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Lisa Kozarits

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Abstract

Das Ziel dieser Literaturübersicht war, für die sedierenden, angstlösenden, beruhigenden und schlaffördernden Wirkungen von ätherischen Ölen und deren Inhaltsstoffen die möglichen Wirkmechanismen zu bestimmen. Anhand der Hauptinhaltsstoffe wird eine mögliche Verbindung zu den $GABA_A$-Rezeptoren und anderen Targets erforscht.

Angstzustände, die mit Unruhe, Schlaflosigkeit und anderen Symptomen und Problemen einhergehen, werden in der Gesellschaft immer häufiger. In der heutigen Zeit, wo der Stress zunimmt, greifen immer mehr Personen zu Einschlafhilfen und Beruhigungsmitteln. Alternativen auf pflanzlicher Basis wie die Aromatherapie oder die orale Einnahme von Lavendelöl Präparaten finden immer mehr Anklang. Denn die Einnahme von Benzodiazepinen als Tranquillantium, Anxiolytika, Sedativa und Hypnotika birgt neben seiner positiven Wirkung auf die Psyche auch einer Reihe an Nebenwirkungen (Sedation, Gewichtszunahme, sexuelle Dysfunktion) und die Gefahr des Missbrauchs. Pflanzliche Produkte werden dadurch immer genauer erforscht um Ihre Wirkungen auszunutzen. Ein gut vertragenes anxiolytisches Medikament könnte deshalb die Bedenken der Betroffenen auflösen und könnte die Grundlage für eine bessere Akzeptanz und Behandlung sein.

Abstract

The aim of this literature review was to describe the possible mechanisms of action for the sedative, anxiolytic, calming and sleep improving effects of essential oils and their ingredients. Based on the main ingredients, a possible link to $GABA_A$-receptors and other targets were explored. Anxiety disorders, which are associated with restlessness, insomnia and other symptoms are becoming more and more common in society. Nowadays stress is increasing, more and more people are using sleep-inducing drugs, tranquillizers and calmative agents. Plant-based alternatives such as aromatherapy or the oral administration of lavender oil preparations are becoming more and more appealing.

In addition to its positive effect on the psyche and its mood-lifting properties, the treatment with benzodiazepines as a tranquillizer, anxiolytic, sedative and hypnotic drug has also a number of side effects and disadvantages (sedation, weight gain, sexual dysfunction) and the risk of abuse and its withdrawal symptoms. Therefore, these herbals are being investigated more and more in order to exploit these effects. A well tolerated anxiolytic drug could resolve the concerns of affected and could be the base for better acceptance and treatment.

Index

Introduction

Essential oils

Essential oils (EOs) are volatile compounds of many different species and parts of aromatic plants, e.g. leaves, peel, barks, flowers, seeds or roots. The method applied for the extraction of EOs is in most cases steam distillation or squeezing of the peel of citrus fruits.

Oily liquids are insoluble in water. The intense smelling oils are complex mixtures consisting of terpenoid and/or non-terpenoid compounds. These include mono- and sesquiterpenoids, sometimes diterpenoids, phenylpropanoids, fatty acids, alcohols, benzoids and some more. EOs are often used as fragrances in the cosmetic industry or perfumery, in food processing, pharmacy or in medicine. They also play an important role in aromatherapy. EOs - possess antimicrobial, anti-inflammatory, sedative, mucous membrane irritating, insecticide, mood changing and other important effects. They can be administered topically, by inhalation or orally.

Nonetheless, internal use is not recommended although the majority of oils are widely considered as safe [1,2]. In the present thesis the sedative, anxiolytic, calming effects of EOs and its ingredients on special receptors and binding sites in the human body are discussed.

Lavender oil, Lavandulae aetherolum

Lavender (*Lavandula spp.*) belongs to the family Labiatae (Lamiaceae), whose EO has a long traditionally use. Records of the

use as a therapeutic agent of lavender oil date back to ancient Rome and to the Greeks [3,4]. Different lavender species are native to the mountainous Mediterranean areas in Southern Europe, growing wildly or cultivated [5]. Lavender EO is obtained by steam distillation of the freshly cut flowering tops of *L. angustifolia* Miller (synonym: *L. officinalis* Chaix) [6]. The chemical composition of the different types is similar on the whole. The general effects of lavender are carminative, antibacterial, antifungal, sedative, antidepressive, insecticidal; moreover, it is also effective for soothing burns. Today, the pure oil is mainly used as a fragrance, in aromatherapy, massage and as an antimicrobial agent. Although lavender oil has been enjoying great popularity for decades and much of its biological activities are known, not all of them have been scientifically tested and proven. Only recently, studies have been performed in larger dimensions.

Chemical composition of lavender essential oil

For the analysis of oil (*Lavandula spp.*) and compositions of true lavender and spike lavender, especially the analytic techniques of gas chromatography coupled with mass spectrometry (GC/MS) or with infrared spectroscopy (GC/IRFT) are used [3, 5].

Lavender EOs are complex mixtures of mono- and sesquiterpenoid alcohols, esters, oxides and ketones (Woronuk et al.) [7]. Lavender oil mainly contains the monoterpenoids linalool and its ester linalyl acetate (up to 47% according to European Pharmacopoeia) [8]. Other monoterpenoid compounds are 1,8-cineole, β-ocimene, terpinen-4-ol and camphor. Also present in trace quantities are sesquiterpenoids, like caryophyllene and nerolidol and other terpenoid compounds, like

perillyl alcohol [7]. These constituents can vary significantly in different oils obtained from various cultivars [3]. Linalool is a naturally occurring terpene alcohol. This cyclic monoterpenoid is a colourless liquid with a fresh floral odour. Due to its sterogenic center at C_3 there are two stereoisomers:

(-)-Linalool is the main compound of the essential oils of *Aniba rosaeodora, C. camphora* leaves, *Bursera delpechiana, Lavandula angustifolia, and Lavandula latifolia*.

(+)-Linalool is up to 60-70% available in Coriander oil (*Coriandrum sativum*) and Neroli oil (*Citrus aurantium*). Linalool represents also a component from cinnamon, basil, thyme and other spices [4,9,10]. Linalyl acetate is the acetate ester of linalool. It is a major compound in petitgrain oil, sage clary oil, bergamot oil, and lavender oil. Because of its fresh sweet odour it is used as an ingredient in perfume and cosmetic products. *In vivo* linalyl acetate is hydrolysed by esterase or carboxylesterase to linalool [4,10].

Therapeutic properties of Lavender oil

Lavender EO has long been considered as remedy for various diseases. By means of its potent calming and sedative effects it is very significantly in aromatherapy. Besides, studies have shown an anticancer and antimutagenic effect of lavender oil constituents [7]. The study of Worunuk et al. [7] has discussed the particular constituents of lavender and their effects on humans as well as on animals. The decreasing anxiety effects of *(R)*-(-)- and *(S)*-(+)-linalool via the olfactory centre have been reviewed in healthy adults.

EOs can be absorbed in the human body by the respiratory system, transdermal or by oral ingestion. Excellent effects have been observed in the context of pain treatment, anxiety, sleep disorders and depressions when consumed by inhalation or dermal contact.

The olfactory uptake of therapeutic agents and the bypass of the blood-brain barrier were confirmed in a survey of Frey et al. [11] by testing the EO on mice as well as on human beings [11]. Mostly lavender aromatics are used to improve sleep both for the elderly and infants.

Inhaling the odour of lavender while sleeping decreases the duration of deep slow-wave sleep phases. Moreover, many studies have shown that anxiety could be reduced and relaxant effects increased, which again have a positive impact on the improvement of sleep. These effects are associated with the olfactory system. Many surveys have examined the anxiolytic potential of lavender EO [7].

