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Abstract

Today, the numbers of people suffering from lifestyle diseases like diabetes, obesity, allergies and depression increases mainly in industrialised states. That does not only lower patients' quality of life but also severely stresses the health care systems of these countries. Essential oils have been in use as therapeutic remedies for centuries against various complaints, but still their effectiveness is being underestimated. In the last decades, a great number of controlled studies have supported efficacy of these volatile secondary plant metabolites for various therapeutic indications. Besides others, essential oils have antidepressant, anti-obesity, antidiabetic, antifibrogenic, antiallergic and antimicrobial effects. In this diploma thesis, the pharmacological mechanisms for selected essential oils are summarised and discussed with the main attention on their impact against public health disorders. Additionally, possible toxic components and drug interactions are described.

Zusammenfassung

Heutzutage leiden zunehmend viele Menschen in den entwickelten Industriestaaten unter Lebensstilerkrankungen wie Diabetes, Übergewicht, Allergien und Depressionen. Dies hat nicht nur eine Minderung der Lebensqualität der betroffenen Patienten zu Folge, sondern wirkt sich auch negativ auf das Gesundheitssystem in den jeweiligen Ländern aus. Ätherische Öle werden seit Jahrhunderten als Heilmittel für verschiedenste Beschwerden angewendet, jedoch wird deren Wirksamkeit noch immer unterschätzt. In den vergangenen Jahrzehnten hat eine große Anzahl kontrollierter Studien die Wirksamkeit dieser flüchtigen sekundären Pflanzenmetaboliten für verschiedene therapeutische Indikationen unterstützt. Neben vielen anderen, haben ätherische Öle antidepressive, anti-adipöse, antidiabetische, antifibrotische, antiallergische und antimikrobielle Wirkungen. In dieser Diplomarbeit werden die pharmakologischen Wirkmechanismen von ausgewählten ätherischen Ölen, besonders im Hinblick auf Zivilisationskrankheiten, sowie mögliche toxische Bestandteile und Arzneimittelinteraktionen, beschrieben.

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1 Introduction

In industrial states several lifestyle diseases increased in recent years mainly due to unhealthy food and limited regular exercise. According to recent publications, lifespan sank in the USA starting from 2014. Hypertension, heart diseases, diabetes mellitus and chronic liver diseases [1] as well as obesity were identified as the main reasons [2, 3]. To avoid the use of synthetic products, the demand of customers and patients for natural drugs also increased mainly because they are believed to provide lesser side-effects. The latest acknowledgment for natural product drug discovery was the Nobel Prize in Physiology/Medicine in 2015 for the research on artemisinin, a sesquiterpene lactone with anti-malaria properties [4].

Essential oils (EO) have already been used since the middle ages for a broad spectrum of biological activities. They are complex mixtures of volatiles, generally composed of terpenes and aromatic structures. The main components are mainly produced by the plants via three biosynthetic pathways: the mevalonic acid pathway for sesquiterpenes, the methylerythritol phosphate pathway for mono- and diterpenes, or the shikimic acid pathway leading to phenylpropanes [5, 6]. According to the European Pharmacopeia, EOs are obtained by steam distillation or by cold pressing of the peels of citrus fruits [7]. These secondary metabolites originally are being produced by the plant for protective benefits. They are known and used for their antimicrobial, antiseptic, analgesic, anti-inflammatory, spasmolytic and sedative effect, but they can serve other less known and underestimated effects [5].

Approximately one billion people worldwide are suffering from arterial hypertension. It is one of the most widespread diseases and can lead to many cardiovascular disorders such as heart failure, coronary artery disease, kidney diseases and stroke [8].

In 2017, more than 264 million people were suffering from depression [9]. Depressive disorders were rated as the third highest cause for disabilities in 2004 [10]. The pathophysiology of depressions is difficult and still not fully clarified. Trigger factors can be psychosocial and biological stressors, imbalance of monoamine neurotransmitters, inflammation, as well as structural and functional brain changes. Additionally, the patient’s personal living condition has an influence

on his/her aetiopathogenesis. Anxiety symptoms often go ahead of depression and about two-thirds of major depressives additionally have to deal with anxiety, although it still comprises two separate disorders [11].

Obesity is one of the most increasing health problems in industrial states. In 2015, the count of obese people from 195 countries rose to 600 million. In about 70 of these countries incidences of obesity had doubled over 25 years. It can trigger many secondary illnesses, such as diabetes, obstructive sleep apnoea, systemic and pulmonary hypertension, and coronary artery disease which all can induce heart failure [12]. Moreover, obesity is part of the metabolic syndrome, which also includes hypertension, hyperlipidaemia, and diabetes mellitus type 2 [13], hereby affecting approximately 60% of patients [14].

Globally type 2 diabetes incidences have increased fourfold between 1980 and 2004. In 2015, 415 million people were diagnosed as diabetics and until 2040, 642 million are being predicted. The deficit of physical exercise, calorie-rich diets and obesity promote this trend [14]. The increase in diabetes also has economic consequences, as the costs for health care systems rise. The costs for diabetics are on average 1.5 to 4.4 times higher than for people without diabetes. In Europe, the average cost per person with diabetes lay between € 1,708 and € 5,899 in 2010 [15]. Depression, obesity and type 2 diabetes can also occur together and influence each other negatively. It has been observed that treatment of depression can have a positive effect on body weight and vice versa [16].

Non-alcoholic fatty liver disease (NAFLD) can lead to non-alcoholic steatohepatitis (NASH) and cirrhosis and even hepatocellular carcinoma (HCC) as these can be triggered by NASH [17]. NASH has a prevalence of 3-5% in the western world [18]. Worldwide liver cancer became the fifth most frequently malignant cancer. Obesity and type 2 diabetes are risk factors for HCC. If they co-exist with NAFLD the negative potential is even higher [17]. Furthermore, oxidative stress seems also to play a role in developing liver diseases [19].

About 20% of the world population are suffering from several allergies [20]. This abnormal reaction of the immune system can manifest to allergic rhinitis, hay fever, asthma, conjunctivitis, angioedema, eczema, urticaria, insect-allergies and anaphylaxis. Nowadays, allergic disorders noticeably increase, mainly in

industrialised countries, leading to a search for new approaches against allergies [21].

Antimicrobial resistances are no new phenomenon but a general reaction of bacteria to their environment. In the last decades improper use of antibiotics has led to an accumulation of resistances against antibiotics reaching an alarming extent in recent years. Resistances can lead to the development of life-threatening infections by germs that have been well treatable to date. According to the WHO, antibiotic resistances are one of the three greatest threats to public health [22]. 671,689 Europeans were infected by antibiotic resistant bacteria in 2015 [23].

Disability	**Number**	**Year**	**Reference**
Hypertension	1 billion	2013	[8]
Depression	264 million	2017	[9]
Obesity	600 million	2015	[12]
Diabetes	415 million	2015	[14]
NASH	3-5 percent	2015	[18]
Allergies	20 percent	2003	[20]
Antibiotic resistances	671,689 antibiotic-resistant infections *	2015	[23]

*Table 1: Prevalence of diseases of the population worldwide and number of antibiotic resistant infections. *in Europe*

The interest in herbal medicine was growing worldwide in the recent years. About 80% of the population in developing states are using traditional herbal medicine for their basic medical care according to the WHO. However, in industrialised countries the use of herbal medicine is also increasing. Throughout the world a respectable percentage of the citizens have used complementary medicine at least once, which includes herbal medicine: in Australia 48%, in Belgium 38%, in Canada 70%, in France 75% and in the USA 42%. The best example for a big place value is China, as traditional herbal medicine counts for 40% of the general health care [24]. There, even 70% combine traditional and conventional medicine [25]. In the USA 16% of the population take prescribed medication in combination with herbal products [26].

This diploma thesis was created to determine the mechanisms of EO for their use against these actual common public health problems. These EO were chosen either for their popularity or for their high effectiveness. In addition, toxic profiles of selected EO compounds and possible EO interactions with drugs are being discussed in this work.

2 Mechanisms of action

2.1 Calcium antagonistic and cAMP releasing activity

Calcium-channel dependent Ca^{2+}-influx can be expected to induce contractions in smooth muscles. This mechanism was also observed in several EOs. Calcium blocking activity often seems to be involved in their spasmolytic effect [27].

2.1.1 *Calamintha glandulosa* essential oil

Calamintha glandulosa Silic (Lamiaceae) has been used in traditional medicine against stomach issues [27]. Ten years ago, a study was performed to proof the mechanism of *C. glandulosa* EO (CGEO) testing 0.003-1 mg/mL *in vitro* on rat ileum against verapamil (0.01-3 µmol/L) and pulegone (0.15-50 µmol/L; Fig. 1A), which was found as the main component of CGEO (37.5%). Concentration dependent relaxation of the ileum basal tonus was observed in spontaneous induced contracting ileum for CGEO, pulegone and verapamil. Additionally, stronger relaxation in potassium-induced (80 mmol/L KCl) contracting ileum was noticed. In Ca^{2+}-free and high K^{+}-depolarised medium all three substances showed concentration dependent inhibition of contractions induced by cumulative added $CaCl_2$ (0.01-10 mmol/L). Results indicated that the spasmolytic effect of CGEO depended on voltage-operated Ca^{2+}-channel blocking activity, comparable to verapamil, so CGEO may contain Ca^{2+}-channel antagonists. Pulegone relaxed the spontaneous contraction of smooth muscles 23 times stronger than CGEO. This suggests that pulegone was mainly responsible for the spasmolytic effect. Authors did not give any declaration of the pulegone-enantiomer in their EO used [28]. Anyway, in general (R)-(+)-pulegone is the mainly contained enantiomer in *Lamiaceae* [29]. Earlier studies have already shown calcium antagonistic activity of other terpenoids [30].

2.1.2 Jasmine absolute

Although an absolute per definition is not an EO, jasmine absolute has to be mentioned in this context due to its spasmolytic effect, its established use in aromatherapy and its well investigated effects. *Jasminum grandiflorum* L. is used as an absolute, which means that it is gained through solvent extraction instead of steam distillation [31]. This technique is also applied for blossoms like rose and violet, that would decompose under distillation conditions [32]. Besides volatile

compounds an absolute includes lipophilic solvent-extractives such as fats, fat-soluble vitamins and pigments. In an *in vitro* investigation on guinea pig ileum, jasmine absolute (JA), acetylcholine and histamine blocked the contractile effect, which suggested a postsynaptic and non-atropine-like effect. To block the spasmolytic effect of noradrenaline on α-adrenoceptors, phentolamine (10 µmol/L) and propranolol (10 µmol/L) were added. No change in the spasmolytic effect of JA was determined. The selective guanylyl cyclase inhibitor oxadiazolo(4,3-a)quinoxalin-1-one (ODQ) inhibited sodium nitroprusside induced relaxation, suggesting that the second messenger cGMP was not participating. In contrast, non-specific phosphodiesterase inhibitor trequinsin increased the effect of JA. This circumstance indicated an intracellular cAMP-rise-affected antispasmodic effect of JA. Similar effects with trequinsin were also shown in previous studies for peppermint oil, geranium oil and its isolated components geraniol (Fig. 1B), citronellol and linalool. These findings suggest that some EOs show effects on Ca^{2+}-receptors and cAMP release. These mechanisms could be of therapeutic benefit against arterial hypertonia [31].

