria strains than against Gram negative bacterial strains and yeast [Andelković 2015].

Against *Salmonella typhimirium*, an ethanolic extract (not further specified) showed an MIC value of 6.25 µg/mL, whereas MIC values for a methanolic and an aqueous extract (not further specified) were 50 and less than 400 µg/mL respectively. Against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *C. albicans* MIC values were between 12.5 and 200 µg/mL for the ethanolic extract, whereas MIC values for methanolic and aqueous extracts were between 50 and 400 µg/mL and 100 and less than 400 µg/mL respectively [Ceyhan 2012].

Anti-inflammatory activity
An aqueous extract (DER ca 4:1), containing 146.3 mg/g of total phenols, 4.3 mg/g of anthocyanin-3-glucoside and 87.09 mg/g of flavonoids, inhibited TNFα-induced IL-8 secretion (IC_{50} = 56.85 µg/mL) and IL-8 promotor activity in human epithelial gastric (AGS) cells dose-dependently (IC_{50} = 9.51 µg/mL).

In AGS cells treated with increasing concentrations of the extract and with TNFα (10 ng/mL), it was shown that the NF-κB pathway was inhibited by interfering NF-κB driven transcription and nuclear translocation with IC_{50} values of 17.01 and 23.55 µg/mL respectively. After i*n vitro* gastrointestinal digestion, the NF-κB translocation was inihibited to a lesser extent with an IC_{50} of 74.25 µg/mL, whereas the NF-κB driven transcription was not significantly changed.

In Caco-2 cells, the extract inhibited TNFα-induced IL-8 promoter activity in a dose-dependent manner (IC_{50} = 2.41 µg/mL) and inhibited the NF-κB pathway by interfering with NF-κB-driven transcription and nuclear translocation induced by TNFα with IC_{50} values of 2.65 and 34.49 µg/mL respectively. After gastric hydrolysis, these effects were suppressed. Before *in vitro* digestion, the extract inhibited IL-8 secretion with an IC_{50} of 96.1 µg/mL, whereas after *in vitro* digestion, this effect was observed at 100 µg/mL. Moreover, no effect was shown on the promoter [Sangiovanni 2015].

Antioxidant activity
An ethanolic extract (not further specified, 70% V/V) exhibited IC50 values of 17.85 ± 0.52 µg/mL and 3.79 ± 0.09 µg/mL in the DPPH and ABTS assays respectively [Antonyan 2014].

An aqueous dry extract (DER 4-6:1) showed an IC50 of 13.4 µg/mL in the DPPH radical scavenging assay [Ahmed 2015].

An aqueous extract containing 63% of flavonoids, at 100 µM, significantly ($p<0.001$) increased endothelial nitrite oxide synthase serine 1177 phosphorylation in HUVEC and in red blood cells (RBC) after 0.5 h. Nitrite concentration in HUVEC medium significantly increased after 0.5 h ($p<0.005$) until 24 h ($p< 0.001$), whereas it decreased in RBC medium at 0.5 h and 1 h ($p<0.05$).

After 30 min, the extract significantly ($p<0.01$) decreased ROS/RNS content compared to control in HUVEC with and without tert-butylhydroperoxide (THBP) (1 mM) added to induce oxidative stress. In RBC, ROS/RNS content was significantly reduced by the extract alone ($p<0.05$ compared to control) and by the extract added with THBP ($p<0.01$ compared with THBP alone).

In living HUVEC, the NO signal was significantly ($p<0.05$) increased after incubation with the extract for 30 min, compared to control. ROS/RNS content was significantly reduced by the extract and THPB (1µM) ($p<0.001$) compared to THBP alone. Superoxide signal was also significantly decreased by the extract alone ($p<0.001$ compared to control) or added with THBP vs THBP alone ($p<0.001$). Compared to control, after incubation with the extract, Cu^{2+} reduction significantly increased in HUVEC cell culture medium ($p<0.001$), in lysed HUVEC ($p<0.01$), in plasma fraction $p<0.001$ and lysed RBC ($p<0.05$). The same effect was observed when samples were incubated with the extract and THBP ($p<0.001$, $p<0.01$, $p<0.001$, respectively).

