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Possible Uses in Medicine“

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ABSTRACT:

In the last few years more and more has been reported on the biological properties of mono- and sesquiterpenes (MTs and SQTs). Although they are already being used widely as flavoring and antimicrobial agents in cosmetics, perfumes, household and cleansing products and food additives, a lot of their pharmacological properties are still undiscovered. Studies report on their anti-cancer, anti-inflammatory, anti-nociceptive, anti-diabetic and antimicrobial activities and effects on the central nervous system which make them potential targets for development of new therapeutics and their usage for medical purposes. This paper is an overview of the biological activities and aromatherapeutical uses of chemical classes of MTs and SQTs which compiles the scientific achievements mostly from 2010, 2011 and the first part of 2012. On account of the fact that there exist hundreds of of MTs and SQTs and their derivatives, only some prominent representatives of MT- and SQT-hydrocarbons, -alcohols, -oxides and -carbonyls are dealt with.

ZUSAMMENFASSUNG:

In den letzten Jahren wird immer mehr und mehr über die biologischen Eigenschaften von Mono- und Sesquiterpenen (MT und SQT) berichtet. Obwohl sie schon vielseitig angewendet werden als Aromastoffe und antimikrobielle Substanzen in der Kosmetikindustrie, Parfümherstellung, in Haushalts- und Reinigungsprodukten und als Lebensmittelzusatz, sind viele ihrer pharmakologischen Eigenschaften noch nicht erforscht. Studien berichten über die antikanzerogenen, entzündungshemmenden, analgetischen, antidiabetischen und antimikrobiellen Eigenschaften sowie über die Effekte auf das zentrale Nervensystem, was sie somit zu therapeutisch wichtigen Zielsubstanzen zur Entwicklung neuer Arzneimittel und zur Anwendung in medizinischen Zwecken macht. Diese Arbeit ist ein Überblick der biologischen Eigenschaften und aromatherapeutischen Anwendungsgebieten von unterschiedlichen chemischen Klassen von MT und SQT, die die wissenschaftlichen Errungenschaften aus den Jahren 2010, 2011 und der ersten Hälfte 2012 kombiniert. Da es hunderte von MT und SQT und deren Derivate gibt, beschäftigt man sich hier nur mit den bekanntesten Vertretern der MT- und SQT-kohlenwasserstoffe, -alkohole, -oxide und -carbonyle.

ABBREVIATIONS:

AC	Adenylate cyclase
AUC	Area under the plasma level/time curve
BAK	BCL-2 homologous antagonist/killer
BCL-2	B cell leukaemia-2
cAMP	Cyclic adenosine monophosphate
CAT	Catalase
CB receptor	Cannabinoid receptor
CDK	Cyclin-dependent kinase
CNS	Central nervous system
COX	Cyclooxygenase
CREB	cAMP response element-binding
CYP	Cytochrome P450
E-BCP	(<i>E</i>)- β -Caryophyllene
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EO	Essential oil
ER	Endoplasmatic reticulum
ERK	Extracellular signal-regulated kinase
GABA	Gamma-aminobutyric acid
G CSF	Granulocyte colony-stimulating factor
GI	Gastro-intestinal
HepG2	Human hepatocellular liver carcinoma
HMG-CoA	3-Hydroxy-3-methylglutaryl-coenzyme A
IL	Interleukine
JNK	C-Jun N-terminal kinase
K _{ATP+}	ATP-dependent potassium channels
L-NAME	N-(ω)-nitro-L-arginine methyl ester
LPO	Lipid peroxidation
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MAPK p38	Mitogen-activated protein kinase p38

MDA	Membrane lipid peroxidation
MPO	Myeloperoxidase
MT	Monoterpene
NMDA	N-methyl-D-aspartate
NFkB	Nuclear factor 'kappa-light-chain-enhancer' of activated B-cells
NO	Nitric oxide
NOS	Nitric oxide synthase
NSAIDs	non-steroidal anti-inflammatory drugs
PG	Prostaglandine
RAF	Rapidly Accelerated Fibrosarcoma
RAS	Rat sarcoma
ROS	Reactive oxygen species
SOD	Superoxid-dismutase
SQT	Sesquiterpene
TNF- α	Tumor necrosis factor alpha
TRP	Transient receptor potential
TRPA1	TRP Ankyrin 1
TRPM8	TRP Melastatin 8
TRPV1	TRP Vaniloid 1

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INTRODUCTION

Monoterpenes and Sesquiterpenes are rather small molecules compared with the majority of drugs used in the classical pharmacotherapy. Also the fact that we cannot find N-containing molecules among them is one of the reasons why we do not encounter terpenic medicines apart from some exceptions, e.g. the MT-ic alcohol menthol as a spasmolytic drug against bile problems. On the other hand, nearly all of the naturally occurring MTs and SQTs are volatile and thus fragrant and render them suspicious for the majority of pharmacologists and physicians. Also another fact hinders the entering of these natural compounds into the pool of established medicaments, namely that they occur as multi-component mixtures in EOs and are therefore not compatible for the so-called “one-molecule-one-target”-dogma^{*} of the classical pharmacotherapy. So, the medicinal uses of these terpenes remain a domain in either complementary or alternative medicinal therapies, if at all. But the question is allowed: “Why should these fragrant, small molecules do not possess other biological, namely therapeutically usable properties?”

As already mentioned, MTs and SQTs are the major components – besides some phenylpropanes and small alkene derivatives, latter often the catabolic products from unsaturated fatty acids – in the EOs which are produced by plants mainly either to protect them against herbivores, insects, mites fungi and bacteria, or to be used as pollinators, or are released in the moment of attack to warn neighbouring plants and/or to “cry for help” in order to allure the enemies of the attacking aggressors [1,2]. The biological properties of EOs and thus also of their constituents are already dealt with in some reviews [3-9]. Therefore, to avoid a repetition of already discussed matters, the present overview tries to put the focus of interest on biological activities and aromatherapeutical uses of chemical classes of MTs and SQTs, strictly

^{*} Hannelore Daniel, Oral presentation, entitled: Genetic & Nutrient Determination of the Metabolic Syndrome (Nutrigenomics), 59th Intern. Congress and Annual Meeting of the Society for Medical Plants and Natural Product Research, Antalya Turkey, 4th-8th September 2001, see also: S. Frantz, Nature (2005), 437:942

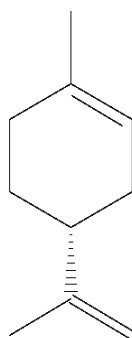
speaking on hydrocarbons, alcohols, oxides and carbonyles. On account of the fact that there exist hundreds of MTs and SQTs and their derivatives, only some prominent representatives of each class will be dealt with. Finally, also the term “biological” has to be defined as it already has been done in [4]. Therefore, in this treatise are not discussed: plant care, inter plant communication (see also [1,2]), pheromones (which can be read in detail in [10]), veterinary therapeutics, cosmetic uses, perfumes, household and cleaning products, flavors for food and drinks and the antimicrobial activities will be just partially mentioned. They have been recently published [7].

MAIN PART

1) HYDROCARBONS

(+)-Limonene

(former: d-limonene, D-limonene)



One of the most prominent MT-hydrocarbons is (+)-limonene which occurs in nearly every EO of the citrus oils, but as a major compound (up to 97 % [11]) in sweet orange oil (from the peel of *Citrus sinensis* (L.) Osbeck, syn. *C. aurantium* var. *sinensis*). Its odor reminds of the typical sweet orange flavour whereas its antipode (-)-limonene possesses an odor which recalls turpentine to ones mind [12]. Sweet orange oil achieves its main importance in the flavour and food industry because it is easily obtainable (yield: ~5% [11])

and on account of its wonderful odor which is accepted by everyone and caused by this “character impact compound” [13] (+)-limonene. In the past years the use of (+)-limonene has experienced a great expansion. Besides its use in the food industry, it is used as flavour and fragrance additive in cosmetics, soaps and perfumes, but also in medicine to mask the bitter taste of alkaloids in pharmaceutical products. (+)-Limonene is being consumed by people mostly as a natural ingredient of commonly used food such as oranges and other citrus fruits, juices, vegetables, coffee, meat and spices [14]. (+)-Limonene made a big progress being used in cleansing and disinfection products as for the industrial use but also in household products.

There is a big increase of interest for the use of these EOs as plant based antimicrobials in the food industry as an excellent alternative to synthetic antimicrobials. This is mainly due to the growing resistance of foodborne microorganisms to synthetic chemicals, but also due to the fact that this plant based antimicrobials are considered as cheaper and more friendly to our environment. The study of Singh et al.[15] can be held as a confirmation for the use of (+)-limonene as a plant based antimicrobial and due to its antioxidative effects as a food preservative. They investigated the antifungal, antiaflatoxic and antioxidant activity of EOs of *Citrus maxima* Burm. (the leaves) and *Citrus sinensis* (L.) Osbeck (the peel) and the 1:1 combination of them. The major components, analyzed by GC-MS, in the oil of *C. maxima* was with 31.8% DL-limonene, followed with 17.7% by *E*-citral and in the oil of *C. sinensis* DL-limonene represented 90.7% followed with 2.8% of linalyl acetate. The 1:1 combination contained 69.8% of DL-limonene. First an antifungal assay was performed due to the fact that fungi are one of the most important destroyers of food that is being stored. The results showed, according to ANOVA and Tukey’s comparison test, that the EOs were in all concentrations effective compared with a control. A broad fungitoxic spectrum was established. Furthermore the efficacy of suppression of the aflatoxin production was investigated and at 500 ppm the EOs of *C. maxima*, *C. sinensis* and their combination showed a complete inhibition of AFB1 production and AFB1.

The antioxidant activity was observed by DPPH radical scavenging assay on TLC and it proved their strong antioxidative effect while tests on mice showed a high value for LD₅₀ that confirmed the safety in oral consumption.

(+)-Limonene is generally recognized as safe (GRAS) by FDA, by oral consumption it has a relative small toxicity, although when applied in high concentrations it may cause dermal irritations [16].

Due to the fact that limonene possesses such strong antioxidant activity, it could be a potential protection from diseases caused by oxidant damage, like cancer for example.

Previous studies in rats and mice showed that limonene prevented the growth of tumors in chemical-induced carcinogenesis models.

Roberto et al. [14] analysed the effect of limonene on proliferation of normal lymphocytes and its connection to the H₂O₂ level and its effect at the cell antioxidant enzymes (catalase, peroxidase and superoxide dismutase). H₂O₂ has a big impact on the process of growth and death of cells. While in small concentrations, it stimulates the cell proliferation, in higher concentrations though, the proliferation is decreased. Also, H₂O₂ stands in connection with damaging the DNA and genetic mutations. The results showed that in low concentrations limonene decreased H₂O₂, while higher concentrations increased the level.

The enzymes peroxidase and catalase reduce the concentration of organic hydroperoxides and hydrogen peroxides while, superoxide dismutase is generating H₂O₂. Limonene presented its activity related to the applied concentrations.

Limonene applied in low concentrations leads to an increase of catalase and peroxidase which then leads to the decreasing of H₂O₂ and converse. In addition, limonene can stimulate cell proliferation, through decreasing the level of H₂O₂ by increasing the activity of the enzymes catalase and peroxidase. Limonene also protected the cells from oxidative damage when H₂O₂ was exogenously added.

Chaudhary et al. [17] investigated the exact mechanism how limonene provides its antitumor effects. The chemopreventive and chemotherapeutic effects of D-

limonene were tested against chemically induced tumors in female Swiss albino mice. The development of the tumors were initiated by DMBA (7,12-dimethylbenz[a]anthracene) and promoted by TPA (12-O-tetradecanoylphorbol-13-acetate). DMBA and TPA are activating a few carcinogenesis pathways. One way is by triggering the RAS-ERK pathway, another is the genetic mutagenesis made by ROS that are generated by TPA. As mentioned in the study above, the activity of antioxidative enzymes is reducing and it comes to an upregulation of proinflammatory genes such as COX-2. Limonene showed significant results by reducing the edemas and hyperplasias that were chemically induced, it reduced the COX-2 expression, the activity of ornithine decarboxylase while the level of antioxidant enzymes was increased and the amount of [3H] thymidine incorporated in the genetic material reduced. A topical treatment with d-limonene, prior to TPA, alleviated the TPA-induced increase of COX-2 enzymes which implies that COX-2 might be a potential target for d-limonene.

A significant inhibition of the RAS/RAF/ERK signalling pathway could be confirmed which is also connected to a suppression of the induced downregulation of Bax and upregulation of Bcl-2. Namely, when ERK is activated, it has activating effects on proteins such as transcription factors and other protein kinases, and by its inhibition, it affects the expression of apoptotic proteins such as Bim, Bax and Bcl-2, leading to an apoptosis. By attenuating the inflammatory process, oxidative stress and RAS-cascade limonene provided its chemopreventive effect and the induced skin tumorigenesis could be delayed.

A recent study showed that d-limonene alleviates the insulin resistance and liver injuries induced by oxidative stress. Santiago et al. [18] performed their investigations on young male Wistar rats that were previously fed a high-fat diet together with L-NAME for 8 weeks and subsequently with 2% (+)-limonene in the last 4 weeks. They examined the effect of (+)-limonene against biochemical and histological alterations of the liver in high-fat diet and L-NAME-induced metabolic syndrome. Dietary d-limonene supplementation improved the biochemical changes in the liver induced by HDF and L-NAME, especially the hepatic lipid accumulation, liver function indicators, circulatory

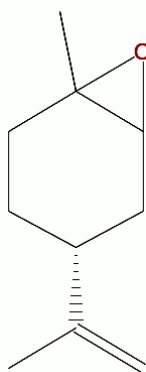
antioxidant, hepatic histology, and insulin resistance. (+)-Limonene restored the pathological changes of liver and pancreas. These findings indicate the potential therapeutic efficacy of (+)-limonene against the development of NAFLD (nonalcoholic fatty liver disease), especially as a promising complementary treatment. NAFLD is most probably the hepatic manifestation of the metabolic syndrome (linked to obesity, insulin resistance, diabetes type 2 and hyperlipidemia).

“d-Limonene is known to inhibit lipid peroxidation, arrest the free radical-induced damage and prevent physical stress, psychological stress, stress-induced hypertension, and stress responses in stroke-prone spontaneously hypertensive rats. d-Limonene is also known to regulate the development of pulmonary hypertension, induce glutathione (phase II detoxification) and inhibit 3-hydroxy-3-methylglutaryl coenzyme A(HMG-CoA) reductase activity. In addition, d-limonene is reported to exert potent biological activities, such as antioxidant properties, chemopreventive or chemotherapeutic properties against many types of cancers, antiinflammatory properties, hepatoprotective activities and immunomodulatory effects.” [18]

Limonene shows also other effects on the cellular metabolism. Park et al. [19] came, in their study, to the conclusion that limonene binds directly to the adenosine A_{2a} receptor. This leads to the activation of receptor-mediated signalling pathways: the increase of the cytosolic cAMP concentration and activation of protein kinase A and further to the phosphorylation of the CREB transcription factor. Through binding on the adenosine A_{2a} receptor, limonene also increased the intracellular calcium level. Both these effects are typical for agonists of the receptor, which leads to the conclusion that limonene also acts as an agonist on the A_{2a} receptor. The ligands of A_{2a} receptors have, in general, an impact on the inflammation process through modulating the release of the pro- and anti-inflammatory cytokines, so they act as a potential protection of tissue injuries. Therapeutically, they can be used as potential sleep inducers, due to their effects in sleep regulation. This implicates on the possible sedative effects of limonene. The activation of the receptor has also influence on cardiovascular system.