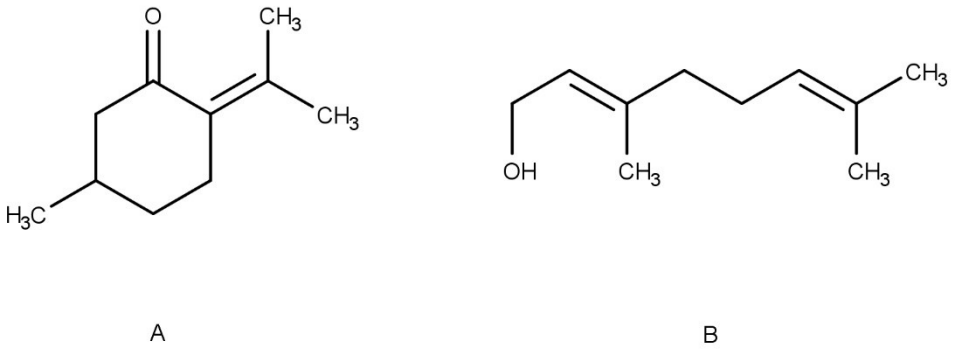

Figure 1: Important cAMP-releasing compounds; (A): pulegone, (B): geraniol

2.2 Antidepressant effect

It is believed that every fifth person has to deal with depressive episodes at least once in his/her lifetime [4]. Depression can be expressed by various symptoms, such as diurnal variation, listlessness, fatigue, loss of appetite and weight as well as insomnia. Some of these symptoms are rather unspecific and consequently not seldom wrong interpreted and diagnosed. Conventional antidepressants often show adverse effects. Specific serotonin reuptake inhibitors (SSRI), for example, can lead to weight gain, sleeping disorders as well as erectile dysfunction besides other side-effects [33]. These circumstances often end up in non-compliance and, subsequently, in poorly treated patients. As the prevalence of depression is increasing, new methods with as few side effects as possible are searched for to effectively improve the patient's quality of life [34].

2.2.1 Bergamot essential oil

Bergamot EO (*Citrus bergamia* (Risso) Wright & Arn; BEO) vapour inhalation was investigated in 41 healthy women, comparing differences between no-treatment and water vapour. BEO treatment yielded in a decrease of salivary cortisol levels as a sign for stress reduction. Additionally, participants indicated a significant decrease of negative mood and an increase of vigour in a standardised mood questionnaire [35]. In a waiting room of a mental health treatment centre the influence of the diffusion of BEO with limonene (36%), linalyl acetate (31%; Fig. 2A), linalool (11%, Fig. 2B), γ-terpinene (8%), and β-pinene (7%) as major constituents, compared to distilled water diffusion periods were measured. After 15 minutes exposure the BEO-group exhibited a 17% higher mean positive effect than the control group. Negative scores increased less, but beside active and proud feelings, an increased nervous feeling was reported , although these findings were not statistically significant [36]. A study on rats treated with BEO (250 µL/kg and 500 µL/kg, respectively) also suggested dose-dependent sedative, relaxant and anxiolytic effects by an open field test, elevated plus maze test, and forced swimming test, which are used in animal studies related to levels of stress and depression. Effects were compared to the benzodiazepine diazepam (1.2 mg/kg, 5 mg/kg) as a positive control. Results indicated different behaviour between BEO and diazepam which could be evidence for different modes of action [37]. It was observed that BEO (20 µL/20 min) perfusion into rats' hippocampus induced

Ca^{2+} independent release of aspartate, glycine, taurine, glutamate and GABA. Therefore, the involvement of the GABAergic system cannot be excluded [38]. This suggestion is in accordance with findings in rats tested with BEO (500 µL/kg i.p.) and flumazenil (3 mg/kg), a benzodiazepine antagonist. Pre-treatment of flumazenil did not affect behaviours in an open field test compared to BEO. Therefore, it is supposed that benzodiazepine receptors are potentially not involved in BEO effects [39].

2.2.2 Clary sage essential oil

Investigations on clary sage EO (*Salvia sclarea* L.; CSEO; 5% in almond oil) in rats were performed by means of the forced swimming test measuring the immobility of the animals after being dropped into water. It is assumed that the shorter the immobility time, the better the antidepressant effect. Compared to a negative control, other EOs (lavender, rosemary, chamomile) and positive controls (imipramine 30 mg/kg, fluoxetine 1.8 mg/kg) CSEO showed the most potent antidepressant-like effect after injection as it resulted in the shortest time of immobility. Comparted to negative control, CSEO (5% in water) resulted in reduced immobility time even after inhalation. The antidepressant-like effect of CSEO injection was blocked by pre-treatment with buspirone (2 mg/kg), a 5-HT_{1A}-receptor agonist, SCH-23390 (0.05 mg/kg), a selective D_1-receptor antagonist, and with haloperidol (0.5 mg/kg), a D_2-, D_3-, D_4-receptor antagonist. These findings indicated dopaminergic and serotonin(5-HT)ergic effects of CSEO [40] and are in accordance with results of a study with CSEO inhalation on menopausal women showing significantly increased 5-HT-plasma levels after inhalation compared to values before inhalation. This effect was found both in the “normal” and “depressive tendency” group of women, whereat the increase in the “normal” group was stronger. Significantly reduced plasma cortisol levels were also found in both groups but to a greater extent in the “depressive” group [41].

2.2.3 Lavender essential oil

A study was performed with lavender flower EO (*Lavandula angustifolia* L., syn.: *Lavandula officinalis*; LAEO), exhibiting linalool (44.7%) and linalyl acetate (42%) as major constituents besides 1,8-cineole (5.3%) and camphor (6.0%). In *in vivo* tests in mice, a sedative effect on the central nervous system was observed for LAEO (600 mg/kg i.p.). 1000 and 1500 mg/kg i.p. even generated a hypnotic effect

[42]. Sedative effects for LAEO with a chemical profile of linalool (37.3%) and linalyl acetate (41.6%) were already described in 1991 in rats after inhalation by decreasing impulse activity [43]. In different assays, LAEO demonstrated moderate serotonin transporter- and potent NMDA (n-methyl-d-aspartate) receptor-binding activity, in which linalool showed the same effects on both receptors, linalyl acetate only for NMDA receptor. Moreover, a neurotoxic preventing effect on neuroblastoma cells against peroxidase was observed for LAEO. Antidepressant effects may partly be explained by these findings [44]. A 2%-diluted blend of *L. angustifolia* and *Rosa* x *damascena* EOs was tested by inhalation or hand m' technique, a standardised massage technique, in 28 *postpartum* women who showed a high risk of anxiety and depression. EO treatment improved scores of the Edinburgh Depression Scale and the Generalised Anxiety Disorder Scale. Results of the m' technique were more significant than inhalation, probably because inhalation may take longer to develop the effect [45].

2.2.4 Basil essential oil

The EO of basil leaves (*Ociumum basilicum* L.; OBEO) was tested on depressive mice. An oral administration of OBEO (0.025 mg/kg) showed a significant decrease in blood cortisol level and an increase of blood serotonin level. Stress leads to a dysregulation in the hypothalamic pituitary adrenal (HPA) axis which further leads to cortisol release. Compared to normal non-depressive (544.9 ng/mL) and untreated depressive mice (367.1 ng/mL), OBEO treated mice showed a higher blood serotonin level of 741.5 ng/mL. Stress and low serotonin levels can be indicators for depression. Due to improvement of these two factors, OBEO can be considered an antidepressant [33]. Chronic unpredictable mild stress (CUMS) induced depression in mice. Administration by inhalation of OBEO (2.5 mL/odour-proofed box) improved behaviour in a forced swimming test, an elevated plus-maze and an open field test in these treated animals. Even neurodegenerative disorders were improved. Under OBEO treatment (linalool (35.9%), 1,8-cineole (11.2%,), α-cadinol (10.4%, Fig. 2D), and farnesyl-acetate (10.2%, Fig. 2E)), negative transformations and increased apoptosis in hippocampus cells of depressive mice were less and the number of astrocytes increased. Furthermore, neurogenesis was improved in the subgranular cell layer of the dentate gyrus. These effects in CUMS

mice were comparable to fluoxetine [46]. The results were supported by the findings in another study under similar conditions [47].

2.2.5 Methyl jasmonate

Methyl jasmonate (Fig. 2C) was observed as an active compound in *J. grandiflorum* L. In mice, 10 mg/kg and 20 mg/kg (i.p.) of methyl jasmonate significantly inhibited the immobility time in the tail suspension test and forced swimming test. The most effective concentration of methyl jasmonate (20 mg/kg) showed an even stronger decrease of immobility time compared to tricyclic antidepressant imipramine (10 mg/kg i.p.). The antidepressant-like effects found seem to be involved in serotonergic, noradrenergic and dopaminergic systems. This assumption is based on results of pre-treatment with p-chlorophenylalanine (100 mg/kg i.p.), a serotonin synthesis inhibitor, metergoline (4 mg/kg i.p.), a 5-HT_2 receptor antagonist, prazosin (62.5 µg/kg i.p.), an α_1-adrenoceptor antagonist, yohimbine (1 mg/kg i.p.), an α_2-receptor antagonist, sulpiride (50 mg/kg i.p.), a D_2 dopamine receptor antagonist and haloperidol (0.5 mg/kg i.p.), a dopamine receptor antagonist, which diminished the anti-immobility effect in the tail suspension test and thus the antidepressant-like effect of methyl jasmonate [48].

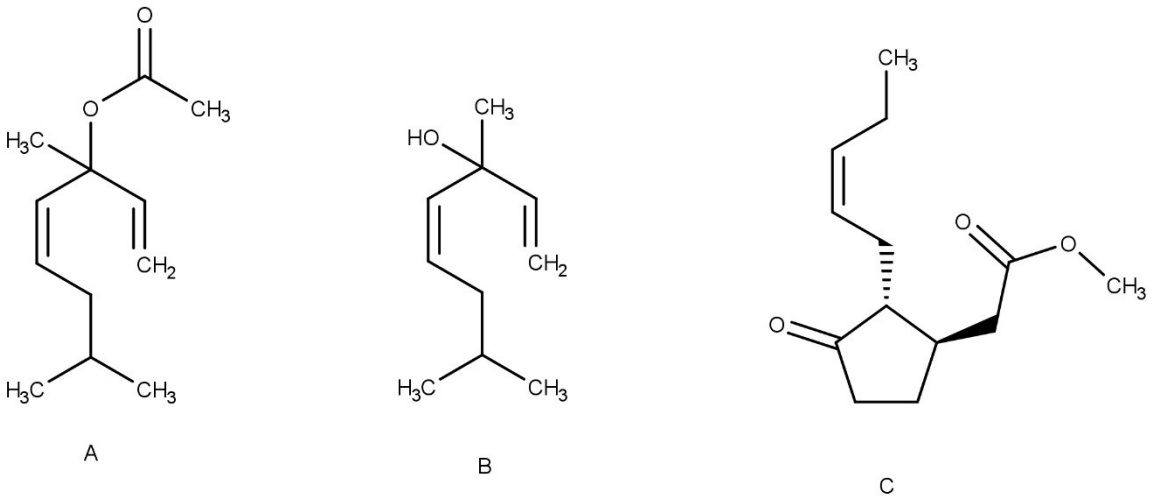

Figure 2: Important antidepressant compounds; (A): linalyl acetate, (B): linalool, (C): methyl jasmonate

2.3 Anti-obesity effect

Body mass in relation to body height is known as body mass index (BMI) and defines obesity, according to the WHO, at a value of 30 kg/m^2. A BMI of 25-29 kg/m^2 is called overweight. An imbalance between energy intake and energy expansion leads to an increase of body weight. The prevalence of death in consequence of a high BMI has increased by 28.3% within 25 years. Additionally, obesity often goes along with several metabolic comorbidities [12]. Drug therapies for obesity mostly effect fat absorption into blood or satiety by implying several side effects and high costs. By searching for alternatives some EO have proven to be promising in the treatment of obesity [13].