Based on studies of *L. angustifolia*, lavender odour exert a similar action to benzodiazepines and enhance the effects of gamma aminobutyric acid (GABA) in amygdala. Gamma-amino butyric acid (GABA) is one of the most important inhibitory neurotransmitters in the central nervous system. Binding the GABA to the $GABA_A$-receptor opens the chlorid-ion channels. The increased influx of negative charged ions results in an inhibitory postsynaptic potential. This prevents the generation of new action potentials. Many physiological actions are modulated by $GABA_A$-receptors, thus deficits of GABA expression can lead to multiple neurological and psychiatric diseases [12,13]. Benzodiazepines, such as diazepam, act as sedative, hypnotic, anxiolytic, anticonvulsive and muscle relaxing drugs. $GABA_A$-receptors possess binding sites for benzodiazepines, barbiturates, anaesthetics, convulsants and many other drugs. These drugs are positive allosteric modulators to $GABA_A$; therefore, they increase the GABA-induced chlorid ion-influx [12,13]. One study showed that dental

patients' anxiety could be alleviated by the smell of lavender. It is assumed that there is a connection between the activity of linalool and anxiolytic effects. By inhalation of lavender oil and linalool positive psychopharmacological effects can be triggered [3,7].

Neurological effects of lavender aromatherapy

During aromatherapy volatile compounds are inhaled and the odour triggers psychological effects. The different compounds act via the limbic system, amygdalae and hippocampus [3]. Many physiological and psychological effects of EOs have been studied on animals, e.g. anxiolytic effects of lavender oil in rats. Already in the 80s effects of lavender were examined: sedative on humans, positive effects on mood and EEG-patterns and math computations [14].

Pharmacological *in vivo*- evaluation of linalool demonstrated dose-dependent sedative effects on the central nervous system (CNS). Therefore, this compound owns hypnotic and anticonvulsive properties too. Neurochemical analysis showed an inhibitory effect of linalool on glutamate binding in rat cortex membranes. NMDA= N-Methyl-D-Aspartat activates NMDA-receptors, which belongs to the ionotropic glutamate-receptors. By its chemical bond, this excitatory cascade is activated [14 - 16]. Furthermore, the natural effects of linalool on the binding of glutamate on the CNS have been examined, as well as the impact of linalool on subcutaneous injected NMDA-induced convulsions. This effect is responsible for anticonvulsive efficacy from linalool on rats [14]. Binding of glutamate decreases with increasing concentration of linalool. The inhibition is dose dependent [16]. Even with neroli and sandalwood oil there is a sedative effect. Citronellal, phenylethylacetate, linalool,

linalyl acetate, benzaldehyde, terpineol, isoeugenol are compounds of EOs of decreasing activity in this order. Many odours of EOs influence the CNS, either in a stimulating or sedative way [14].

As mentioned above, one author suggested that lavender acts like benzodiazepine. It has a similar action in effecting gamma-aminobutyric acid (GABA) neurotransmission. The anxiolytic and sedative effect is related to the olfactory influence of lavender oils to the CNS [3,7]. The calming effects of lavender herb pillows are already in a long traditionally usage to enhance sleep.

A wide selection of EOs has shown a cortical brainwave (EEG) response after aromatherapeutic use. More precisely, (*R*)-(-)-linalool reduces β-brainwave activity associated with alertness and being awake. It also intensifies α-wave activity associated with relaxation. These observations have been confirmed in clinical trials. As well as the sedative and local anesthetic effects were shown years ago in animal studies [17]. The study from Tanida et al. [18] suggested that the odour of lavender and its major constituent, linalool, influences autonomic neurotransmission and reduces blood pressure via the central histaminergic nervous system and the hypothalamus [17,18]. In the course of these studies behavioural models were demonstrated which also show an anxiolytic effect of other EOs. For instance thyme (*Thymus broussonetti Boiss*) exerts an anxiolytic effect on animals in light/dark box [19].

Shaw et al. [17] compares in an animal model the already pre-tested anti-anxiety effect of chlordiazepoxide, a benzodiazepine, with lavender oil inhalation on openfield behaviour in rats. In 3 of 4 experiments a sedative and anxiolytic effect could be confirmed. An increasing immobility was shown. This confirms previous findings that linalool and linalyl acetate exhibit dose dependent anxiolytic effects, because lavender oil increases the $GABA_A$-receptor-response at low concentrations and inhibits the response at higher concentrations. Especially the results of Shaws´

experiment 4 supported that lavender oil exerts an effect of the CNS similar to other anxiolytic drugs, provided lavender oil had to be inhaled before testing behaviour [17].

A few years later the study of Takahasi et al. [20] showed that linalyl acetate potentiates the anxiolytic effect of linalool in mice tested in the elevated plus maze. This reference evidenced the anxiolytic effect of lavender EO again, but the full pharmacological properties of this anxiolytic mechanism are not fully explained yet. It is not exactly clear which neurotransmitter is involved. Generalized anxiety disorders (GAD) are most frequently treated with benzodiazepines (BDZs) that interact with the $GABA_A$-receptor/BDZ complex, antidepressants, mostly selective serotonin reuptake inhibitors (SSRIs) that control serotonin neurotransmission and the 5-HT1A-receptor agonist buspiron. In the previous studies the anxiolytic effect of the interaction was assigned with the $GABA_A$-receptors. However, serotonin is another relevant neurotransmitter that is associated with anxiety. Serotonin receptors such as the 5HT1A-receptor play an important role in anxiety. The aim of the study of Chioca et al. [21] was to evaluate the involvement of $GABA_A$/BDZ and serotonin neurotransmission and its pharmacological mechanism of action.

Again, lavender oil inhalation in mice by the marble-burying test in maze was examined. The anxiolytic effect of lavender was consistently confirmed. Though, it appears that the serotonergic neurotransmission plays a more important role in this anxiolytic treatment of lavender oil and that the $GABA_A$-receptor complex has a smaller participation [21].

Other scientists expected that linalool inhibits acetylcholine (ACh) release. This results in alteration of the ion channel function at the neuromuscular junction. Linalool and linalyl acetate reveal a rapid topical absorption through the skin. Linalyl acetate has narcotic effects, while linalool acts sedative [3]. According to studies from Re et al. [15], linalool

shows an inhibitory effect to ACh release and to the open probability of the neuromuscular junction in mice. The results of this study demonstrate that linalool reduces the efficacy of the nerve impulse in ACh release. The reduced ACh release results in a continuous/steady decay of the miniature endplate. Consequently, the inhibitory effect introduces the local anaesthetic action [15].

Melissa officinalis vs. Lavandula angustifolia EOs

EOs from *L. angustifolia* and *Melissa officinalis*, singly or combined are the most established EOs in clinical trials for usage in patients with agitation. The major constituents ((*E*)-caryophyllene, caryophyllene oxide, geranyl acetate, linalyl acetate, linalool, nerol, oct-1-en-3-ol, 3-octanone, myrcene, all-ocimene, p-cymene and α-terpineol) of the two oils were used in an experiment by Mahita et al. [22] to identify the $GABA_A$-receptor ligands via radioligand binding assay to $GABA_A$-receptors in rat forebrains. Neurotransmitters from the serotonergic, noradrenergic, dopaminergic and GABA-system are responsible to regulate behaviours. Therefore, the pharmacological intervention can interact on these targets. The allosteric interaction at the $GABA_A$-receptor is used by benzodiazepines, barbiturates and anesthetics. Because of the great molecular diversity of multi-subunit hetero-oligomeric $GABA_A$-receptors many opportunities exist to develop new drugs. Relevant molecular targets for receptor subtype specific action are used to create novel drugs for sleep-disorders, anxiety, epilepsy, dementia and even more.

In order to identify the components of lavender and melissa EOs probably responsible for binding to the $GABA_A$-receptor, the study of Mahita et al. [22] experiments with well-washed rat forebrain membranes were determined. The effects of the 12 major oil constituents were investigated on the

channel-side of $GABA_A$-receptors. Three different concentrations (0.001, 0.01, 0.1 mg/ml) of each constituent (linalool, limonene, (*E*)- caryophyllene, caryophyllene oxide, nerol, p-cymene, geranyl acetate, 3-octanone, oct-1-en-3-ol, myrcene, ocimene and α-terpineol) were used. A dose-dependent inhibition for many of these constituents could be observed. The higher concentrations attained up to 50% inhibition between 0.01-0.1 mg/ml of the substances on $GABA_A$-receptors. Linalool and nerol from lavender showed an inhibitory effect only at the highest concentration (0.1 mg/ml), while the melissa constituents demonstrated beneficial effects even at lower concentrations (0.001 mg/ml). At these concentrations both oils showed an antagonistic effect on voltage-gated sodium channels as well. It is also known from previous studies. Demonstrated antagonistic effects of melissa constituents need to be investigated furthermore according to Mahita et al. [22]. Bilobalide, a sesquiterpene trilactone derived from *Ginkgo biloba*, which has been used for the treatment of Alzheimer disease (AD) showed a similar inhibitory effect on $GABA_A$-receptor and also neuroprotective effects. The conclusion of Mahita´s study identified trans-ocimene (= β-ocimene), a compound of both oils, is the $GABA_A$-receptor inhibitory component [22].

