

# Actions of essential oils on the central nervous system: An updated review.

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**ABSTRACT:** The administration of essential oils or their constituents in aromatherapy, complementary medicine, and folk medicine has been known for a long time, and its relevance is steadily growing. In the last few years, many scientific studies were conducted to investigate the effect and the mechanisms of action of these compounds on the central nervous system. The aim of this article is to summarize the literature on this topic published in the period 2008–2010, upgrading a comprehensive review. The major actions discussed are pain, anxiety, learning, memory, attention, arousal, relaxation, sedation and sleep. Furthermore, the effects on mood, behaviour and perception as well as the application of essential oils in the treatment of epilepsy, stress, dementia and Alzheimer's disease are discussed. Copyright © 2011 John Wiley & Sons, Ltd.

**Keywords:** Alzheimer's disease; analgesic effects; anxiolytic effects; arousal; attention; dementia; epilepsy; learning; memory; relaxation; sedation; sleep; stress

## Introduction

The therapeutic use of essential oils (EOs) and/or hydrolates has a long tradition. Even in the Bible they are mentioned for 'mental, spiritual and physical healing'. Nowadays the pharmacological and/or psychological properties of odourants are acknowledged and in many cases the combination of both play an important role.<sup>[1]</sup> However, the utilization of EOs is spread widely. They are used as an adjunct in school medicine, folk medicine, Chinese medicine,<sup>[2]</sup> alternative medicine, aromatherapy and massages, as well as in the cosmetics and perfumes industries, and in food flavourings and cleaning products.<sup>[3]</sup>

A great number of studies exist which refer to the effects of EOs on the central nervous system (CNS).<sup>[1,4]</sup> Several biological properties such as peripheral antinociceptive effects, anticancer effects, antiphlogistic effects, antiviral activity, antioxidative activity, and penetration enhancement are also documented in the literature.<sup>[5]</sup> An update of the latter topics was recently discussed in detail by Adorjan and Buchbauer.<sup>[6]</sup> The scope of this present review is to make a similar update referring to activities of EOs on the CNS, just as analgesic effects, anxiolytic effects, effects on the treatment of stress, effects on learning, memory, attention and arousal, effects on relaxation, sedation and sleep, effects on mood, behaviour and perception, anticonvulsive effects and the treatment of epilepsy, and finally effects on the treatment of Alzheimer's disease and Parkinson's disease. It should be mentioned that this essay is not complete as the goal was to present an overview. Relevant studies within the period 2008–2010 are discussed.

## Analgesic Action

The aim of analgesic drugs is to alleviate pain, acting therefore in various ways either on the CNS or peripheral nervous system. In this section the effects of EOs on the CNS will be treated as Adorjan has already discussed the peripheral antinociceptive activity of essential oils.<sup>[6]</sup>

Analgesics are classified into two main groups: opioids (e.g. morphine, codeine) which bind on opioid receptors in the CNS, and non-opioids (e.g. aspirin and other non-steroidal anti-inflammatory drugs, NSAIDs), which inhibit the cyclooxygenase enzyme (COX) in the periphery. Opioids are very effective analgesics; however, they have many undesirable side effects such as nausea, vomiting, pruritus, constipation, miosis, drowsiness, respiratory depression and, finally, the drug tolerance.<sup>[7]</sup> So, there are a few studies which try to find alternatives to the opioid treatment of pain, such as the application of EOs.

(–)-Linalool, a monoterpene alcohol with a woody lavender note, is a component of the EO in several aromatic plants, for example *Lavandula angustifolia* Mill., and *Salvia sclarea* L. (less than 20%), both Lamiaceae.<sup>[8]</sup> Recent studies examined the anti-inflammatory, antihyperalgesic and antinociceptive activities of (–)-linalool, but the following study by Batista *et al.*<sup>[9]</sup> analysed the contribution of the glutamatergic system in the antinociception caused by (–)-linalool in mice. The authors characterized the nociceptive answer by observing how often the mice licked the injected hind paw or bit the target organ following glutamate receptor agonist injections. After (–)-linalool had been applied, whether intraperitoneally (i.p.) (10–200 mg/kg), orally (5–100 mg/kg) or intrathecally (0.1–3 µg/site), a dose-dependent inhibition of glutamate-induced nociception was observed (70 ± 4; 72 ± 7 and 74 ± 8%). Intraplantar injection of (–)-linalool showed less inhibition of glutamate-induced nociception (49 ± 9%). Moreover, (–)-linalool (200 mg/kg) applied i.p. significantly reduced the biting responses of the mice caused by intrathecal injection of glutamate,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA), substance

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P (SP), *N*-methyl-D-aspartic acid (NMDA) and kainate with inhibitions of  $89 \pm 6\%$ ,  $739 \pm 11\%$ ,  $85 \pm 4\%$ ,  $98 \pm 2\%$ ,  $52 \pm 15\%$ , respectively. Nevertheless, (–)-linalool did not inhibit nociception caused by intrathecal injection of *trans*-amino-cyclopentane-dicarboxylic acid, an agonist of glutamate-induced release of pain mediators. Therefore, the authors concluded that (–)-linalool produced marked antinociception against glutamate induced pain in mice, probably by mechanisms operated by ionotropic glutamate receptors AMPA, NMDA and kainate.<sup>[9]</sup>

The monoterpene ketone (–)-carvone is the main active substance of *Mentha spicata* L. (Lamiaceae). Research by Gonçalves *et al.*<sup>[10]</sup> demonstrated its effect on pain using different experimental models of pain in order to find out whether (–)-carvone might be involved in the nervous excitability provoked by other monoterpenes. Mice treated with (–)-carvone showed a significant reduction of writhes in the acetic acid-induced writhing test when doses of 100 and 200 mg/kg were applied. It was also observed that (–)-carvone prevented the licking response of the injected paw after doses of 100 and 200 mg/kg were applied i.p. to mice in the formalin test. Naloxone (an opioid antagonist) applied subcutaneously at a dose of 5 mg/kg did not show any influence on the antinociceptive activity of (–)-carvone (100 mg/kg). It follows from this observation that the opioid system did not participate in the analgesic effects of (–)-carvone, whereas (–)-linalool (a monoterpenoid as well) is involved in a central antinociceptive effect associated with glutamate NMDA receptors, as mentioned in the study above. So, a clear statement would be the involvement of (–)-carvone in such a nonopioid central mechanism.<sup>[10]</sup>