In RBC, S-nitrosylation significantly ($p<0.05$) increased after extract incubation. RBC deformability was increased by the extract alone ($p<0.01$) compared to control and by the extract with THBP ($p<0.01$) compared to THBP alone. In contact with the adhesion protein laminin at 20 µg/well, adhesion of HUVEC was significantly decreased by the extract alone ($p<0.001$ compared to control) and with THBP ($p<0.001$ compared to THBP) [Grau 2016].

An IC_{50} of 11.2 ± 0.12 µg/mL was obtained in the DPPH assay with an ethanolic extract (DER 7-9.5:1, 80% V/V) containing approx. 137 mg/g of quercetin and 58 mg/g of rutin [Aouey 2016].

Cytotoxic activity
An ethanolic extract (not further specified, 70% V/V) showed IC_{50} values of 1.03 ± 0.2 mg/mL and 80 ± 5 ng/mL, inhibiting proliferation of mouse peripheral blood leucocytes and mice Ehrlich ascites carcinoma cells respectively [Antonyan 2014].

In vivo experiments

Antihyaluronidase activity
A hydroalcoholic dry extract (50-80% V/V) from red vine leaf or a red vine leaf infusion were administered orally to mice for 3 days. Hyaluronidase and Indian ink were injected i.p. 105 minutes after the last administration. Hyaluronidase alone induced diffusion of the Indian ink, while hyaluronidase at 1-2 IU/kg and 3-4 IU/kg b.w. was inhibited by the hydroalcoholic dry extract at 10 mg/kg and 25 mg/kg respectively. Hyaluronidase at 1-3 IU/kg was inhibited by red vine leaf infusion at 250 mg/kg [Abeles 1962].

Action on capillary resistance
In guinea pigs with reduced capillary resistance (measured using an angiopraximeter) due to vitamin C deficiency, i.p. administration of a hydroalcoholic dry extract (50-80% V/lV) for 3 days at 20-50 mg/kg b.w. increased capillary resistance more effectively than similar administration of a red vine leaf infusion at 250-500 mg/kg [Abeles 1962].

Hepatoprotective and nephroprotective activity
Nephrotoxicosis was induced in mice by intragastric administration of 25 µg of the mycotoxin citrinin twice a week for 12 weeks. One group was also given 0.5 mL of an aqueous extract (50 g of fresh leaf in 200 mL of water) for 20 weeks. The control group received the toxin only. Blood analysis and histopathological examination of the kidneys revealed positive effects in the extract-treated group, estimated as up to 41% recovery from renal damage [Bilgrami 1993].

The hepatoprotective effects of an ethanolic extract (80% V/V, DER 6:1) and its $CHCl_3$, EtOAc, *n*-BuOH and water fractions containing 89.4 ± 2.4, 216.0 ± 5.1, 91.2 ± 2.1 and 68.6 ± 1.4 mg/g of total phenolics expressed as GAE equivalents were investigated against CCl_4-induced hepatotoxicity in male Wistar-albino rats. At a dose of 125 mg/kg of the extract, the plasma and liver MDA levels were reduced by 45.7% ($p<0.05$) and 14.0% respectively. GSH content in the liver was increased by doses of 250 and 125 mg/kg as compared to the CCl_4 and control groups. At these doses, severity of histopathological injury was decreased. A dose of 62.5 mg/kg did not produce any hepatoprotective or antioxidant effects.

Among the four fractions, the n-BuOH fraction at 83 mg/kg demonstrated antioxidant activity by decreasing the ALT and significantly the AST ($p<0.001$) activities by 9.9% and 31.8% respectively, and the hepatic MDA level by 29.2% ($p<0.05$), as well as by increasing hepatic GSH levels by 50.8% ($p<0.001$), as compared to the CCl_4 and control groups. Improvements

of histopathological parameters were also observed. The water fraction at 73.64 mg/kg significantly ($p<0.001$) reduced the plasma MDA level by 82.5%, but did not improve histopathological parameters [Orhan 2007].