Anxiolytic properties of lavender oil using modulating voltage dependent calcium channels

The most widespread mental disorders in the European Union are anxiety disorders, insomnia and major depressions. Generalized and persistent anxiety is often associated with panickiness and other symptoms (GAD, Generalized Anxiety Disorder). More than a half of the patients suffering by anxiety disorders do not receive the relevant treatment. Benzodiazepines have been the first-line treatment for decades. In addition, there are many different available treatments

including antidepressants (selective serotonin reuptake inhibitors, SSRIs), buspiron, hydroxyzine, gabapentin and pregabalin. However, these substances have some side effects: sedation, amnesia, attention problems, delirium, withdrawal syndrome and a high potential for drug abuse [23-25].

In patients with subsyndromal anxiety the clinical use of oral lavender oil is supported. Silexan® is a patented EO produced from *L. angustifolia* flowers by steam distillation composed of the main active constituents linalool and linalyl acetate. It accounts for about 70% of the ingredients. The soft gelatine capsules of Silexan® contain 80mg of EO. It is at present the only pharmaceutical quality lavender oil preparation for oral use at the market. As a prerequisite, it has to confirm with the monograph Lavender oil of the European Pharmacopeia [24-26,28].

Current clinical trials showed distinct effects in patients with subsyndromal or subthreshold anxiety disorders as well as in patients with GAD after oral using of Silexan® capsules. According to the studies of Schuwald et al. [25] and Kasper [27] the effects of Silexan® base on a potent anxiolytic inhibition of voltage-dependent calcium channels (VOCCs). It is a highly selective drug target. Schuwald et al. [25] showed for the first time that lavender oil possesses some similarities with the anxiolytic pregabalin. It inhibits VOCCs in synaptosomes, primary hippocampal neurones and overexpressing cell lines in the same area like pregabalin [25,27]. Pregabalin is a S-configured isobutyl derivative of GABA. Its active profile is similar to gabapentin. Despite the structural similarity to GABA, the effect of pregabalin is due to its binding to the $\alpha_2\delta$-subunit of the voltage dependent P/Q-type calcium channel and not to the mechanism of GABA [29]. However, Silexan® does not bind essentially to P/Q-type calcium channels such as pregabalin and does not interact with its binding site, the $\alpha_2\delta$-subunit. Lavender oil decreases

the influx of calcium ions through several different types of VOCCs such as the N-type, P/Q-type and the T-type non-selectively [25].

Another mechanism according to Kasper [27] is the significant reduction of serotonin-1A-receptor (5-HT1A) binding potential in some brain clusters. Schuwald et al. [25] demonstrated that the inhibition by lavender oil in the hippocampus is mainly mediated via the N-type and P/Q-type VOCCs. The hippocampus is a brain region that is important for anxiety disorders. This survey provided a pharmacological and molecular motive for the clinical use of the oral lavender oil preparation in patients suffering from anxiety and GAD [25].

More orally properties of lavender oil preparations

Since the anxiolytic properties of lavender oil have been demonstrated in previous pharmacological and clinical trials, the following studies postulated a positive effect in patients with GAD [24,28]. In the study of Woelk et al. [24] the efficacy of a 6-week treatment of Silexan® in comparison to lorazepam a benzodiazepine acting on the gamma-aminobutyric acid/benzodiazepine-receptor-complex were tested in adults with GAD. It was a multi-center, double-blind, randomized study with the primary outcome to identify a change in the Hamilton Anxiety Rate Scale (HAM-A-total score). This scale is an objective measurement of the severity of anxiety. After a wash out phase with placebo, adults were randomised divided in a Silexan®-group and a lorazepam-group. Both lorazepam and Silexan® act via the $GABA_A$-receptor. The mean of the HAM-A total score decreased significantly and to a similar extent in both groups. About 45% in the lorazepam group and 46% in the Silexan®-group at the beginning of the treatment. The two HAM-A-subscores “somatic anxiety” and “psychic anxiety” also decreased in both groups during the active treatment. The

results finally showed that Silexan® improves GADs just as effectively as common benzodiazepines. The safety of Silexan® has also been confirmed. No serious side effects and potential of drug abuse were discovered. According to Woelk et al. [24] Silexan® is a well-tolerated alternative to benzodiazepines for the treatment of GAD. The randomized, double-blind study of Kasper et al. [28] compares the efficacy of Silexan® to placebo and paroxetine, a selective serotonin reuptake inhibitor (SSRI) in four parallel groups. The Silexan®-groups received 160 mg or 80 mg, the paroxetine-group 20 mg or placebo once daily. The efficacy endpoint of this study was also a reduction of the HAMA total score between baseline and treatment end. A secondary objective was the anxiolytic efficacy of Silexan® compared to paroxetine. During the 10-week treatment all 4 groups demonstrated a constant decrease of the HAMA total score. Most pronounced in the Silexan®-groups followed by paroxetine and placebo. Already after 4 weeks of treatment Silexan® (160 mg) was significantly superior to placebo and after 6 weeks also Silexan® 80 mg. The HAMA total score reduction at the end of the treatment of each group was: 14.1± 9.3 points for Silexan® 160 mg/d, 12.8 ± 8.7 points for Silexan® 80 mg/d, 11.3 ± 8.0 points for paroxetine, and 9.5 ± 9.0 points for placebo. In conclusion the anxiolytic efficacy of the orally use of lavender oil preparation Silexan® was investigated in GAD in comparison to placebo and paroxetine. Silexan® also showed an antidepressant effect and improved general mental health. In GAD Silexan® is more efficient than placebo [28].

Coriandrum sativum EO and its effects

Coriandrum sativum L., belongs to the family Apiaceae and is cultivated world-wide for medicinal, culinary use and the production of EOs. Its long traditionally use, especially in the Iranian traditional medicine, includes

digestive disorders, loss of appetite, insomnia and anxiety. Coriander essential oil (CEO) is a complex natural mixture of concentrated volatile compounds, extracted from coriander seeds. It has been demonstrated that the extracts and EOs possess antimicrobial, antioxidant and anti-diabetic activities. Because of the long history in dietary use, coriander oil is considered as safe. The main constituents of coriander volatile oil are linalool (65-78%), α-pinene, γ-terpinene, geranyl acetate, camphor and geraniol [30-32]. The aim of Emamghoreishi et al. [30] was to figure out if the EO and the aqueous and hydro-alcoholic extracts of coriander seeds exert a sedative-hypnotic activity. They were using prolonging pentobarbital-induced sleeping time as an index of sedative-hypnotic activity. Pentobarbital is a short-to-intermediate acting barbiturate that uses its pharmacological effect on the CNS by increasing inhibition of GABA-mediated neurotransmission. Before, there were no pharmacological scientific studies that evaluated its sedative and central depressant effects. To male albino mice, different concentrations of aqueous and hydro-alcoholic extracts or EO of coriander seeds (100-600 mg/kg) were administered intraperitoneally (i.p). Diazepam hydrochloride was used as a reference drug. The control-group received olive oil respectively saline, the vehicles of each administration (EO or extracts). The latency to sleep, of sleeping duration and the loss of the righting reflex were recorded for each mouse. Both extracts and EOs of coriander seeds demonstrated a sedative-hypnotic effect. However, the aqueous extract showed much profound effects than the groups of hydro-alcoholic extracts and EOs. The increased pentobarbital-induced sleeping time in the CEO-group was only observed at the highest dose (600 mg/kg). Therefore, this study assumes that components, which are mainly present in aqueous extracts, are responsible for the hypnotic effect. In this study it was suggested that $GABA_A$-acting compounds prolong pentobarbital-induced sleep duration. In future studies, the mechanism of action of coriander must be examined more closely [30].