A synthetic intermediate of (–)-carvone, namely hydroxy-dihydrocarvone (HC), can be obtained by hydration of (–)-carvone. HC shows chemical and structural similarity to (–)-carvone and other monoterpenes with psychopharmacological activity and for this reason a study was designed to determine the possible central analgesic effect of i.p. applied HC as well as any possible effects on the opioid system in mice. Therefore, de Oliveira and colleagues<sup>[11]</sup> used the tail immersion test (the tail was kept in water at 50 °C and the reaction time was measured), the hot plate test (animals were placed on a hot plate at 47 °C and the responses were observed), the formalin test (formalin was injected into the right hind paw and duration of paw licking was measured) and the catalepsy test (forepaws were placed on a 6 cm high horizontal bar while the hind paws stayed on the floor; latency to step down was recorded). In the immersion test, the reaction time was longer in HC-treated mice (200 mg/kg). Similarly, in the hot plate test HC (100–200 mg/kg) prolonged the time mice remained on the plate. In the formalin test HC showed central antinociceptive activity and was effective with significant dose-dependent response (50–200 mg/kg). However, HC did not provoke catalepsy (25–200 mg/kg). In order to analyse the mechanism of action, mice received 5 mg/kg naloxone s.c. before the treatment was started. The effect of HC on the formalin and the hot plate test was not antagonized by naloxone, thus showing that HC exerted an antinociceptive effect on the CNS without producing catalepsy. However, non-participation of the opioid system in the modulation of pain was assumed.<sup>[11]</sup>

Opioids and NSAIDs are also used for treatment after surgical interventions, e.g. after laparoscopic adjustable gastric banding of morbidly obese patients. To avoid serious side effects, complementary analgesic techniques could be utilized. Therefore, a randomized placebo controlled study carried out

by Kim *et al.*<sup>[12]</sup> investigated the effectiveness of lavender aromatherapy in decreasing the opioid demand after laparoscopic adjustable gastric banding. Fifty-four patients participated. The aroma group was treated with lavender oil applied via an oxygen face mask, whereas the control group received unflavoured baby oil. Post-operative pain was medicated with morphine. Numerical rating scores from 0 to 10 were used to measure the level of pain. Even the sedation was estimated using the Observer Assessment of Alertness/Sedation scale (OAA/S, from 0 to 5). The amount of opioids received, numerical rating scores, OAA/S, post-discharge time from the anaesthesia care unit and possible side effects were considered. At the end of the study more patients from the placebo group asked for analgesics to treat the post-operative pain (22 of 27, 82%) than those of the lavender group (12 of 26, 46%). No influence on the demands for post-operative antiemetics, antihypertensives or discharge time was observed. With this explanation the authors stated that lavender aromatherapy can be utilized to reduce the application of opioids in the immediate post-operative period. However, further studies are needed to evaluate the effect of this complementary therapy on the incidence of respiratory complications, constipation, discharge time or the influence of lavender oil therapy on other operations.<sup>[12]</sup>

As mentioned before, morphine tolerance is a serious problem during treatment with opioids. Haghparast *et al.*<sup>[13]</sup> tried to identify the influence of fruit essential oil (FEO) of *Cuminum cyminum* L. (Apiaceae) on the development and expression of morphine tolerance and dependence in mice. Thus, morphine was injected three times daily for 3 days. For the experiment either FEO (0.001, 0.01, 0.1, 0.5, 1 and 2%, 5 ml/kg, i.p.) or Tween-80 (5 ml/kg, i.p.) were administered 60 min before each application of morphine (for acquisition) or before the last application of morphine on the test day (for development). On the fourth day (test day) the authors measured morphine tolerance by tail flick, before and after administration of a single dose of morphine (50 mg/kg, s.c.), and by counting the jumps after injection of naloxone (5 mg/kg, i.p.). The findings revealed that FEO can only decrease morphine tolerance and dependence at the dose of 2%, whereas a significant effect on the expression of morphine tolerance (1 and 2%) and dependence (0.5, 1 and 2%) was dose-dependent. It should be mentioned that solely a FEO injection did not show any analgesic effects. However, these findings justified the conclusion that the VO of *C. cyminum* L. may improve morphine tolerance and dependence in mice.<sup>[13]</sup>

In traditional Chinese medicine the EO of the rhizome of *Ligustici Chuanxiong* Hort. (Apiaceae) has been applied for many years to treat headache, but a chemical analysis has been carried out only recently.<sup>[14]</sup> According to this study, ligustilide – a phthalide derivative and the most abundant constituent – could be detected in rat brain upon nasal administration even after 5 min. Thus, the analgesic and neuroprotective effect of this EO can be explained. Research by Peng *et al.*<sup>[15]</sup> tested the pharmacodynamic action of the volatile oil of this drug in mice and used not only the hot-plate test and the acetic acid writhing test, but also observed the locomotor activity and a prolongation in sleeping time (induced by sodium phenobarbital, 35 mg/kg). The hot-plate test and the writhing test showed a significant elevation of pain threshold; furthermore, sleeping time was prolonged and a reduction in locomotor activity was measured. Further studies in rabbits pre-treated with nitroglycerin to cause headache, and rabbits with headache due to hot radiation,

showed a significant increase of the pain threshold. In addition, expression of the c-fos gene in the brain stem and hypothalamus was remarkably inhibited and plasma calcitonin gene-related peptide was decreased after the volatile oil of Ligustici Chuanxiong (LCVO) had been administered. Following dosages also raised the level of plasma 5-HT. This seemed to confirm the potential of LCVO in the treatment of headache.<sup>[14]</sup>

Pain is a common reaction of the body upon hurt, damage and inflammation which originally acts as a warning system. However, in many cases long-lasting, unopposed pain often renders itself independent and thus becomes a problem that has to be combatted with constantly increasing doses of an analgesic. Therefore, the search for a complementary (even daily) therapy which avoids synthetic drugs is a worthwhile goal. In this respect EOs play an important role, which has been emphasized put in this section. Remarkably, there is only one study with human subjects;<sup>[12]</sup> the remainder of the cited papers used animals to find complementary methods of alleviating pain. In all of these studies the possible mechanism of the analgesia has been discussed by the authors. Single EO constituents were administered to animals in the studies by Batista *et al.*<sup>[9]</sup> and Gonçalves *et al.*,<sup>[10]</sup> whereas de Oliveira *et al.*<sup>[11]</sup> dealt with hydrogenated hydroxycarvone, a synthetic product. Aromatherapy is sometimes regarded doubtfully; however, we hold the study by Kim *et al.*<sup>[12]</sup> in esteem on account of the sound performance of the assessment. On the other hand one of the cited papers<sup>[14]</sup> deals only with a volatile oil (and not an EO) within traditional Chinese medicine – another complementary and often, also, alternative medicinal method – and reports on the analgesic effect of an extract used for centuries against headache by inhibiting expression of the c-fos gene in the brain stem and hypothalamus.