Healthy male adult Wistar rats were pretreated with 100 mg/kg p.o. of an aqueous dry extract (DER 4-6:1) for 17 days before i.p injection of 1.25 mL/kg of CCl_4. In the liver the extract significantly restored the CCl_4-induced elevated levels of serum alanine aminotransferase ($p<0.001$), ALP, γ-glutamyl transferase and bilirubin ($p<0.01$) as well as the CCl_4-induced reduction in levels of serum albumin ($p<0.001$) and total protein ($p<0.01$). CCl_4-induced changes in TC, triglycerides, VLDL-cholesterol ($p<0.001$), HDL-cholesterol and LDL-cholesterol ($p<0.01$) were also ameliorated. The increased hepatic MDA level was significantly decreased by the extract ($p<0.001$). In the kidney, the extract significantly inhibited the CCl_4-induced raised levels of creatinine, uric acid, calcium and MDA ($p<0.001$). Restoration of the histopathological features in both organs' tissues were also observed [Ahmed 2015].

Anti-inflammatory activity
In a carrageenan-induced rat paw oedema test in Wistar rats, a methanolic extract (not further defined) demonstrated significant ($p<0.01$) inhibition of inflammation at oral doses of 200 and 400 mg/kg, with a reduction of paw volume of 58.6% and 67.3% respectively after 3 h, when compared to control. In the histamine-induced paw oedema test, the extract significantly ($p<0.01$) reduced the paw volume by 46%, 66.7% and 76% at 100, 200 and 400 mg/kg b.w. p.o respectively [Singh 2009].

In the carrageenan-induced hind paw oedema model in Swiss albino mice, maximal anti-inflammatory activity was observed 5h after oral administration of doses of 100 and 200 mg/kg (34.62 and 36.20%) and 4h after administration of 400 mg/kg (50.02%) of an ethanolic extract (80% V/V, DER 7-9.5:1) containing 137.3 ± 10.3 mg/g of quercetin and 58.03 ± 4.2 mg/g of rutin. Oral doses of 100, 200 and 400 mg/kg of this extract also significantly ($p<0.001$) inhibited acetic acid-induced vascular permeability by 37.16%, 59.89% and 68.18% respectively, when compared to control [Aouey 2016].

Antinociceptive activity
In Swiss Albino mice, oral doses of 100, 200 and 400 mg/kg b.w. of the same extract significantly ($p<0.05$) and dose-dependently reduced the number of acetic acid-induced writhings by 48.15%, 57.97% and 68.09% respectively. In the early phase of the formalin test in mice, the extract reduced the licking time in a dose-dependent manner. In the late phase, the doses of 200 and 400 mg/kg significantly ($p<0.001$) inhibited the licking response with licking times of 33.35 ± 2.29 s and 23.79 ± 1.43 s respectively, when compared with the control group (43.69 ± 3.92 s) [Aouey 2016].

In Swiss albino mice, oral doses of 100, 200 and 400 mg/kg b.w. of a methanolic extract, administered 60 min before formalin injection to induce paw licking, demonstrated significant and dose-dependent reductions in pain compared to control, exhibited by inhibition of licking times of 2.89% ($p<0.05$), 30.97% and 50.71% ($p<0.01$) respectively. The extract also demonstrated a significant ($p<0.05$ to $p<0.01$) increase in the latency time for thermal stimulation in the tail flick test after 30, 60 and 120 min [Singh 2009].

Antipyretic activity
Doses of 200 and 400 mg/kg p.o. of an ethanolic extract (80% V/V, DER 7-9.5:1), containing 137.3 ± 10.3 mg/g of quercetin and 58.03 ± 4.2 mg/g of rutin, significantly ($p<0.01$) reduced rectal temperature 23h after yeast-induced pyrexia in Swiss Albino mice when compared to control, demonstrating activity comparable with 100 mg/kg p.o. of paracetamol ($p<0.001$) [Aouey 2016].