2.3.1 Sweet orange essential oil

Sweet orange EO (*Citrus x sinensis* (L.) Osbeck; SOEO) with (R)-(+)-limonene (=D-limonene, Fig. 3A) as major component (93.7%), was tested on obese rats to investigate its impact on obesity. To ameliorate solubility and bioavailability, SOEO was applied orally in microcapsules. Under high-fat diet, weight gain of rats treated with SOEO microcapsules (19 mg SOEO in 630 mg microcapsules) was 41.3% less than without treatment. In comparison to SOEO in saline (19 mg SOEO), the microcapsule formulation showed better efficacy. More ameliorating effects in SOEO microcapsule treated rats compared to negative control were the reduction of TC (total cholesterol) (by 17.2%) and LDL-c (low-density-lipoprotein cholesterol) (1.46 mmol/L), no fat accumulation in liver tissue and a reduced size of adipocytes. Additionally, peroxisome proliferator-activated receptor-γ (PPAR-γ) expression was reduced, which lowered the differentiation of preadipocytes to adipocytes. PPARs are ligand-controlled nuclear receptors that have a regulatory effect on transcription and cell metabolism. The effect of SOEO on the regulation of these PPAR target genes could be responsible for the caused weight loss. UCP2 (uncoupling oxidative phosphorylation process of respiratory chain), HSL (lipid mobilisation in triglyceride catabolism) and CPT 1 (fatty acyl-CoA transport for β-oxidation into mitochondria) expression was upregulated and ACC (rate limiting of fatty acid synthesis) expression was downregulated. Therefore, triglyceride β-oxidation was stimulated by SOEO microcapsules. Same effects were observed for SOEO in saline, but in a less potent manner. This suggests better bioavailability of SOEO application in microcapsules [13].

2.3.2 Bitter orange essential oil

For the EO of bitter orange (*Citrus aurantium* L.; BOEO) a probable anti-obese effect was observed. The oil was obtained as cold pressed oil from side products of bitter orange juice production and contained limonene ((+)-99.3%; (-)-0.7%; 2300 µmol/L) as the main compound, followed by linalyl acetate (56.7 µmol/L), linalool (13.7 µmol/L), (±)-carvone (13.6 µmol/L), geranyl acetate (2.9 µmol/L), osthole (2.2 µmol/L), geranyl propionate (1.8 µmol/L), neryl acetate (1.2 µmol/L) and citral (0.7 µmol/L). The aim of the study was to prove TRP (transient receptor potential) channel activity of BOEO. Main focus of attention was led on terpenes of the oil, because they already showed TRPA1 activation in previous studies. TRP channels, with several subfamilies, are non-selective Ca^{2+}-permeable cation channels which are expected among others to regulate gastrointestinal motility and energy metabolism. In human TRPV1, hTRPA1, or hTRPM8 expressing T-REx™-293 cells, BOEO (1000 µg/mL) produced Ca^{2+}-response only in hTRPA1-expressing cells (EC_{50} 7.3 µg/mL). In the same conditions in hTRPA1-expressing cells, all ten above described terpenes showed concentration dependent Ca^{2+}-response compared to allyl-isothiocyanate (positive control; EC_{50} 0.2 mmol/L, response 90%). From that, osthole showed the strongest maximum response with 92% and an EC_{50}-value of 6 mmol/L. After comparing EC_{50} with the concentrations in the oil, limonene and linalyl acetate seemed to be the main agonists of TRPA1. TRPA1 is expressed in sensory neurons and nonneuronal cell types. In previous studies its agonists showed improvement of energy metabolism. All these results suggest that BOEO could be an agent with good prospects for obesity therapy, although further studies are needed to verify this assumption [49]. Further TRP channel activities from EOs are known for cinnamon-oil (cinnamaldehyde TRPA1 agonist), clove-oil (eugenol TRPV1-, TRPA1-, and TRPM8-agonist), and eucalyptus-oil (1,8-cineole TRPM8 agonist, TRPA1 antagonist) [50, 51].

2.3.3 Lime peel essential oil

Obesity often leads to hyperlipidaemia. A disorder of lipid metabolism like elevated triglyceride (TG), total cholesterol (TC), low-density-lipoprotein cholesterol (LDL-c), very-low-density-lipoprotein-cholesterol (VLDL-c) and reduced high-density-lipoprotein cholesterol (HDL-c) values is defined as dyslipidaemia or hyperlipidaemia. In this context, results on lime peel EO (*Citrus aurantifolia*

(Christm.) Swingle; LPEO), containing limonene (42.4%), γ-terpinene (15.4%), and β-pinene (12.6%) as major components, seem to be promising. LPEO was tested in radical-scavenging assays and *in vivo* on obese rats with high fat diet (0.2% cholesterol, 9.8% lard) in different amounts (0.74% and 2.23%). Free-radical-scavenging effect in DPPH and ABTS assays were determined for LPEO with IC_{50} values of 2.23 mg/mL and 0.26 mg/mL. These results indicate that LPEO possessed antioxidant activity and may inhibit peroxidation of lipids which can prevent tissue and organ damage. Results of LPEO treated obese rats showed a significant decrease of TG, TC, LDL-c and atherogenic index (CVD incidence) and a slight increase of HDL-c in comparison to rats with the same high-fat diet without LPEO. Moreover, faeces were investigated on neutral sterols and the liver was histopathologically examined, demonstrating a reduced cholesterol absorption and an improved hepatic fat infiltration in LPEO treated rats compared to high-fat diet animals. AST (aspartate aminotransferase) and ALT (alanine aminotransferase) serum levels were significantly decreased in a dose-dependent manner, although no improving effects were described on inflammatory cell infiltration. These results indicated a possible lipid-lowering effect of LPEO [52].

2.3.4 (R)-(+)-Limonene

As described above, (R)-(+)-limonene (Fig. 3A) seems to play a leading role in anti-obese activity. This was confirmed by investigations on 3T3-L1 adipocytes demonstrating blocking effects on lipid accumulation and inhibitory effects on adipocyte cell differentiation by (R)-(+)-limonene (0.5 µmol/L). After twelve weeks high fat diet, obese mice were treated with 0.6 g/kg (R)-(+)-limonene (p.o., added to food) for two weeks. They showed no difference of body weight, but TG and LDL-c were decreased whereas HDL-c was increased (by 36.1%, 20.4%, and 18.3%, respectively). Potentially, (R)-(+)-limonene prevents consequences of high fat diet, by reducing the size of white and brown adipocytes and diminishing the lipid deposition in liver tissue. Liver X receptors (LXR) are nuclear receptor transcription factors. LXRβ is involved in controlling lipid homeostasis. Since LXR target gene expression was supressed by (R)-(+)-limonene these receptors may be involved in the lipid lowering effect. These results confirm the effect of (R)-(+)-limonene on dyslipidaemia [53].

2.3.5 Spearmint essential oil

Besides EO of citrus fruits, also labiate like spearmint (*Mentha spicata* L.) may have positive effects on obesity. A study investigated the possible lipid lowering impact of the EO of *Mentha spicata* L. (MEO), with carvone (36.9-76.8%, Fig. 3B), limonene (6.2-9.8%), and dihydrocarveol (2.3-13.8%) as main components, by testing the inhibition of porcine pancreatic lipase (PPL). Additionally, pure carvone, orlistat (a pancreatic lipase inhibitor, used as reference) and their combinations were spectrophotometrically tested *in vitro* by two-dimensional checkerboard method. This method allows to test synergism of two diluted compounds. MEO and carvone showed a lipase inhibitory effect with a MIC_{50} of 12.5 µL/mL. MIC_{50} for orlistat was 0.032 µg/mL. In combination, MIC_{50} was reduced to 0.078 µL/mL (MEO/carvone + orlistat), 0.0039 µg/mL (orlistat + MEO) and 0.0019 µg/mL (orlistat + carvone). Thereby a synergistic effect on PPL inhibition was found for both latter combinations. These results hypothesize that the high concentration of carvone is responsible for the lipase inhibiting effect in MEO. Both, MEO and carvone, could probably occur as anti-obesity agents [54].

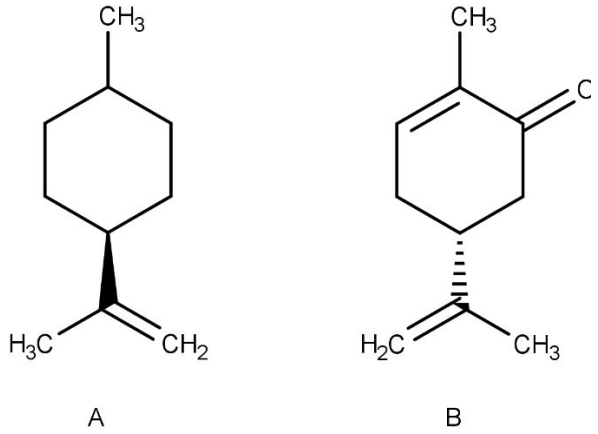

Figure 3: Important anti-obese compounds; (A): (R)-(+)-limonene, (B): (R)-(-)-carvone

2.4 Antidiabetic effect

Type 2 diabetes is distinguished by dysfunctional β-cells of the pancreas, which are responsible for insulin production. This leads to insulin deficiency or even resistance [14]. B-cell function is likely to be affected by diabetes-induced oxidative stress [55]. Even the liver can be impaired due to diabetes as it is the main organ for storage and metabolism of glucose [56]. Diagnostic parameters for diabetes are the fasting plasma glucose, HbA_{1c} (glycolyzed haemoglobin), oral glucose tolerance test and C-peptide (circulating plasma insulin marker) [14]. Improvement of these parameters could be achieved by specific EOs and single compounds.

2.4.1 (R)-(+)-Limonene

In mice, high-fat diet increased blood glucose was significantly reduced by (R)-(+)-limonene (0.6 g/kg; p.o., added to food) by 29.1%. The same effect (30.3% blood glucose reduction) was observed in obese mice which had developed mild hyperglycaemia. Even on glucose tolerance, beneficial effects of (R)-(+)-limonene were documented. On PPAR-γ transcription activity, (R)-(+)-limonene showed only a weak inhibitory effect. It, thereof, has probably no effect on insulin resistance The blood-glucose-regulating impact seems to be interesting for type 2 diabetes patients and for reducing hyperglycaemia [53].

2.4.2 Cumin essential oil

The antidiabetic effect of green cumin seeds (*Cuminum cyminum* L.) was described in several animal studies. In a trial on diabetic rats, cuminaldehyde (Fig. 4A) and cuminol (Fig. 4B), two main components of *C. cyminum*, were identified as most potent stimulants for insulin secretion at a dose of 0.25 µg/mL, respectively. This effect was attributed to K^+-ATP-channel closure [57]. In 2005, a study on male rats reported the inhibitory effect on aldose reductase and α-glucosidase of cuminaldehyde (IC_{50} 0.00085 mg/mL; 0.5 mg/mL) [58]. In a study on humans suffering from type 2 diabetes (fasting blood sugar (FBS) 126-200 mg/dL), the effect of 50 and 100mg capsules with cumin EO (CEO; p.o.) daily, was investigated on glycaemic and inflammatory indices. Results showed significant dose-dependent improvements. In CEO treated participants levels of FBS, HbA_{1c}, serum insulin, TNF-α (tumour necrosis factor) and hsCRP (high sensitive C-reactive protein) were decreased, insulin sensitivity (HOMA-IR) and adiponectin were increased compared to a placebo group [59]. Especially the effect

on inflammatory mediators seems to be promising, because previous studies indicated an increase of TNF-α and hsCRP and a decrease of adiponectin in type 2 diabetes patients [60, 61]. In another investigation, effects of the orally application of 75 mg/day of CEO for 10 weeks were monitored in pre-diabetic patients with impaired fasting glucose (fasting plasma glucose (FPG) 100-126 mg/dl) and impaired glucose tolerance. Cuminaldehyde (41.9%), γ-terpinene (16.5%), ρ-cymene (16.2%), and β-pinene (10.9%) were found as major constituents by GC-analysis. Compared to the placebo group, FPG, HbA_{1c} and β-cell function (HOMA-B) did not improve significantly, and fasting serum insulin and HOMA-IR were just reduced marginally significant. These non-significant results might possibly be due to testing pre-diabetic instead of diabetic patients. In addition to glycaemic markers, lipid profile and anthropometric indices (body weight, BMI, waist circumference) were also collected and improvement was determined [62].