## Anxiolytic Action

Anxiety is explained as a psychological and physiological state marked by cognitive, somatic, emotional and behavioural elements. These components, together, provoke a disagreeable emotion associated with fear, worry and concern, as well as uneasiness. Anxiety can appear suddenly without any triggering stimulus and therefore can be a huge barrier in everyday life. In the state of anxiety the body prepares to cope with a menace; as a result, the symptoms are heart palpitations, tension, nausea, chest pain, shortness of breath, sweating, trembling, pale skin, papillary dilatation and so on. In this moment the body is in 'fight or flight' response and in severe situations this can be experienced as panic.<sup>[16]</sup> Generalized and persistent anxiety often leads to the use of benzodiazepines, even though these drugs have several side effects (e.g. sedation) and a high potential for drug abuse. Therefore alternatives (e.g. aromatherapy) are needed.<sup>[17]</sup> Popular anxiolytic oils are, for example, lavender (*Lavandula angustifolia* Mill., Lamiaceae), rose (*Rosa damascena* Mill., Rosaceae), orange (*Citrus sinensis* L.), bergamot (*Citrus bergamia* Risso.), lemon (*Citrus limon* L.) (which are all from the Rutaceae), sandalwood (*Santalum album* RBr., Santalaceae), clary sage (*Salvia sclarea* L., Lamiaceae), roman chamomile (*Anthemis nobilis* L., Asteraceae) and geranium (*Pelargonium* spp., Geraniaceae).<sup>[18]</sup>

Lavender remedies are the most used phytotherapeutics in the treatment of anxiety. One of the main components of lavender essential oil is linalool, a monoterpene alcohol. Cline *et al.*<sup>[19]</sup> started research on male Sprague–Dawley rats to

investigate the anxiolytic effects of linalool. Therefore, 44 rats were separated into four groups: a control, a linalool, a midazolam (a benzodiazepine, for positive control) and a linalool and flumazenil (a benzodiazepine antagonist) group. The authors used the elevated plus maze test (EPM) to examine the anxiolytic behaviour. Thereby, rodents are placed into a plus-shaped elevated apparatus with two enclosed and two open arms. Anxiety reduction is measured by an increase of time spent on the open arm, because rodents instinctively avoid open spaces.<sup>[20]</sup> The neurohormonal–physiological component was measured by determining the levels of serum catecholamine and corticosterone. The results of the study did not show linalool-induced anxiolysis by modulation of  $\gamma$ -aminobutyric acid (GABA<sub>A</sub>) receptor, but linalool probably modulated motor movements and locomotion.<sup>[19]</sup>

Another randomized double-blind study dealt with the effects of orally administered lavender essential oil on responses to anxiety provoking film clips. Lavender capsules (100, 200  $\mu$ l and placebo) were administered on 97 test persons, who viewed neutral and anxiety evoking film clips. Mood, Positive and Negative Affect Scale, anxiety, State Trait Anxiety Inventory, heart rate, galvanic skin response as well as heart rate variation were measured. In neutral films the 200  $\mu$ l lavender dose could decrease anxiety, galvanic skin response and heart rate, and increase heart rate variation. In anxiety-evoking film clips lavender was mildly beneficial in females (only on heart rate variation), whereas in males galvanic skin response increased. So it can be concluded that lavender showed moderate anxiolytic effects on humans, but did not have any effect on high anxiety.<sup>[21]</sup>

Aromatherapy is also often used for patients who are petrified of surgical interventions or dental treatments. Therefore, lavender EO is a good choice as proved by several studies. One of these studies aimed to determine whether lavender oil is more effective than standard care to reduce pre-operative anxiety in surgical patients. One hundred and fifty adults were divided randomly into either a control group (standard care), an experimental group (standard care including essential oil) or a non-aroma group (standard care plus jojoba oil). The anxiety on admission and operating room transfer was evaluated via visual analogue scales. As a result, the lavender group showed a remarkable lower anxiety on operating room transfer, which suggested that lavender was a simple, economical and safe alternative and helped to ameliorate pre-operative anxiety and enhanced patient satisfaction. The authors mentioned that further studies concerning the use of lavender in the post-operative phase are welcome.<sup>[22]</sup>

Hoya *et al.*<sup>[23]</sup> investigated the use of a new non-pharmacological intervention (an optimal soothing environment, OSE) to decrease anxiety in patients undergoing gastroscopy. Fifty subjects were randomly divided into a control group (24 patients) and an OSE group (26 patients). The study lasted for 6 months and took place in a 150 bed acute-care hospital in Japan. Patient anxiety was evaluated via the Face Scale score. For each patient, systolic blood pressure was measured before and after gastroscopy and was compared with blood pressure upon arrival at the hospital. For the OSE, a safe essential oil burner with lavender aroma and a digital video disk programme entitled 'Flow' (manufactured by NHK software) were provided to the patients waiting for gastroscopy. In the control group the score for self-assessed anxiety level was remarkably higher before gastroscopy but returned to baseline afterwards and values for systolic blood

pressure before and after the gastroscopy were significantly higher than those on arrival at the hospital and the baseline values. In contrast, the score was not enhanced before gastroscopy and systolic blood pressure was not increased in the OSE group. Hence, providing an OSE before and during the gastroscopy could minimize patient anxiety and would be a simple, economic and low-risk method to alleviate anxiety.<sup>[23]</sup>

The effects of lavender scent on anxiety levels dental patients were investigated in a cluster randomized controlled trial by Kritsidima *et al.*<sup>[24]</sup> Anxiety in 340 patients was evaluated either under the influence of lavender or without treatment while waiting for dental treatment. Current anxiety was assessed via State Trait Anxiety Indicator and generalized dental anxiety via the Modified Dental Anxiety Scale. The evaluation detected comparable moderate levels of generalized dental anxiety in both groups, but only the lavender group showed remarkably lower current anxiety compared to the control group. So, even if anxiety about future dental visits cannot be avoided, lavender scents could help to reduce the anxiety state in dental patients.<sup>[24]</sup>

The efficacy of Silexan, an innovative oral lavender oil capsule preparation, versus a benzodiazepine was studied recently in a controlled clinical trial by Woelk and Schläfke.<sup>[17]</sup> Adults had to take Silexan or lorazepam for 6 weeks. The severity of anxiety was objectively measured by the Hamilton Anxiety Rating Scale (HAM-A total score) between baseline and week 6. At the end of the study it was demonstrated that Silexan was able to alleviate generalized anxiety and was comparable to a common benzodiazepine such as lorazepam. 'Somatic anxiety' and 'psychic anxiety', two HAM-A sub-scores, were also decreased in both groups. Other sub-scores, for example the Self-rated Anxiety Scale, the Penn State Worry Questionnaire, the Clinical Global Impressions of Severity Disorder and a sleep diary showed equal positive effects of the two medications. All in all, these data confirmed that Silexan was as effective as lorazepam in adults. It showed no sedative effects or potential for drug abuse and therefore could be a safe and well-tolerated alternative to benzodiazepines in the treatment of generalized anxiety.<sup>[17]</sup>