Effects on endurance
An extract (not further defined), containing 59% of polyphenols, 0.6% of anthocyanins and 0.02% of resveratrol, was administered to male BALB/c mice for 10 weeks at 0.2 or 0.5% (w/w) of a standard diet. Compared to control (standard diet alone), the extract at 0.2% and 0.5% dose-dependently increased swimming endurance capacity by 11.4% and 34.3% respectively. Mice fed with 0.5% of the extract had significantly ($p<0.05$) longer swimming times than controls at week 10. Plasma lactate was significantly ($p<0.05$) lower at the end of 10 weeks treatment in the 0.5% extract group when compared to control. The concentration of non-esterified fatty acids after treatment was 21% higher than in the control group ($p<0.05$). Significant differences in plasma glucose and triglyceride levels were not observed. A significant ($p<0.05$), dose-dependent increase of fatty acid oxidation enzyme activity was also observed as compared to the control group. mRNA expression levels of several lipid metabolism and mitochondrial function related genes and transcription factors in soleus muscle, as well as hormone-sensitive lipase in mesenteric adipose tissue, were significantly ($p<0.05$) upregulated in the 0.5% extract group compared to control [Minegishi 2011].

Antidiabetic and antioxidant activity
An aqueous dry extract (DER 5:1), and various fractions, were assessed for their effect on blood glucose in normoglycaemic, glucose-hyperglycaemic and streptozotocin-induced (STZ) diabetic rats, as well as for renal, hepatic and cardiac antioxidant activity. The extract was administered at 250 and 500 mg/kg b.w. p.o. as a single dose (acute) or daily for 15 days (subacute). After the single dose, significant hypoglycaemic activity was demonstrated in the glucose-hyperglycaemic rats after 2h at 250 mg/kg ($p<0.001$) and 500 mg/kg ($p<0.05$), and after 4h at 500 mg/kg ($p<0.001$) in the streptozotocin-induced (STZ) diabetic rats, when compared to control, with no significant activity in normoglycaemic rats. In the subacute test, blood glucose levels were significantly ($p<0.001$) and increasingly reduced on days 5, 10 and 15, at both doses, in STZ-diabetic rats as compared to control. The extract at both doses also exhibited greater hypoglycaemic activity than the positive control tolbutamide at days 5, 10 and 15.
Liver, kidney and heart were assessed for MDA and GSH levels on day 15. Liver MDA was significantly ($p<0.05$) reduced at 500 mg/kg, kidney MDA at both doses ($p<0.001$) and heart MDA at 250 mg/kg ($p<0.01$) in STZ-diabetic rats compared to control diabetic rats. GSH levels were most significantly increased ($p<0.001$) in the liver at 500 mg/kg (68.8%), less significantly ($p<0.05$) in the heart and not significantly in kidney. However, GSH levels decreased in the liver, heart and kidney at 250 mg/kg, in the STZ-rats compared to control diabetic rats [Orhan 2006].

Pharmacological studies in humans
No data available.

Clinical studies

Chronic venous insufficiency
After a 2-week placebo run-in period, 260 patients with CVI (Widmer's stages I-II) were randomly assigned to double-blind treatment with 360 mg (n = 87) or 720 mg (n = 85) of an aqueous dry extract (5-7:1), or placebo (n = 88), once daily for 12 weeks, followed by a 2-week follow-up period on placebo. The mean lower leg volume (measured by water displacement plethysmography) increased by 33.7 mL after 12 weeks in the placebo group and decreased significantly ($p = 0.0001$)

by 42.2 and 66.2 mL in the groups treated with the extract at 360 mg/day and 720 mg/day respectively. Reductions in ankle circumference followed a similar pattern but were less marked. Significant (p = 0.0001) improvements in key CVI symptoms (tired and heavy legs, sensation of tension, tingling and pain) were observed in both of the active treatment groups compared to the placebo group [Kiesewetter 2000].