2.4.3 Cinnamon essential oil

Several cinnamon products are known for their antidiabetic effects. The EOs from the barks of Chinese cinnamon (*Cinnamomum cassia* Presl.; CCEO), and *C. zeylanicum* Blume (CZEO) as well as from the leaves of Indigenous cinnamon (*C. osmophloeum* Kanehira; COEO) were reported to show antidiabetic effects. The different species had different chemical compositions of volatile compounds. The main compound was cinnamaldehyde (Fig. 4C) for CCEO (78.5%) and CZEO (> 98%), whereas COEO showed linalool (40.2%), *trans*-cinnamyl-acetate (11.7%), and camphor (9.4%) as major constituents. Cinnamaldehyde appeared at a concentration of 6.9% [63-65].

In diabetic KK-A^{y} mice, oral administration of 50 and 100 mg/kg, CCEO significantly decreased blood glucose levels compared to 25 mg/kg CCEO or control (no EO). All three concentrations, especially 100 mg/kg, showed amelioration in an oral glucose tolerance test (OGTT). Reparation of diabetic damaged β-cells and improved insulin sensitivity were determined with 50 and 100 mg/kg CCEO. Additionally, significant reduced C-peptide levels were measured for 100 mg/kg CCEO which, again, indicated improved insulin sensitivity [63].

In diabetic rats 5, 10, and 20 mg/kg of CZEO also decreased fasting blood glucose. A dose of 20 mg/kg was as potent as antidiabetic agent glipizide. Histological

recovery of glomerular expansion was also comparable. The high content of cinnamaldehyde is considered to be responsible for these effects as it is known for its antioxidant property [64].

In another study COEO was tested via gavage in concentrations of 12.5, 25 and 50 mg/kg in male Wistar rats. Interestingly, the lowest dose showed the highest insulin secretion as well as the highest increase of OGTT, GLP-1 (glucagon like peptide 1) and GIP (gastric inhibitory peptide). These effects were even higher compared to cinnamaldehyde (40 mg/kg) and equal to glibenclamide (1.2 mg/kg; positive control). In fasting, blood glucoses 12.5 mg/kg of COEO significantly showed the fastest improvement. This untypical non-dose-dependent effect might derive from the higher camphor concentrations in higher doses which seem to supress metabolic glucose oxidation. The comparable effect with gliblenclamide may be due to the oil's higher antioxidative effect in β-cells. The outperforming efficacy over cinnamaldehyde suggests effective synergistic effect of other EO compounds with cinnamaldehyde [65].

2.4.4 Peppermint essential oil

In an investigation on peppermint EO (*Mentha* x *piperita* L.; MPEO), 40 and 80 mg/kg orally applied oil on diabetic and non-diabetic albino rats were tested against glibenclamide (3 mg/kg). The oil's main components were menthol (31.5%), menthone (18.4%), carvone (13.0%), anethole (7.6%), p-menthan-3-one (6.2%). Blood glucose was lowered, and insulin and C-peptide values were significantly increased. At 80 mg/kg, MPEO showed even higher effects than glibenclamide. Additionally, MPEO stimulated the expression of CAT (catalase activity) and GSH (glutathione), two antioxidant enzymes which are reduced by diabetes. This antioxidative effect, which is probably due to phenolic constituents in the oil, may be responsible for antidiabetic effects such as restoration of pancreatic β-cell tissue, regeneration of hepatic dysfunction and an antiapoptotic effect [66].

2.4.5 Sage essential oil

Sage EO (*Salvia officinalis* L.; SEO; thujone (29%; Fig. 4D), 1,8-cineole (12%), camphor (7.2%), β-caryophyllene (6.4%)) was able to inhibit α-amylase *in vitro* (IC_{50} 38 µg/mL) and *in vivo* in rats by 47% at an oral dose of 12 µL/kg compared to

control. This mechanism prevents starch and glycogen (long-chain carbohydrates) degradation and thereof less glucose is available for admission. Fasting blood glucose was reduced by 71%, liver glycogen storage, which is often reduced in diabetics, was lifted by 44%, diabetic increased lipase activity was inhibited by 53.3% (glimepiride 32.1%) and diabetic induced ALT, AST, LDH (lactate dehydrogenase) levels were normalised. SEO also ameliorated fat accumulation in hepatic sinusoids as well as fat disposition in renal tissue and glomeruli size. Moreover, urination and accordingly uric acid and creatinine levels were normalised by SEO treatment. These observed effects of SEO were comparable to those of glimepiride. As diabetes often comes along with obesity, the lipase inhibitory effects of SEO seems to be very promising for therapeutic treatment [67].

2.4.6 *Rhaponticum acaule* essential oil

For the EO of *Rhaponticum acaule* (L.) DC (*Asteraceae*; RAEO), with germacrene D (49.2%; Fig. 4E), methyleugenol (8.3%), β-ionone (6.2%), and β-caryophyllene (5.7%) as main compounds, a strong antioxidant activity was determined *in vitro*, although it contained rather low amounts of phenolic compounds. Additional *in vitro*, RAEO showed a 42-fold more potent α-glucosidase inhibition (IC_{50} 6.7 µg/mL) compared to positive control acarbose. This antidiabetic mechanism may potentially be referred to sesquiterpene germacrene D. Turkey pancreatic lipase was irreversible inhibited by 2 mg/mL of RAEO to a maximum of 80% (IC_{50} 0.22 mg/mL). Compared to positive control tetrahydrolipstatin (IC_{50} 0.16 mg/mL), it was less potent. These promising results, especially α-glucosidase inhibition, make RAEO interesting as a treatment of metabolic disorders like type 2 diabetes [68].

2.4.7 Geranium essential oil

Pelargonium graveolens L'Hér EO (PGEO), known as geranium, containing (R)-(+)-citronellol (31.6%;4F) as major component, was tested on alloxan-induced diabetic rats. After 21 days of intraperitoneal PGEO (42.5 mg/kg) admission, significant reduction of blood glucose levels compared to non-treated diabetic rats was shown. Furthermore, the lipid profile was normalised, antioxidant enzymes were elevated, lipoperoxidation was decreased, hepatic and kidney functions as well as histological changes were protected by PGEO treatment. These results might refer to the oil's antioxidant properties and its regulation of insulin secretion,

leading to regulation of hyperglycaemia and lipoprotein lipase activation by insulin which might be responsible for the lipid level regulation [69]. Similar antidiabetic results were found for PGEO (150 mg/kg) administered orally to diabetic rats, confirming previous findings [70]. The underlying antidiabetic effect is probably due to synergistic effects of PGEO components. Furthermore, a previous study identified monoterpenes as potentially responsive antidiabetic constituents [71]. In another investigation, citronellol (100, 200, 400 µmol/L) showed dose-dependent lipopolysaccharide (1 µg/mL) induced COX-2-inhibitory and PPAR-α- and PPAR-γ-activating effects in bovine arterial endothelial cells. These results suggested anti-inflammatory effects and may be interesting for lifestyle related diseases, like diabetes and inflammatory diseases [72].

2.4.8 Eucalyptus essential oil

The EO of leaves from *Eucalyptus globulus* also showed antidiabetic effects. *In vitro* it inhibited α-amylase (IC_{50} 27.29 µg/mL) with a nearly double fold activity compared to acarbose (IC_{50} 14.88 µg/mL). Additionally, pancreatic lipase inhibition (IC_{50} 17.85 µg/mL) and free radical scavenging effect, which describes antioxidant efficacy, were observed. The investigated EO mainly contained 1,8-cineole (43.2%; Fig. 4G), α-pinene (13.6%), aromadendrene (10.1%) and 4-carene (6.9%). Again, phenolic components were suggested to be responsible for the observed effects [73].

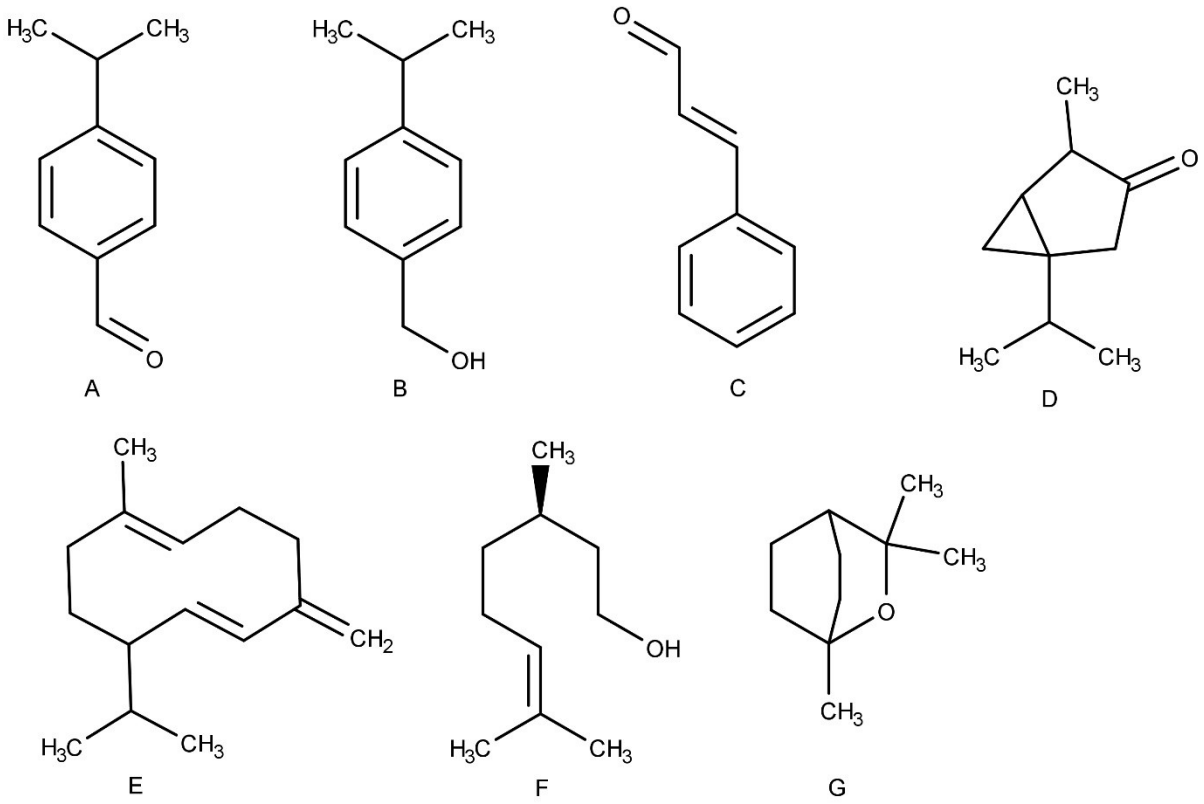

Figure 4: Important antidiabetic compounds; (A): cuminaldehyde, (B): cuminol, (C): cinnamaldehyde, (D): thujone, (E): germacrene D, (F): (R)-(+)-citronellol, (G): 1,8-cineol

2.5 Antifibrogenic effect

Treatment options for liver fibrosis are rare to missing. Oxidative stress seems to be a major influencing factor for this disability. Especially two EOs seem to be promising for improvement of liver fibrosis and may lead to new target agents [74].