Fletcher [20] investigated the effects of (+)-limonene and its metabolite perillyl alcohol, perillaldehyde and perillic acid on the membrane lipid bilayer. The effect was assessed by bilayer-spanning gramicidin (gA) channels using two methods. The first one was a fluorescence assay, which showed that at micromolar concentrations (+)-limonene decreased the gA channel activity and all its metabolites, except perillic acid which had no effect, increased the activity. The second method using single-channel electrophysiology showed though, that each terpene increased the lifetime and occurrence of the gA channel. So, disagreements appeared between (+)-limonene and perillic acid using these two methods, but nevertheless, these terpenes have confirmed to have significant bilayer-modifying potential.

Limonene possesses also an effect on the central nervous system. Further studies have shown the relaxant properties and anxiolytic effect of EO of *Citrus sinensis* suggesting a possible depressant activity of these constituents [21]. De Almeida et al. [22] analysed the effects of (+)-limonene epoxide on the CNS on male Swiss mice.



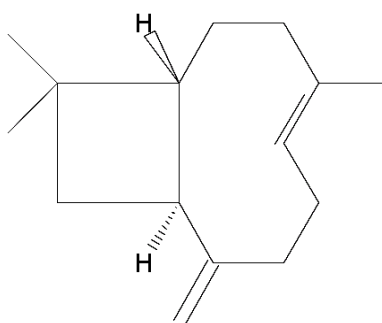
(+)-Limonene epoxide is synthesized from (+)-limonene and it is a mix of cis and trans isomers that is found in many plants. It showed to have antitumor and antinociceptive activities. In the study, the acute toxicity of (+)-limonene epoxide in mice was examined, showing, dose dependent, a relatively high safety, though falling into the group of slightly toxic substances. Furthermore, the anxiolytic, sedative and motor coordination effects were investigated using diazepam as a positive control. (+)-Limonene epoxide was able to decrease

significantly the number of crossings, grooming and rearing. It increased the percentage of open arms entries and the time spent in those arms.

At higher doses, it produced an inhibition of the motor coordination, presented through a muscle relaxation effect. Flumazenil reversed the diazepam and (+)-limonene epoxide effect, suggesting that its mechanism might be involved in an action on the GABA_A receptor complex. These findings suggest (+)-limonene epoxide as a therapeutical approach in the treatment of anxiety, due to the fact that it is relatively safe to a great extend. Also, the results indicated that (+)-limonene epoxide might be responsible for the effects of limonene on the CNS.

Caryophyllene:

Synonym: (–)-trans-Caryophyllene



Another prominent representative of the SQT-hydrocarbons with significant scientifically proven biological activities is (*E*)-β-caryophyllene. It occurs in large amounts as a major plant volatile in the EOs of spice and food plants like *Origanum vulgare* L. (oregano), *Cinnamomum spp.* (cinnamon) and *Piper*

nigrum L (black pepper). (*E*)-BCP in the nature is found together with small amounts of its isomers (*Z*)- β -caryophyllen (*Z*-BCP) and α -humulene (former name α -caryophyllene) or with its oxidation product β -Caryophyllene oxide[23]. β -Caryophyllene possesses a woody, spicy aroma, and traditionally it is used in the fragrance and cosmetic industry. But, due to the fact that by scientific studies its antibiotic, anesthetic, anti-inflammatory, antioxidant and other effects have been established, there is a big interest in using this natural product as a starting point for the development of new drugs.

In the present time, β -caryophyllene is being isolated by various methods of purification from oleoresins extracted from huge amounts of plant materials. In order to avoid this wasteful way of producing, Reinsvold et al. [24] performed engineering on phototropic microorganisms with SQT-synthase genes. The β -caryophyllene synthase gene from *Artemisia annua* was inserted into the genome of the cyanobacterium *Synechocystis* sp..

The experiment was successful and the synthesis of β -caryophyllene could be confirmed in the transgenic strain using GC-FID and GC-MS analysis. This was an important step to develop alternative ways of synthesizing relevant terpenoids, as for pharmaceutical researches, but also as biofuels.

(*E*)-BCP is a selective agonist of the cannabinoid receptor type 2. The investigations were performed back in 2008 by Gertsch et al.[23] in the EO of *Cannabis sativa* L., which contains (*E*)-BCP up to 35%. It was the first *Cannabis*-derived CB receptor ligand with a basically different structure than the one of typical cannabinoids.

Traditional cannabinoids are agonists of CB₁ and CB₂ receptors, and despite their potential therapeutical effect by activating the CB₁ receptor, they cannot be taken for a pharmacological development because of their central CB₁ receptor activity. In this study a CB₂ receptor-selective agonist was discovered, that provided all the potential therapeutic effects of a CB₂ receptor activator but without the psychoactive effects associated with a CB₁ receptor activation. This makes (*E*)-BCP an excellent candidate for the development of new drugs for treatment of inflammations and pain, atherosclerosis and osteoporosis. (*E*)-BCP binding of the CB₂ receptor initiates a complete stimulation program:

-inhibition of the adenylate cyclase which leads to calcium transiency in the cell

-a weakly activation of the mitogen-activated kinases ERK1/2 and p38 in primary human monocytes. Three major MAPK pathways are known: ERK1/2, JNK and p38 and they can further on phosphorylate cytoplasmic and nuclear targets. ERK is mostly activated by mitogenic factors, while JNK and p38 is usually activated by stress-inducing stimuli such as UV-light. MAPKs have, in general, an important role in cell proliferation [25].

-inhibition of LPS-induced proinflammatory cytokine expression in peripheral blood

-alleviation of LPS-stimulated ERK1/2 and JNK1/2 phosphorylation in monocytes, because these pathways are critical for expression of IL-1 and TNF- α (both cytokines involved in inflammation processes in the body). The experiment confirmed that (*E*)-BCP provides its effect also *in vivo*.

After this discovery, the interest for further investigations of (*E*)-BCP was awoken.

Horváth et al. [26] investigated the possible therapeutic effects of BCP in a cisplatin-induced murine nephropathy model. Cisplatin is a chemotherapeutical agent often used in cancer therapy but with nephrotoxicity as a side effect. This side effect is probably caused by oxidative and nitritive stress and inflammations, thus a solution for preventing or reducing this complications is in great demand. β -Caryophyllene showed to attenuate the cisplatin-induced kidney disfunction and morpholocial damage, inflammatory response in the kidney, the increased oxidative and nutritive stress and the enhanced cell death. All of these effects were provided in a CB₂-receptor-dependent manner, which was proven by the fact that the protective effect of BCP was absent in CB₂ knock-out mice.

CB₂ receptors also exist, in low levels, in cells of the gastrointestinal and cardiovascular system, bone and neuronal cells, liver tissue and other cell types [26]. CB₂ is up-regulated in inflamed colonic tissue of colitis patients. It is believed that the CB₂ receptors are in close interaction with the PPAR γ receptor, and both of them are considered targets for treatment of inflammatory

bowel diseases. That was the motivation for Bento et al. [27] to investigate the effect of oral BCP in DSS (dextran sulphate sodium)-induced colitis experimental models. The results showed that BCP inhibits the influx of inflammatory cells and decreased the damage on the colon, reduced the production of inflammatory mediators and cytokine release from LPS-stimulated macrophage. It also inhibited the activation of transcription factors NFkB, CREB and ERK $\frac{1}{2}$ and activation of colonic caspase-3 but not claudin-4. The effects of BCP could be reversed by CB₂ and PPAR γ selective antagonists. That leads to the conclusion that BCP activates the CB₂ receptor and reduces the inflammation of the colon by directly or indirectly interacting with the PPAR γ receptor. The examination showed, with small significant differences though, that a preventive treatment was more effective than the therapeutic treatment, so BCP exhibits both preventive and therapeutic effects in DSS-induced colitis models. A preventive treatment with BCP also improved oxazolone-induced colitis by reducing weight loss and increasing the surviving rate. Investigation also showed that at applications of BCP in high concentrations induced an antiedematogenic effect in CB₂ knock-out mice, which could suggest that BCP acts not only on the CB₂ receptor exclusively.

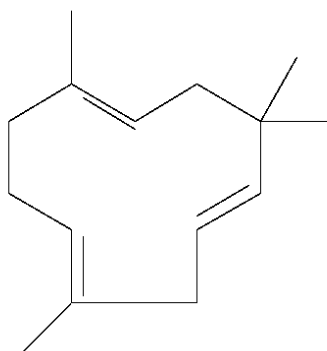
β -Caryophyllene showed to have also antispasmodic activity. Leonhardt et al. [28] examined the effect of BCP as the main constituent of the EO of *Pterodon polygalaeflorus* F. on the isolated ileum from rats. Both BCP and the EO of *P. polygalaeflorus* F. showed to have a dose-dependent relaxant effect on the ileum and they were able to inhibit the acetylcholine and KCl-induced contractions of the ileum and to alleviate the CaCl₂-induced contractions. This effect on the muscle contractility is provided by an intracellular mechanism and it is myogenic. That leads to the conclusion that BCP plays a crucial role in the relaxative and antispasmodic effect that the EO of *P. polygalaeflorus* F. provides in the ileum.

β -Caryophyllene shows a potential anti-cancer effect. Previous studies have demonstrated that BCP possesses a strong antimutagenic activity against 2-nitrofluorene mutagen [29] and that it has a potentiating effect in the anticancer activity of α -humulene, isocaryophyllene and paclitaxel against

tumour cell lines [30]. To investigate if BCP provides its antitumor effect and the possible mechanism of it, di Sotto et al. [31] studied *in vitro* the effects of BCP at chromosomal level by using human lymphocytes. The cultured lymphocytes were exposed to the genotoxic effects of two different mutagens: the alkylating agent EMS (ethyl methanesulfonate) and the aneugenic agent COL (demethylcolchicine). The treatment with BCP was performed three times: pre-treatment before the treatment with the mutagens (to examine the capability to prevent the damage), a co-treatment (to see if BCP can directly interfere with the mutagen) and after the damage was made by mutagens, a post-treatment (to see if BCP is capable to repair the genotoxic damage). The results showed that in comparison to a control BCP by itself did not provoke any cytotoxic nor genotoxic effect. BCP provided its anticlastogenicity potential exclusively in the pre- and co-treatment with EMS significant, but not dose-dependent. The post-treatment could not assert any antimutagenic effect of BCP, which means that it could not promote a reversion of the damage made on the DNA. Also the testing in the presence of COL could not confirm a protective effect. This could lead to the conclusion that BCP acts as a desmutagen, it is an active pre- or co-treatment antimutagen, which means that it deactivates the mutagens before they attack the DNA. The exact mechanism is not yet clear. The anticlastogenic activity could be involved in the antioxidant effect that BCP provides, or a chemical interaction with the mutagens is possible. Another hypothesis would be a destabilizing effect on the cellular membrane. Nevertheless, due to the lack of genotoxic effects and the anticlastogenic activity, BCP gave a valid reason for further investigations and interests as a potential chemoprotective agent.

α -Humulene

Synonym: Humulene, α -Caryophyllene



α -Humulene is a naturally occurring monocyclic sesquiterpene and its structure is built of three isoprene units. The name α -Humulene is derived from *Humulus lupulus*, in whose EO it is found. Humulene and its oxidation products play an important role for the hoppy beer flavour [32]. In plants it is often found together with its isomer β -caryophyllene, like in the EO of *Cannabis sativa* L. where they are the major sesquiterpenes [33] and were it is contributing to the characteristic odor of this plant.

α -Humulene is also an important constituent of *Cordia verbenacea*, which is used in folk medicine for its anti-rheumatic, anti-inflammatory, analgesic and healing properties. It is believed that the oral-inflammatory actions, that *C.verbenacea* provides, are related to the presence of the SQTs α -humulene and β -caryophyllene.

α -Humulene showed to have a rapid and relatively good absorption by oral and topical administration which plays an important role for the topical and systemic anti-inflammatory and antinociceptive effects it provides. Chaves et al. [34] published in their writing that the oral anti-inflammatory effects of α -humulene and β -caryophyllene, isolated from *C. verbenacea*, could be compared to the effects observed in animals treated with dexamethasone. They examined the inhibitory effects of these two compounds in different inflammatory models in mice and rats

Fernandes et al. [35] reported that these SQTs were able to inhibit the activation and/or release of inflammatory mediators like bradykinin, platelet activating factor, histamine, IL, IL-1 β , TNF α and PGE₂. They were also able to inhibit the up-regulation of the enzymes COX-2 and iNOS (inducible nitric

oxide synthase). α -Humulene stood out in the study with the fact that only this compound could, in a systematic treatment, reduce the histamine-induced mouse paw edema and largely prevent both TNF α and IL-1 β generation in carrageenan-injected rats, while β -caryophyllene reduced only TNF α release.

Based on these findings, Rogerio et al. [36] investigated the anti-inflammatory properties of α -humulene, in order to identify potential targets that could prevent or treat inflammatory diseases like allergic diseases or asthma. α -Humulene was applied preventively and therapeutically in an allergic airway inflammation murine model. The examination results revealed that this SQT reduced the eosinophilic migration into the bronchoalveolar lavage fluid and lung tissue, similar to that reported by corticosteroids.

It is believed that the mechanism is related to a reduction of inflammatory mediators, adhesion molecule expression and activation of transcription factors. Namely, by modulation of the Th1/Th2 (T-helper1/T-helper2) balance, reduced production of mucus, inhibition of IL-5, CCL11 (chemokine (C-C motif) ligand 11 (eotaxin)) and LTB₄ (leukotriene B₄) levels and P-selectin expression. All this probably by inhibiting the NF- κ B and AP-1 pathways. An interesting fact was that the animals treated with α -humulene gained weight similar to animals from the control, while dexamethasone-treated animals suffered from weight loss. This implicates on the minor collateral effect of this compound. α -Humulene was successful, by orally or aerosol treatment, though the application through aerosol was more effective.

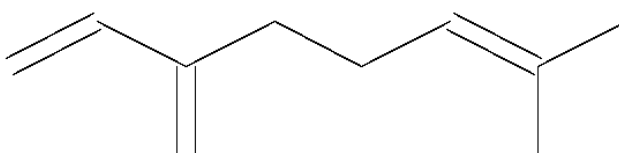
It is reported that α -humulene provides also an anti-tumor effect. In the study α -humulene inhibited the growth of MCF-7 breast cancer cells by about 50%, respectively. This effect could be potentiated by β -caryophyllene through increasing the inhibition up to 75% [30].