In an open, multicentre observational study, 65 male and female patients suffering from CVI (Widmer's stages I or II) received 360 mg of an aqueous dry extract (4-6:1) daily for 42 days. All four subjective symptoms evaluated (tired and heavy legs, sensation of tension in the legs, tingling sensations in the legs, pain in the legs) improved compared to baseline. Global efficacy of the treatment was rated by both patients and investigators as good or satisfactory in approximately 80% of the patients [Schaefer 2003].

A randomized, double-blind, placebo-controlled, crossover study was carried out to determine the effect of an aqueous dry extract (4-6:1) on cutaneous microvascular blood flow, transcutaneous oxygen pressure ($tcpO_2$) and leg oedema in patients with CVI (Widmer's stages I or II). After a 2-week run-in period the treatment consisted of either 360 mg of extract (n = 36) or placebo (n = 35) once daily for 6 weeks, followed by a 4-week wash-out period with placebo and then another 6-week treatment period. Microvascular blood flow and $tcpO_2$ values increased significantly ($p<0.0001$) after verum treatment. Significant ($p<0.0001$) reductions in calf and ankle circumferences were also observed [Kalus 2004].

In an open observational study, 39 patients with CVI (Widmer's stages I or II) received 2 × 180 mg of an aqueous dry extract (4-7:1) daily for 6 weeks. The calf volume measured by water plethysmography, the circumference of the leg at a fixed height, and the intensity of sensation of heaviness and pain on a visual analogue scale significantly ($p<0.001$) decreased after 2, 4 and 6 weeks of treatment [Monsieur 2006].

In a randomised, double-blind, placebo-controlled study, patients with CVI (CEAP stages 3-4a) received, after a 2-week run-in period on placebo, either 720 mg of an aqueous dry extract (4-6:1) (n=126) or placebo (n=122) for 12 weeks. The extract significantly (p=0.0268) reduced lower limb volume compared to placebo. No significant difference was observed in calf circumference between groups. At day 84, leg pain (assessed by VAS) was significantly (p=0.047) decreased in verum treated patients compared to placebo, whereas no significant difference was observed for other subjective symptoms between the groups [Rabe 2011].

In an open, single blind, placebo-controlled study, 17 healthy women suffering from swollen legs received 600 mg of an ethanolic dry extract (not further specified) daily for 6 weeks, followed by placebo for 6 weeks after a 2-month wash out period. After 6 weeks of verum treatment, swelling of the right leg had decreased significantly ($p<0.018$) compared to placebo. Swelling of the left leg was significantly reduced compared to baseline, both in the morning and in the evening, but this effect was not significant when compared to placebo [Perrinjaquet-Moccetti 2013].

In an open study involving 62 women with orthostasis-dependent phlebopathy of the lower limbs and a history of long term hormone replacement therapy, 360 mg of an aqueous dry extract (DER 4-6:1) was administered daily for 6 months. During the first 3 months, calf circumference decreased significantly (p=0.001) from 36.77 ± 2.89 cm to 35.98 ± 2.83 cm and then remained stable for the following 3 months. Ankle circumference also decreased, but to a lesser extent. The evening diameter of the great saphenous vein significantly (p=0.001) decreased from 0.7 ± 0.11 cm to 0.65 ± 0.99 cm. A decrease in the intensity of symptoms of venous insufficiency was observed with a VAS. The effects were most pronounced in the first 3 months of the study but were maintained for the 3 subsequent months [Tsukanov 2014].

Pharmacokinetic properties

No data are available on pharmacokinetic properties of red vine leaf extracts or corresponding preparations. Some data exist on absorption and metabolism of relevant individual flavonoids [Zuo 2006; Manach 2004; Manach 2005; Williamson 2005].

Preclinical safety data

Acute toxicity

A single dose of 2 g/kg b.w. p.o. of a methanolic extract (not further specified) did not cause any mortality in albino Wistar rats after 14 days. The LD_{50} was greater than 2 g/kg [Singh 2009].

No toxic effects or pathological anatomical changes were observed after oral administration of an aqueous dry extract (4-6:1) to mice and rats at up to 10 g/kg b.w. [Petrini 2003].

Repeated dose and chronic toxicity studies

No adverse effects were observed in rats after oral doses of the same extract at 250 mg/kg b.w. for 90 days [Petrini 2003].