Inhalation of coriander volatile oil

The study of Cioanca et al. [31] examined the potential anxiolytic, antidepressant and antioxidant properties of inhaled CEO. The oil was extracted from *Coriandrum sativum* var. *microparum.* The anxiety-like behaviour and depressive-like reaction was analysed after several exposures to CEO in β-amyloid (1-42) rat model of Alzheimer's disease (AD). Prior to this, these effects of inhaled CEO were studied by *in vivo* experiments (elevated plus maze and forced swimming test). AD is characterised by the attendance of β-amyloid in the senile plaques in the brain and blood vessels. Aβ (1-42) is neurotoxic and results in the pathogenesis of AD. By preventing plaques the symptoms of AD improve. Recent evidence suggested a connection between depression and AD because AD patients often possess psychiatric symptoms along with the cognitive decrease. Anxiety and depression are both worsening AD and its cognitive decline, leading to abnormal behaviour and a limited daily life. In the trial of Cioanca et al. [31] sixty male Wistar rats separated in six groups were used: 1. Control group received saline treatment (0.9% NaCl), 2. Diazepam alone-treated group, 3. Tramdol alone-treated group, 4. Aβ (1-42) alone-treated group, 5. Aβ (1-42) + CEO 1% (Aβ (1–42) + CO1%) and 6. Aβ (1-42) + CEO 3% (Aβ (1–42) + CO3%). Groups 1-4 were caged in the same conditions without oil. The control group were injected with diazepam respectively tramadol before testing. CEO inhalation (1% or 3%) was performed via an electronic vaporizer placed at the bottom of chamber. Two different tests were carried out. The elevated plus-maze test, which is considered to be an etiologically valid animal model of anxiety, and the forced swimming test, which is validated as a suitable tool for prognosticating the antidepressant properties of drugs. The elevated plus-maze test evaluates in which of the arms (enclosed or laid open) the rats

entered after inhalation, the time spent and rats behaviour. From this the test concludes anxiety and motoric activities. An anxiolytic agent increases the time spent in open arms and the incidence to enter. In the forced swimming-test, the behaviour regarding immobility (time with required swimming movements) and swimming (time with active movements) is first compared without inhalation and then with inhalation. The chemical composition of CEO was analysed by GC-MS and GC-FID. Monoterpenes were the most prevalent components, namely linalool (69.4%), γ-terpinene (7.7%) and α-pinene (6.5%). The β-amyloid (1-42)-treated rats showed a decrease of the locomotor activity and the number of entries in the open arms during the plus-maze test. At the forced swimming test there was a decrease of swimming and immobility times. The inhalation with CEO seriously improved these parameters, suggesting anxiolytic-like and antidepressant effects. The high-linalool containing volatile oil showed - in accordance with the report that the anxiolytic and antidepressant activities were mediated by the $GABA_A$-receptor complex - a decreasing effect relating to locomotor activity and anxiolytic-like behaviour in rats. Due to the fact that linalool is the main component, it is probably responsible for the mentioned effects. It was proposed that inhaling linalool rich volatile oils could be adjuvant to treat anxiety. The results of this study indicate that multiple exposures to CEO can prevent anxiety, depressions and oxidative stress in Alzheimer's patients. So it is a useful alternative for the prevention and treatment of dementia [31].

Central administration of Coriander essential oil

In the study of Gaston et al. [32] the effects and the behaviour of neonatal chicks (1 day old) were tested in an **o**pen-**f**ield test. The OF-test has been widely validated in the detection of anxiolytic and sedative drugs, such as

benzodiazepines. The EO from coriander seeds (GC-analysis: >80% linalool) was administered intracerebroventricular. The locomotor activity and emotionality in neonatal chicks were analysed. Chicks received CEO or linalool, both at doses of 0.86 µg, 8.6 µg and 86.0 µg per chick, or saline and diazepam, both at doses 17.5 µg per chick. On the one hand they wanted to explore the anxiolytic-like behaviour and on the other hand, whether the effects are due to the main component of linalool. The results showed a linalool-dose-dependent effect in neonatal chicks. At doses of 8.6 µg and 86 µg from CEO and linalool the following parameters of the OF-test were significantly decreased ($p<0.05$): square crossed number, attempted escapes, vocalizations and defecation number. Moreover, it increased the sleeping posture on an open field compared to the saline treated group and similar to the diazepam group. The intracerebroventricular injection of CEO revealed a dose-dependent sedative effect, which is also due to the monoterpene linalool. Therefore, it could be used as a potential therapeutic agent similar to diazepam. Already prior *in vitro* studies have demonstrated that linalool may act via the NMDA-receptor (=N-methyl-D-aspartate) as a competitive antagonist and not via the $GABA_A$-receptor. According to Gaston [32] linalool and CEO could be used in clinical therapy similar to diazepam, although further studies should be carried out to identify the molecular mechanism of CEO and it constituents especially linalool [32].

Effects of *Citrus aurantium* L. EO

Scents, administered via inhalation, acting on the brain and resulting in behavioural changes are well known. The EO from peel of *Citrus aurantium* L. (Rutaceae) is widely used for insomnia, anxiety, depressions and epilepsy [33,34]. The anxiolytic effect of the Citrus EOs has been

demonstrated in mice by the study of Carvalho-Freitas et al. [34] and de Moraes Pultrini et al. [35].

Carvalho-Freitas et al. [34] used male mice (35-45 g) treated with EO from peel (EOP) or hydroethanolic extract from fresh leaves of *Citrus aurantium* each 0.5 g or 1.0 g/kg. The following were evaluated: sedative-hypnotic activities by pentobarbital-sleeping time model (duration of sleep), anxiolytic activities by elevated plus-maze test (time spent in open or closed arms) and anticonvulsant activity by convulsing test (using PTZ= pentylenetetrazole s.c. and maximal electroshock corneal). The oil was obtained by steam distillation with his main component determined by GC-MS, 90.4% limonene. This monoterpene is the most important compound of Citrus EO. The results showed a non-dose-dependent effect in matters of the increased latency period of tonic seizures. But there were no anticonvulsant effects compared to PTZ and MES. Moreover, mice treated with EOP (only at the dose of 1.0 g/kg) showed a significant prolonged sleeping time inducted by barbiturates and the time spent in open arms in the elevated plus-maze. These effects indicate an anxiolytic effect of the substances. However, the control group treated with diazepam showed clearer effects. The results of the study are consistent with the traditional use of Citrus EO [34].

De Moraes Pultrini et al. [35] used two other experimental procedures: the light-dark box, to evaluate GAD and the marble-burying test, which is cohesive to compulsive disorder. They also used the EOP (0.5 or 1.0 g/kg) orally administered by gavage in mice single or multiple. The EO used here mainly contains the monoterpenes limonene (98.8%) and myrcene (1.4%) as well as an aldehyde, octanal (0.45%). The positive control group was also treated with diazepam and the negative group with tween. In that survey the behavioural profiles from the EOP treated group were similar to the diazepam treated group. However, this effect decreased after repeated treatment. The mechanism of action was not discussed here [35,36].

Lima´s et al. [33] objective of their study was to evaluate the anxiolytic-like potential of inhalation with (*R*)-(+)-limonene in mice and to value the correlation of pharmacological data of GC/MS.

The exposure with (+)-limonene, a chemical constituent of various bioactive EOs (*Citrus aurantium, Citrus juno, Citrus sinensis, Lippia alba*) significantly modified all parameters evaluated in the elevated plus-maze test. Again, the same concentrations were used as in the previous studies, 0.5 or 1% of (*R*)-(+)-limonene with 97% purity and also diazepam or tween/saline in control groups. Flumazenil was used as a benzodiazepine receptor antagonist. Thus, the pharmacological effect of inhaled limonene (1%) was not blocked by the previously administered flumazenil. This indicates that the benzodiazepine receptor-binding site does not mediate this effect. Nevertheless, other GABAergic sites could be involved. At the concentrations of 0.5 and 1% (+)-limonene the number of mice entered the open arms in the maze-test increased. An anxiolytic-like effect of *in-vitro* inhalation of (+)-limonene similar to diazepam was supposed. Additional investigations to explain the anxiolytic mechanism are necessary. The GABAergic effect could not be excluded. According to the volatility of (+)-limonene the data showed a high bioavailability. Therefore, a potential clinical benefit was expected. Only volatile psychoactive substances interact with chemoreceptors. This allows a stimulus to be delivered to the brain and triggered the olfactory sense [33].