El Hadri et al. [37] investigated the cytotoxic effect of both α -humulene and β -caryophyllene from *Salvia officinalis* on breast cancer MCF-7, colon cancer HCT-116, and murine macrophage RAW264.7 cellular lines by the MTT assay (a colorimetric assay to assess the viability and cell proliferation and also the cytotoxicity of substances).

The results showed that the subfraction of *S.officinalis* EO, containing α -humulene, provided the highest activity at the RAW264.7 and HCT-116 cell line, while the subfraction with β -caryophyllene showed less activity on the same cell lines. This suggested that both SQTs were able to inhibit the growth of tumor cells.

Myrcene:

Synonym: β -myrcene



Myrcene is an unsaturated acyclic MT, which exists in two isomeric forms: β -form, which can be found in nature and the α -isomer, which does not occur naturally, but can be synthesized easily. The EO possesses the pleasant odor of geranium, but in pure form it is rather not used as a flavour, a solution with at most 5% would be recommended for smelling. Myrcene tends to polymerize, which is why it is unstable in the air [38]. It has a reactive diene structure which makes it an eclectic starting material for flavoring agents and fragrances. It has its use also in cosmetics, soaps, detergents, vitamins and pharmaceuticals and as a flavoring agent in food and beverages. It is also the main constituent of hop and bay oils, which are used in the production of alcoholic drinks [38][39].

Myrcene can be found in *Humulus lupulus*, *Pimenta racemosa*, *Rosmarinus officinalis* and *Salvia officinalis*.

Behr and Johnen reported in their paper [38] that myrcene is an important starting point for the synthesis of menthol, nerol/geraniol and linalool. Further derivatives are citral, citronellal and citronellol and they are used because of their lemon-like smell. Based on the diene structural component it can be used for Diels-Alder reactions with unsaturated structures, which leads to synthesis of amberlike flavors and anti-cancer therapeutics. By a C-C linkage

geranylacetone and β springene can be obtained, derivatives which can be used to synthesize side chains of vitamin E.

Another use of myrcene is the synthesis of pheromones that can be used as traps for insects.

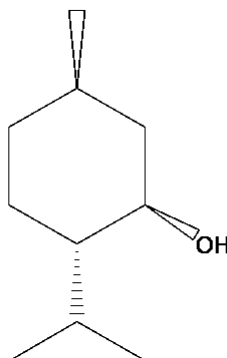
Myrcene can be found in many plants, but its extraction would not be economical, so industrially, it is being obtained by pyrolysis of β -pinene, which is contained in turpentine oil.

The National Institute of Environmental Health Sciences [39] studied myrcene for its cancerogenic activity, due to the fact that it is produced a lot and showed structural connections to limonene, which could induce tumors in male rats kidneys. The study was performed on male and female rats and mice by force-feeding with myrcene for either 3 months or 2 years. The results of the 2-year studies showed that myrcene possesses a carcinogenic effect based on increased incidence of renal tubule neoplasms in male rats and renal tubule adenomas in female rats. Increased incidence of hepatocellular adenoma, hepatocellular carcinoma and hepatoblastoma in male mice and marginally increased incidences of hepatocellular adenoma and carcinoma in female mice.

ALCOHOLS:

(-)-Menthol:

Synonym: *Levomenthol*



Menthol belongs to the group of monocyclic terpenes, which can be found as a major compound in the EO of leaves of mentha species like *Mentha piperita* and *Mentha arvensis*.

Because of the presence of three asymmetric C-atoms in its structure, menthol occurs in four pairs of optical isomers, the (-)-menthol and (+)-menthol, (+)- and (-)-neomenthol, (+)- and (-)-isomenthol, (+)- and (-)-neoisomenthol. (-)-Menthol is the isomer that mostly occurs in nature and besides its characteristic odor, it possesses a cooling effect on the skin and mucosa. It can be obtained synthetically or from peppermint or other mint oils, or from essential oils such as citronella oil, eucalyptus oil and Indian turpentine oil. Due to its minty smell and flavor it is used in pharmaceuticals, soaps, hygiene products like toothpaste, cosmetics, chewing-gum, teas, sweets and tobacco products[41][40].

Because of its antispasmodic, carminative, choleric and cholagogic effects it is traditionally used for treating of gastrointestinal disorders and also in mucus-dissolving and broncholytic preparations. In pharmaceuticals it is a compound in antipruritic, antiseptic and cooling preparations [40].

The cooling effect and tingling sensation of menthol by topical application is related to its stimulation of cold-receptors. This stimulation is caused by inhibiting Ca⁺⁺-currents of neuronal membranes, since Ca⁺⁺-channel blockers are connected to painkilling properties [40]. Both (+)- and (-)-menthol show

equiactive local anaesthetic activity, but only (–)-menthol elicits also an analgesic effect [40].

Kahner et al. [41] reported in their paper about the effects of menthol in tobacco-products. Approximately a quarter of the world's expenditure on menthol can be traced back to its usage in tobacco products and according to the tobacco industry it gives the tobacco a more intensive and pleasant taste. Actually, the addition has a quite strong pharmacological effect, like easing the inhalation and increasing the addiction potential, which can further on lead to numerous chronic diseases and death. They explained extensively in the paper the mechanism how menthol interacts in the body. Menthol interacts with channels responsible for perception of hot, cold and pain, so called TRP ion channels, particularly with TRPM8, which reacts on cold. Menthol inhibits TRPA1, responsible for pain perception, so it works analgesic and anesthetic. Upon longer and recurrent consumption it comes to a desensibilisation in the mouth which affects the perception of irritant substances such as nicotine. Menthol also increases the transdermal and transbuccal absorption of substances and it prolongs the time the breath can be held and suppresses the need of coughing. Some other studies reported that menthol inhibits the oxidation of nicotine to cotinine, so nicotine stays longer in the body. Subsequently, there have been reports of menthol pyrolysis-products containing the cancerogenic agent benzopyrene and menthol inducing the absorption of benzopyrene.

For conclusion, menthol in tobacco products is definitely not just a flavour, increasing the tobacco taste, but affecting the sensoric perception, smoking manners and addiction-potential.

The mechanism of menthol interaction in the body, as mentioned above, could be confirmed by other studies as well. Willis et al. [42] came to the conclusion that menthol in mentholated cigarettes, acts as a counterirritant that diminishes the chemosensory responses of irritants that are inhaled. Irritations were elicited in mice by irritants that occur in cigarette smoke (acrolein, acetic acid, cyclohexanone) and menthol abolished the irritation responses caused by these

irritants, which are agonists of the TRP channel family. Menthol effects could be reversed by a TRPM8 antagonist.

The effect of menthol in mentholated cigarettes on nicotine pharmacokinetics is an important topic for investigation for the tobacco industry. Abobo et al. [43] performed their examination by exposing rats to the smoke of mentholated and nonmentholated cigarettes, then collected blood samples and analyzed the nicotine and cotinine concentrations. The results showed that mentholated cigarettes decreased the maximum concentration (C_{max}) of nicotine in plasma and the plasma AUC compared to nonmentholated cigarettes. The values for cotinine were reduced by menthol as well. These results showed, in conclusion, that menthol in mentholated cigarettes decreased the absorption and increased the clearance of nicotine.

Kreslake and Yerger [44] reported that menthol, besides its use as a flavour and easing the inhalation also reduces the irritations from inhaling smoke and modulates the subjective effects, like smoke harshness and increased smoothness.

But besides the use in tobacco industry, menthol provides a lot of biological activities, which make it extremely attractive in pharmacy and medicine. It has been reported that menthol showed anti-cancer effects by being effective in treating prostate cancer in vitro. Menthol induced cell death in prostate cells through TRPM8 activation and the resulting increase in Ca^{++} [45].

Bhadania et al. [46] reported in their writing that menthol had a protective effect on β -amyloid peptide induced cognitive deficits in mice. The fact that menthol was able to interfere in cognitive actions, opened another field in medicine interested in the effects of this MT. The examination was taken on young and on aged mice, using interceptive and exteceptive memory models (modified elevated plus-maze test and Morris water maze test, which are used in behavioral neuroscience to study spatial learning and memory processes in rodents) and various biochemical parameters were assigned (brain glutamate, glycine, glutathione and thiobarbituric acid reactive substances). The nootropic effect of menthol on learning and memory was evaluated with piracetam as a

control. Menthol was able to maintain the glutamate concentration in the mouse brain throughout its antioxidant activity. It triggers the glutamate release through acting directly on the presynaptic Ca^{++} stores of sensory neurons to release Ca^{++} . It is believed that the glutamate concentration plays an important role in cognitive functions. The results showed a significant enhancement in learning and memory. Menthol did not modify the level of glycine in the brain, but it increased the glutamate level, which leads to the conclusion that the effect is most probably based on the glutamatergic neuronal effect.

As previously mentioned, menthol is known for a long time as a treatment of gastrointestinal disorders, due to the fact that it is able to relax the GI smooth muscles. Based on that knowledge, the usage of menthol as an antispasmodic agent before GI endoscopy is being examined. The application of the EO in form of a spray on the gastric mucosa, was able to inhibit gastric peristalsis, having the advantage of being connected with fewer undesired drug effects than the substances usually used. Hik et al. [47] investigated the mechanism and pharmacokinetics of menthol while used for GI-endoscopy. Menthol showed to be fast absorbed and excreted mainly through the urine in form of menthol-glucuronide. It had a good safety, only a few adverse effects, which relation to the GI treatment can not be excluded. The C_{max} and AUC of menthol increased dose-dependently, but the elimination half life showed to be dose-independent. The study showed that the c_{max} can be obtained faster through spraying on gastric mucosa (0.17-1.00h) than taken orally.

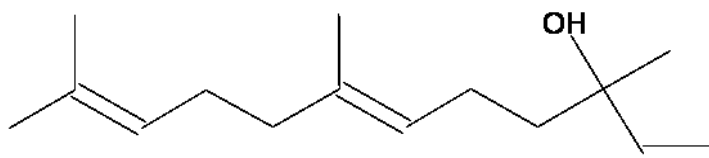
Menthol is traditionally used to relieve the pain caused by exercising because of its cooling and relaxing effect. Topp et al. [48] examined the mechanism by testing the effect of menthol on blood flow and arterial diameter. The investigation was performed on a small group of 8 males and 8 females, and with two doses of menthol (3.5% and 10%), assessing the blood flow and arterial diameter before and after MVMC (maximum voluntary muscular contraction) were performed on the quadriceps and hamstrings. Exercise with high intensity and short duration showed to increase the blood flow of the surrounding tissue and menthol acts by stimulating the thermoreceptors leading to a vasoconstriction and localized cooling. The results showed that application

of both doses decreased the local as the generalized blood flow after a MVMC. This effect may be attributed to an inhibition of local NOS and NO, but also to an increasing of systemic α_2C adrenergic tone.

The authors [49] compared in another study the effect of ice and a menthol gel (3.5 % menthol) on blood flow and muscle strength of the lower arm. The results suggested that menthol provides a fast-acting but short-lived reduction of the blood flow, while with topical application of ice, the similar vasoconstrictive effect can be achieved only by a longer application of ice.

Nerolidol

Synonym: Peruvicol



Nerolidol is a natural occurring, aliphatic SQT-alcohol which possesses two chiral centres in its structure and it prevails as a mixture of its cis and trans-form. It is an isomer of farnesol, from which it is distinguishable by a different position of one double bond and the hydroxyl-group. Nerolidol is a major component of EO extracted from many plants [50][51][52], it has a woody aroma which reminds on tree barks. It is used to enhance the flavor and aroma and used as a fragrance in perfumes, cosmetics, shampoos, toilet soaps, but also in household products [53].

It has been reported that this long chain SQT is an enhancer for the transdermal delivery of therapeutic drugs and for substances that permeate the human skin membranes in general [54] and that it reinforces the bilayers, possibly by orientating alongside the lipids of stratum corneum [55]. Nerolidol also exhibits antineoplastic activity, probably by having an impact on protein prenylation or affecting the mevalonate pathway [56]. The antibacterial effect

of nerolidol was confirmed in several studies, for example on *Staphylococcus aureus*, reporting that the mechanism of this action is probably the damaging of the cell membrane [57]. Other studies accounted the antifungal effect against *Microsporium gypseum* [58] and its antileishmania effect by inhibiting the growth of *Leishmania amazonensis*, *L. braziliensis*, and *L. chagasi* promastigotes and *L. amazonensis* amastigotes [59]. Nerolidol displays also an anti-ulcer activity. As a main constituent from the essential oil of *Baccharis dracunculifolia* DC it inhibited the formation of ethanol-, indomethacin- and stress-induced ulcer models in rats [60]. Already 1986 nerolidol and farnesol were classified as active ingredients in biochemical pesticides [61].

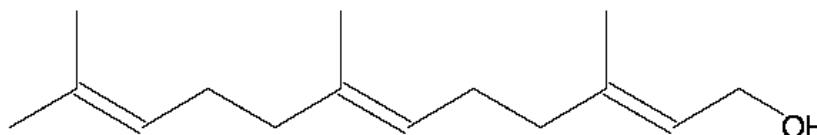
Ferreira et al. [62] investigated the effect of nerolidol in mitochondria and also correlated the results with its cytotoxic effect on HepG2 cells. The mitochondria might be a target for compounds of EO, provoking changes, possibly leading further on to enzyme inhibition or cell death. And nerolidol can, as a hydrophobic compound, cross the plasma membrane easy and interact with cellular proteins and intraorganelle sites. Nerolidol showed to increase the respiratory chain activity and decrease the phosphorylative efficiency. The inhibitory effect in phosphorylative system is related to a reduced concentration of ATP in cells, through inhibition in the ATP-ase enzyme activity. Nerolidol also showed, dose dependently, to induce a decrease of the mitochondrial transmembrane electric potential. The permeability transition in the mitochondria was delayed, most probably as a result of a Ca^{2+} -uniporter reduced activity. The decrease of the calcium-induced permeability transition susceptibility, can be a consequence of the decreased membrane potential and modifications of the mitochondrial membrane fluidity. In connection to the HepG2 cell line, nerolidol presented hepatic cell cytotoxicity. It induced cell death and inhibited cell growth.

Nerolidol (and farnesol) is classified in “Toxicity Category IV” for acute oral toxicity, “Toxicity Category III” for acute dermal toxicity, primary eye irritation and primary dermal irritation, and “Toxicity Category II” for acute inhalation toxicity, according to American Environmental Protection Agency (EPA) [61]. Considering this and the mentioned cytotoxicity, nerolidol may present a possible risk in the use as a therapeutic agent, or as a flavour

enhancer at high doses, therefore a differentiation between the therapeutic and toxicological effect is important.

Pículo et al. [63] assessed the genotoxicity of nerolidol in vivo. The authors investigated if a single treatment with this compound was able to induce DNA damage in peripheral blood and liver cells of mice and micronuclei in polychromatic erythrocytes of their bone marrow cells. The comet assay was used for assessing the genotoxicity and *N*-nitroso-*N*-ethylurea was a positive control for the comet and micronucleus assay. In both peripheral blood and liver cells, nerolidol induced weak, dose dependent DNA damages in comparison to the control. Nerolidol also induced a clastogenic effect on bone marrow cells of mice by enhancing the average number of micronucleated cells in high doses tested. For conclusion, the study pointed out the clastogenic and weak genotoxic effects of nerolidol.