2.5.1 Peppermint essential oil

Peppermint is a widespread used herbal plant and known, besides others, for its analgesic, antifungal and antibacterial impact. Hepatoprotective effects have been documented and further investigated in a study using MPEO (50mg/kg; i.p.) *in vivo* in rats with CCl_4-induced liver fibrosis. Values were determined for percentage of fibrosis, liver enzymes (ALT, AST, MDA, NO), antioxidant profile (GSH, SOD, CAT, TAC), α-SMA-protein-expression, desmin protein expression, TGF-β1 protein expression, SMAD3 protein expression, p53 (tumour-suppressor) protein expression and CYP2E1 mRNA expression level. MPEO and CCl_4 administered intraperitoneal on rats, showed significant better results compared to the rats only treated with CCl_4, including improved liver histopathology, lower values of liver enzymes, higher levels of antioxidants, reduction of α-SMA-, desmin-, TGF-β1-, SMAD3- and p53-proteins and increased CYP2E1 expression. Suppression of hepatic stellate cells (HSC) and proliferation have also been described [74].

2.5.2 Basil essential oil

In comparable conditions similar effects were detected for basil EO (*Ocimum basilicum*, OBEO). In addition to the mechanisms described above for MPEO, stimulation of hepatocellular growth factor was determined [75].

Radical scavenger properties were awarded to menthol and menthone, as they possess an additional hydroxyl radical (•OH). They are among others also the major constituents of MPEO (menthol (46.7%; Fig. 5A), menthone (18.3%; Fig. 5B), iso-menthone (5.3%)). Investigated OBEO was mainly composed of iso-menthone (38%), 1.8-cineole (13.9%), *trans*-sabinene (12%), methyleugenol (7.5%) and pulegone (6.4%) [74, 75].

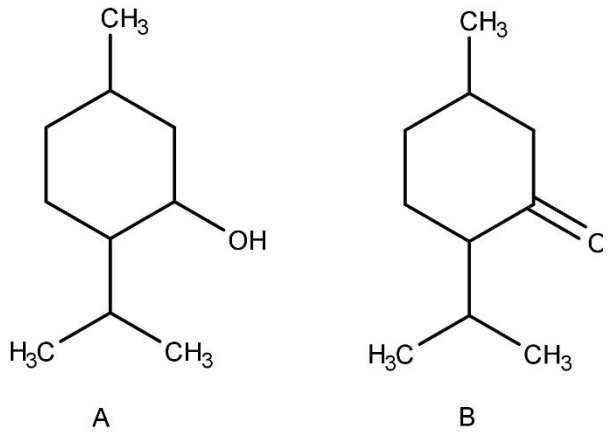

Figure 5: Important antifibrogenic compounds; (A): menthol, (B): menthone

2.6 Antiallergic effect

Allergies can lead to chronic inflammatory diseases and lower quality of life of the patients. Common antiallergic medications are antihistamines. For asthma inhaled corticosteroids and β-adrenergic-receptor-agonists more frequently are used, causing various side-effects. Understanding the antiallergic mechanism of EOs may lead to new, compliant complementary drugs against allergic diseases [21, 76].

2.6.1 *Minthostachys verticillata* essential oil

Minthostachys verticillata (Griseb.) Epling is common in South America and primary used for its digestive, antispasmodic and antirheumatic effects. In addition, an *in vitro* study conducted in 2007, detected the antiallergic effect of *M. verticillata* EO (MVEO). In this experiment, basophiles and leukocytes were extracted from 30 participants, at the age of one to 27 years, with allergies like asthma, rhinitis, eczema, prurigo, sinusitis and otitis. MVEO (1.0; 0.8; 0.16 and 0.001 mg/mL) was tested *in vitro* in comparison to dexamethasone (0.04 mg/mL) and theophylline (0.2 mg/mL), two substances often used against asthmatic diseases which stimulate cAMP production and prevent degranulation of basophils and mast cells. To determine the inhibition of degranulation in basophils of allergic patients, a β-hexosaminidase (degranulation marker)-assay was performed with and without environmental fungi allergen stimulation. There was no significant enzyme release by MVEO itself without the allergen. Allergen addition lead to enzyme release in all basophiles. Regardless of concentration, the release of β-hexosaminidase was decreased by MVEO (32.15%-39.72%) as well as by dexamethasone (39.75%) and theophylline (41.63%). This suggests a membrane protective effect of MVEO. Lymphocyte proliferation was slightly stimulated whereas the proportion of CD8(+)-T-cells was 40% in MVEO stimulated cells (20-25% in normal cells). As MVEO mainly contained terpenes pulegone (63%) and menthone (16.4%) it was assumed that they were responsible for the effect [76]. In a further study, the impact of the main components of the MVEO were investigated. Pulegone (63.4%), menthone (15.9%), and limonene (2.1%) were again identified as main components. In an *in vitro* assay the levels of cytokine IL-13 (interleukin-13) and lymphocyte proliferation were measured from lymphocyte culture supernatants of allergic patients after stimulation with MVEO (10 µg/mL for IL-13; 6 µg/mL for lymphocyte proliferation)

and pure main components (pulegone (62 µg/mL), menthone (60 µg/mL), and limonene (55 µg/mL)). From that, limonene showed the highest efficacy in inhibiting IL-13 levels and lymphocyte proliferation assay. MVEO (10 µg/mL) showed the highest inhibitory effect on β-hexosaminidase release in basophiles. This inhibition was stronger compared to single compounds or combined monoterpenes (pulegone 40 µg/mL, menthone 40 µg/mL, limonene 20 µg/mL). Also *in vivo* in a murine passive cutaneous anaphylaxis reaction (PCA), limonene provided best results as it showed the highest dose-dependent inhibition (100, 200, 250 mg/kg; i.p.) compared to MVEO (50, 100, 200 mg/kg; i.p.) and monoterpenoids (pulegone and menthone; 100, 200, 250 mg/kg; i.p.). In summary, the most potent immunomodulatory activity was determined for limonene. The results indicated antiallergic effects of MVEO and its main components comparable to anti-inflammatory drugs, demonstrating higher antiallergic effects compared to desloratadine. Therefore MVEO and its single compounds could be used to supplement antiallergic agents and to treat allergic disease - particularly as an alternative to corticosteroids, since they stimulate the proliferation of T-lymphocytes [77]. Toxicity and genotoxicity of MVEO after long term (90 days) repeated oral application was tested in a study on Wistar rats. No negative changes like mortality, adverse effects or cytogenotoxicity were found, suggesting the safety of MVEO without toxic risk [78].

2.6.2 Geranium essential oil

Citronellol (39.6%), citronellyl-formate (9.9%), geraniol (5.9%), iso-menthone (5.4%), and isoledene (3.9%) were identified as the main components of geranium EO (*P. graveolens;* PGEO). PGEO was tested in a study, indicating dose-dependent (44-176 µg/mL) inhibition of IgE-induced degranulation of murine cultured mast cells (CMC), via *in vitro* β-hexosaminidase assay. Testing 1 mmol/L of each EO compound, the main ingredients citronellol (54.6%) showed a strong, and citronellyl formate (33.8%) a slight rise of degranulation-inhibition compared to PGEO (32.2%). At a concentration of 0.5 mmol/L, (S)-(−)-citronellol (=L-citronellol, 69.4%; Fig. 6A) exhibited a stronger effect on degranulation than (R)-(+)-citronellol (=D-citronellol, 21.3%). Moreover, citronellol supressed IgE-stimulated MAPK phosphorylation (regulates proinflammatory agents) and TNF-α production (immune and inflammatory mediating cytokine). As degranulation and cytokine

production effect allergic mechanisms, PGEO and especially citronellol are suggested to exert anti-allergic activity without any cytotoxic effects [79].

2.6.3 *Mentha arvensis* essential oil

Another study also investigated IgE-related and inflammatory mechanisms. Special attention was paid to an anti-asthmatic effect. Therefore, albino mice were sensitised (i.p. and by aerosol inhalation) with ovalbumin (causes allergic reactions) and treated i.p. with *Mentha arvensis* EO (MAEO; 200 µL/kg; menthol (72.6%), menthone (8.5%), limonene (3.3%), and menthyl acetate (2.4%)). The levels of serum IgE, blood eosinophils, BALF (broncho alveolar lavage fluid) eosinophils as well as BALF neutrophils were significantly decreased. Histopathology of lung sections showed that MAEO treated samples were nearly comparable in reducing inflammation with normal control and dexamethasone (2 mg/kg i.p.) treated ones. Additionally, 400 µL/kg MAEO increased the pre-convulsive time of histamine-induced bronchoconstriction in guinea pigs similar to dexamethasone. These results indicated that MAEO exhibited anti-allergic effects and can be recommended for improvement of allergen-induced asthma [80].

2.6.4 *Zanthoxylum coreanum* essential oil

In a study testing anti-allergy effects of 15 EOs, the oil of the fruits of *Zanthoxylum coreanum* Nakai (ZCEO), which grow in China and Korea, had the highest supressing effect on mast cell degranulation (0.0025, 0.005, and 0.01%). In an *in vitro* study, dose-dependent anti-inflammatory effects like TNF-α-release reduction (IC_{50} 0.0068%), IL-6 reduction (IC_{50} 0.0023%) and decreasing NO-production (IC_{50} 0.0025%) were observed. Mast cell mediated inflammatory responses, such as IL-4 production which is supposed to be an important point of attack in allergic treatment, was inhibited by ZCEO (IC_{50} 0.0034%; IC_{50} 0.0059%) in differently stimulated RBL-2H3 cells. 0.01% of the oil significantly reduced the LPS (200 ng/mL)-induced pro-inflammatory proteins iNOS and COX-2, in comparison to COX-2 inhibitor celecoxib (10 µmol/L). NF-κB transcription and translocation (both LPS stimulated 1 µg/mL) from cytosol to nucleus were decreased by ZCEO (0.01%). In mice chloro-2,4-dinitrobenzene-induced ear swelling and atopic-dermatitis-like reactions were reduced by dermal application of ZCEO (1%, 2%), which was comparable to dexamethasone (1%) *in vivo*. The Monoterpene β-ocimene (24.5%; Fig. 6B) was identified as major compound of

ZCEO, (-)-α-pinene (16.5%) had the second highest percentage. In summary, it seems ZCEO could be a promising method for treatment of allergic diseases by inhibition of mast cell degranulation and inflammation [81].

2.6.5 Cumin essential oil

Under similar conditions *in vitro*, similar effects for iNOS, COX-2, NF-κB and MAPK pathway were shown on RAW 264.7 cells for the EO of cumin seeds (CEO) (0.001%, 0.01%). Furthermore, CEO reduced the expression of the proinflammatory cytokines IL-1β and IL-6 to 30.2% and 1.3%. Cuminaldehyde (48.8%), 3-caren-10-al (14%), β-pinene (11.4%) and γ-terpinene (10.7%) were identified as main components [82].