In a further study, the pharmacophysiological effects of Shirodhara with medicated sesame oil which was enriched with the EO of lavender were investigated. Shirodhara is a common Ayurvedic oil-dripping treatment which makes use of medicated herbal sesame oils. Previous reports had already shown that Shirodhara with plain sesame oil had anxiolytic effects and an altered state of consciousness (ASC) on healthy subjects. Sixteen healthy participants were randomly divided into either a plain sesame oil treatment group (plain Shirodhara), an aroma sesame oil group (0.3 vol%, lavender Shirodhara) or a control group in the supine position. The oil was applied by a robotic oil-dripping system. To evaluate the psycho-physiological changes caused by Shirodhara, heart rate, skin temperature of the back of hands and feet, anxiety and ASC were monitored. In the aroma group the most anxiolytic and ASC effects and the largest increase in foot skin temperature were observed. The relation between anxiolysis and ASC and the relation between psychological effects and the increased foot skin temperature were larger in the aroma Shirodhara group than in the other two groups. The authors presumed that the psycho-physiological effects of lavender Shirodhara were based on three mechanisms: first, the well-established relaxing action of lavender EOs mediated by olfactory nerves; second, the pharmacological

action of substances when absorbed through the skin in the sesame or lavender oil; and third, the physiological effect of sesame oil dripping on the forehead induced by the somato-autonomic reflex through thermosensors or pressure sensors via the trigeminal cranial nerve. This complicated pharmacophysiological action of Ayurvedic oil-dripping treatment could be a helpful model for future therapies.<sup>[25]</sup>

The influence of inhaled linalool on anxiety, social interactions and aggressive behaviour in mice was the focus of research by Linck and his Brazilian co-workers.<sup>[26]</sup> Moreover, the influence on memory was assessed. With linalool, social interactions were enhanced whereas aggressive behaviour was reduced. The light/dark test (observation whether the animals stay in the dark or in the lit chamber; the stay in the lit chamber represents anxiolysis because mice have an innate aversion to illuminated areas) confirmed an anxiolytic effect. A bad influence on memory was only observed at higher doses of linalool. These findings were interpreted as indicating anxiolysis and relaxation by linalool.<sup>[26]</sup>

Besides lavender EO, neroli EO is well known for its anxiolytic action. Neroli oil is a distillate obtained from the flowers of bitter orange (*Citrus aurantium* L., Rutaceae).<sup>[27]</sup> Chen *et al.*<sup>[28]</sup> reported on its anxiolytic effects on gerbils when inhaled. To assess the levels of anxiety, forced swimming tasks and locomotor activity were measured. Therefore, the duration time of the forced swimming test was compared to the total distance as well as the duration time in the central and peripheral areas between the aroma and the control group. The 1,4-benzodiazepine derivative Xanax<sup>®</sup> (in the USA; Alprazolam in Europe), an anxiolytic substance, served as positive control. Although inhalation of neroli oil as well as the treatment with Xanax<sup>®</sup> showed anxiolytic actions in the behavioural tests, the mechanisms of anxiolytic effect responses were not understandable for both. However, with this study the use of neroli in the treatment of anxiety is evidence based.<sup>[28]</sup>

Another study was designed to assess the effect of either music alone, aromatherapy with neroli essential oil alone, or a combination of both on anxiety levels of adults who accompanied their children to a paediatric appointment. The study lasted 28 days and adults were either assigned to a non-intervention group, a classic music group (60–70 beats per min), an aromatherapy group or a group with both aromatherapy and music. A survey was made of the test persons, including the Spielberger State Anxiety Inventory with questions of whether they took notice of aroma and music or not. A total of 1104 questionnaires were completed. Anxiety levels were significantly lower when adults listened to music, but there was no difference in anxiety levels on those days when aromatherapy was present compared to those days when aromatherapy was not present. So these results suggested that music was a simple and helpful method to alleviate anxiety of patients in an emergency department waiting room. The absence of the aromatherapy potency could be explained by environmental conditions or the inexact application of the EO. So the author suggested future studies to either confirm or not confirm its effectiveness in this setting.<sup>[29]</sup>

Anxiolytic-like effects of sweet orange aroma (*Citrus sinensis* L., Osbeck, Rutaceae) in Wistar rats were recently analysed.<sup>[30]</sup> After the male rats had been exposed to 100, 200 or 400 µl of orange aroma they had to carry out behavioural tests, namely the EPM and the light/dark test. The results revealed anxiolysis at all doses of sweet orange aroma at least in one test; at 400 µl



even anxiolysis in both tests could be observed, because the exploration activity in the open-arms region, respectively the stay in the lit chamber was enhanced. Further tests with the EO of *Melaleuca alternifolia* Maiden & Betche, Cheel, Myrtaceae, showed that these results were not influenced by any other exposure, because no anxiolytic effects were observed, so the anxiolytic activity of sweet orange EO was shown.<sup>[30]</sup>

The EO of the shell flower leaves (*Alpinia zerumbet* [Pers.] B.L. Burt and R.M. Sm, Zingiberaceae, EOAZ) is used in phytotherapy to treat neuropsychiatric symptoms such as anxiety, depression and stress when associated with and imbalance of reproductive hormone in women. Murakami *et al.*<sup>[31]</sup> carried out gas chromatography–mass spectroscopy (GC/MS) analysis and identified the composition of EOAZ via retention indices, mass spectra and comparison with standards and thus found five main components (Table 1). The influence of EOAZ on mice was examined by behavioural observations and the anxiolytic activity was tested in the EPM test. Results demonstrated that inhaled EOAZ led to unique jumping behaviours in the animals. To analyse the behavioural regulatory mechanism of EOAZ, either 5-hydroxytryptophan (which increases serotonin concentrations and therefore lightens mood) or fluoxetine (an antidepressant) were injected i.p. at doses of 10 mg/kg before the inhalation of EOAZ. After this pre-treatment jumping frequencies significantly decreased. Furthermore, experiments in the EPM confirmed the anxiolytic action of EOAZ.<sup>[31]</sup>

A similar author team continued further investigations concerning EOAZ and anxiolytic effects in 2010. The EPM, the light/dark test and the open-field test were used. The last test is based on the natural behaviour of rodents, which avoid open fields (i.e. there are no hiding places). All behavioural tests indicated anxiolytic effects after inhaling EOAZ. This was at most observed in the EPM test. Regarding pharmacokinetics, the EO of the shell flower mainly accumulated in the kidney, so mostly it was not necessarily distributed to organs throughout the body in the same ratio. It would be essential to consider tissue distribution in order to explore the effects of EO inhalation.<sup>[32]</sup>

The behavioural effects of the monoterpene phenol carvacrol (CVC) were examined in several animal models such as the EPM test, the open-field test, the barbiturate-induced sleeping time test or the Rotarod test. The last test measures the influence of a substance on the locomotor system by determining how long animals can stay on a rotating cylinder. CVC at 12.5, 25 or 50 mg/kg was administered orally while diazepam was used as a standard and flumazenil was used to determine the mechanism of action of CVC. At all three doses, CVC did not influence the spontaneous motor activity in the Rotarod test, or the number of squares in the open-field test, but it decreased the amount of

grooming. The EPM test demonstrated that flumazenil was able to reverse the effects of diazepam and CVC, but CVC did not prolong sleeping time and did not alter sleep latency. Taken together, these findings suggested that CVC really had anxiolytic effects but it did not impair locomotor activity.<sup>[33]</sup>