Farnesol



Farnesol is an acyclic SQT-alcohol which possesses a bloomy odor, some people report it reminds them on *Convallaria majalis*. It is often used to enhance the odor and flavour of sweet floral perfumes and as an antibacterial compound in cosmetics. Farnesol showed to decrease biofilms of *Staphylococcus epidermidis*, which cause often infections and is resistant to antimicrobial agents, so farnesol showed to be a potential therapeutic for clinical *S. epidermidis* biofilm infections [64]. There is a big increase of interest in the use of this compound as an antifungal agent. In *Candida albicans*, it caused a downregulation of the expression of some aspartyl proteinase genes, provoking morphological changes that way [65]. Farnesol is believed to be endogenously

produced by dephosphorylation of farnesyl-PP, a metabolite of the cholesterol biosynthetic pathway [66].

Hyuck Joo and Jetten [67] reported in their writing about the anti-cancer and chemoprotective effects of farnesol and they made a summary of the mechanisms of its apoptosis-inducing activities. Farnesol showed *In vitro* to inhibit cell proliferation and induce apoptosis in different types of malignant cells, noticing that tumor cells were more sensitive to the growth inhibition induced by this compound, than normal cells. Cells treated with farnesol showed to have a G_0/G_1 cell cycle arrest, reduction in CDK2 activity and an increased generation of the cyclin-dependent kinase inhibitor $p27^{Kip1}$ with cyclin E/CDK2 complexes. The inhibitory effect of this terpene is suggested to be dependent on these CDK inhibitors ($p21^{Cip1}$ and $p27^{Kip1}$), because a down-regulation of them showed to provide a protection from the proliferation-inhibitory effect. Farnesol also proved its anti-tumor effects *in vivo*. Liver of farnesol treated rats had a number of phase I and phase II enzymes increased, which metabolize drugs and carcinogens, so farnesol might interfere in the metabolism, toxicity or carcinogenesis of drugs. The fact that farnesol has inhibitory effects on HMG-CoA reductase can be related to its anti-cancer effect. Tumor cells need an increased cholesterol biosynthesis, thus by inhibiting it, farnesol might provide its growth suppressing activity. A farnesol-induced endoplasmatic reticulum stress is a major factor leading to cell death. It can activate ERK1/2 and MAPK p38, and by activating this MEK-ERK-pathway the ER-stress is most probably induced. The authors also reported about farnesol inhibiting the phosphatidylcholine synthesis by changing the subcellular localization and activity of CCT α (CTP: phosphocholine cytidyltransferase α), which catalyzes its biosynthesis. Phosphatidylcholine is important in maintaining the structure of membranes and it is a precursor of a few second messengers, which control several cellular processes, also including proliferation and cell death. Namely, under the treatment of farnesol, CCT α translocates to the inner nuclear envelope, following an further export to the cytoplasm and causing an inhibition of the phosphatidylcholine synthesis. Apoptotic stimuli can lead to an assembly of an apoptosom, a protein complex, which includes, among others, also caspase. Farnesol activates caspases 3, 6, 7

and 9, but not caspase 8, which leads to the conclusion that the apoptosis is mediated by the intrinsic, mitochondrial-dependent pathway and not the extrinsic pathway. A higher level of expression of the pro-apoptotic protein Bak and a lower level of anti-apoptotic proteins BCL2 and BCL-X are also related to a farnesol-induced apoptosis. Farnesol activates the NF- κ B signaling pathway and expression of inflammatory genes and increases the level of ROS. Studies in vivo demonstrated also that this substance can reduce oxidative stress, inflammations and injuries in rat lungs exposed to intratracheal installation of cigarette smoke extract. For conclusion, the anti-tumor effects are probably involving a few mechanisms, and farnesol can act at the initiation phase (reducing the DNA strand breaks and formations of DNA adducts) or at the progression phase of tumor development.

Qamar et al. [68] investigated the chemopreventive effects of farnesol on rats which were intratracheally exposed to the cancerogene benzo(a)pyrene. A pretreatment with farnesol was able to alleviate the inflammation, edema, surfactant dysfunction and injuries caused by this cancerogene. Farnesol showed to have effect on the benzo(a)pyrene metabolizing enzymes (NADPH-cytochrome P450 reductase, microsomal epoxide hydrolase (mEH), and glutathione S-transferase (GST)) and it was able to normalize the reduced levels of the lung surfactants.

Farnesol seems to be a very interesting and promising compound for its antioxidant, anti-inflammatory and chemopreventive properties. Khan and Sultana [69] explored in their study its anticipatory effect against DMH (1,2-dimethylhydrazine) -induced oxidative stress, inflammatory response and apoptotic tissue damage in the colon of Wistar rats. The study showed that a prophylactic treatment with farnesol increased the antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase and quinone reductase and the cellular antioxidant-reduced glutathione. Farnesol showed to have protective effect against DMH-induced lipid peroxidation in colonic tissue. The pretreatment could also down-regulate the caspase-3-activity, which was upregulated by DMH, a colon specific cancerogene. Farnesol showed to suppress the initial stages of colon

cancerogenesis, and the mechanism is, according to these findings, probably by ameliorating the oxidative damage, inflammatory processes and apoptotic responses.

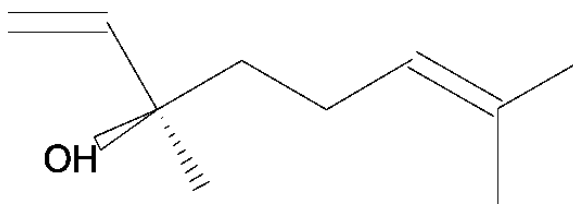
Due to the fact that farnesol proved its antioxidant effect, and antioxidative agent can have a protective effect against neurotoxicity, de Oliveira Júnior et al. [70] investigated the antinociceptive effect of farnesol and its effect on the brain of adult mice. Mice were treated with doses of 50, 100 and 200mg/kg, injected intraperitoneally. In the group treated with the highest dose, 16% of the mice had a brain injury that affected 12% of the hippocampus, but no lesions were found on mice treated with doses of 50 and 100 mg/kg. This leads to the conclusion that farnesol provides an antinociceptive effect, with no significant neurotoxicity.

A number of studies have previously shown that farnesol has an impact on the metabolism of lipids and can regulate the serum lipid concentrations. This effect is a consequence of farnesol up-regulating the PPAR α and genes of fatty acid oxidation, as well as down-regulating the synthesis of fatty acid in liver cells, which result from a decreased mRNA and protein level and activity of fatty acid synthase. Farnesol proved to lower the serum triglyceride levels, contributing to be a potential protective factor to hypertriglyceridemia. [71]

Goto et al. [72] made further investigations on farnesol being a ligand of PPARs and its effect on metabolic abnormalities. PPARs control energy homeostasis. Farnesol showed to improve metabolic abnormalities by decreasing plasma glucose concentration, glucosuria and the hepatic triglycerids. The study confirmed the previously mentioned mechanism of action, noticing that farnesol could not up-regulate the mRNA expression of PPAR γ target genes in adipose tissues. This showed that an up-regulation of fatty acid oxidation genes requires the function of PPAR α , but farnesol showed to act on two types of receptors, next to PPAR α , there is also the FXR (farnesoid X receptor). FXR is regulating genes important for bile acid homeostasis, lipid and glucose metabolism [65]. The decrease of the hepatic triglycerides is probably related to activation of both receptors, with FXR presenting a PPAR α -independent way of acting.

Linalool:

Synonym: β -linalool



Linalool is a MT-alcohol which possesses one chiralic C-atom, so it occurs naturally in form of two enantiomeres (-)-linalool and (+)-linalool. This compound is widely spreaded in plants, the most commonly used *Lavandula* species are *L. angustifolia*, *L. latifolia*, *L. stoechas* and *L. x intermedia* [73]. Other prominent linalool producing species would be *Citrus bergamia* Risso, *Melissa officinalis* L., *Rosmarinus officinalis* L., *Cymbopogon citratus* DC, and *Mentha piperita* L. [74]. Linalool is one of the best examined terpenes. Even from ancient times it was used as a compound of these plants, providing sedative, analgetic and anxiolytic effects, which has been later on proved in scientific studies. Studies also reported about the strong anti-oxidative, antibacterial, antifungal, anti-convulsive and anti-hypercholesterinemic effect. For its pleasant scent it is used as a flavour and fragrance, being incorporated in soaps and cosmetics, hygienic products, used in aromatherapy and it is a common compound of herbal essential oils and teas.

Linck et al. [74] investigated the effect of inhaled linalool on anxiety, aggressive behaviour and social interactions in mice. The results showed that inhaled 3% linalool extended anxiolytic effects on mice, due to the fact that it increased the time spent in the lit area in the light/dark test. A step-down inhibitory avoidance test was performed, showing that linalool possesses amnesic effects. All results were compared to diazepam as a control. Linalool decreased aggressive behaviour and increased social interactions but at a

concentration of 1%. 3% inhaled linalool showed a lack of effect in the social interaction test, most probably related to the fact that linalool acts as an antagonist on NMDA receptors, which is a common effect of NMDA antagonists in general.

The same team of authors showed in a previous study already that inhaled linalool can bolster up the pentobarbital induced sleep, also decrease body temperature and locomotion. These results can be taken as another proof for the psychopharmacological effects of inhaled linalool and EO containing this compound.

Takahashi et al. [75] compared the EO from six *Lavandula* species, investigating how the interspecies differences affect the expression of their anxiolytic activity. The result showed a qualitative as well as a quantitative compositional variance between the EOs, leading to significant differences in the provided anxiolytic effect. The authors also investigated the influence of the major constituents of the EOs of these species, suggesting that linalyl-acetate acts synergistical with linalool, and that the presence of both compounds is required for the anxiolytic effect of the inhaled EOs.

Linalool is, as already mentioned, a competitive antagonist of the NMDA receptor, for which is believed to have an important role in the building of memory. Coelho et al. [76] evaluated the effect of (–)-linalool on the acquisition of long- and short-term memories by using three types of behavioral models: recognition task, inhibitory avoidance test and habituation in a new environment. With an open field test, the effect on motivation, locomotion and exploration level was investigated. The test was performed on more than 200 male Wistar rats, using a glutamate antagonist as a positive control. (–)-Linalool showed different effects in the three types of tests. In the object recognition task, (–)-linalool impaired the formation of long-term memory without having impact on short-term memory. The building of both STM (short term memory) and LTM (long-term memory) showed to be impaired in the inhibitory avoidance test, while in the habituation test the LTM was impaired. In the open field test, the tested rats showed no difference in the crossing and locomotion, but higher concentrations of (–)-linalool decreased

rearing behavior. But besides, the fact that the effect was different in each assay, the compound still showed to impair memory acquisition in every assay. This suggests that (-)-linalool, probably due to its antagonistic effect on the NMDA receptor, affects the memory, like others antagonists of this receptor also do.

The anxiolytic and anticonvulsant effect of linalool is related to its mechanism of acting in the CNS, blocking glutamatergic NMDA receptors, stimulating GABA receptors and blocking voltage dependent ion channels. Sampaio et al. [77] investigated the inhibition of adenylate cyclase by rosewood oil (*Aniba rosaeodora* Ducke), due to the fact that an increased cAMP concentration plays an important role in development of seizures in epilepsy, so inhibitors of the AC could be potential anticonvulsant therapeutics. Rosewood oil, (-)-linalool and the racemate ($\pm$)-linalool were tested against the increase of cAMP concentration, also involving the effect on adenosine receptors (adenosine decreases cAMP concentration through binding to the A1 receptor). Chick retinas were used as a CNS model and a phosphodiesterase inhibitor and an adenosine receptor antagonist as a control to determine the involvement of these receptors in the resulting effect. Rosewood oil and the linalool isomers showed to inhibit the accumulation of cAMP but only when the AC was activated by a forskolin stimulus, suggesting that they act on the forskolin binding site of the AC. The effect was provided even when the adenosine receptor were blocked, showing that the antagonist did not interfere in the effect of the EO in the AC activity.

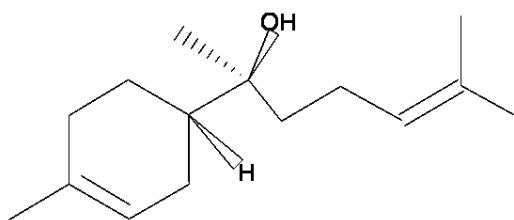
De Sousa et al. [78] investigated the difference in the anticonvulsant activity between the two linalool enantiomers and the racemate. The results showed that a pretreatment with all types of linalool could increase the latency of convulsions, but racemic linalool was more effective, providing the effect at lower doses applied, than the enantiomers. Also, all types of linalool could inhibit convulsive actions, with effects comparable with diazepam. (-)-Linalool showed to be, in general, more potent than (+)-linalool, but still less potent than the racemic linalool. When it comes to preventing tonic convulsions, both enantiomers were equipotent and racemic linalool showed to be even more

effective than phenytoin. The study reported the presence of a chiral influence, noticing that the two enantiomers have similar anticonvulsant effects, but different potencies.

Cho et al. [79] investigated the hypocholesterolemic effect of linalool in high-fat fed mice and in HepG2 cells. A treatment with linalool on high-fat fed mice reduced the total- and LDL-cholesterol level, with an accompanying reduction in the hepatic lipid concentration. The levels of HDL cholesterol showed to increase. In hepatocytes, linalool extended a dose-dependent reduction of cholesterol and triglyceride concentration. Linalool showed to decrease cholesterol by decreasing the expression of the sterol regulatory element binding protein-2 and an accompanying decrease of the HMG-CoA reductase protein expression through transcriptional and posttranscriptional mechanisms. The reduction of expression of HMG-CoA reductase is a result of the reduced binding of SREBP-2 (sterol regulatory element binding protein-2) to its promoter and the induction of an ubiquitin-dependent proteolysis of the HMG-CoA reductase.

Nevertheless, the antitumor effect of linalool should be mentioned. Gu et al. [80] investigated the antitumor effect of linalool on different hematopoietic tumor cell lines but also the effect on healthy blood cells. Linalool showed to inhibit proliferation and induce rapid apoptosis on different human leukemia cells, but it spared normal blood cells. The effect is associated with an activation of the tumor suppressor gene p53 and cyclin-dependent kinase inhibitors.

Bisabolol:



The name bisabolol includes both α - and β -isomeres of this compound, with each of them consisting in two enantiomeric forms. In nature, the most common form is $(-)\text{-}\alpha$ -bisabolol. $(-)\text{-}\alpha$ -Bisabolol is a not saturated, optically active SQT alcohol which possesses a delightful bloomy odor [81]. It is part of the EOs of a variety of plants, the most commonly utilised source is *Chamomilla recutita* L, but to be noticed is also *Salvia runcinata*, *Plinia cerrocampanensis* [82] or *Vanillosmopsis erythropappa* [81]. $(-)\text{-}\alpha$ -Bisabolol is used in different formulations, and due to its antiseptic effect often in cosmetics, aftershave lotions, moisturisers and creams for sensitive skin. Previous studies reported about the anti-inflammatory, antibiotic, anti-ulcerative, anti-oxidative, anti-tumor and other effects of this compound [82], a few of them will be discussed here.

