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MONOGRAPHS

The Scientific Foundation for Herbal Medicinal Products

Melissae folium
Melissa Leaf

2013

E/S/C/O/P
EUROPEAN SCIENTIFIC COOPERATIVE
ON PHYTOTHERAPY

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ONLINE SERIES

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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.

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

MELISSAE FOLIUM

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.

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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 [³H]-(*N*)-nicotine and [³H]-(*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 [³H]-(*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 [³H]-(*N*)-nicotine and [³H]-(*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 [³H]-nicotine, and 1.46 to 4.31 mg/mL for the displacement of [³H]-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 [³H]-nicotine and a fraction containing ionizable alkaline material provided IC_{50} values of 102.0 to 162.9 µg/mL for the displacement of [³H]-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 [³⁵S]-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 [³H]-ketanserin to $5\text{-}HT_{2A}$ receptors, of [³H]-8-hydroxy-N,N-dipropyl-2-aminotetralin to $5\text{-}HT_{1A}$ receptors, of [³H]-pirenzepine to muscarinic receptors and of [³⁵S]-TBPS to the $GABA_A$ site channel and of [³H]-clobenprobit to histamine H_3 receptors. The binding of [³H]-muscimol at the $GABA_A$ receptor agonist site was increased as well as the [³H]-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 ($p≤0.05$) 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-methylenedioxymeth-amphetamine (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 in 100 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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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
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CINNAMOMI CORTEX	Cinnamon	Second Edition, 2003
CRATAEGI FOLIUM CUM FLORE	Hawthorn Leaf and Flower	Second Edition, 2003
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ECHINACEAE PURPUREAE RADIX	Purple Coneflower Root	Supplement 2009
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GINKGO FOLIUM	Ginkgo Leaf	Second Edition, 2003
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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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MONOGRAPHS

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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