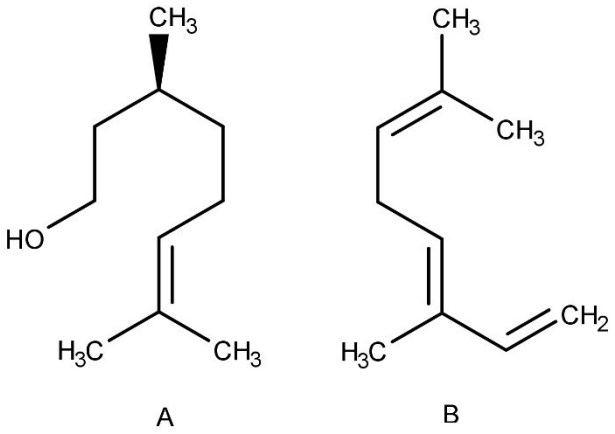

Figure 6: Important antiallergic compounds; (A): (S)-(-)-citronellol, (B): β-ocimene

2.7 Antimicrobial effect

The good antimicrobial impact of many EOs has been described in the last decades. This could lead to new active substances and starting materials for the development of antibiotics. Additionally, it could play an important role in the global fight against antibiotic resistance. Due to a less complex membrane, EOs have a higher efficacy against Gram-positive than Gram-negative bacteria. The mechanism behind this activity seems to be the penetration of the EO into the phospholipid bilayer of microorganism cell membranes. In addition to a disturbed cell membrane, other cell functions can also be affected, such as the EOs ability to reduce the activity of the efflux pump as well as the cell's respiratory activity [83].

2.7.1 Cinnamon essential oil

Cinnamon EO with its main component cinnamaldehyde (85%) was tested on *Map* (*Mycobacterium avium* subsp. *paratuberculosis*) cells, a bacterial pathogen causing Johne's disease (paratuberculosis). Cinnamaldehyde (minimal inhibitory concentration (MIC) 24 mg/L) induced a membrane leakage in a dose and time dependent manner, determined by an increase of extracellular potassium level. Furthermore, cinnamon EO and cinnamaldehyde incubation lead to intracellular ATP reduction. This probably occurred to a self-protection mechanism of *Map*. Due to a decrease of the cinnamon aldehyde peak at 290 nm in the absorbance scan, admission of cinnamaldehyde into cell or cell surface was observed. The absorbance scan also showed hints of nucleic acid leakage or leakage of any other substance from the bacterium by cinnamaldehyde incubation [84].

2.7.2 Oregano essential oil

For oregano EO with carvacrol (85%; Fig. 7A) as main component similar but weaker effects were observed, which was also reflected in a higher carvacrol MIC of 74 mg/L. Other than by membrane permeability due to potassium leakage, as described above, it appears that the antimicrobial effects of cinnamaldehyde, carvacrol, and geraniol are due to changes in the lipid cell membrane. Incorporation of EO compounds into the lipid monolayer could be a possible mechanism. In previous studies cinnamon and oregano EO even showed efficacy against resistant bacteria [84].

2.7.3 *Trachyspermum ammi* essential oil

Trachyspermum ammi EO increased the cytoplasm membrane permeability which made it porous to ions and protons. This effect was probably responsible for the antibiotic effect against *Bacillus subtilis* ATCC 6633 cells. Phenolic compounds like thymol and eugenol, one of the major compounds of *T. ammi* EO (thymol (49.64%; Fig. 7B), β-cymene (16.33%), eugenol (3.04%) and β-pinene (2.51%)), probably also contribute to the antibiotic effect [85].

2.7.4 Black pepper essential oil

Black pepper EO showed a MIC of 1 µL/mL against *Eschericha coli*. It also impaired bacterial cell membrane and cell walls. Electrolyte leakage increased the longer *E. coli* cells were incubated with black pepper EO and the higher the concentrations were. The EO treatment also led to a release of nucleic acid and protein. The membrane damage was further confirmed by induced potassium and phosphate leakage. The resulting death led to a reduction in *E. coli* cells [86]. With about 28%, caryophyllene (Fig. 7C) was determined as major compound in the black pepper EO [87].

2.7.5 Lemongrass essential oil

Lemongrass (*Cymbopogon citratus*) EO and its effective main component citral (Fig. 7D) lead to modifications of Gram-negative double membrane shaped cell walls of *Desul fovibrio alaskensis*. The induced cytoplasmatic permeability ended in cell lysis. A MIC of 0.17 mg/mL was observed under this condition. Beside cytotoxic activity, lemongrass EO also inhibited biofilm emergence and corrosion. The EO seems also to affect cell metabolism as granules near the cytoplasmic membrane could be detected in survived cells [88].

2.7.6 Tea tree essential oil

Tea tree (*Melaleuca alternifolia*) EO inhibited the viability of *E. coli* (MIC 0.25%), *Staphylococcus aureus* (MIC 0.25%), and *Candida albicans* (MIC 0.125%) cells in a dose and time dependent manner. Respiratory was inhibited in all three cell types. The provoked increase of cell permeability was proven by potassium leakage and propidium iodide absorbance, a nucleic acid stain which is only possible with damaged cell membranes. Potassium leakage could only be determined for *E. coli* and *S. aureus.* Multilamellar liposomes permeability was

determined by a leakage of carboxyfluorescein and it confirmed the assumption of tea tree oil membrane-toxicity [89]. Terpinen-4-ol (35%; Fig. 7E), eucalyptol (15%) and α-pinene (12%) are most contained in the essential oil of *M. alternifolia*. Tea tree oil showed potent bactericide properties even against multidrug resistant microorganisms. MICs of tea tee oil for methicillin-sensitive *S. aureus*, *E. coli*, methicillin-resistant *S. aureus*, extended-spectrum beta lactamases producer carbapenem-sensitive *Klebsiella pneumoniae*, carbapenem-resistant K. pneumoniae, *Acinetobacter baumannii*, and *P. aeruginosa* reached from 0.25% to 1%. In the vapor assay tea trea oil showed very potent efficacy on carbapenem resistant *A. baumannii*, a common pathogen of pneumonia. These findings and additional synergistic effects with antibiotics on these bacteria, are promising prospects for treatment [90].

2.7.7 *Isodon melissoides* essential oil

Isodon melissoides EO also effected protein-, DNA- and RNA-leakage in a dose-dependent manner. Thus, it resulted in cell membrane damage and increased membrane permeability in *S. aureus*, *S. epidermidis*, and *Enterococcus faecalis*. Even on methicillin resistant variants of *S. aureus*, *S. epidermidis* the oil showed antibacterial activities. The main compounds in the oil were found to be carvacrol (45.4%), p-cymene (11.6%) and thymol (11.3%) [91].

2.7.8 Orange and bergamot essential oil

A 2% blend of orange (*Citrus sinensis*) and bergamot (*C. bergamia*) EO lead to vacuoles in *E. faecium* and *E. faecalis* probably containing the EO. Cell membrane permeability increased and consequently lead to cell lysis. Additionally, reduced intracellular pH and ATP values were observed. It seems that the EO uptake into the cell and its effectivity coming from the inside of the cell might be a possibly hypothesis about the antimicrobial mechanism of this essential oil blend [92].

2.7.9 Anti-quorum sensing essential oils

Quorum sensing is a mechanism of bacteria whereby they can measure their cell density and allows them to communicate information like transcription regulation [93]. It may also be responsible for bacterial resistance and pathogenicity. N-acyl homoserine lactones (AHLs) are mediators of quorum sensing [94]. In *Chromobacterium violaceum,* clove EO (*Syzygium aromaticum*), cinnamon EO

(*C. verum*), lavender EO (*L. angustifolia*), and peppermint EO (*M. piperita*) presented anti-quorum sensing activity. From these, clove oil showed the most potent effect as it inhibited pigment production in *C. violaceum* with an antimicrobial concentration of 20 µl in the disc diffusion method. In *Pseudomonas aeruginosa,* clove oil inhibited swarming motility which is also an indicator for anti-quorum sensing activity [93]. Furthermore, rose EO (*R. damascena* L.), lavender EO, geranium EO (*Geranium robertianum* L.), rosemary EO (*Rosmarinus officinalis* L.) [94] and black tea tree EO (*M. bracteate*) also demonstrated strong quorum sensing inhibition [95].

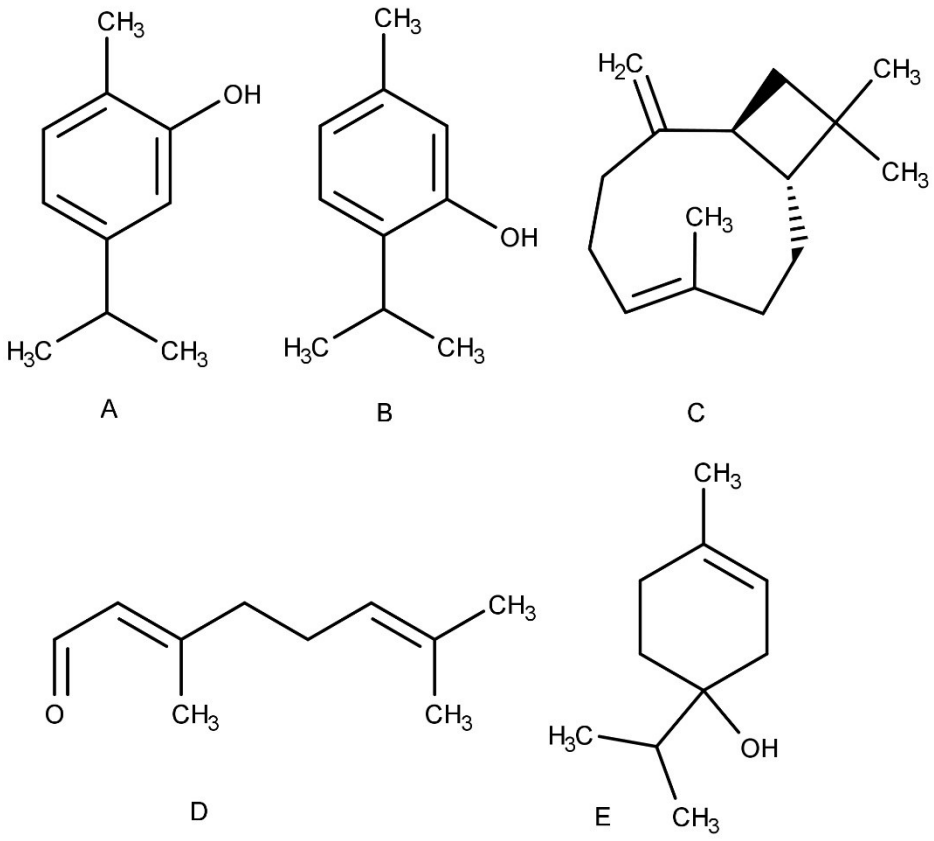

Figure 7: Important antimicrobial compounds; (A): carvacrol, (B): thymol, (C): β-caryophyllene, (D): citral, (E): terpinene-4-ol

3 Toxicity

Most of the above described oils have GRAS (generally recognised as safe) status [96]. Nonetheless, EOs are often underestimated and seen as “harmless” as they are natural products [97]. However, several compounds can lead to undesired reactions due to their toxic profile.