Rocha et al. [81] were one of the first teams who examined the anti-nociceptive and anti-inflammatory potential of $(-)\text{-}\alpha$ -bisabolol as an isolated drug and not just as a plant containing this compound. The examinations were performed on male Swiss mice and male rats in classic models of pain and inflammation. The study showed that $(-)\text{-}\alpha$ -bisabolol reduced carageenan and dextran induced paw oedemas, and at higher doses also reduced edemas produced by direct application of 5-HT. This suggests that the substance does provide anti-inflammatory effects. Bisabolol showed to be a peripheral anti-nociceptive and anti-inflammatory drug. This finding is assessed by the fact that the anti-nociceptive test on hot-plate response did not suggest central analgesic activity and also the formalin test supported this conclusion. The formalin test includes two phases, the first phase confirmed the peripheric mechanism of action, the second phase proved the anti-nociceptive activity of the substance by

influencing inflammatory mediators (histamine, serotonin, prostaglandins and bradykinin). Also worth to be mentioned is that a pre-treatment with (-)- α -bisabolol which decreases leukocyte migration, protein concentration and myeloperoxidase activity on rats with peritonitis. It could also decrease TNF- α in the peritoneal fluid of rats with carrageenan-induced peritonitis. The effects of (-)- α -bisabolol might be related to the effect on TNF- α , but also the effect on other inflammatory mediators cannot be excluded. The study proves that the substance exhibits anti-inflammatory effects but without ulcerative potential like the commonly used analgetics and inflammation therapeutics (diclofenac, indomethacin). In contrast (-)- α -bisabolol provides rather gastroprotective effects.

Moura Rocha et al. [83] investigated the gastroeffective effect of isolated (-)- α -bisabolol in ethanol and indomethacin-induced ulcers in mice. The substance showed anti-ulcerative activity in both ulcer-models. The authors assessed the possible mechanisms involved in this action. (-)- α -Bisabolol was able to protect the gastric mucosa from lesions caused by NSAIDs, similar to ranitidine. Thus, to examine the role of prostaglandins in the effect of this substance in ethanol-induced ulcer models, mice were pretreated with indomethacin, but this did not prevent the effect of (-)- α -bisabolol, so an increased prostaglandin synthesis is not the way of action. The involvement of K_{ATP+} channels and (-)- α -bisabolol in gastric functions was investigated, but it showed not to be related in the mechanism of action, due to the fact that there was no difference in the gastroprotective effect of (-)- α -bisabolol in animals pre-treated with glibenclamide (glibenclamide closes the ATP-dependent potassium channels) or not. Also, the nitric oxide pathway is not involved, because the anti-ulcerative effects could not be reversed by L-NAME, an inhibitor of the nitric oxide synthase. Finally, the effect showed to be probably related to a decreased reduction of non-protein sulfhydryl groups, which leads to an increase of their occurrence and strengthening their protective effects on gastric tissue and leading to a reduction of gastric oxidative injuries induced by ethanol and indomethacin. Namely, ethanol is able to diminish the levels of non-protein sulfhydryl groups, such as reduced glutathione in gastric tissue,

which provides its gastroprotective effects by scavenging free radicals and preventing the gastric damage made by free-radicals accumulation.

In the previous study, (-)- α -bisabolol showed to have the ability to reduce gastric ulcer in response to absolute alcohol, but the way of acting was not cleared yet, although a few mechanisms could be excluded. A year later Moura Rocha et al. [84] performed further experiments, evaluating the gastroprotective effect in ethanol-induced lesions on the gastric mucosa. The methods they used were histopathological determination, measuring the membrane lipid peroxidation, myeloperoxidase, superoxide-dismutase and catalase activity and the nitrite level. Ethanol produces characteristic necrotic gastric lesions, but the damage is also related to a massive production of free radicals. That is why the authors investigated the connection between the capability of (-)- α -bisabolol to reduce oxidative stress and inflammations, and the anti-ulcerative effect on ethanol-induced lesions.

The study showed that (-)- α -bisabolol prevented the ethanol-induced increase of MDA, showing its antioxidant activity. The substance increased the SOD activity and the dismutation of superoxide anion and it prevented the reduction in CAT activity. (-)- α -Bisabolol also reduced the influx of neutrophils in the gastric lesions. In agreement with the findings, mentioned in the previous study, the pathway of nitric oxide is not related to the effect, because the substance did not significantly modified the nitrite levels.

Seki et al. [82] reported that (-)- α -bisabolol is capable to suppress proliferation and lead to death in pancreatic cancer cell lines. The substance was effective and did not cause significant side effects. The mechanism of action includes the inhibition of Akt activation (one of the most often activated serine/threonine kinases in pancreatic cancer) and an up-regulation of the expression of the tumor suppressor early growth response-1 (EGR1). The authors did not exclude that other mechanisms, next to those two mentioned, are involved in the activity. They report that (-)- α -bisabolol might be a potential therapeutic in treatment of pancreatic cancer.

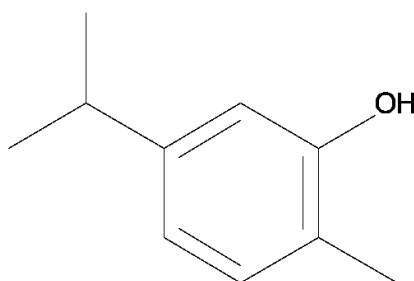
Cavalieri *et al.* [85] gave first evidence of α -bisabolol being a pro-apoptotic substance for primary human acute leukemia cells. The cells used in the study, were Philadelphia-negative and -positive B acute lymphoid leukemias (Ph-/Ph+B-ALL), acute myeloid leukemias (AML), normal leukocytes and bone marrow stem cells. α -Bisabolol showed to be effective in ALL cells at all concentrations and duration of the treatment and it spared normal leukocytes and bone marrow cells. At a bit higher concentration, it was acting apoptotic also against primary AML cells. The apoptotic activity was present even in imatinib mesylate-resistant Ph+B-ALL. The mechanism of acting might be involved in disrupting the mitochondrial membrane potential, which goes along with the decrease of oxygen consumption in presence of glutamate/malate and by the unpursued respiration levels in presence of succinate/glycerol-3-phosphate.

De Siqueira *et al.* [86] investigated the pharmacological effect of (-)- α -bisabolol in various smooth muscle preparations of rats. The substance showed to be biological active in smooth muscle but it had different effects depending on the tissue and applied concentration. For example, in preparations that were electromechanically or pharmacological pre-contracted, (-)- α -bisabolol had a relaxing effect. At concentrations of 30–300 $\mu\text{mol/L}$ (-)- α -bisabolol relaxed duodenal strips, contracted endothelium-intact aortic rings and urinary bladder strips, but relaxed the same tissues at higher concentrations (600–1000 $\mu\text{mol/L}$). On tracheal or colonic tissue the effect was relaxing but with a lesser potency than in mesenteric vessels. (-)- α -Bisabolol alleviated the increase of carbachol in tracheas of ovalbumin-sensitized rats challenged with ovalbumin, but could not interfere with the decreasing responsiveness of urinary bladder strips in ifosfamide treated mice. The authors suggested that a possible mechanism of acting is the inhibition of voltage dependent Ca^{++} channels.

Alvesa *et al.* [87] studied the pharmacological effect of (-)- α -bisabolol on the peripheral nervous system of mice. The examination was performed *ex vivo*, observing the effect on the compound action potential characteristics, using a modified single sucrose-gap method. (-)- α -Bisabolol was, dose-dependent, able to decrease the neuronal excitability. The effect was similar to lidocaine,

but not to 4-aminopyridine, both of them are known as inhibitors for sodium and potassium voltage-gated channels. In contrast to lidocaine, the (–)- α -bisabolol action showed a irreversible and non-use-dependent pathway. Based on this finding, the effect might be provided through irreversible inhibition of voltage-dependent sodium channels.

Carvacrol:



Carvacrol is a member of MT phenols which occur in many EOs of the family *Labiatae* including *Origanum*, *Satureja*, *Thymbra*, *Thymus*, and *Coridothymus* species. This alcohol possesses a status as a generally recognised as a safe, so it is commonly used as a flavoring substance in our daily life. Carvacrol is described to have a pungent and warm scent reminding on oregano. The EO from *Origanum vulgare* contains carvacrol at the highest naturally occurring concentration (up to 80%) [88]. Carvacrol showed, in rat models, to be metabolized and excreted very fast. After 24h, only small amounts could be found in urine, suggesting the excretion is almost complete in one day. The substance is mostly excreted unchanged, but an oxidation of the methyl and isopropyl group can also occur, leading to benzyl alcohol and 2-phenylpropanol and their carboxylic acids [89].

Carvacrol showed to strongly activate and sensitize TRPV3 channels, which are warm-sensitive Ca⁺⁺-permeable channels, often occurring in skin and neural tissues, causing the sensation of warmth. Carvacrol also activates and desensitizes TRPA1, a pain receptor, giving a possible explanation for the

pungent taste of oregano [90]. Years after this finding, Parnas et al. [91] found carvacrol to inhibit the non-thermoTRPs, TRPL and TRPM7 channels. TRPM7 channels are mediators of anoxic neuronal death so by inhibiting the expression, a protection from ischemic cell death is provided.

Hotta et al. [92] report about carvacrol acting on other receptors. They assessed that this substance, as a major part of the EO of thyme is an activator of PPAR α and γ , leading to an inhibition of COX-2 expression. This finding is a strong indicator for the anti-inflammatory effect of carvacrol since COX-2 is known to play important roles in inflammation processes and circulatory homeostasis.

Liu et al. [93] recently examined in vitro the anti-inflammatory effect of seven plant extracts, including carvacrol, on alveolar macrophages collected from pigs. Carvacrol showed to significantly suppress TNF- α and decrease IL-1 β secretion from LPS-treated macrophages. Carvacrol also suppressed TGF- β from macrophages with LPS stimulation. An even more detailed report about the effect of this substance was given by Guimarães et al. [94]. The authors evaluated the effect of carvacrol on inflammatory hypernociception and inflammation on different mice models, and also on stimulated murine macrophages. The inflammations were induced by carrageenan, TNF- α , PGE2, and dopamine. The effect on leukocyte-accumulation and production of TNF- α in carrageenan-induced pleurisy, as well as the effect on the NO building in murine macrophages was also examined. A carvacrol-pretreatment showed to be successful reducing hypernociception and edema induced by carrageenan and TNF- α , but with no effect when induced by PGE2 and dopamine. In agreement with the study mentioned before, the TNF- α concentration was decreased and a accumulation of leukocytes could be inhibited. Carvacrol inhibited the LPS-induced nitrite production. The authors suggest that the suppression of TNF- α production and NO release play the most important roles in the anti-inflammatory effect of carvacrol.

Carvacrol elicits an inhibitory effect on histamine receptors, as Boskabady et al. [95] report. They examined the effect of an aqueous-ethanolic extract of

Zataria multiflora Boiss (Labiatae) and its constituent carvacrole on H1 (histamine 1) receptors in tracheal chains of guinea pigs. The results confirmed the inhibitory effect of both extract and carvacrol on H1 receptors with no significant difference when different concentrations of extract and carvacrol were applied.

Aristatile et al. [96] investigated the effect of carvacrol on mitochondrial enzymes, oxidative stress and DNA damage in hepatic tissue in a model of D:-galactosamine (D:-GalN)-induced hepatotoxicity. The studies were performed on male Wistar rats and silymarin was used as a control drug. Carvacrol showed to normalize the changes that were induced, providing antioxidant and defensive effects against mitochondrial enzymes and DNA damage. Carvacrol was able to bring the hepatic mitochondrial enzymes isocitrate dehydrogenase, α -ketoglutarate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, NADPH dehydrogenase and cytochrome C oxidase after they got decreased by D:-GalN-, to normal levels again. Also the increased concentration of thiobarbituric acid reactive substances could be decreased. Carvacrol was able to modulate the enzymatic antioxidants SOD, glutathione peroxidase and the non-enzymatic antioxidants vitamin C, vitamin E and reduced glutathione back to higher concentrations. Carvacrol decreased the DNA damage, probably due to the scavenging of free radicals before they cause the damage.

The effect of carvacrol on the mitochondrial pathways plays an important role in its anti-hepatocarcinogenic activity as the study of Yin et al. [97] found. The study was performed on HepG2 cells showing that carvacrol was able to induce apoptosis and suppress further growth of cancer cells. The apoptosis mechanism involved an activation of caspase-3, PARP cleavage (a marker for apoptosis in tissue sections) and reduction of Bcl-2-gen expression. An important mechanism in the antitumor activity might be the influence in the mitogen-activated protein kinase pathway, by reducing phosphorylation of ERK1/2 and activating the p38 phosphorylation, but not interfering with JNK MAPK.

The effect of carvacrol on hepatocellular carcinoma has been also the occupation of Jayakumar et al. [88]. The authors examined the preventive effect of the substance against carcinoma induced by diethylnitrosamine in rats. The thematic priority of the authors was the strong antioxidant effect and free radical elimination as anti-cancer mechanism. Carvacrol modulated the LPO levels and enhanced the endogenous antioxidant defence in the cancerogenesis, and decreased the high levels of serum markers.

An interesting fact for the application of carvacrol and carvacrol-containing plants is that this substance exerts inhibitory properties on UGTs (UDP-glucuronosyltransferases). UGTs are responsible for metabolizing about 35% of all drugs metabolized by phase II enzymes. An inhibition of it could result in serious drug-drug-interactions and cause metabolic disorders. Dong et al. [98] investigated the inhibition of main isoforms of UGT using a nonspecific probe substrate 4-methylumbelliferone and recombinant UGT enzymes as enzyme resources. Carvacrol was capable to inhibit UGT1A9, one of the most important UGT isoforms, with an irrelevant effect on other UGT isoforms.

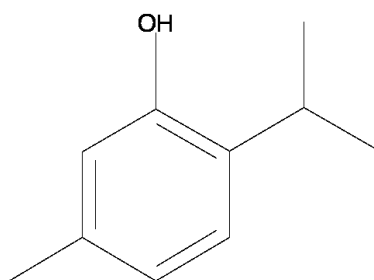
Yu et al. [99] investigated the neuroprotective potential of carvacrol against cerebral ischemia and/or reperfusion damages in mice, using the middle cerebral artery occlusion model. The study showed the protective effect of carvacrol by decreasing the infarct volume and the level of neuronal cell death. Noticeably, a post-treatment was also able to provide protection. The author's suggestion is that the PI3K/Akt pathway is related to the protective mechanisms of carvacrol on cerebral I/R damages. With the intracerebroventricular treatment after cerebral I/R damages, carvacrol showed to have a wide therapeutic window, by still providing its protection even when applied 6h after reperfusion. The therapeutic window was shortened up when carvacrol was intraperitoneally applied, so this method might affect its protective efficiency. The authors suggest the usage of carvacrol as a therapeutic drug, better yet as nanoformulations, which would make it even more efficient and easier to apply for an infarct treatment.