Rashidi-Fakari et al. [37] and Namazi et al. [38] tried to determine the efficacy of aromatherapy with *Citrus aurantium* EO in reducing anxiety during childbirth for example, contraction of the uterus, fetal hypoxia. A stressed condition can have a negative impact on childbirth. Pregnant women were randomly assigned in two groups (orange oil or placebo). In these clinical trials, different parameters are measured such as blood pressure, pulse rate, respiratory rate. Based on the Spielberg questionnaire, the levels of anxiety are evaluated at the beginning and after the inhalation.

In the Rashidi-Fakari et al. [37] study, there was no significant change in the measured parameters in the intervention group, but only a minor improvement. In the study of and Namazi et al. [38] the level of anxiety after treatment in the aromatherapy group was significantly lower compared to the control group. The results of both studies indicate that the inhalation of orange essential oil may be a useful alternative to reduce anxiety, stress and tension during childbirth. Women with less anxiety have less pain during birth [37,38].

Citrus aurantium L. EO and its interference with serotonergic and GABAergic pathways

The study of Saketi et al. [39] investigated the anxiolytic effect of *Citrus aurantium L.* EO and its interference with the serotonergic pathway. Serotonin, also known as 5-HT (= 5-hydroxytryptamine), a neurotransmitter, which is involved in expressing anxiety, disorders and depressions. 60 mice were assigned into control groups and treated group with the EO (0.5, 2.5 and 5% for 5 days). After i.p injection some of the therapy group were treated also with fluoxetine (2 mg/kg) to examine its modulator properties. Fluoxetine (= a SSRI) is one of 5-HT3-receptor antagonists used in treatment of depression. The trial has also used the elevated plus-maze test to assess anxiety. In comparison to the saline and olive oil treated control group, the therapy group (EO with/without fluoxetine) resulted in a significantly increasing number of entries in open arms ($p<0.001$). It was concluded that *Citrus aurantium* L. EO reduced anxiety in male mice. Due to the fluoxetine potentiation it was observed that the EO exerts its anxiolytic effects partly via the serotonergic system. Serotonin plays a role in the emergence of anxiety, which is already known from previous studies. The

reduction and thus a lack of serotonin in the synaptic cleft leads to anxiety disorders and depression. It has been shown that 5-HT3-receptor antagonists are responsible for anxiety disorders and depressive moods. In the study of Saketi et al. [39] fluoxetine (i.p) acted as a 5-HT agonist. The authors concluded a serotonergic amplification by certain compounds. This increased effect of serotonin in the synaptic cleft leads to the maintenance of rest and reduced anxiety [39].

As mentioned before, GABAergic systems also play a significant role in the development of anxiety and disorders. In the study of Khosravi et al. [40] the interaction of *Citrus aurantium* L. EO on GABAergic pathways was investigated. The trial conditions were the same as in the study of Saketi et al. [39]. Same model, same doses, same procedure. The only difference was the treatment with diazepam (0.1 mg/kg) instead of fluoxetine.

Similar results have shown that EOs can reduce anxiety-related disorders that affect the GABAergic system in mice. In mice lacking the GAD65 (glutamic acid decarboxylase) enzyme, anxiety disorders were observed. This enzyme converts glutamate to GABA. The level of GABA increases the pathogenesis of anxiety, disorders and depression. The therapy group (EO) revealed a significant increase of the percentage time spent in the open arms ($p<0.001$), similar to the diazepam group. These effects were probably the result of an interaction with GABA pathways. Limonene reduced the activity of neurons in the CNS and attached itself to $GABA_A$-receptors. In this way, limonene reduced anxiety-related activities. The result of Khosravi´s et al. [40] study confirmed previous studies on the anxiolytic effect of diazepam and *Citrus aurantium* L. EO [40].

Citrus aurantium subspecies *bergamia* (Bergamot)

Bergamot EO (BEO) from peel of *Citrus aurantium* ssp. *bergamia* (Rutaceae) has also been used as an anxiolytic acting oil in aromatherapy. From earlier studies it is known that BEO increases the level of GABA in the hippocampus. The major compounds of BEO were identified via GC-MS as limonene, linalool and linalyl acetate. They also exert an effect of the GABAergic system. In the study of Saiyudthong et al. [41] the potential anxiolytic effect of inhaled BEO in rats has been investigated. The BEO was tested in different concentrations (1%, 2.5%, 5%) using two methods (EPM-test =elevated plus maze test and hole-board test), according to the anxiety-related behaviour. The hole-board test is widely used to assess anxiety, emotionality and stress in animals. The board is made of wood with holes in certain distances. In this test, the number of head-dips and time spent with head dipping were monitored. This behaviour reflects an anxiolytic state. Furthermore, the BEO was compared to diazepam in stress-induced levels of plasma corticosterone. BEO and diazepam showed a significant increase at the percentage of open arm entries in the EPM at higher concentrations. At the dose of 5% of BEO there was also a significant increase of total arm entries. Therefore, the study concluded a raised locomotor activity. 2.5% BEO and diazepam showed a significantly ($p< 0.05$) increase in the number of head dips in the hole-board test and lessen the corticosterone reaction on acute stress burden. These results suggested an anxiolytic-like behaviour and a stress reduced response. Both tests showed an anxiolytic effect of inhaled BEO similar to the diazepam treated rats. The lowest dose has not shown any significant result. The mechanism of action of BEO is not yet known but is due to a change in GABA in the brain. These results are supported by previous studies. In this study, Saiyudthong et al. [41] were referring to earlier studies with lavender oil, since the main components are very similar. However, the mediated GABA reaction must be investigated more closely [41].

Effects of sweet Orange Aroma

Based on previous assumptions that citrus fragrances could treat anxiety symptoms the study of Faturi et al. [42] investigated the potential anxiolytic effects of inhaled *Citrus sinensis* (sweet orange) EO on male rats. The behaviour of the rats was examined in the EPM test followed by the light/dark paradigm. The volatile main component of the oil determined by GC/MS analysis is the monoterpene limonene (97.7%). Thus, limonene is assumed to be the component responsible for the anxiolytic effect. However, previous studies using i.p administered EO of *Citrus sinensis* showed no anxiolytic effect. It may be due to the different ways of administration. The rats were exposed to the orange odour (100 µl, 200 µl, 400 µl) for 5 minutes and the behaviour tests were then carried out. By inhalation, volatile molecules can enter the lungs and diffuse from there into the blood, thus activating the brain via systemic circulation. It could also be that these volatile molecules bind to olfactory receptors and mediate emotional reactions via the limbic system. At all doses, an anxiolytic activity could be detected in at least one test. At the highest dose of 400 µl the experiment showed a significant effect. As already mentioned above, this effect is shown in the EPM test by an increased percentage of entries and time spent in open arms and of the lit chamber of the light/dark paradigm (also time spent in it). The control group was exposed to the odour of *Melaleuca alternifolia* - an antiseptic acting EO to make this outcome possible only on the orange fragrance. No anxiolytic activity could be observed in this control group. Irrespective of the mechanism, which still needs to be clarified in more detail by clinical investigations, an anxiolytic-like effect of the sweet orange essence could be observed [42]. Only shortly after this study, there was a similar study with 40 healthy adult subjects exposed to the aroma of sweet orange. The objective of the randomized double-blind trial of Goes et al. [43] was to investigate the potential

anxiolytic effect of *Citrus sinensis* EO in male students exposed to an anxiogenic situation. The volunteers were separated in different groups for the inhalation of sweet orange essential oil (2.5, 5 or 10 drops on a surgical mask), tea tree essential oil (aromatic control group) and water (non-aromatic control group). Immediately after inhalation, a standardized method was used to induce anxiety (Stroop-Colour-Word-Test). It is proven that this method significantly increases anxiety symptoms during the task. After treatment in the anxiogenic situation the volunteers received a standardized questionnaire to measure anxiety with a points-based system (State-Trait Anxiety Inventory). Also parameters such as heart rate, subjective tension and sedation were examined and determined after, during and before the treatment. Here, too, the main component was analysed as limonene.