3.1 Pulegone

Besides in EOs, pulegone is also an ingredient for example as flavouring substance in mouth washes, tooth pastes, food and perfumes as flavouring substance [29, 98]. In EOs, especially of *Lamiacea*, (R)-(+)-pulegone is the mainly contained enantiomer [29]. The toxicity of pulegone has to be differentiated between (R)-(+)-pulegone and (S)-(-)-pulegone, as the stereoisomers have different toxicological potential. (R)-(+)-pulegone shows a threefold higher hepatoxic potential than (S)-(-)-pulegone. The metabolism of pulegone is decisive for the toxicity, as the metabolites are often blamed for it. CYP1A2, 2B6 and 2C19 enzymes seem to be responsible for the metabolism from pulegone to menthofuran and further metabolic products [99]. Glutathione plays a former role in the metabolism of pulegone. Pulegone and its metabolites deplete glutathione, which may inhibit detoxification, and this could further increase the hepatotoxic potential. Menthofuran is assumed to have less influence in this case [100]. Especially since glutathione is an important antioxidant, a deficiency can lead to oxidative stress and thus, beside others, have a negative influence on liver diseases [101]. Although most of the studies were performed on rodents, one investigation tested pulegone, menthofuran and acetaminophen *in vitro* on human liver slices. Interestingly, pulegone showed the highest hepatoxicity from the three compounds tested. This outcome deviated from previous information, since menthofuran as a metabolite was always held responsible for the hepatotoxic effect [102]. There are indications that menthofuran as a metabolite of pulegone plays a lesser role in the human metabolism as only small amounts of menthofuran were found in the urine of human volunteers after pulegone administration [103]. However, these findings are controversial as other studies have described the conversion of pulegone to menthofuran by human liver cytochromes, mainly by CYP2E1 and moreover CYP1A2 and CYP2C19 [104]. The different toxicity of the stereoisomers may be due to the double bond orientation of (R)-(+)-pulegone which allows less enzymatic

reduction. This could promote oxidative metabolites [103]. Moreover, the α-isopropylidene ketone seems to be necessary for a hepatotoxic effect and the configuration of the methyl group also has influence on the hepatotoxic potential [105]. Rats treated orally with (S)-(-)-pulegone and (R)-(+)-pulegone (250 mg/kg) showed a higher level of *p*-cresol and piperitenone after (R)-(+)-pulegone administration. In contrary, the urinary values of unmetabolized pulegone, piperitone and benzoic acid were higher after (S)-(-)-pulegone administration [106]. The different metabolization could be another explanation for the different toxicity. γ-Ketoneal is a degradation product of piperitenone and preceding R-(+)-menthofuran metabolism. γ-Ketoneal is a known strong hepatotoxic substance. The resulting formation of *p*-cresol is also related to this degradation pathway [107]. Although urinary bladder neoplasms were observed in a study on rats by pulegone administration, carcinogenicity in humans does probably not pose a relevant risk because there is no regular and prolonged exposure. This is additionally enhanced by high volatility and irritant properties [108]. In 2018, the FDA (Food and Drug Agency) evaluated the data on the effects of pulegone and the MOE (margins of exposure) were calculated to be able to relate the results of rodent studies to humans. Pulegone was classified as non-genotoxic. The investigations leaded the FDA to conclude, that the MOE of pulegone on humans as an additive in food, is a negligible problem [98]. Although pulegone has GRAS status, its tolerable daily intake was regulated to 0.1 mg/kg bw (bodyweight) per day by the European Union [109]. As already described in chapter 2.6.1, rats orally treated with MVEO for 90 days, showed no adverse or toxic effects after an intake of 460 mg/kg bw/day. The used EO had a content of 65% pulegone and 24% menthone, resulting in a daily intake of pulegone of approximately 299 mg/kg bw/day [78]. Interestingly, the observed NOEL (No-observed effect level) for pulegone was 20 mg/kg bw/day in another investigation [110]. This discrepancy could be explained by the fact that EOs are mixtures of different compounds. Therefore, other substances in MVEO seem to influence the hepatotoxic potential of pulegone [78]. Another explanation could be the amount of menthone and its antioxidant properties which were already described above [74]. In this context, pennyroyal oil (*Mentha pulegium*) has to be mentioned, as it can have a pulegone content of more than 80%. It showed several toxic effects depending on the dose and also on the varying composition of the oil. The side

effects of pennyroyal oil, which was used for abortion in former times [103], reached from gastritis and central nervous system toxicity after an intake of less than 10 ml, up to even more serious side effects as coma, hepatic and renal failure for 10 ml intake [109].

3.2 Methyleugenol

The metabolism of methyleugenol (Fig. 8A) plays a crucial role in its toxification. After hydroxylation by CYP1A2, CYP2C9, CYP2C19 and CYP2D6, the sulfotransferase SULT1A produces 1-sulfoxymethyleugenol. Due to its electrophilic property this metabolite reacts with amino bases of DNA bricks like adenosine and guanosine, forming methyleugenol-DNA adducts [111]. These adducts were found in a high percentage in human liver tissues and related to carcinogenicity. Investigated tissues were non-tumourous, derived from liver-operated Caucasian persons showing neither hepatitis nor cirrhosis or extensive alcohol consume [112]. Methyleugenol and its metabolites, 1′-hydroxymethyl-eugenol, methyleugenol-2′,3′epoxide and 3′-oxomethylisoeugenol exhibited DNA strand breaks in human colon adenocarcinoma cells HT29, whereas the metabolites showed stronger DNA damaging properties than methyleugenol itself, probably due to the high enzymatic activity of HT29 cells. Moreover, 3′-oxomethylisoeugenol was identified as topoisomerase I- and human histone deacetylase-inhibitor, which additionally contributes to DNA damage [113]. In several *in vitro* and *in vivo* studies on rodents, methyleugenol showed genotoxic and carcinogenic effects. Beside others, liver neoplasms and neuroendocrine tumours of the glandular stomach were observed in mice and rats. Even the lowest investigated dose (37 mg/kg bw/day) caused liver cancer [114]. Therefore, methyleugenol has been forbidden by the European Union for the use in food flavouring since 2011 [115]. The recommendations of the Canadian government limit the daily intake of methyleugenol as a component of EO to 200 µg/kg bw [116]. The EOs described in this review, in chapter 2.4.6 (*Rhaponticum acaule*, 8.3% methyleugenol) and 2.5.2 (*Ocimum basilicum*, 7.5% methyleugenol), would therefore allow a daily intake of 3.36 drops of RAEO and 7.46 drops of OBEO for a person with 70 kg [68, 75, 117].

3.3 Eugenol

Although eugenol (Fig. 8B) is already known for its positive antioxidant, anti-inflammatory and antimicrobial activities, its toxicological profile cannot be ignored [118]. Indications of genotoxicity and DNA-damaging effects were found. Anyway, these findings were contradictory as genotoxic and gene-repairing effects were found: on the one hand, eugenol (0.62, 1.24, 2.48 mg/mL) induced DNA damage in the macrophages of mouse peritoneum, on the other, hand doxorubicin induced DNA damage was repaired. The differences could be explained by different mechanisms of the phenoxyl radical in different circumstances, such as duration of application and concentration [119]. Contrary results were also found in dental pulp fibroblasts in humans. Eugenol (0.06-5.1 µmol/L) showed DNA damaging properties, whereas at higher concentrations (320-818 µmol/L) they were no longer present [120]. Eugenol was reported to induce allergic contact dermatitis and sometimes urticaria, asthma and rhinitis. These contact allergies can occur occupationally for hairdressers, cleaners, dental staff and drugstore employees as several cleaning products, fragrances, perfumes and medicinal products contain eugenol [121].

3.4 Camphor

The toxic properties of camphor (Fig. 8C) have been known for decades. Especially children can react very sensitively. Major systemic toxicity has not been reported with ingestion of up to 30 mg/kg of camphor. Toxic symptoms usually only occur at doses starting from 30 mg/kg, 50-150 mg/kg can be enough to cause lethal consequences. A fatal dose was observed at 500 mg/kg [122, 123]. Symptoms can reach from gastrointestinal disorders to disabilities of the central nervous system. Confusion, hallucinations as well as seizures were observed. Even the respiratory and cardiovascular system can be affected by apnoea and asystole. Therefore, products in the USA are restricted only to contain less than 11% of camphor in medicinal used products since 1983 [122]. The neurotoxic effects appear very fast, as they already occur at 5-20 minutes after exposure of more than 50 mg/kg camphor. The concentration peak is reached after 90 minutes. Seizures and respiratory arrest are the mainly responsible incidents for a fatal outcome [123]. Actual findings showed effects of camphor on reproduction of Japanese medaka after 28 days of exposure: concentrations of 50 and 500 µg/L diminished the

spermatogenesis, and 500 µg/L negatively affected fecundity and fertility. These findings may be especially interesting for environmental conditions [124].

3.5 Thujone

(-)-α-Thujone and (+)-β-thujone are neurotoxic agents, when used orally in high doses [125]. They act as GABA-$_A$ and 5-HT$_3$ receptor antagonists [126, 127], inducing hyperreflexia, seizures, tonic-clonic convulsions and spasms as neurotoxic side effects [125]. The main metabolites are 7- and 4-hydroxy-thujone, in which the α-diastereomer seems to be predominantly hydroxylated to 7-hydroxy-thujone, the β-diastereomer to 4-hydroxy-thujone. CYP2A6 is mainly responsible for its metabolization which is further supported by CYP3A4 and CYP2B6 [128, 129]. The reduction results in detoxification. Indeed, thujone is quickly available in the organism and therefore early effective but also very rapidly reduced [127]. The National Toxicology Programme tested α,β-thujone on rats (12.5, 25, 50 mg/kg) and mice (3, 6, 12, 25 mg/kg) for two years, administered via stomach gavage. Animals with α,β-thujone doses of 25 mg/kg showed seizures, whereas rats treated with 50 mg/kg didn't survive the study. In addition, an increased occurrence of preputial gland cancers and adrenal gland cancers in male rats was observed [130]. In case reports, tonic-clonic spasms of a child and a new-born after unintentional exposure to SEO (*Salvia officinalis* L.) were discussed. In contrast to the infant, the cramps occurred more than once in the new-born, but both weathered it well [131]. However, it has to be considered that thujone is not the only convulsant compound in SEO, since camphor (discussed in chapter 3.4) and 1,8-cineol are contained, too [67, 125, 131]. Although human studies are very rare and often inconclusive [132], the Scientific Committee of Food (SCF) decided that the sensitivity of humans to thujone is comparable to that of animals. Thujone is forbidden as flavouring food additive in the European Union and the United States [133]. Different calculations resulted in an ADI (acceptable daily intake) of thujone from 0.05 mg/kg over 0.1 mg/kg to 0.11 mg/kg, leading to a maximum of 3.5-7.7 mg per day for a 70 kg person [129, 132]. Although thujone restrictions on sage preparations have been abolished by the European Union, the EMA (European Medicines Agency) refers for *S. officinalis* to a recommended maximum intake of 5 mg thujone per day [132]. For SEO (29% thujone), discussed in chapter 2.4.5, this would allow a daily intake of less than one drop [67, 117].