Another medical field, where carvacrol proved to be useful, is the fact that it improves cognitive activity. Azizi et al. [100] examined the effect of carvacrol

and thymol in two rat models of dementia: deficits caused by amyloid β and by scopolamine. The method they used was the Morris water maze test and they also assessed the acute toxicity of both carvacrol and thymol. The result showed that both substances could reverse and alleviate the induced cognitive impairments, for example the escape latency and reduction in target quadrant entries. Both substances also showed to be relative safe, with LD50's of thymol (565.7 mg/kg) and carvacrol (471.2 mg/kg) which was significant higher than the therapeutic concentration. The authors also suggest that the antioxidative, anti-inflammatory, and anti-cholinesterase activity could be involved in these activities.

Nevertheless, carvacrol possesses antibacterial, antifungal and anti-insecticidal effects, which is worthy to notice. The antibacterial potential of this substance has been ascribed to its effect on the structural and functional integrity of the cytoplasmic membrane. Due to this effect, carvacrol has its usage to extent the time food gets spoiled by bacteria [101]. For example, carvacrol showed to inhibit, in sub-lethal concentrations, the virulence of *Salmonella typhimurium* by reducing the motility and invasion in porcine epithelial cells, which makes it an important finding due to the fact that carvacrol is commonly used in sub-lethal concentrations [102].

Thymol:



Thymol is a MT-ic phenol derivate of cymene, which can be found in EOs of thyme, *Thymus vulgaris* or *Thymus zygis* L. var. *gracilis* Boissir. Thymol is with up to 80% the major compound of thyme EO, but it can be found in various citrus plants as well [103]. Thymol possesses a well-known

antimicrobial and antiseptic activity, and also because of its pleasant taste it is used in mouthwashes or toothpastes for many years [104].

Thymol is a ligand of odorant receptors which are expressed in the intestinal mucosa, and by binding to those receptors, serotonin secretion is being stimulated. These receptors belong to the group of chemical receptors which play an important sensoric role and can modulate functions of the GI system. Due to the fact that the ion transport, as a result of the binding, has not been evaluated until then, Kaji et al. [105] investigated the effect of thymol on ion transport in human and rat colonic epithel-cells by using an Ussing chamber (used to measure the short-circuit current to determine the ion transport taking place across an epithelium). The results showed that thymol could interfere in the permeability and anion secretion in colon cells. The mucosal application of thymol induced dose dependently an anion secretion which is probably related to an activation of TRPA1 channel. The authors came to this conclusion because the anion secretion could be reversed either under Ca^{++} -free conditions or application with a blocker of TRPA1.

Thymus vulgaris L and/or *Thymus zygis* L. extracts from leaves and flowers have been traditionally used for diseases of the respiratory tract due to its broncholytic, secretomotoric and anti-spasmodic effects. Thymol is known to relax the trachea and binds with α_1 -, α_2 and β -receptors of smooth muscles, so this effect is believed to be related to the activity of thymol and carvacrol, the main phenolic compounds in the extract. To get evidence of this hypothesis, Engelbertza et al. [106] investigated the spasmodic effect of thymol-deprived thyme extracts and determined which compounds are responsible for the actual effect. The thyme extract was splitted into fractions, the compounds were isolated from them and the anti-spasmodic effect was determined on smooth muscle trachea model of rats with papaverin as a control. The results showed that thymol possesses antispasmodic effect, but for the complete effect it was not responsible just by itself, but probably in synergistic effect with the flavone luteolin.

The pro-apoptotic and anti-cancer effects of thymol are reported in several studies. Xuan et al. [107] investigated the effect of thymol on the immune response, by examining the effect, survival and function of dendritic cells. Dendritic cells are important for inducing an immune reaction against pathogens, but also to prevent not necessary immune reactions against harmless antigens and it plays important roles in the intestine. The study was performed on dendritic cells either from wild-type mice or from mice lacking acid sphingomyelinase, treated and untreated for 24h with thymol. The treatment with thymol showed to stimulate sphingomyelinase and a formation of ceramide, downregulation of Bcl-2 and Bcl-xL expression, activation of caspase-3 and -8 and suicidal death of the cells in the end. This finding is interesting, due to the fact that thymol showed to protect from suicidal cell death in erythrocytes, which is also triggered by sphingomyelinase stimulation and ceramide formation. So, there is an opposite effect on erythrocytes and dendritic cells. The authors suggest, besides the fact that the thymol-induced apoptosis could induce anti-inflammatory actions, a caution with the use in infectious diseases, because there is a possibility it might induce the pathway of an infectious disease.

Hsu et al. [108] examined the effect of thymol on Ca^{++} and the viability in human astrocytes, using glioblastoma cells for model. The study showed that thymol induced a rise of the Ca^{++} concentration and cell death in the glioblastoma cells. The Ca^{++} rise was thymol-dose-dependent and realised through releasing Ca^{++} from the intracellular stores and inducing Ca^{++} entry from extracellular medium via non-store operated Ca^{++} channels.

The mechanism is, according to the authors, related to the phospholipase C- and protein kinase C-dependent release from stores from the ER. Thymol induced cell death that was not triggered by the rise of Ca^{++} concentration. The cell death most probably involves apoptosis and necrosis.

Chang et al. [109] investigated the same topic, using MG63 human osteosarcoma cells. The results were partially similar. Thymol provoked a Ca^{++} rise by triggering the phospholipase C-dependent release from the stores in the

ER and the Ca^{++} entry through kinase C- dependent store-operated Ca^{++} channels. Thymol was also able to induce cell death, probably related to apoptosis via mitochondrial pathways.

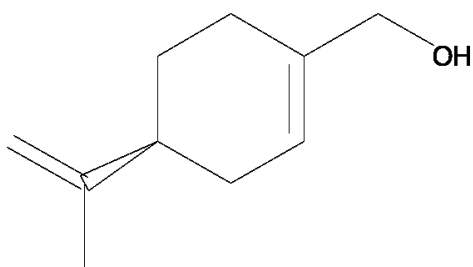
Satooka and Kubo [110] investigated the inhibitory effect of thymol on the formation of melanin. They came to the conclusion that this effect is due to the radical scavenging activity of thymol. Thymol inhibits the redox reaction between dopaquinone and leukodopachrome without any interaction with tyrosinase, although, tyrosinase is the key enzyme in melanin synthesis. This finding is important due to the fact that a high melanogenesis produces free radicals and can be further on related with development of diabetes mellitus, cardiovascular diseases of cancer. One year later, the same authors investigated [104], on base of the previous findings, the effect of thymol on B16-F10 murine melanoma cells. They wanted to examine if thymol is capable of inhibiting melanogenesis in cultured melanocytes, but without interfering the cell growth. Thymol showed to exhibit moderate cytotoxicity but not an antimelanogenic activity. With vitamin C and D the moderate cytotoxic activity could be inhibited and the cell viability enhanced. Actually, B16 melanoma cells that were cultured with thymol showed a significant increasing of oxidative stress. For conclusion, at high concentrations, thymol acts as a pro-oxidant rather than an antioxidant, so the authors suggest rather caution when using it as a food additive.

The anti-cancer effect of thymol is related to its ability to scavenge free radicals, but besides these antioxidant properties, other effects exist. That is why Deb et al. [111] investigated the anti-cancer effect of thymol on acute promyelotic leukemia HL-60 cells. Thymol exerted a cytotoxic effect on HL-60 cells but not on normal human peripheral blood mononuclear cells. The authors suggest that the different effect on normal and cancer cells can be related to a different gene expression or an activity-modulation of thymol, and in general, the case that a plant extract acts on one tissue pro-oxidative and on another tissue antioxidative is known from before. The cytotoxic effect of thymol is related to an increase of ROS production, mitochondrial H_2O_2 generation, depolarization of the mitochondrial membrane potential, decrease

in Bcl-2 protein and increase in Bax protein expression and activation of caspase. So, thymol was able to induce the cell death in HL-60 cells both caspase dependently and independently.

Archana et al. [103] investigated the role of thymol against radiation-induced DNA damage, determined by micronuclei and comet assay in Chinese hamster lung fibroblast cells. Radiation is being commonly used for cancer treatment, but the DNA damaging it causes can also affect normal tissue. That is one of the reasons that the author team was interested in finding new effective substances as a protection against damage of healthy tissues while exposed to radiation. Thymol showed to protect the cells against genotoxicity and apoptosis that are induced by radiation. This effect is most probably related again to its antioxidant and radical scavenging properties. On base of these informations, the same team of authors investigated the radioprotective effect of thymol in Swiss albino mice [112]. Besides the radioprotective, the anticlastogenic effect of thymol was investigated against a whole-body gamma radiation. The results showed that with a pre-treatment with thymol on gamma radiation-sensitized mice, a decrease in LPO levels and increase of the antigenotoxic, anticlastogenic and radioprotective effects and an increase in viability of the animals could be caused. This effect is again due to the antioxidative and free radical scavenging activity, but the existence of other mechanism can not be excluded.

Perillyl alcohol:



Perillyl alcohol is a MT-ic alcohol which can be found in the EOs of lemon, lavender, mint, ginger, and some vegetables. Perillyl alcohol is most famous for its anti-cancer activities and there are several studies reporting of its mechanism of action.

For example, Garcia et al. [113] reported in their paper about the inhibitory effect of this substance on the Na/K-ATPase from guinea pig kidney and brain tissues, and from A172 human glioblastoma cells. They suggest that the anti-cancer activity could be related to its Na/K-ATPase binding properties. The study showed a non-competitive inhibition of perillyl alcohol to Na(+) and K(+) and an un-competitive inhibition towards ATP. The authors also suggest that the drug is probably acting in the initial phase of the catalytic cycle of the enzyme, differently to the standard inhibitor ouabain (binding and inhibiting on the plasma membrane Na⁺/K⁺-ATPase).

The results of many recently published studies are pointing out the anti-cancer effect on glioblastoma cells. Those discoveries are important due to the fact that glioblastoma multiforme is known to be the most deadly primary brain tumor in human. Da Fonseca et al. [114] investigated the efficacy of perillyl alcohol, when intranasally administered, on the surviving rate of patient with recurrent malignant glioma. The study was performed in comparison to historical untreated patients. The intranasal administration was chosen due to the evidence that the substance takes its route through perineural and/or perivascular channels along the olfactory and the trigeminal nerves. The study showed that perillyl alcohol could increase the overall survival of recurrent glioblastoma patients, when comparing to the historical control group. The effects were outstanding on patients with secondary glioblastoma multiforme.

and patients who had tumors in deep areas of the brain. It is also important to notice that there were almost no side effects, even in patients that were treated for 4 years. The mechanism of action of perillyl alcohol is believed to be related to the inhibition of RAS/RAF/ERK pathways, NFkB; and also the isoprenylation of the RAS small GTPase superfamily of proteins that induce tumor-related angiogenesis. In the primary glioblastoma multiforme, the EGFR and its mutant EGFRvIII are overexpressed, so by alterations of the EGF/EGFR pathway, perillyl alcohol might probably influence the development of the disease.

The previous study was a further work appended to earlier findings of the same group. In 2008, their study proved that perillyl alcohol was able to extend the average life more than 8 months in recurrent glioblastoma patients, decrease the tumor growth and reduce the size of it. But, after 7 months, the tumor became resistant to perillyl alcohol and continued to grow [115]. Based on these findings de Saldanha da Gama Fischer et al. [116] investigated the molecular changes that finally lead to the resistance of the tumor to perillyl alcohol. For the purposes of the study, a new glioblastoma cell culture was generated heretofore as A172r, which was able to tolerate doses of perillyl alcohol with which the standard cell line would die. The result was a list of protein markers that are representative in resistant or the non-resistant cell line. The proteins are related to cellular growth, negative regulation of apoptosis, RAS-pathway and other functions of the cell.

Malignant gliomas are related to alterations in the EGF/EGFR signaling. So, da Silveira et al. [117] examined the influence of an EGF+61A>G gene polymorphism on the development of the disease and the different responses to an intranasal administered perillyl alcohol-therapy. The study showed that patients, who had lower EGF levels, survived longer after the perillyl alcohol treatment, probably due to the fact that high levels of EGF can be related to bigger tumor sizes and with the malignancy degree. So, the EGF level in the serum could be used for a prediction of the treatment response. The authors also suggest that perillyl alcohol, as a lipophilic substance, can cross the blood-

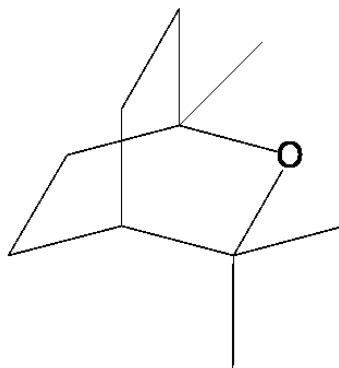
brain barrier and induce cell death even in cells with low rates of EGF/EGFR signalling

Khan et al. [118] reported on perillyl alcohol exerting antioxidant effects, modulating the TNF- α release and NF κ -B activation. By providing those activities, which are related to inflammation processes and damaging of the cells, perillyl alcohol showed protective effects in models of ethanol induced liver injuries in Wistar rats. The study demonstrated that perillyl alcohol has the capability to prevent liver toxicity by boosting the endogenous antioxidant system, inhibition of lipid peroxidation, suppressing the inflammatory cytokines and NF κ -B activation. The authors recommend this MT-ic alcohol for a possible role in the prevention of liver toxicities.

ETHER:

1,8-Cineole

Synonym: Cineole, Eucalyptol



1,8-Cineole is a MT compound that can be abundantly found in nature. It is the major compound of the *Eucalyptus* EO with up to 80%, but it can be also found in *Rosmarinus off.*, *Salvia off.*, *Mentha sp.* and in other plants as well. It

is used in different cosmetic products, such as tooth paste, soaps and creames, but also in household products such as air-refresheners or cleansing products [119]. The substance is a colorless liquid, which possesses a fresh, camphor-like odor. It is reported that with appropriate handling of 1,8-cineole, no toxic effects can be expected, but after admission of high concentrations, systematic effects such as blood pressure drop down, CNS disturbance and somnolence could be caused [120].

The pharmacological effects of 1,8-cineole, that have been published in studies in the past, are mostly focused on the therapeutic activity of this substance on the respiratory tract and inflammations in general.

A long-term systematic treatment can have therapeutic, mucolytic effects in asthma, sinusitis and COPD and in diseases of the lower and upper airways in general. The normalizing effect of the mucus hypersecretion by 1,8-cineole is related to its ability to inhibit the arachidonic acid metabolism and generation of cytokines in human monocytes. So, the substance showed to exhibit a steroid-saving effect on steroid-depending asthma [121]. 1,8-cineole is also an inhibitor of TNF- α and IL-1 β [122].

Worth et al. [123] investigated if 1,8-cineole can, due to its mucolytic, bronchodilating and anti-inflammatory activity, reduce the exarcerbation rate and improve the health status when applied as a concomitant therapy on COPD patients. The substance possesses positive effects on the beat frequency of the cilia in the mucus. 1,8-Cineole showed to reduce the exacerbation rate and improve the lung function by improving the airflow obstruction and reducing severity of dyspnoea. Due to its positive effect on the health status, lack of side effects and relative low cost, the concomitant therapy can be recommendent in therapy of the rather costly COPD, in the opinion of the authors.