The tea tree oil test aroma group showed no significant change in the anxiogenic situation ($p>0.05$). The results revealed a significant acute anxiolytic activity of sweet orange aromatherapy due to the lack of a significant increase in anxiety level during the Stroop-Task. Also a decrease of the rest with the aroma group remained. However, significant results were not found in subjects exposed to the mean dose (5 drops). The higher and lower doses showed more statistical significance. Therefore, the result is to be questioned. Nevertheless, further studies are needed to evaluate the clinical relevance and therapy. The authors identified anxiolytic properties in humans, but there was no statistical difference between the orange aroma group and the tea tree oil group and no mechanism of action was enlightened [43].

Terpenoids from *Sideritis* extracts

Sideritis species are the basis for evening tea preparations consumed in Mediterranean areas because of its sedative properties. The genus *Sideritis*

comprises more than 150 species. In earlier studies it has already been demonstrated that receptors of the Cys-loop family (nicotinic Ach-receptors, glycine receptors and $5HT_3$-receptors) are the target of volatile odorants such as linalool and geraniol. In the review of Kessler et al. [44] various odorants were analysed, which were identified in *Sideritis* species. The aim of the study was to detect single substances from *Sideritis* extract, which are responsible for $GABA_A$-receptor modulation. One of the sedative acting constituents of *Sideritis* could enhance GABA-gated streams an effect, which is similar to benzodiazepines. On the basis of a wide variety of GABA-subunits, there is a great variability of subunit combinations that can be responsible for the effect. Most heteromeric receptors in the brain contain α and β subunits with the composition α1β2γ2. Depending on the subunit combination, a sedative or anxiolytic effect or both may occur. $GABA_A$-receptors also interact anticonvulsants and anesthetics. The binding site for diazepam is located between the α and the γ2 subunits. Diazepam and other benzodiazepines pass the blood-brain barrier because of their hydrophobic properties. However, it is not yet clear how odorants that bind to odorant receptors reach the brain. The individual volatiles of *Sideritis* determined by GC were tested in 2 expression systems: human embryonic kidney cells (HEK293-cells) and *Xenopus* oozytes. By expressing α1β2γ2 or α1β2 receptors in these systems, the individual components could be tested separatedly. *Sideritis* extracts are rich in terpenes, specifically the compounds α- and β-pinene, terpinen-4- ol, α-terpineol, β-caryophyllene and its oxide, sabinene, α- phellandrene, linalool, eugenol, carvone, 1,8-cineole, 1-octen- 3-ol, and carvacrol were found as quantitatively dominant or clearly detectable compounds. On oocytes the activity of $GABA_A$-receptors was measured with the two-electrode voltage clamp technique. For the HEK cells, current amplitudes were measured using the patch clamp technique in the presence of GABA [44]. The study by Kessler et al. [44] was able to identify which structural features of the above-mentioned

compounds are important for their modulatory properties. Some terpenes revealed a potent GABAergic reaction. Pinenes did not indicate modulatory effects and sabinene, α- phellandrene and β-caryophyllene have not increased the response. A significant increase was observed at linalool, 1-octen- 3-ol, and carvacrol. Therefore, the phenolic monoterpene carvacrol was tested in a dose-dependent way using concentrations from 30 µM to 1 mM. According to Kessler et al. [44] carvacrol showed at concentrations of 100 µM (1.8 fold potentiation) or higher significantly advanced GABAergic currents compared to GABA alone. Thus carvacrol was among the tested compounds of *Sideritis* the most potent $GABA_A$-receptor modulator comparable to linalool, of which already an evidence for its $GABA_A$ modulating effect was found. However, the review also wanted to determine if these effects were due to structural features within the terpenes. Positive modulation was noticed in substances containing hydroxyl groups and increased with mono- or bicyclic character. Furthermore, data showed a modulation independent from the presence of the γ2 subunit, like alcohols and anesthetics. Myrtenol, a volatile pinene metabolite, is able to enhance GABA-evoked current response compared to GABA application alone. Myrtenol was identified as the volatile compound with the strongest effect on the GABA-dose-response curve (7.5 fold decrease), similar to the diazepam effect. The decrease for carvacrol and linalool were only 4 fold. Moreover, the hypothesis that the proximity of an isopropyl group to the hydroxyl group on a ring structure (such as propofol and menthol) might share similar modulatory sites at $GABA_A$-receptors could be shown recently. The results revealed that some volatiles could be identified as $GABA_A$-modulators, which provide an explanation for the observed sedative effects [44].

Lippia alba

Lippia alba Mill. (Verbenaceae) is widely used in different regions of Central and South America as a tranquillizer. In the study of Hatano et al. [45], the effects of the EO of *L. alba* on rats in the elevated T-maze test (ETM), derived from the EPM - consisting of one enclosed and two open arms - were investigated. The EPM test has already been used in previous studies and has not produced any significant results. The ETM test evaluated two reactions, inhibitory avoidance and one-way escape, which are due to GAD and panic disorders. The EO from chemotype II was administered daily (for 14 days) i.p (12.5 mg/kg or 25 mg/kg). The main components of chemotype II are 55% carvone and 23% limonene. Diazepam was administered as an effective positive control and saline and tween80 as placebo. Furthermore, the effects of a treatment with (*R*)-(-)-carvone (25 mg/kg) were tested in the ETM test. Carvone was previously not tested for anxiolysis. Results showed that treatment with the chemotype II from leaves of *L. alba* at both doses exerted an anxiolytic effect due to a reduced ETM avoidance latency. The same significant results were observed in the carvone group ($p< 0.05$) and the diazepam group. Chemotype II acted selectively on inhibitory avoidance in clinical terms related to GAD. It is supposed due to previous studies with carvone that the mechanism of action of the monoterpenes interacts with $GABA_A$-receptors in the brain after the blood-brain barrier has been passed. In addition, the study suggested that carvone inhibits neurons which modulates the behavioural inhibition in several brain areas such as amygdala and hippocampal system. A defensive behaviour triggered by LA in rats, which is related to GAD in humans, suggested that carvone is responsible for the effect as a tranquillizer. This main compound of *L .alba* EO is specific for the anxiolytic-like effect in rats [45].

Anxiolytic like and neurobehavioral effect of the EO of *Cymbopogon citratus* (lemongrass)

Cymbopogon citratus (Poaceae) is worldwide known as lemongrass and also a popular used herb in traditional medicine as an infusion or decoction. Despite of its long use there are few controlled studies confirming its biological activity in central nervous system.

The main components of the EO from fresh leaves from *Cymbopogon citratus* were identified by GC/MS as the monoterpene citral (a mixture of the stereoisomers neral and geranial) followed by β-myrcene and geraniol [46-48]. In previous studies by Costa et al. [46] and Blanco et al. [47] if was indicated that the acute treatment with the EO of *C. citratus* is effective against anxiety disorders and epilepsy in mice. The authors of Blanco et al. [47] administered the EO (0.5 or 1.0 g/kg) orally to male mice to evaluate the sedative and hypnotic activity (by pentobarbital sleeping time), the anxiolytic activity (by EPM-test and light/dark box test [LDB]) and the anticonvulsant activity (seizures induced by pentylenetetrazole and maximal electroshock. The positive control group received chlordiazepoxide or diazepam. The acute toxicity and the behaviour of the groups treated with EO at different doses were evaluated after the treatment and during 15 days after the treatment. The EO caused an effective outcome on increasing sleeping time, the percentage of entries in open arms and time spent in it at the EPM-test, as well as the time spent in the light area of the LDB-test. Furthermore, the EO exerted also an anticonvulsive effect since it delayed induced clonic seizures. All these results of anxiolytic and anticonvulsant effects were obtained by acute treatment [36, 46, 47].

A few years later another study of Costa et al. [48] has investigated the effect from EO of *C. citratus* after a long-term treatment (21 days) and the potential synergistic effect of EO and diazepam. The anxiolytic-like activity

was examined in LDB-test and marble-burying test (MBT), the antidepressant activity in the forced-swimming test (FST). To determine the mechanism of action of the EO, flumazenil, a BZD-receptor competitive antagonist or WAY100635, a highly selective 5-HT_{1A}-receptor antagonist were used. Based on the blockade of the GABAergic and serotonergic pathway, they wanted to infer the mechanism and the pharmacological activity of the EO to validate its use in combination with BZD. Such a combination could be a new approach for the treatment of anxiety. In the LDB-test an anxiolytic activity was observed at an administered concentration of 10 mg/kg EO.