Pharmacological studies have already shown that black cumin seeds (*Nigella sativa* L., Ranunculaceae) have effects in the CNS. Perveen *et al.*<sup>[34]</sup> investigated its anxiolytic effects in rats and therefore made use of the EPM and the open-field test. The oil was administered daily for 4 weeks and the rats exhibited an increase in open-field activity; anti-anxiety effects were observed in the EPM as well. Moreover, the authors noted that orally administered black cumin oil enhanced the cerebral concentration of serotonin but lowered the cerebral concentration of 5-hydroxyindole acetic acid (5-HIAA), the main metabolite of serotonin in the human body. Brain and plasma tryptophan levels increased as well. This demonstrated the anxiolytic activity of black cumin oil.<sup>[33]</sup>

As mentioned in the literature, aromatherapy massages can relieve anxiety in cancer patients. Between 13.9% and 25% of all cancer patients also suffer from anxiety disorders. Seventy-five per cent of these anxiety disorders are non-pathological anxieties which can seriously influence the quality of life.<sup>[35]</sup> Recently, an Italian research team reported on the neuropharmacology of the essential oil of *Citrus bergamia* Risso (Rutaceae, BEO). This oil is commonly used in aromatherapy to alleviate symptoms of cancer pain, mild mood disorders and stress-induced anxiety. The results of the study showed that BEO released exocytotic and carrier-mediated discrete amino acids with neurotransmitter function in the mammalian hippocampus. This supported the deduction that BEO could interfere with normal and pathological synaptic plasticity. There was also some neuroprotection in the course of experimental brain ischaemia and pain. These data explain the common use of BEO in complementary medicine, but further research and translation into clinical settings is needed.<sup>[36]</sup> Imanishi *et al.*<sup>[37]</sup> examined the influence on psychological and immunological parameters in 12 patients with breast cancer in an open semi-comparative study. The probands received a total of eight aroma massages which lasted 30 min over a period of 4 weeks. The results during the aromatherapy massage and 1 month after the massage sessions were compared to a waiting control period 1 month before the massages. The State-Trait Anxiety Inventory test showed that anxiety was reduced in just one aromatherapy massage lasting 30 min, and also was reduced in the eight sessions using the Hospital Anxiety and Depression Scale test. Moreover, the immunological state was improved. Nevertheless, the authors suggested that further studies were needed in order to confirm the anxiolytic properties of aromatherapy massages in patients with breast cancer.<sup>[37]</sup> The effects of aroma hand massage on pain, state anxiety and depression in hospice patients with terminal cancer was the focus of other research.<sup>[38]</sup> Fifty-eight probands participated in the test with a non-equivalent control group pre-test–post-test design. Twenty-eight subjects with terminal cancer were in the experimental group and received an aroma hand massage; the other 30 subjects were in the control group and received a general oil hand massage. The aroma hand massage lasted 5 min on each hand for 7 days. The aroma blend was a mixture of bergamot, lavender and frankincense in the ratio of 1:1:1 and was diluted with sweet almond carrier oil (1.5%). Likewise, the control group received 5 min massages for 7 days on each hand

**Table 1.** Chemical composition of *Alpinia zerumbet* essential oil, according to Murakami *et al.*<sup>[31]</sup>

Constituent	Per cent
<i>p</i> -Cymene	28.0
1,8-Cineole	17.9
Terpinen-4-ol	11.9
Limonene	6.3
Camphor	5.2
Other compounds	30.7

but this time the sweet almond carrier oil did not contain any aroma. As a result, the aroma group showed more remarkable differences in the changes of pain score and depression than the control group. Therefore the authors concluded that the aroma hand massage even had a helpful effect on pain and depression in hospice patients with terminal cancer.<sup>[38]</sup> Another study<sup>[39]</sup> investigated whether aromatherapy has a positive effect on health problems and complaints such as sleeping disorders, anxiety, sadness and depression, as well as pain, colds and flu, etc. Thirty-six nurses (all women, aged 25–63 years) from two psychiatric hospitals in Trondheim participated in a randomized, controlled study. The effect of the treatment was measured by a Norwegian version of the Ursin's Subjective Health Complaints Questionnaire. Because of financial reasons, the selection of essential oil was limited (Table 2). The nurses were treated with a full body massage lasting 90 min. There was a significant decrease in Subjective Health Complaints (SHC) score in the aroma group but an increase in the control group. These data emphasized that in the treatment group fewer health complaints were reported compared to the control group. These results could possibly have consequences for health problems in the workforce and a certain economic value.<sup>[39]</sup>

Treatment of anxiety is often the field of complementary or even alternative therapies, especially aromatherapy. Therefore, it was very important to evaluate the large number of papers (found in data bases) as to their scientific background and performance. Scientifically weak studies were not accepted for this treatise. Nevertheless, 11 of the cited papers deal with aromatherapeutic practices and we regard them as valuable complementary therapies.<sup>[17,21–25,35–39]</sup> If an aroma massage with an EO or an EO mixture is able to significantly reduce the anxious feelings of cancer patients (either before surgical intervention or especially afterwards during the healing process wished for), in particular, then all arguments against such an aromatherapeutic treatment have lost their justification.<sup>[35–39]</sup> Also a paper reporting on an Ayurvedic treatment shows the remarkable anxiolytic effects of EOs<sup>[25]</sup> (especially lavender EO) which could be attributed to an altered state of consciousness and a somato-autonomic reflex via the trigeminal cranial nerve. Some of the other papers of this section tested the anxiolytic activity mainly of linalool and carvacrol using animal experi-

ments. Two studies contradict each other, in one case when gerbils (in particular, recently more often used rodents) showed less timid behaviour after treatment with neroli oil;<sup>[28]</sup> and the other when children were treated with the same oil but were also listening to music. Here the 'music therapy' helped to alleviate the anxiety of the young patients in an emergency department waiting room.<sup>[29]</sup> Summarizing all these results it is evidenced since a long time that, especially on this somatic-emotional field, EOs proved to be a very helpful and a simple, cheap and gladly accepted complementary and/or alternative therapy.

## Effects of the Treatment of Stress

The term 'stress', itself, was first mentioned by Hans Selye in 1930. In psychology and biology 'stress' means a situation in which an organism cannot cope with emotional or physical demanding situations, whether real or imagined.<sup>[40]</sup> Sustained stress can cause illness and mental disorders and concerns humans as well as animals. The symptoms are increased alarm and adrenaline production, abnormal fatigue, muscular tension, excitability, lack of concentration and also several physiological reactions like headache and raised heart rate.<sup>[41]</sup>

Toda and Morimoto<sup>[42]</sup> reported on the effect of lavender aroma on salivary endocrinological stress markers. As the title suggests, the team was working on the abatement of stress by lavender EO and therefore measured the sensitive salivary endocrinological stress markers cortisol and chromogranin A (CgA).<sup>[43]</sup> Thirty healthy students divided into an aroma group and a control group had to carry out arithmetic tasks for 10 min followed by a break of 10 min. During the tests, the aroma group (16 students) were exposed to EO of lavender in the air. Saliva samples were taken directly before and after the arithmetic tests as well as 5 and 10 min after them; finally, cortisol and CgA levels were determined by an enzyme-linked immunosorbent assay. The results of the aroma group showed that levels of CgA determined 10 min after the tasks were significantly lower compared to these assessed directly after the tasks, whereas the control group showed no similar changes. Concerning the levels of cortisol, no changes were noticed in both groups. Thereupon the authors concluded that the EO of lavender had an anti-stress effect.<sup>[42]</sup>