Mutagenicity and carcinogenicity

In the Ames test, no mutagenic effects of an aqueous extract were evident at concentrations ranging from 8 up to 5000 µg per plate on *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 [Anonymous 1991].

Oral administration of an extract at 1.0, 3.0 and 10.0 mL/kg did not cause any mutagenic effects in the micronucleus test in mice [Anonymous 1992].

Concentrations from 10 up to 400 µg/mL and 1 up to 5 mg/mL of an extract had no mutagenic effect on Chinese hamster V79 cells [Anonymous 1993a].

Reproductive toxicity

No teratogenic effects were observed in animal studies using an aqueous dry extract (4-6:1) [Petrini 2003].

After administration of an extract (containing 1.92% of quercetin 3-glucuronide, 0.8% of isoquercitrin and 2.96% of total flavonoids) by gavage to pregnant rabbits at 300, 1000 and 3000 mg/kg b.w. from the 6th to the 18th day of pregnancy no teratogenic effects were observed [Anonymous 1993b].

Clinical safety data

Daily doses of 360-720 mg of an aqueous dry extract (4-7:1) taken for periods of 6-12 weeks by over 400 patients in clinical studies were generally well tolerated; reported adverse events were usually mild in nature, the most common being gastrointestinal disorders [Kalus 2004; Shaefer 2003; Kiesewetter 2000]. In two placebo-controlled studies more adverse events were reported in placebo groups than in verum groups [Kalus 2004; Kiesewetter 2000]. In at least 1% of patients in treated (n=126) and placebo (n=122) groups, nasopharyngitis, hypercholesterolemia, oropharyngeal pain, urticaria and back pain were also observed [Rabe 2011].

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E/S/C/O/P
MONOGRAPHS

MOST RECENT VERSIONS

Title	Common name	Publication
ABSINTHII HERBA	Wormwood	Second Edition, 2003
AGNI CASTI FRUCTUS	Agnus Castus	Second Edition, 2003
AGRIMONIAE HERBA	Agrimony	Online Series, 2019
ALCHEMILLAE HERBA	Lady's Mantle	Online Series, 2013
ALLII SATIVI BULBUS	Garlic	Online Series, 2019
ALOE BARBADENSIS	Barbados Aloes	Online Series, 2014
ALOE CAPENSIS	Cape Aloes	Online Series, 2014
ALTHAEAE RADIX	Marshmallow Root	Online Series, 2018
ANGELICAE RADIX	Angelica Root	Supplement 2009
ANISI FRUCTUS	Aniseed	Online Series, 2014
ARNICAE FLOS	Arnica Flower	Online Series, 2019
ARCTII RADIX	Burdock Root	Online Series, 2016
BALLOTAE NIGRAE HERBA	Black Horehound	Online Series, 2015
BETULAE FOLIUM	Birch Leaf	Online Series, 2015
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ECHINACEAE PALLIDAE RADIX	Pale Coneflower Root	Online Series, 2018
ECHINACEAE PURPUREAE HERBA	Purple Coneflower Herb	Supplement 2009
ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
ELEUTHEROCOCCI RADIX	Eleutherococcus	Supplement 2009
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HIPPOCASTANI SEMEN	Horse-chestnut Seed	Second Edition, 2003
HYDRASTIS RHIZOMA	Goldenseal rhizome	Online Series, 2013