The antioxidant properties of the EO and methanolic extracts of *Eucalyptus loxophleba* Benth. subsp. were evaluated by Rahimi-Nasrabadi et al. [124], where 1,8-cineole presents, with 39.4%, the major compound. For the examination, the DPPH, β -carotene/linoleic acid and reducing power assays were used. The results showed that the methanolic extracts are very effective

antioxidants and that the compounds of *E. loxophleba* exhibit antioxidant activities.

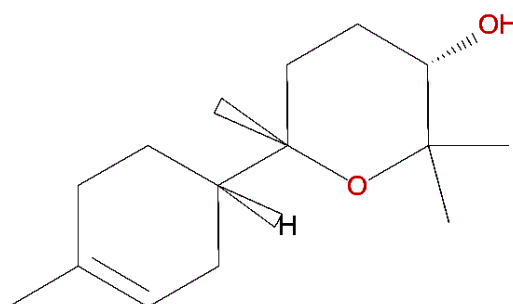
It has been known that EOs with MT compounds such as 1,8-cineole, can be used to epileptic seizures. That is the reason why Ćulić et al. [125] investigated the effect of this compound in the camphor EO using wavelet and fractal analysis to quantify the electrocortical changes. Animals were intraperitoneally treated with camphor EO or 1,8-cineole and the by wavelet analysis, the frequency bands in pre-ictal, ictal and inter-ictal stages were examined. The properties of the acute, epileptic-like seizures, caused by either the EO or 1,8-cineole, could be described through frequency bands in wavelet analysis. δ frequency bands showed to dominate in brain activity, with $\approx 45\%$ mean relative wavelet energy (MRWE) in the control group (no treatment) and growing up to $\approx 76\%$ MRWE after drug application. The effect seems to be concentration-dependent.

Kirscha et al. [119] investigated the flavor changes in breast milk after the oral intake of a preparation that contains 1,8-cineole. The background of this investigation was the fact that odorants in breast milk can potentially affect the breastfed child. Newborns are extremely sensitive to olfactory stimuli, so throughout this influence, hope is set that it could affect the food preferences in the years to come and be a potential preventive of nutrition-related diseases. The study showed that after ingestion of a preparation containing 100mg 1,8-cineole, the substance was transferred into the milk in a time-dependent manner. The change of the flavor of the milk could lead to rejection of the milk, and looking in long-terms, potentially have effect on the food preferences later in life.

Yoshimura et al. [126] examined the influence of 1,8-cineole on proliferation and elongation in plant cells by using BY-2 suspension-cultured tobacco (*Nicotiana tabacum*) cells. 1,8-cineole showed to inhibit cell elongation more efficiently than cell proliferation; The authors suggest that the inhibitory effect of this substance is not specific, but it seemed to affect several cellular activities in an almost non-specific way by direct contact with the cells.

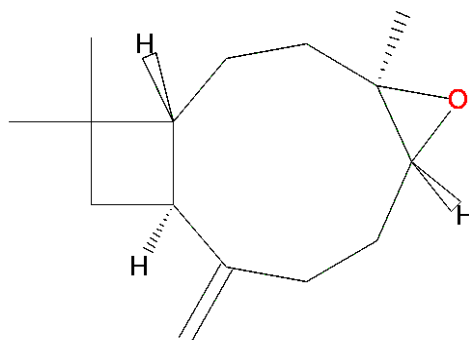
Nevertheless, it is worthy to notice that Tomsheck et al. [127] found that 1,8-cineole can be produced in *Hypoxylon sp.*, an endophyte of *Persea indica*. This way, a novel source is found which makes it easier for the use and application in medicine, industry and even as a fuel additive, due to the fact that it is a derivate of octane.

Bisabolol oxide



Can et al. [128] investigated the effect of the EO of *Matricaria recutita* L., on the CNS by performing a few psychopharmacological tests. α -Bisabolol oxide A is with 28% the major compound of this EO, followed by α -bisabolol oxide B with 17.1%, and (Z)- β -Farnesene (15.9%) and α -bisabolol (6.8%). The results showed that the EO exhibits stimulant effects on CNS, comparable with the psychostimulant caffeine. The authors suggest that the effect is related to the major compounds, or due to a synergism between them. At an administration of 50 and 100 mg/kg, the EO increased the number of spontaneous locomotor actions, showed to have anxiogenic effect in the open field test, elevated plus-maze and social interaction test and decreased the time of immobility in tail-suspension test.

Caryophyllene oxide:



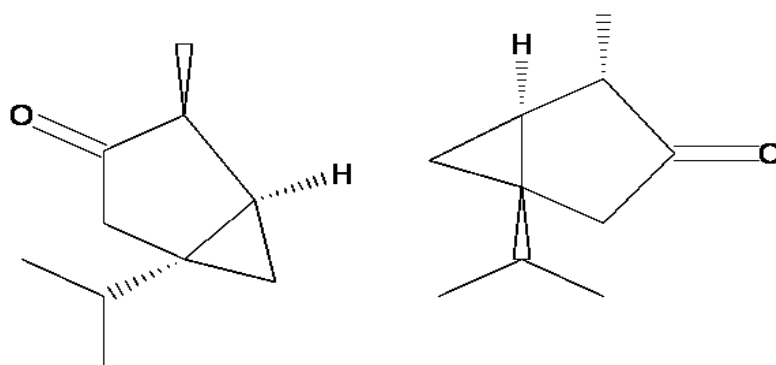
β -caryophyllene oxide can be found in the EO of guava (*Psidium guajava*), oregano (*Origanum vulgare* L.), cinnamon (*Cinnamomum* spp.) clove (*Eugenia caryophyllata*), black pepper (*Piper nigrum* L.) and other medicinal used or edible plants. β -Caryophyllene oxide possesses several pharmacological effects. It has been reported about its antibacterial, antifungal, immunomodulatory, anti-inflammatory, anti-oxidative and even anti-proliferative and anti-cancer effects. To investigate the mechanism how β -caryophyllene oxide provides its anti-cancer effect, Park et al. [129] investigated the effect of this sesquiterpene on the PI3K/AKT/mTOR/S6K1 and MAPK activation pathways in human prostate and breast cancer cells. The results demonstrated that β -caryophyllene oxide can inhibit the PI3K/AKT/mTOR/S6K1 pathway and induce ROS-mediated MAPK activation. This leads further on to suppression of the cell proliferation and down-regulation of different gene products that are related to processes of cell survival, proliferation, metastasis, and angiogenesis in human prostate and breast cancer cells. β -caryophyllene oxide showed to inhibit mTOR activation in PC-3 and MCF-7 cells. mTOR has been related closely to the development of cancer.

Chavana et al. [130] reported about the mechanism of the analgesic and anti-inflammatory activity of caryophyllene oxide, which was isolated from the extract of *Annona squamosa* bark. The study was performed on rats and mice and caryophyllene oxide was applied intraperitoneally. The study showed that the compound exhibits anti-nociceptive effects through various mechanism that may include both central and peripheral pathways. The central action can be

related to an inhibition of the central pain receptors and in the peripheral action, an inhibition of COX and/or lipxygenase is probably involved.

CARBONYLS:

Thujone:



Thujone belongs to the group of bicyclic MT ketons and it is occurring in two stereoisomeric forms, the α -thujone and β -thujone. It is an ingredient of the EOs of *Salvia spp.*, *Thuja spp.*, *Artemisia spp.* and some others [131]. Thujon is used as a compound in aromatic plants that are used for flavoring food and beverages. The first association with this substance is usually related to absinthe, the spirit flavored with *Artemisia absinthium* L.. Many discussions have been lead for the possible role of thujone in causing adverse psychoactive effects in the “absinthism” syndrome. But the symptoms may have been wrongly attributed to thujone, but rather be caused by ethanol or other toxic adulterants. Nowadays, thujone-containing plants can be used in food without restrictions, but in pure form, thujone is still forbidden to be directly added to food. That is a decision made in 2008 by the European Union Regulation on flavourings.[132] Thujone is believed to be neurotoxic, by providing a convulsant effect and α -thujone being even more toxic than β -thujone. The EO

of *Salvia off.*, which is rich in thujone (35–50%, mainly α -thujone) is known to be abortifacient and an emmenagogue agent [133].

Lachenmeier and Uebelacker [132] made in 2010 a re-evaluation of the toxicological evidence of thujone by making a new risk assessment using the benchmark dose (BMD) approach. The limits that are set for thujone in food products at the present time, are based on short-term animal studies from the 1960s and they estimated the acceptable daily intake (ADI). As mentioned above, the restrictions for food had been lowered in 2008, but the opposite happened in for the use in medicine, so in 2009 the European Medicines Agency (EMA) introduced limits for the substance. *Artemisia absinthium* L. and *Salvia officinalis* L. are often used in medicine in form of various preparations. Besides the lack of toxicological data on thujone, it was not possible to set a right value of ADI for thujone, so the ADI for *A. absinthum* has been determined on 3.0 mg/person for maximum two weeks use and 5.0 mg/person for *S. officinalis*. The results of the evaluation showed to be similar to the previous short-term studies, so the authors propose an ADI of 0.11 mg/kg bw/day which would not be possible to reach even when consuming high levels of food containing thujone and that between 2 and 20 cups of wormwood or sage tea would be required to reach this ADI.

The National Toxicology Programm [134] published the results of their study where the effects of α,β -thujone on male and female rats and mice were examined. The aim was to determine the potential toxic and cancer-related activity of thujone. All rats receiving 50 mg/kg, died and all other rats, which received 25mg/kg, had seizures. Similar results were obtained dose dependently with mice. Male rats showed high frequency of preputial gland cancer and slight increase of pheochromocytomas in the adrenal gland, but no increases in cancer was shown in female rats or male and female mice.

Abass *et al.* [135] described in their writing the metabolism of α -thujone, using human hepatic preparations in vitro. Their aim was also to determine the relevance of cytochrome P450 and the possible interference of other enzymes in the metabolism of α -thujone. The substance showed to have two major

metabolites (7- and 4-hydroxy-thujone), two minor metabolites (2-hydroxy-thujone and carvacrol) and glutathione and cysteine conjugates could also be detected. CYP2A6 showed to be responsible for 70-80% of the metabolism, followed by CYP3A4 and CYP2B6.

To answer the controversy about the psychoactive effects of thujone in absinthe, the content in this alcoholic drink is too low to produce such effects, but still, modern studies report that at high concentrations, thujone can indeed induce seizures, which is one of the symptoms of absinthism. Because this effect can be attenuated by benzodiazepines, an interaction with GABA_A receptors has been suggested. But, because the effect of thujone on GABAergic synaptic transmission and also the mechanism of GABA_A modulation was unknown, Szczot et al. [136] investigated this effect. They used cultured hippocampal neurons and compared the effect of thujone and dihydroumbellulone on GABAergic miniature inhibitory postsynaptic currents and on responses caused by rapid exogenous GABA applications. α -Thujone showed to reduce miniature inhibitory postsynaptic currents frequency and amplitude and it also showed to modulate their kinetics, suggesting to have both pre- and postsynaptic mechanisms. The current response on exogenous GABA showed to have reduced amplitude, modulated onset, desensitization and deactivation, which indicates a receptor gating. Dihydroumbellulone was ineffective or showed much smaller effects. α -Thujone confirmed to exhibit a specific action on GABAergic activity, indicating the existence of a MT-recognition site on GABA_A receptors. The authors suggest further systematical investigations.

Thujone is a major compound in several plants that are believed to have antidiabetic properties. That is the reason why Alkhateeb and Bonen [131] examined the use of thujone per se in the therapy of insuline resistance. In the study, an insuline resistance was rapidly induced with high concentrations of palmitate in the skeletal muscle and the restore of the insulin sensitivity with thujone was assessed. The study showed that this substance was able to recover completely the insulin sensitivity, even when palmitate was continuously present. This effect is related to the complete restoration of AS160

phosphorylation and palmitate oxidation. Thujone showed improvement in the insulin-stimulated glucose transport and GLUT4 (glucose transporter type 4) translocation (an insulin-regulated glucose transporter), but those two effects were not totally parallel to each other. The authors suggested a possible improvement of the intrinsic activity of GLUT4, as a second mechanism, next to the modulation of GLUT4 translocation. Thujone was able to activate AMP-activated protein kinase (which usually stimulates fatty acid oxidation via inhibition of acetyl-CoA carboxylase activity) but, not every restorative effect was related to this activation, like the oxidation of palmitate for example. The insulin-stimulated AS160 phosphorylation and glucose transport showed to be related to AMP-activated protein kinases though. The finding that thujone is capable of improving the insulin sensitivity in skeletal muscle, suggests it as a potential, relatively cheap, therapeuticum, although the exact mechanisms and its safety should be more examined.

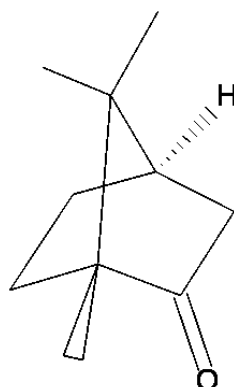
Although, as already mentioned, thujone showed to induce cancer when applied in high doses, it seems that in opposite, it can also provide anti-cancer effects. The ethanolic extract of *Thuja occidentalis* is commonly used, in form of a mother tincture (TOΦ), in homeopathy but also in traditional medicine in treatment of moles and tumors. The EO of fresh leaves from this plant contains approximately 65% thujone, related to the MT fraction. Biswas et al. [137] investigated the anti-cancer effect of the mother tincture and a thujone rich fraction (TRF), which was separated from it, on the cancer melanoma cell line A375. TOΦ had four fractions, chromatographically separated, of which the TRF showed to have the best anti-cancer and pro-apoptotic effect. The TRF might be actually the key-component for this effect in general. The anti-cancer activity was provided by inducing a pro-apoptotic pathway via activation of Bax, caspase-3 and cytochrome 3. Both TOΦ and TRF also caused a reduction in cell viability, induced DNA fragmentation, mitochondrial transmembrane potential collapse and higher ROS production.

The study of Siveen and Kuttan [138] gave another proof for the anti-cancer, more precisely, antimetastatic effect of thujone. The examination was performed on mice, where the metastasis was induced by injecting highly

metastatic B16F-10 melanoma cells through the lateral tail vein. Administration of thujone, either prophylactically or parallel to tumor induction, suppressed the tumor nodule formation in lungs and increased the surviving rate. The parameters that thujone influenced were extensively discussed. The treatment led to an inhibition of pro-inflammatory cytokines (TNF- α , IL-1 β , IL6, GCSF), downregulation of the matrix metalloproteinase 2 and 9, tissue inhibitor of metalloproteinase 1 and 2, VEGF, ERK-1 and ERK-2 in the lung of the animals. The invasion of the melanoma cells across the collagen matrix in a Boyden chamber was suppressed by thujone treatment, as well as the adhesion of the cancer cells to collagen-coated microtiter plate wells and the migration of the melanoma cells across a polycarbonate filter.