Likewise the above-mentioned paper, the 'effects of aromatherapy on stress and stress responses in adolescents' were studied by Seo,<sup>[44]</sup> who used a two-group cross-over design. The aroma group inhaled EO, whereas the control group was treated with a placebo as they inhaled carrier oil using a necklace. Thirty-six female high-school students had to solve Fisher's exact test, a *t*-test as well as a paired *t*-test using the SPSS/WIN program to investigate the effects of aromatherapy. The author discovered that the stress levels were significantly decreased by students who received the aroma treatment compared to the placebo group, inhaling the carrier oil. The stress reaction apart from salivary IgA levels decreased after the students were treated by aromatherapy. Therefore, the author rated aroma inhalation as a possible method of effective stress management for students at high school and recommended that this programme was also used in clinical practice.<sup>[44]</sup>

Since it is known that nurses have a very high-stress job, there exist several studies which refer to the management of occupational stress in this position. In an interesting study, Cooke *et al.*<sup>[45]</sup> described the influence of aromatherapy

**Table 2.** Essential oils used in the experiment by Hansen and Hansen<sup>[39]</sup>

Oil	Plant of origin
Benzoin	<i>Styrax benzoin</i> Dyrand., Styracaceae
Bergamot	<i>Citrus bergamia</i> Risso, Rutaceae
Black pepper	<i>Piper nigrum</i> L., Piperaceae
Clary sage	<i>Salvia sclarea</i> L., Lamiaceae
Fennel	<i>Foeniculum vulgare</i> (L.) Mill., Apiaceae
Geranium	<i>Pelargonium graveolens</i> L'Hér., Geraniaceae
Juniper berry	<i>Juniperus communis</i> L., Cupressaceae
Lavender	<i>Lavandula angustifolia</i> Mill., Lamiaceae
Lemon	<i>Citrus limon</i> (L.) Burm f., Rutaceae
Patchouli	<i>Pogostemon patchouli</i> Pill., Lamiaceae
Peppermint	<i>Mentha x piperita</i> L., Lamiaceae
Rosemary	<i>Rosmarinus officinalis</i> L., Lamiaceae
Rosewood	<i>Aniba roseaodora</i> Ducke, Lauraceae

massage, including music, as a possible way of coping with job-related stress and anxiety encountered by nurses in an emergency department. Moreover, the study tried to determine whether there are any differences between a summer and winter 12-week massage plan because, normally, the winter months are much more stressful, because people with cardiac and respiratory diseases are hospitalized more often in winter months. The design of the study was a one-group pre-test and post-test with random assignment. The staff's stress was analysed before and after 12 weeks of aromatherapy massage with music and the stress was measured before and after each session. Sick leave was assessed as well. All in all, 365 massages were performed over two 12-week periods, one in summer and one in winter. The results showed that aromatherapy with music significantly decreased anxiety for both seasonal periods. Anxiety before the massage was higher in winter compared to summer. The authors did not find any changes in sick leave or workload, or any difference in the working stress level of nurses following the two 12-week periods of massage. The results showed that emergency nurses were more anxious in winter compared to summer but this might not be related to sick leave or workload. In conclusion, aromatherapy massage with music could significantly decrease emergency nurses' anxiety. Increased levels of anxiety and stress can cause physical or emotional diseases of emergency nurses and there should be a possibility of a support mechanism such as on-site massages in the hospitals.<sup>[45]</sup> An American scientist-team observed in another study that examinations in nursing schools partly provoked stress, which could prevent the students from realizing their goals of graduation. But this stress caused by tests could be lowered when the 40 nursing students used a combination of EOs from the common lavender (*Lavandula angustifolia* Mill.), and rosemary (*Rosmarinus officinalis* L.) both Lamiaceae plants. The students had to pass some examinations with a grade of 80% to complete the master's programme in advanced nursing practice. The first test was without aromatherapy to obtain some control data. In the second examination lavender aromatherapy was used before and during the test. In the third examination the students inhaled rosemary EO with a camphor phenotype before and during the test. The stress reduction was proved by lower scores on Test Anxiety Scale, pulse rate, blood pressure, just as personal statements. The students themselves preferred the rosemary aromatherapy in comparison to the lavender aromatherapy, because the latter 'made things more relaxed and even fuzzy at times'.<sup>[46]</sup> Pemberton *et al.*<sup>[47]</sup> investigated the effects of EOs of *L. angustifolia* Mill. and *Salvia sclarea* L. on work-related stress of nurses working in an intensive care unit. As a result, the perception of stress in the intervention group was significantly lower while the nurses had to do three 12-h shifts.<sup>[47]</sup>

Lavender EO also has an influence on the stress of babies.<sup>[48]</sup> Newborns already show aroma preferences, such as breast milk aroma, but show a negative reaction to acetic acid odour. For the study, mothers and their babies were randomly divided into three groups: a lavender bath oil group (group 1), a non-aroma bath oil group (group 2), and a lavender bath oil group of mothers who received instructions that the aroma bath would help babies to calm down, would reduce stress and crying and could enhance sleep (group 3). While the mothers were bathing their babies, the mother-child interactions were video-taped, just as the sleeping behaviour of the babies after the bath. For cortisol assays saliva samples were taken immediately before

and 20 min after the bath. Cortisol was examined to value the potential stress reducing properties of the lavender bath oil. Saliva samples were taken from both the mother and child. The analysis showed that the mothers in the aroma group were less stressed, but smiled more and had a more intensive parent-child relationship during the bath. Apart from that, the babies looked at their mothers more often and cried less. Furthermore, they spent more time in deep sleep after having the aroma bath. The cortisol levels of mothers and babies were significantly lower, which is a measure of more relaxation. These data emphasized that the EO of lavender not only had a stress-decreasing effect, but also relaxing and sleep-inducing properties.<sup>[48]</sup>

As cited in Domingos *et al.*,<sup>[1]</sup> but not discussed in detail, are citrus essential oils, including lemon essential oils, which have a long tradition in aromatherapy and alternative medicine. A Japanese working team carried out a study with the aim of estimating the effects of citrus EOs on physical or psychological stress. After i.p. application of EO components such as limonene,  $\gamma$ -terpinene and citral, stress was provoked and serum corticosterone and monoamines in brain tissues were measured. Three hours after the administration perillidic acid (a limonene metabolite) was found in serum (1.5–2.5  $\mu\text{g/ml}$ ) and in brain tissue (0.4–0.6  $\mu\text{g/g}$ ). An interesting result was that the lemon components (*R*)-limonene, citral and  $\gamma$ -terpinene kept down the concentrations of serum corticosterone and cerebral monoamines. (*S*)-limonene (a stereoisomer of (*R*)-limonene) had apparently stronger activity compared to other monoterpenes and inhibited monoamines induced elevation of psychological stress. These results showed that the use of lemon EO containing components such as limonene and citral could decrease both physical and psychological stress.<sup>[49]</sup>