3.6 (R)-(+)-Limonene

(R)-(+)-Limonene holds GRAS status and has low toxic properties. Nonetheless, the substance showed some adverse effects and its toxicological profile has to be discussed. The biggest amount of the compound in the human organism after application was found in liver, blood and kidneys and the subsequent urinary excretion was 60% [134]. The metabolites of (R)-(+)-limonene in humans after oral intake are dihydroperillic acid, perillic acid and its methylesters as well as limonene-1,8-diol [135]. (R)-(+)-Limonene was observed to lead to skin irritation in rats and mice after dermal application. The compound itself is non-allergenic, but due to oxidation, limonene oxide, limonene peroxide and (R)-(-)-carvone can emerge as allergenic products which have a higher irritant potential than (R)-(+)-limonene. Other possibilities are oxidation with ozone or free radicals in the environment [136]. Even carcinogenicity was established on male rats as they developed renal adenomas. However, this seems to be specific for this species and sex, as male rats are the only ones producing the urinary protein α_{2u}-globulin which seems to be the cause for the urinary carcinogenicity of (R)-(+)-limonene. Therefore, the nephrotoxicity seems to be irrelevant for humans [137]. Investigated NOAEL (No observed adverse effect level) and LD_{50} values showed a wide range. In an acute oral toxicity study LD_{50} was reported to be 4400 mg/kg bw in rats and 6600 mg/kg bw in mice. In another study under same conditions LD_{50} for rats was determined to be 5 g/kg, for mice 6 g/kg and for rabbits 5g/kg after dermal application. In chronic toxicity testing studies on rodents, lower NOAEL-values were determined, reaching from 75 to 500 mg/kg bw per day [134, 137]. ADI value for the human consumption is not specified [134]. Especially long-term intake and exposure at higher dose should be observed carefully and further investigations are needed.

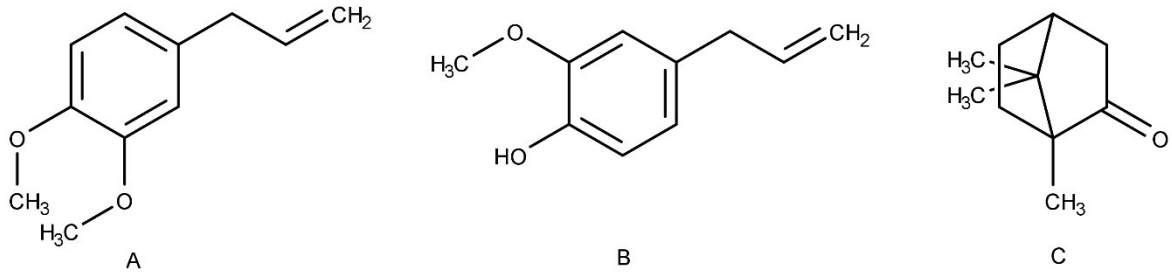

Figure 8: Important toxicological compounds; (A): methyleugenol, (B): eugenol, (C): camphor

4 Interactions

On the one hand the rising popularity of herbal medicine is very gratifying, on the other hand it also entails risks. The powerful potential of herbal medicines, such as EOs, and their influence on metabolism as well as interaction with other remedies, is often underestimated or unknown [138]. Additionally, the intake of herbal products is often concealed so that physicians have no possibility to take interactions into account [139]. Cytochromes 450 enzymes play a major role in metabolising xenobiotics. As oxidising agents, CYP450 are responsible for drug metabolism in 80%, and in 50% they perform as eliminating agents [140]. UDP-glucuronosyltransferases are another essential group of enzymes for the degradation of drugs [141]. The frequent involvement of these enzymes in drug metabolism can lead to adverse interactions of different orally administered compounds which need the same enzyme for degradation or metabolism. This competition in pharmacokinetic and pharmacodynamic can lead to decreased or increased levels of activity and effects of certain medications [142].

4.1 Bitter orange essential oil

In human participants, bitter orange juice (240 ml) was found to inhibit intestinal CYP3A4 as the AUC (area under the curve) of felodipine (10 mg, extended release) increased by 76% when taken orally at the same time. Bergamottin (5 µmol/L), 6',7'-dihydroxybergamottin (36 µmol/L) and bergapten (31 µmol/L) were the involved furanocoumarins. Of these cytochrome affecting compounds, 6',7'-dihydroxybergamottin was determined to be the most potent CYP3A4 inhibitor. In contrast to grapefruit juice, which also contains furanocoumarins and is a well-known gut inhibitor of CYP3A4 and P-gp (P-glycoprotein) efflux pump, bitter orange juice is assumed to inhibit only intestinal CYP3A4. This was concluded, as ciclosporin, an immunosuppressant, was only altered significantly by grapefruit but not by bitter orange juice [143]. Therefore, it can be assumed, that although ciclosporin is a CYP3A4 substrate, bitter orange oil (BOEO; chapter 2.3.2) primarily affects the oral bioavailability of drugs, which are mainly metabolised by intestinal CYP3A4 and not removed by P-gp [144]. Contrary to the above claim, another study investigated the influence of bitter orange juice (200 ml) on the antitussive dexamethasone (30 mg, p.o.) in 11 human subjects. Dexamethasone urinary excretion was induced by bitter orange and grapefruit juice in comparison

to water. The explanation can be seen in the intestinal inhibition of CYP3A4 and P-gp that increases the absorption. Thereby, the bioavailability and thus the metabolization and excretion rate are also increased. As the pharmacokinetic profile of dexamethasone with bitter orange juice did not significantly differ to grapefruit juice, and CYP3A4 substrates are usually P-gp substrates at the same time, it can be concluded that BOEO inhibits CYP3A4 as well as P-gp. CYP2D6 activity was also investigated but not influenced [145]. In an *in vivo* study of bitter orange capsules and their effect on CYP activity, no effect was found. The bitter orange extract used in this investigation did not contain any furanocoumarins and thus no 6'-7'-dihydroxybergamottin [146]. Thus, it can be concluded that the CYP3A4 inhibiting potential of BOEO is present in case it contains furanocoumarins. Although these are non-volatile compounds, residues of furanocoumarins and coumarins can be included in bitter orange EO, due to the cold pressing procedure of citrus peel EOs [147]. The CYP3A4 inhibition only counts for intestinal and not for liver cytochromes. The latter were observed not to be affected [145]. However, the bioavailability of orally administered drugs could be concerned by orally taken BOEO.

4.2 Peppermint essential oil

MPEO (chapter 2.4.4; 2.5.1) was found to be a possible CYP3A4 inhibitor, an enzyme of the phase-I-metabolism. Menthol and menthylacetate may be the responsible constituents. An *in vitro* investigation, in human liver microsomes on both single components as well as MPEO, showed moderate inhibitory effects of nifedipine metabolism, which is performed by CYP3A4. In twelve human participants, felodipine (10 mg) and its metabolite dehydrofelodipine values, together with a single dose of MPEO (600 mg) administered orally were investigated. Water served as control condition. The concentrations of both substances were higher after MPEO exposition compared to water. The fact that the concentration of the metabolite also increased, indicated an additional inhibition of further degrading enzymes. An about ten times higher content of menthol (54%) than menthylacetate (5%) in MPEO suggests, that menthol seems to be mainly responsible for the effect [148]. Anyway, another study observed that menthol (100 mg at the beginning, 50 mg after two hours, 25 mg after five and seven hours) had no influence on felodipine (10 mg) efficacy level after a single oral dose in ten

subjects. Felodipine concentrations and cardiovascular parameters (heart rate, blood pressure) were not significantly influenced. Therefore menthol might not be responsible for increasing bioavailability, it seems rather to be due to the complex composition of MPEO [149]. On male rats, MPEO (100 mg/kg) increased the concentration of ciclosporin (25 mg/kg), an immunosuppressive agent, for about three times. Although ciclosporin is metabolised via CYP3A4, simultaneous administration of ciclosporin with ketoconazole (10 mg/kg, 20 mg/kg), a CYP3A4 inhibitor, had no effect on the concentration levels of ciclosporin. The increase of the ciclosporin bioavailability after MPEO co-administration is probably not only due to an altered CYP3A4 metabolism. Therefore other metabolic pathways could be affected too [150]. In another investigation, mice were pre-treated either acute (an hour before) or chronically (five days before) with MPEO (0.1 mL/kg, 0.2 mL/kg; p.o. gavage). Thereafter changes in parameters of codeine (25 mg/kg; i.p.), pentobarbital (40 mg/kg i.p.) and midazolam (5 mg/kg; i.p.) were examined. A significant reduction of the analgesic effect of codeine was observed for chronic pre-treatment mice at the higher dose by a decrease of the AUC and a decreased maximum possible effect (% MPE) in the hot plate test. A higher % MPE indicates a better antinociceptive effect. Since codeine needs to be metabolised into morphine via CYP2D6 in order to develop its analgesic properties, an inhibition of this mechanism by MPEO could be a possible explanation. Chronic MPEO pre-administration of 0.2 mL/kg prolonged the effects of midazolam and phenobarbital, as motor coordination and sleeping time were increased, respectively. As midazolam is a CYP3A4 substrate, the increased effective period is probably due to the inhibition of this enzyme, which is a further confirmation of the CYP3A4 inhibitory effect of MPEO. The extended pentobarbital activity may also be due to the influence in the cytochrome system like CYP2B6 and CYP2D6, as they are the metabolising cytochromes. In comparison to a control (saline) and a loperamide (1.5 mg/kg, 3 mg/kg; i.p.) group, the highest dose of MPEO (0.2 mL/kg) showed an increase in gut motility in mice 60 minutes after charcoal meal administration. The effect on intestinal motility can also affect the bioavailability of drugs due to the variability of time the drug remains in the intestine to be absorbed into the bloodstream [151].

4.3 Rosemary essential oil

1,8-Cineol is one of the major components of rosemary essential oil (*R. officinalis* L.; chapter 2.7.9). It can induce enzyme activities such as CYP2B, especially CYP2B1, CYP3A2 and UDP-glucuronosyltransferase. By influence on the cytochrome enzymes, it can possibly modify drug metabolization. Testing the efficacy of rosemary EO on mice in combination with paracetamol and codeine, two analgesic substances, showed higher additive analgesic effects of the EO in higher concentrations. Thus, it can be assumed, the varying concentrations of the EO may have different effects on the CYP450 enzymes [152]. In another study on rats, a two-week dietary rosemary EO intake (0.5%) induced CYP2B1, CYP2B2, EROD (ethoxyresorufin-O-deethylase), MROD (methoxyresorufin-O-demethylase) and PROD (pentoxyresorufin-O-dealkylase) phase-I-enzymes. The phase-II-enzymes UDP-glucuronosyltransferase (UGT), especially UGT1A6, UGT2B1, PNP-UGT (p-nitrophenol-UGT) and 4-OH-biphenyl-UGT, were also enhanced by the EO of rosemary [153]. As estimated 40-70% of metabolised xenobiotics, like drugs and herbs, become glucuronidated, oral rosemary EO application could affect the active pharmaceutical ingredient level due to an increased metabolic rate [141, 152, 153]. The impacts on specific medication on humans has not yet been clarified.

5 Conclusion

The increasing number of people affected by public health issues, the demand for natural remedies and sometimes unsatisfactory therapies provide the basis for the search for alternative active substances. In this thesis, the mechanisms of action of selected interesting EOs in relation to common lifestyle diseases like depression, obesity, diabetes, allergies and furthermore hypertension and liver fibrosis were described. In the case of antibacterial active EO, interesting mechanisms and effects against resistant germs could be promising in the fight against antibiotic resistance, whereas the aim was to give an overview and not to provide completeness. Promising effects and effectiveness have been identified and the modes of action clarified. To know how the EOs operate is important to be able to treat diseases specifically. The active ingredient responsible for the efficacy could not always be detected. However, EOs are often effective precisely because of their complex mixture of ingredients. Despite the good compatibility of described EOs, those with a high content of potentially toxic ingredients or possible CYP3A4 drug interactions should be used carefully.

The insights gained, give hope for an alternative and supportive treatment option for people affected by the discussed diseases. As many findings are based on *in vitro* or rodent studies, some questions remain unanswered and further investigations and studies, especially *in vivo* in humans, are necessary to ensure safety of use in relation to dosages and best way of application, as well as possible side effects and interactions.

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