HYPERICI HERBA	St. John's Wort	Online Series, 2018
JUNIPERI PSEUDO-FRUCTUS	Juniper	Second Edition, 2003
LAVANDULAE FLOS/AETHEROLEUM	Lavender Flower/Oil	Supplement 2009
LEONURI CARDIACAE HERBA	Motherwort	Online Series, 2019
LICHEN ISLANDICUS	Iceland Moss	Second Edition, 2003
LINI SEMEN	Linseed	Online Series, 2017
LIQUIRITIAE RADIX	Liquorice Root	Second Edition, 2003
LUPULI FLOS	Hop Strobile	Second Edition, 2003
MALVAE FLOS	Mallow Flower	Online Series, 2016
MARRUBII HERBA	White horehound	Online Series, 2013
MATRICARIAE FLOS	Matricaria Flower	Online Series, 2020
MELALEUCAE AETHEROLEUM	Tea Tree Oil	Supplement 2009
MELILOTI HERBA	Melilot	Second Edition, 2003
MELISSAE FOLIUM	Melissa Leaf	Online Series, 2013
MENTHAE PIPERITAE AETHEROLEUM	Peppermint Oil	Second Edition, 2003
MENTHAE PIPERITAE FOLIUM	Peppermint Leaf	Online Series, 2019
MENYANTHIDIS TRIFOLIATAE FOLIUM	Bogbean Leaf	Online Series, 2013
MILLEFOLII HERBA	Yarrow	Supplement 2009
MYRRHA	Myrrh	Online Series, 2014
MYRTILLI FRUCTUS	Bilberry Fruit	Online Series, 2014
OLIBANUM INDICUM	Indian Frankincense	Supplement 2009
ONONIDIS RADIX	Restharrow Root	Online Series, 2015
ORTHOSIPHONIS FOLIUM	Java Tea	Online Series, 2014
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PAULLINIAE SEMEN	Guarana Seed	Supplement 2009
PELARGONII RADIX	Pelargonium Root	Online Series, 2015
PIPERIS METHYSTICI RHIZOMA	Kava-Kava	Second Edition, 2003
PLANTAGINIS LANCEOLATAE FOLIUM/HERBA	Ribwort Plantain Leaf/Herb	Online Series, 2013
PLANTAGINIS OVATAE SEMEN	Ispaghula Seed	Second Edition, 2003
PLANTAGINIS OVATAE TESTA	Ispaghula Husk	Online Series, 2016
POLYGALAE RADIX	Senega Root	Second Edition, 2003
PRIMULAE RADIX	Primula Root	Second Edition, 2003
PRUNI AFRICANAE CORTEX	Pygeum Bark	Supplement 2009
PSYLLII SEMEN	Psyllium Seed	Online Series, 2017
RATANHIAE RADIX	Rhatany Root	Online Series, 2017
RHAMNI PURSHIANI CORTEX	Cascara	Online Series, 2015
RHEI RADIX	Rhubarb	Online Series, 2018
RIBIS NIGRI FOLIUM	Blackcurrant Leaf	Online Series, 2017
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ROSMARINI FOLIUM	Rosemary Leaf	Second Edition, 2003
RUSCI RHIZOMA	Butcher's Broom	Online Series, 2017
SALICIS CORTEX	Willow Bark	Online Series, 2017
SAMBUCI FLOS	Elder flower	Online Series, 2013
SALVIAE OFFICINALIS FOLIUM	Sage Leaf	Second Edition, 2003
SALVIA TRILOBAE FOLIUM	Sage Leaf, Three-lobed	Online Series, 2014
SENNAE FOLIUM	Senna Leaf	Second Edition, 2003
SENNAE FRUCTUS ACUTIFOLIAE	Alexandrian Senna Pods	Second Edition, 2003
SENNAE FRUCTUS ANGUSTIFOLIAE	Tinnevelly Senna Pods	Second Edition, 2003
SERENOAE REPENTIS FRUCTUS (SABAL FRUCTUS)	Saw Palmetto Fruit	Second Edition, 2003
SERPYLLI HERBA	Wild Thyme	Online Series, 2014
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TARAXACI RADIX	Dandelion Root	Second Edition, 2003
THYMI HERBA	Thyme	Second Edition, 2003
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URTICAE RADIX	Nettle Root	Online Series, 2015
UVAE URSI FOLIUM	Bearberry Leaf	Online Series, 2012
VACCINII MACROCARPI FRUCTUS	Cranberry	Supplement 2009
VALERIANAE RADIX	Valerian Root	Supplement 2009
VERBASCI FLOS	Mullein Flower	Online Series, 2014
VIOLAE HERBA CUM FLORE	Wild Pansy	Online Series, 2015
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