A similar paper<sup>[50]</sup> described the effects of (*S*)-limonene on brain neurotransmitter levels and behaviour of rats. Several neurotransmitters like dopamine, serotonin (5-HT), GABA, glutamic acid as well as some of their metabolites such as dihydroxyphenylacetic acid and 5-HIAA were detected by high-performance liquid chromatography-high-performance-liquid-chromatography-electron-capture-dissociation (HPLC-ECD) and amino acid analysis. (*S*)-limonene was applied for 1 week at concentrations of 0, 5, 25 or 50 mg/kg. Significant changes concerning, for example GABA, 5-HIAA and 5-HT, were observed. Basal hypothalamic-pituitary-adrenal (HPA) activity was determined by corticosterone after administration of (*S*)-limonene for 1 week. Due to the increased concentration of GABA and the changes of other neurotransmitters, an anti-stress effect was assumed. After that, the results showed that (*S*)-limonene inhibits HPA activity under physical stress and the stress-alleviating effect possibly acted by the GABA<sub>A</sub> receptor.<sup>[50]</sup>

Lime essential oil (LEO) is extracted from lime peel (*Citrus aurantifolia* [Christm.] Swingle, Rutaceae). Lime is a plant that is cultivated in many tropical countries. Limonene and citral are the main components of LEO and it is used as alternative treatments for stress, infection, inflammation, cancer or fatigue. Saiyudthong *et al.*<sup>[51]</sup> analysed the effect of aromatherapy massage with LEO on stress using 40 healthy office women between 25 and 45 years of age. All probands were randomly allocated to either the control group or the treatment group. The non-volatile vegetable sweet almond oil was used as carrier oil for the placebo group and in the aroma group 10% LEO was used. The effects of a single 1 h LEO massage and the effects of repeated LEO massage (1 h a week for 4 weeks) were observed. Cortisol levels, blood pressure, heart rate and body temperature

were measured as stress markers before and after the massage. Additionally, the Thai General Health Questionnaire 28 (GHQ-28) was used to evaluate mental parameters. There was no significant difference between the aroma group and the placebo group concerning the cortisol levels and the blood pressure before the aroma massage. However, the single LEO massages remarkably reduced blood pressure in comparison to the control group, probably because of the stimulation of the parasympathetic activity. On the other hand, repeated LEO massages decreased the GHQ-28 almost equal to the control group.<sup>[51]</sup>

Komori *et al.*<sup>[52]</sup> investigated the effect of aromatherapy before and after social skill training (SST), which is an important method in psychiatric care. Some patients are very stressed and tense during the SST. Six patients suffering from either schizophrenia or depression participated in the experiment. During a session of SST (1 h), hand baths, aroma massages and aroma inhalation (10 min) were applied to reduce tension. For the massage and the inhalation one of six different blends was used (Table 3). Saliva samples were collected in order to measure the cortisol and immunoglobulin A (IgA) levels. Stress can increase cortisol levels, but the direction of change of IgA (increase or decrease) depends on the manipulated stressor. During SST a clear increase in salivary cortisol concentrations was measured, but the increase was lower when the probands had a treatment with EOs prior to SST. IgA levels increased in subjects who received such an aroma treatment compared to those who had no aroma massage or inhalation. This study showed that EOs possess anti-stress and relaxant effects, but future research is necessary to confirm these findings.<sup>[52]</sup>

Summarizing the studies of this section it is noteworthy that with the exception of two papers (see Fukumoto *et al.*<sup>[49]</sup> and Zhou *et al.*<sup>[50]</sup>) all other assessments were done with human subjects. Especially remarkable is the result of an experiment with mothers and their babies<sup>[48]</sup> where a lavender bath influenced the mother–child interactions, the sleeping behaviour of the babies and the smiling time of both very positively. The video-taped recordings were supported by determination of the cortisol concentration in saliva samples from both. In all these human studies EOs or EO mixtures were used; single fragrances were administered only to animals.<sup>[49,50]</sup> With the exception of Zhou *et al.*<sup>[50]</sup> no explanation was given for the possible stress-reducing mechanism using EOs; the striking results – either assessed in various tests or by determination of the concentration of the stress indicator cortisol in saliva samples and evidenced by placebo experiments – clearly speak

for themselves. In particular, lavender oil possesses distinct stress-reducing activity. For the individual person in stress situations it does not matter if the effect is merely a psychological one or is the outcome of a neurotransmitter interaction.

## Influence on Learning, Memory, Attention and Arousal

In this section, the stimulating effects of essential oils on the central nervous system will be discussed. The special focus will be on their influence on the cognitive ability; for example, how probands can concentrate in difficult exercises, their attention during the tasks and the influence on their performance. Improving memory will also be mentioned, but will be treated referring to Alzheimer's disease in the last section as well. Additionally, exciting and hallucinogenic actions will be discussed.

A variety of researches have shown psychophysiological aspects of odours, but the cognitive functions are not fully understood until now. Shimizu *et al.*<sup>[53]</sup> tried to determine the effects of flavours on sustained attention in a vigilance task. 'Vigilance' means a state of being alert over a long period of time. In the worst case, a decrease of vigilance can be the reason for fatal accidents in everyday life. In the study there were three different situations: in two situations odours were present, either the essential oil of *Lavandula angustifolia* Mill. (Lamiaceae) or the essential oil of *Eucalyptus globulus* Labill. (Myrtaceae); in the third situation no odour was present (control). While the subjects had to carry out the vigilance task, the odour was presented to them via a constant-flow olfactometer; no odour was presented in the control situation respectively. The observers had to detect a target digit '0' for 30 min among other distracter digits that were shown every second; thereby, the reaction time could be measured. In the lavender aroma group reaction time was significantly decreased compared to the control group. These results led to the conclusion that the administration of lavender essential oil helped to maintain sustained attention during longer and challenging exercises.<sup>[53]</sup> The same authors published a further study in the same year, and again they examined the effects of lavender and eucalyptus odour during a long-term vigilance task in humans. The authors mentioned that sedative odours such as lavender are more effective than stimulating odours in demanding exercises, because too much alertness can impair vigilance. There again, stimulating odours are more helpful in less-demanding tasks, because they support the workers in