Nevertheless, the possible antioxidative effect should be mentioned. Laciari et al. [139] tested the EO of *Artemisia echegarayi* for its antioxidant activity. It inhibited, with one exception, the growth of Gram-positive and -negative bacteria. It had the lowest minimal inhibitory concentration against *Listeria monocytogenes* and *Bacillus cereus*. Thujone and camphor are believed to be responsible for the antibacterial activity.

Camphor:



Camphor is naturally occurring in the camphor laurel tree (*Cinnamomum camphora*), but it can be obtained synthetically from turpentine oil. It possesses a cyclic turpentine structure, so it is very lipophilic, which is the reason why it is so good distributed in the body and can make crossings through mucus.

membranes and probably attract to myelinated axons. Camphor has its use in medicine for its local anaesthetic, antipruritic and antiseptic activities and is used as an expectorant in pharmaceutical preparations [140].

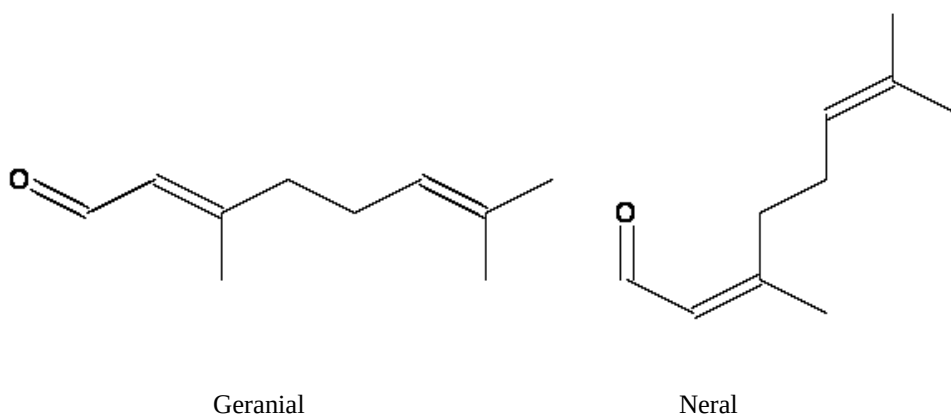
Camphor has been cherished for its medical uses for ages in Asia, it remained less known in other parts of the world. The camphor vapor is not irritating the eyes, so it is used in cosmetical products, but also in room fresheners or in food as a disinfection [141].

Even in the 18th century, Leopold Auenbrugger reported about camphor in the treatment of psychosis by inducing epileptic seizures. Camphor was considered to be similar to opium in pain or quinine in malaria fever [142]. Indeed, camphor can cause seizures, but besides seizures, camphor poisoning could result with apnoea, renal insufficiency, high hepatic enzyme levels and vomiting which could end in pneumonitis due to the aspiration or even death. Many every-day used products contain this substance. For example, several cases of camphor poisoning by ingestion of camphor mothballs for persisting headaches are known [143]. The most common symptoms in serious poisoning are neurological, such as irritability, hyperreflexia, tonic muscle contraction, confusion and coma. The only care there is a supportive help, since no antidotes are present. During acute camphor toxicity, changes in the axonal excitability are happening. There is an excessive response to hyperpolarising currents in the threshold electrotonus and the current-threshold relationship, which leads to a decrease in the conductance that was initiated by the hyperpolarisation [140].

Camphor has showed to act on two members of the TRP family, TRPV1 and TRPV2. That is the reason for the modulating sensation of warmth in humans by this substance [144]. Recently Marsakova et al.[145] investigated the molecular mechanism of this action and the possible interaction site on TRPV1. The results showed that camphor acts on the channel by affecting the gating equilibrium of the outer pore helix domain of the channel. Camphor might also induce changes in the spatial distribution of phosphatidylinositol-4,5-bisphosphate on the inner leaflet of the plasma membrane, since it is known that the substance can decrease fluidity of the plasma membrane.

Worth of mentioning is also the finding of Nikolic et al. [146] that camphor (eucalyptol and thujone as well) can stimulate error-free DNA repair processes and act as a bioantimutagen. The examinations were performed on prokaryotic and eukaryotic cells. The results showed the antimutagenic potential of these MT, although at higher concentrations these substances induced DNA strand breaks.

Citral:



Citral is a naturally occurring aliphatic MT-ic aldehyde mixture. The name citral stands for the mixture of cis and trans isomers, called geranial and neral. With approximately 80%, it is the major component of lemongrass oil (*Cymbopogon citratus*), but it can be found in all other citrus fruits as well. Citral possesses a fresh, intensive lemony scent, which is why it is extensively used in food, cosmetics or in household products. *Cymbopogon citratus*, which is the most prominent source of this MT, is an evergreen plant growing widely in Asia and traditionally used in oriental households. Citral is believed to be non-toxic and does not induce cancer in animal models [147]. Reports on the pharmacological activities of citral are made continuously, so a few recent discoveries will be mentioned within the next lines.

C. citratus is traditionally used against GI disorders and citral is believed to be responsible for most activities of this plant. That is why Devi et al. [148]

investigated the extract of different parts of *C. citratus* (leaves, stems and roots) and citral on the visceral smooth muscle in the rabbit ileum. The aim of the study was to examine the spasmolytic activity of citral, so the effect was tested on acetylcholine (ACh) and KCl- induced contractions. The study showed that citral and the leaf extract (LE) were capable of inhibiting spontaneous contraction in a dose-dependent manner, while the extracts of the root and stem did not show results worth noticing. Citral and LE were also able to reduce the contractions induced by ACh, which was similar, although less strongly, to atropine, an antagonist of the muscarinic receptor, suggesting the possible mechanism of action. Furthermore, citral was able to inhibit contractions induced by high concentrations of K^+ , very similar to verapamil (80% inhibition with citral compared to 90% by verapamil). The inhibitory effect of citral on the visceral smooth muscle was the strongest on spontaneous contractions, and then KCl- and ACh-induced contractions. The mechanism of relaxation is probably by interfering in the NO-pathway and inhibiting calcium channels, due to the fact that the spasmolytic effect of citral could be reduced by L-NAME, an inhibitor of the nitric oxide synthase. A possible other mechanism would be the blockage of muscarinic receptors, as mentioned above, but also by inhibiting IP₃, resulting in a relaxation of the smooth muscle.

It is believed that citral provides anti-inflammatory and analgetic activities, which was examined and proved in several studies. Katsukawa et al. [149] evaluated this effect using established assays for COX-2 and PPAR. Citral showed to be a dual activator of PPAR α and γ and also as a PPAR γ -dependent inhibitor of the COX-2 expression. This finding was assessed by the fact that the NF- κ B site of the COX-2 gene was involved in the inhibition of LPS-induced COX-2 promoter activity by 15d-PGJ₂, a natural PPAR γ ligand, as well as by dexamethasone. In U937, human macrophage-like cells, citral was able to inhibit dose-dependently both LPS-induced COX-2 mRNA and protein expression. Citral is known to act on the TRP channels, particularly TRPM8 and TRPA1. The TRP channels are believed to be related to inflammation processes and even cancer, therefore this mechanism can not be excluded. The authors suggest that this finding of citral is useful for the consumption as a

compound of the daily diet, but rather not as a pharmacological drug, because the activity after all is still lower than compared with standard synthetic drugs.

Macrophages present an important source of inflammatory cytokines and they have the possibility to control an overproduction of these products, protecting by that way development of immunopathologies. Bachiega and Sforcin [150] investigated the effect of lemongrass and citral on the production of the cytokines IL-6, IL-1 β and IL-10 by peritoneal macrophages in vitro. The effect was determined before and after macrophages where incubated with LPS. The study proved the anti-inflammatory effects of citral, suggesting that the possible mechanism is involved with the inhibition of the transcription factor NF- κ B. Citral inhibited the release of IL-1 β , both before and after the LPS challenge, the same effect with IL-6 and IL-10. Lemongrass was in comparison to citral not so effective, it could only inhibit LPS action after the macrophage challenge with LPS.

The synergistic action of a NSAIDs with plants that provide the same effects, can increase the anti-nociceptive activity with even lower rates of side effects and using lower doses. Ortiz et al. [151] investigated the anti-inflammatory effects and the gastric damage of the application of citral, naproxen and their combination in rat models. The substances were orally applied and the effect was assessed on carrageenan-induced paw edemas and gastric damages, while the interaction type was assessed by isobolographic analysis. Naproxen, citral and their combination showed to exhibit anti-inflammatory effects. The advantage of their combination was that the gastric damage, that naproxen significantly produced when administered by itself, was not obtained when applied with citral, suggesting a possible minimal gastric damage in the therapeutic use. Naproxen and citral showed to have a synergistic interaction, how the isobolographic analysis demonstrated. This interaction is most probably provided by citral acting on TRP channels and inhibiting NO production, while naproxen, on the other side, suppresses the prostaglandin production.

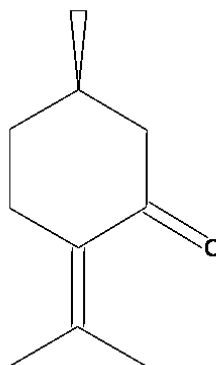
Another interesting pharmacological effect of citral is its antiadipogenic and antidiabetic activity, recently investigated by Modak and Mukhopadhyaya [144]. This finding was based on the fact that citral acts as a competitive inhibitor of retinaldehyde dehydrogenase, leading to higher levels of retinaldehyde in adipocytes. Retinaldehyde is known to suppress adipogenesis and increase the metabolic rates and also influences the glucose tolerance. The study showed that citral was able to decrease the body weight gain and abdominal fat mass in rats, which were held on a high-energy diet. The effect was dose-dependent. The food intake of the rats has not changed while citral was administered, suggesting that the lower weight gain is related to a lower fat absorption or higher energy expenditure. An increased metabolism is most probably the involved mechanism because an increased metabolic rate, temperature and respiratory quotient were determined. Citral also showed to affect insulin, by decreasing its levels which is related to an improvement of glucose tolerance and lower fasting plasma glucose levels. Taken all this findings together, the authors suggest citral having a possible role in alleviating lifestyle diseases like obesity or diabetes.

Chaimovitsh et al. [152] reported on the effect of citral on mitotic microtubules on models of tobacco BY2 cells and wheat roots. Citral showed to disrupt mitotic microtubules and suppress the cell cycle and also increase the occurrence of asymmetric cell plates in those cells. The effect seemed to be dose-dependent. The authors propose that at lower concentrations, citral influences the cell division by disruption of the mitotic microtubules and cell plates but, at higher concentrations it suppresses the cell elongation through disrupting cortical microtubules.

The antibacterial activity of citral should be shortly mentioned. Citral inhibits swarming and virulence factor expression of *Proteus mirabilis*, which can cause urinary tract infections, preventing that way development of these infections [153]. Citral also showed to be effective against four pathogenic strains of isolated bovine mastitis, including *Staphylococcus aureus*, *Streptococcus agalactiae*, *Bacillus cereus* and *Escherichia coli* and also effective in destroying *S. aureus* biofilms [154]. The inactivation of *E. coli* cells

is related to inducing a damage in the cell envelope [155]. In addition, citral showed to be the responsible compound in the anti-leishmania activity of *C. citratus*, by providing a significant inhibition of *L. infantum*, *L. tropica* and *L. major* [156].

Pulegone:



Pulegone is a MT ketone, which can be found as a compound in pennyroyal EO (*Mentha pulegium*). It is used in low doses as a flavoring agent in food, beverages and hygienic products. In high doses, it is reported to cause gastritis, seizures, hepatic and renal damages, toxicity of the CNS and coma. It was even used to induce menstruation or even abortion [157]. The National Toxicology Program published the results of the toxicology and carcinogenesis gavage study of pulegone. The study was performed on rats and mice receiving pulegone and the data assessment was performed after 2 weeks, 3 months, or 2 years. Genetic toxicology studies have been performed on *S.typhimurium*, *E.coli* and mouse erythrocytes. The toxic effects of pulegone on rats and mice increased dose dependently. Even in the 2-week-treatment group, several cases of animal death occurred when pulegone was applied in high concentrations. In the group of rats and mice treated for 3 months, besides changes in blood parameters, most damages or death cases were attributed to liver toxicity. The rats and mice treated for 2 years with pulegone, showed symptoms like thinness, lethargy and ruffled fur. In comparison to vehicle controls there was an increase in pathological developments in the liver (oval cell hyperplasia, bile duct hyperplasia, hypertrophy, hepatocyte necrosis, portal fibrosis), kidneys and urinary tract (hyaline glomerulopathy, nephropathy), osteoma and

osteosarcoma, degeneration of the olfactory epithelium and inflammations, hyperplasia and ulcerations of the forestomach [158].

Based on the findings of the above study, da Rocha et al. [157] investigated the mechanism of action of pulegone on the urinary bladder of female rats. It was concluded that female rats showed an increase of urinary bladder neoplasms, while male rats did not show an increased incidence of neoplasms that type. The metabolism of pulegone includes hydroxylation, reduction or conjugation with glutathione and the metabolites identified are piperitone, piperitenone, menthofuran and menthone. The results of the study were in agreement with those from the previous study, with rats losing body weight, bloody nasal mucus, and alopecia in the mouth and urogenital area. Scanning electron microscopy showed damages on the surface of the bladder induced by pulegone. The authors suggest that the tumors are induced due to chronic exposure to high doses of pulegone, its metabolism, excretion and concentration of it and its toxic metabolites, especially piperitenone in urine, the urothelial cytotoxicity, cell proliferation and ultimately development of tumors in the end.

De Sousa et al. [159] reported in their writing about the pharmacological effects of (R)-(+)-pulegone on the CNS. Pulegone showed to have a central depressant effect, increased the latency of convulsions and showed to inhibit both chemical and thermal models of nociception. The authors suggest therefore, that pulegone is a psychoactive substance with activities of analgesic drugs.

De Cerqueira et al. [160] investigated the ionotropic effects of R(+)-pulegone in mammalian myocardium. They wanted to examine the effect of pulegone on L-type Ca^{++} channels, due to the assumption that it might decrease the Ca^{++} influx and so change the heart contractility. The results showed that pulegone was able to decrease the myocardial contractility and reduce the intracellular Ca^{++} transient and L-type Ca^{++} current. The negative inotropic effect is very similar to nifedipine, a L-type Ca^{++} channel inhibitor, which indicates that this is the mechanism of action, but the authors do not exclude

other mechanisms being possibly involved. The effects of pulegone were almost reversible, so a possible myocardial damage was unlikely.

Umezu [161] examined in his study if dopamine is involved in a pulegone-induced ambulation in ICR mice. The results indicate a possible involvement of dopamine and pulegone. A co-administration of pulegone and bupropione (a dopamine agonist) showed to increase the effect on ambulation-promoting actions and antagonists of dopamine (chlorpromazine, fluphenazine, haloperidol and spiperone) were able to alleviate the effects of pulegone. A pretreatment with reserpine (a dopamine depletor) eliminated the sensitivity to the effect of pulegone, which implicates that pulegone may not be a direct dopamine receptor agonist.

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