To determine whether this effect was modulated by the GABAergic system, flumazenil was administered to mice treated with EO or diazepam. Flumazenil was able to reverse the anxiolytic effect of the EO and diazepam in the LDB-test, indicating that the EO activity occurs via the $GABA_A$-receptor complex rather than (and not via) the serotonergic system (5-HT_{1A}). But WAY100635 could not reverse the effect and did not affect the EO activity. Therefore, it was suspected that the $GABA_A$-receptor complex was involved because it was antagonised by flumazenil. The significant increase of time spent in the light part of the box, demonstrated by co-treatment with ineffective doses of diazepam and the EO a synergistic effect. After long-treatment in the LBD-test there was a lack of activity, which is possible related to a tolerance induction. Otherwise, no differences could be detected between acute and repeated treatment with the EO in rota-rod test. In summary these results confirmed the traditional use of *C. citratus* in folk medicine and suggested a $GABA_A$-BZD-complex mediated anxiolytic activity of the EO [48].

The anti-stress effect of lemon oil vapour and its regulatory mechanism

The study of Komiya et al. [49] examined the anti-stress action of the vapour of EOs of lemon, lavender and rose using EPM-test, FST and the open-field test (OF-test) in mice. *Citrus limon* EO from the leaves, containing limonene (52.7%) and linalool (1.7%), possessed the strongest anti-stress effect in all 3 tests [36, 49]. Here, too, a special focus was placed on the regulatory mechanisms of the EO to find out which neurotransmitter activity is associated with the anti-stress effect. The mechanism of action of the EO was investigated by pre-treatment with agonists or antagonists of BZD-receptor, 5-HT-receptor, dopamine-receptor and α-2 adrenaline receptors in the EPM test and FST and measured the metabolic turnovers in the brain by HPLC. Flumazenil and apomorhine, a nonselective dopamine-receptor agonist, significantly blocked the anti-stress effect from lemon oil. Compared, agonists and antagonists on the 5-HT-receptor and the α-2-receptor did not affect the anti-stress effect of the EO. Considering that flumazenil, WAY100635, and apomorphine, all blocked lemon oil's effect in the EPM-test the effect may be meditated by either $GABA_A$/BZD, serotonergic, and/or dopaminergic neurotransmissions. In the group treated with lemon EO, there was a significant increase in the number of entries in open arms and the time spent in it ($p<0.0001$). In the same study, lemon oil inhalation reduced locomotion and exploration in the OF-test, which would be suggestive for a sedative effect. These results revealed an anxiolytic, antidepressant-like and sedative effect of inhaled lemon oil vapour in the EPM test, FST and OF-test in mice [36,49].

The anxiolytic-like effect of the EO from *Spiranthera odoratissima*

Spiranthera odoratissima A. St. Hil. (Rutaceae) is known in Brazil as manacá. Its leaves and roots are used in folk medicine for different diseases. β-Caryophyllene, the major component of *S. odoratissima* EO is a sesquiterpene compound with anti-inflammatory properties that has been found in essential oils derived from several medicinal plants. The study of Galdino et al. [50] have examined the behavioural effects of oral treatment with the EO derived from *S. odoratissima* leaves and its major component, β-caryophyllene, using well-validated animal models, the rota-rod, OF, pentobarbital-induced sleep, hole-board, EPM and LDB tests. The main objective of this study [50] was to evaluate the neuropharmacological activity of the EO in the CNS with a special attention to possible anxiolytic-like effects. The authors wanted to assess as well the roles of $5HT_{1A}$ and $GABA_A$/benzodiazepine receptors in these effects. The identification of the active components is necessary in the development of a herbal drug. Swiss male mice received the EO, extracted from dried leaves by hydrodistillation. The major compounds were indentified by GC/MS as β-caryophyllene (20.6%), γ-muurolene (17.7%), bicyclo- germacrene (14.7%), δ-cadinene (13.4%), γ-cadinene (4.6%) and cubenol (3.1%). The positive control group was treated with diazepam 1 mg/kg for anxiolytic effects and 5 mg/kg for sedative and myorelaxant effects. Primarily animals were pre-selected in a training session with the rota-rod test 24 hours before treatment to test on their ability to remain on the bar (at 12 rpm) for 2 minutes, because a deficit of the motor coordination would probably influence the performance in the behavioural tests. Then the mice were treated (p.o) with substance, vehicle (2% Tween 80, 10 mL/kg), EO (125, 250 or 500 mg/kg), β-caryophyllene (50, 100, 200 or 400 mg/kg) or diazepam (1 or 5 mg/kg) respectively. Sixty minutes later, the mice were placed on the bar and the numbers of falls

were evaluated. The results showed that the EO had no significant influence on the motor coordination, contrary to diazepam. In the OF-test EO (500 mg/kg) and β-caryophyllene (50, 100 and 200 mg/kg) increased the crossing frequency ($p<0.05$) as well the time spent in the centre by EO (250 and 500 mg/kg) and β-caryophyllene (200 mg/kg) ($p<0.05$). EO and β-caryophyllene have not changed the number of falls in the rota-rod test. The latency to sleep was reduced by these substances and the sleep time significantly increased depending on the dose of EO and β-caryophyllene. The head-dipping behaviour and number of transitions in the hole-board test, the time spent in open arms in the EPM-test and the time in the light area of the LDB-test had increased significantly in these anxiety tests. These results indicated an anxiolytic effect. To investigate the mechanism of action the animals were pre-treated with flumazenil or NAN-190 (1-(2-Methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine), a 5HT1A –antagonist using the EPM- and LDB-test. These anxiolytic-like effects of EO were significantly reduced by the pre-treatment with NAN-190 ($p<0.05$) but not flumazenil ($p>0.05$). The anxiolytic effect of β-caryophyllene was not blocked by either NAN-190 or flumazenil. The results indicated that the EO of *S. odoratissima* exerted an anxiolytic effect which is mediated by $5HT_{1A}$- but not via the BZD-receptor complex. In addition, β-caryophyllene also acted an anxiolytically contributing to the effects of EO. This anxiolysis, however, did not appear to be mediated by either $5HT_{1A}$, or the BZD-receptor. The effects on the general behavioural test observed in the EO treatment, e.g. impaired spontaneous ambulation, presence of analgesia, are suggestive of centrally effective depressive drugs. As no toxic effects were observed, further doses of up to 1000 mg/kg could be used [50]. Galdino et al. [50] concluded that this EO and its main component showed anxiolytic-like effects without altering motor coordination and seemed to be mediated by 5-HT_{1A}-receptors, while the anxiolytic-like effects of β-caryophyllene seemed to be independent of both receptors [50].

The Menthol and related monoterpenoids effect on $GABA_A$ and Glycine receptors

Positive modulation of LGICs (=ligand-gated ion channels), $GABA_A$- and glycine-receptors, has a profound influence on neuronal activity, often resulting in sedation and anaesthesia. Monoterpenoids, like menthol, camphor, and thujone, are naturally occurring oxygenated C-10 compounds. According to previous works, these ingredients have neuroactive properties by preventing the neuronal intracellular signaling or by modulation of neurotransmitter-gated currents. The study of Hall et al. [51] investigated the modulation of $GABA_A$- and glycine-receptor-currents by stereoisomeres of different monoterpenoid ketones (menthone, camphor, thujone and carvone) and alcohols (menthol and borneol). The receptors were expressed by *Xenopus oocytes*. Approximately 1-3 days after injection in oocytes with the provided cDNA encoding the respective subunits of $GABA_A$- and glycine-receptors, the oocytes were screened for $GABA_A$ or glycine-evoked currents. The two-electrode voltage clamp technique was used to record the results. Oocytes were placed under tension (50 mV) and the respective dissolved drugs were applicated. The measured currents were digitised at 200 Hz, recorded and analysed with the patch clamp software. Utilization with 30 µM GABA and 50 µM glycine induces EC_{20} (EC_{20}= effective concentration that evoked 20% of maximal current) currents. Many different reactions were elicited.

The application of 10 µM monoterpenoid alcohols resulted in a significantly improved EC_{20}-$GABA_A$ response. However, the effect of the ketones was minimal. Small potentializations were showed by camphor, menthone and carvone. Thujone resulted in current inhibition. Menthol showed the most intense positive modulation. The dose dependence on the activity of the receptors must be further investigated. The reactions on the glycine-receptor were positively modulated by the application of the menthol enantiomers.

The current increase of EC_{20}-glycine was thus ordered (starting with the most intense); (-)-menthol and (+)-menthol > (-)-borneol. The menthol stereoisomers also showed the strongest effects on the glycine-receptor. The authors concluded a structural dependence of the monoterpenes and their functional groups (-OH or =O) on the action of the receptors.
In summary, menthol is a potent and stereoselective modulator on the $GABA_A$-receptor, menthol enantiomers enhance the glycine receptor response and thujone diastereomers are $GABA_A$ inhibitors. The effects of the different monoterpenes are an important component of the neuroactive properties and their widespread medical use [51].

Rose Oil

Natural rose oil extracted from rose flowers (*Rosa centifolia* produced in Morocco) possesses anticonflict effects as shown in previous studies, suggesting that the oil exhibits also anti-anxiety properties. Rose Oil contains many ingredients that may be responsible for the pharmacological effects. By GC/MS identified chemicals were myrcene, benzyl alcohol, 2-phenethyl alcohol, citronellol, geraniol, citronellyl acetate, eugenol, geranyl acetate and methyl eugenol. Umezu et al. [52] evaluated the effects of rose oil (i.p) and diazepam (s.c), the positive control substance, in the Geller conflict- and Vogel conflict test, two well-established methods. Both treatments increased the methods of punishment in male mice, used in both experiments. Furthermore, the study examined the effects of the individual constituents of rose oil on the basis of these two tests by treating the animals i.p with authentic standards (myrcene, 2-phenethyl alcohol, citronellol, geraniol and so on). In the Geller-test mice can obtain the food by pressing a lever - in the safe period without electric shock, in the alarm period, every 20th lever press was punished with a shock (50-90V) and a

continuous warning signal was heard all the time. Rose oil or its constituents were administered 20 min before the test. There were also days without treatment to check stability and behaviour. The access to food was limited, to cause a withdrawal situation. In the Vogel test, the mice were individually placed in separate chambers after 2 days of water dehydration and allowed free access to water for 40 minutes. After one week, water was withdrawn (for 2 days) to the same animals again, then rose oil (100-800 mg/kg) or their constituents were administered to them, and the same test was carried out 20 minutes later. Then the mice had free access to water and the number of water licks was recorded. Over 40 minutes the animals were punished with an electroshock every 20th lick, the number of shocks the mice received was also recorded. The experiment with diazepam showed, as expected, significant effects in both tests, namely diazepam increased the response rate during the alarm period and the number of electro-shocked mice. In the case of rose oil, apparent effects could also be observed in both tests. The Geller conflict test indicated that rose oil significantly resulted in an alteration of the response rate during the alarm period in mice ($p<0.05$). The number of electro-shocked mice increased with the application of rose oil ($p<0.01$). All this indicates an anti-anxiety-like effect of the oil, which can explain the use of rose oil to relieve anxiety in aromatherapy. In the other experiment, the effects of the individual components of rose oil were determined using the same two conflict tests. Myrcene, benzyl alcohol and citronellyl acetate did not produce any effects. Geranyl acetate and methyl eugenol exhibited no effect in the Geller conflict test. Geraniol and eugenol resulted in a decreasing response rate during the safe period, but did not influence the response rate during the alarm period. However, 2-phenylethyl alcohol and citronellol, as observed with rose oil, produced an increasing effect on the response rate during the alarm period and increased the number of electro-shocked mice similar to the EO. Since these two substances produced the same anti-conflict effects as EO in both

trials, Umezu et al. [52] concluded that these are the active constituents, which are responsible for this anti-anxiety-like effect. Since rose oil and its cultivation is very expensive and the flowers contain not much EO, it is beneficial to synthesize the two active substances individually, in order to use them as an alternative to relieving anxiety [52]. One year later the study by Almeida et al. [53] investigated the potential anxiolytic effect of inhaled essential rose oil compared to anxiolytic drugs. The behavioural effects of inhalation were assessed in male rats in various concentrations of rose oil emulsion (1%, 2.5% and 5%) in the EPM test and compared with the anxiolytic activity of the diazepam (1.0 and 2.0 mg/kg) treated reference group. The negative control group received saline (i.p). Diazepam was administered i.p to the animals 30 minutes before the experiment. Before the EPM test the animals were placed in odourless chambers. Each rat was exposed to the aroma for 7 minutes. During the 5- minute behavioural test, 5 measurements were made to infer anxiolytic effects. Rose oil produced a similar anxiolytic effect as the reference diazepam. It significantly increased the number of entries and time spent in open arms in the EPM test. The observed behaviour was consistent with other clinical trials. No significant differences between the different EO concentrations and the diazepam treatment were noticed. The results of the EPM test in the study of Almeida et al. [53] suggested the anxiolytic activity of the inhaled EO treatment. However, further studies are necessary to determine the exact mechanism.

Anxiolytic-like effect of Carvacrol

Carvacrol (5-isopropyl-2-methylphenol) is a monoterpenic phenol present in the EO of many plants. It is the main component of the EO fraction of oregano and thyme. Previous studies already showed the antibacterial,

antifungal, analgesic, antioxidant, antimutagenic, antigenotoxic, antispasmodic, antiinflammatory and hepatoprotective effect of carvacrol. But there are no published studies on the central actions of carvacrol. The study of Melo et al. [54] investigated the behavioural and pharmacological effects of carvacrol using the EPM test, rota-rod test, OFT and barbiturate-induced sleeping time test in male mice. Carvacrol was administered orally in different single doses (12.5, 25 or 50 mg/kg) in male swiss mice, one hour before the experiments. The effects were compared with the anxiolytic standard diazepam (1 or 2 mg/kg) and were also evaluated in the EPM test. Flumazenil (2.5 mg/kg), a BZD-antagonist, was administered to determine the possible anxiolytic mechanism of action of carvacrol. The substance had no influence on the spontaneous motor activity in the rota-rod test or on the events in the OFT. In the EPM test, carvacrol significantly increased the observed parameters at all doses. In the barbiturate-induced sleeping time test, carvacrol did not change the sleep latency and sleeping time. However, it is important that flumazenil was able to reverse the effects of diazepam and also carvacrol, indicating that both drugs might exert a similar mechanism of action. Thus, the results showed an anxiolytic potential in the EPM test, which was not influenced by the locomotor activity in the OFT. In conclusion, this study suggested that acute treatment with carvacrol at doses of 12.5, 25 and 50 mg/kg shows anxiolytic effects, which are mediated by the $GABA_A$-receptor. However, carvacrol did not show sedative or myorelaxant properties [54].

Conclusion

The already considerable attention directed at the use of natural EOs from plants is continuing to increase. The usage of most EOs has its origin in folk medicine. On the basis of this study the mechanisms of action of different Eos were explained and their effectiveness regarding to anxiolytic, antidepressant, anticonvulsant and sedative effects examined. The exact mechanism of action could not yet be proved at all EOs, but there are some assumptions that need further research. Lavender oil and its active constituents e.g. linalool, have been investigated by many studies and showed clear anxiolytic effects by both oral and topical application. In various behavioural animal-models the effect of the lavender EO, compared to positive control groups, could be confirmed that possibly acts via the $GABA_A$-receptor/BDZ-complex. However, studies also indicated an effect on VOCCS in patients suffering in GAD. Further studies should also be made with CEO, as one expects an anxiolytic effect of linalool via the NMDA-receptor. The widely used EO of *Citrus aurantium* and its main component limonene also showed an anxiolytic effect in different trials similar to diazepam. Here, too, the $GABA_A$-receptor is believed to be involved. Carvone, the main compound of *L. alba* EO, is specific for the anxiolytic-like effect in rats and suggested the responsibility for the effect as a tranquillizer. It is also supposed that carvone interacts with $GABA_A$-receptors. The demand and the great interest in plant products that exhibit this range of effects are growing apparently on a multitude of different studies in the recent years. Mechanisms of action are further investigated for alternatives to anxiolytic drugs with many side effects. A promising product, Silexan®, is already on the European market.

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