**Table 3.** Blends of essential oils used in an aromatherapy treatment<sup>[52]</sup>

Blend number and use	Essential oil			
	1	2	3	4
Aroma massage				
Blend 1	Labdanum	Sweet orange	—	—
Blend 2	Lavender	Orange	Neroli	—
Blend 3	Lavender	Geranium	Sweet orange	—
Aroma inhalation				
Blend 4	Neroli	Bergamot	—	—
Blend 5	Neroli	Lavender	—	—
Blend 6	Neroli	Rosewood	Ylang ylang	Bergamot



keeping alert. In the study two different pure essential oils (as mentioned before, lavender and eucalyptus) and in addition the major constituents of lavender (linalyl acetate) and of peppermint (–)-menthol, *Mentha piperita* L., Lamiaceae) were presented via a direct odour delivery system to keep the inhalation volume as constant as possible. Two experiments were started; in the first experiment (lavender and eucalyptus odour) seven healthy adult males and in the second experiment (linalyl acetate and (–)-menthol) eight healthy adult males from 20 to 24 years participated. For the vigilance task a laptop computer was used. The subjects were requested to push a button when they saw '0' on the screen, although numbers between 0 and 9 were shown every second for 30 min. In the first experiment the increase in reaction time was remarkably lower with the lavender odour compared to the control (non-odour). The second experiment showed no effect, even though the tranquilizing effect of linalool and the stimulating effect of (–)-menthol are known. For this reason the authors assumed that the single fragrances did not have the same action mechanism as the essential oil. These findings suggest that the common lavender oil helped to restrain the decrease of vigilance.<sup>[54]</sup>

It is well established that visual stimuli can affect olfactory perception, but less is known about the reverse case. So Seo *et al.*<sup>[55]</sup> recently started a study to investigate the influence of smell on visual performance to determine whether smells can increase attention towards visually presented pictures which correspond to the smell. For this reason four flavours – namely orange, lavender, coffee and liquorice – were presented to 60 healthy subjects before and during a photographic slide show which contained one congruent and three incongruent pictures with the presented flavour. An eye-tracking system was utilized to evaluate the visual attention of the test persons as the total number and time of eye fixations. It could be noticed that the probands looked more often and longer at an according object when they smelled an odour in comparison to non-aroma conditions. Consequently, these results verified an olfactory effect on visual selective attention. It was evidenced that a flavour can enhance attention towards a corresponding visual object compared to a non-flavour circumstance.<sup>[55]</sup>

Rosemary (*Rosmarinus officinalis* L., Lamiaceae) is a common household and spicy plant and often added to diet because of its antioxidant properties. Moreover, it also possesses very important biological activities; for this reason it is even used as natural animal feed additive. Faixova and Faix<sup>[56]</sup> discussed the antimicrobial, antifungal, antioxidant, anti-carcinogenic and glucose level lowering properties of rosemary EO in a review. In addition it can also be used as natural alternative to antibiotics and could inhibit bone absorption in a trial. Furthermore, under sympathetic control rosemary EO can stimulate the nervous system and therefore improves memory and concentration abilities. An increased performance in tasks due to the olfactory impact of rosemary could be observed in a study: the overall quality of memory as well as the secondary memory factors increased whereas the speed component of memory decreased. In another study, peppermint (*Mentha piperita* L., Lamiaceae) and rosemary EO could enhance the activity level of mice and with the help of rosemary diffusion dogs were more alert. Finally, in a further experiment, rosemary oil could moderately inhibit the acetyl cholinesterase through the synergic interaction between 1,8-cineole and 2-pinene. Therefore, on account of its important biological activity this EO

could be used in the treatment of Alzheimer's disease, senile dementia and myasthenia gravis. Despite all these therapeutic actions, even adverse effects of rosemary are known. It was reported that it could lower sperm motility and density in male rats and could reduce fertility of female rats.<sup>[56]</sup>

Hongratanaworakit<sup>[57]</sup> investigated the effect of rosemary essential oil massage (*Rosmarinus officinalis* L., Lamiaceae) on emotional and autonomic levels in healthy test persons after trans-dermal administration. It is noteworthy that aromatherapy is the fastest developing complementary medicine and is widely used to ameliorate the quality of life. Rosemary oil is applied for cerebral efforts, inactivity or lack of energy. Moreover, it helps to improve alertness, memory and cognitive functions and strengthens the heart. It can also release the stress hormone cortisol. Animal studies even showed that rosemary could increase locomotor activity and had stimulating effects on the brain cortex. It is supposed that 1,8-cineole is the main active component (Table 4). In the study, rosemary oil was diluted in sweet almond oil and applied to the skin of the lower stomach. Then, unaided, 35 volunteers massaged the oil into the skin for 5 min. The control group used simple sweet almond oil. Afterwards, the participants assessed their emotional responses such as alertness, attentiveness, vigour, mood, relaxation or calmness with the help of rating scales. Autonomic parameters, for example blood pressure, breathing rate, pulse rate and skin temperature were assessed. Concerning the emotional state, volunteers in the aroma group felt more attentive and alert, more lively and more joyful than before the administration of rosemary oil. Compared to the placebo group, rosemary oil increased breathing rate and blood pressure, which suggested an increase of autonomic arousal. All in all, these findings demonstrated that rosemary oil had a stimulating effect and therefore the application in the complementary medicine is justified.<sup>[57]</sup>

One year later, in a second study, Hongratanaworakit<sup>[58]</sup> concentrated on exploring the influence of aromatherapy massage with the volatile oil (absolute) of jasmine (*Jasminum sambac* L., Oleaceae) on humans. Jasmine oil is known for its stimulating and arousing properties; rosemary and peppermint show similar properties. Jasmine absolute was administered topically to the skin of the stomach, afterwards 40 healthy participants had to assess their emotional state in terms of alertness, attentiveness, relaxation, vigour, calmness and mood so that subjective behavioural arousal could be evaluated. Additionally, several autonomic parameters like blood pressure, pulse rate, blood oxygen saturation, breathing rate and skin temperature were collected as indication for the arousal level of

**Table 4.** Composition of rosemary oil obtained from Thailand Flavours and Fragrances Industry Co. Ltd, Thailand<sup>[57]</sup>

Constituent	Per cent
1,8-Cineole	50.9
$\alpha$ -Pinene	14.4
Camphor	9.1
Sabinene	7.1
Camphene	5.0
Caryophyllene	2.5
Other compounds	11.0

the autonomic nervous system. A significant influence of this absolute was observed: breathing rate, blood oxygen saturation and systolic as well as diastolic blood pressure increased compared to placebo: again an increase of autonomic arousal. In relation to the emotional level, the human subjects in the jasmine aroma group felt more attentive, more energetic and less tranquilized than the subjects in the control group, which showed an increase of subjective behavioural arousal. Also, these findings revealed a stimulating and activating effect and verified the use of jasmine absolute in aromatherapy.<sup>[58]</sup>

It is already known that a person's expectation when she/he ingests some medicine plays an important part and may have an enormous influence on the real effectiveness. As the effects of manipulating probands expectations of an essential oil on memory have not yet been fully investigated, Robbins and Broughan<sup>[59]</sup> started a study on this topic. For the experiment the essential oil of Spanish sage (*Salvia lavandulifolia* Vahl, Lamiaceae) was used. Actually it is not very often used in commercialized beauty products but its improving effect on memory has already been acknowledged. In the study, 60 subjects from 19–45 years were randomly divided into three groups and each of them had to do a memory test before and after they received any specific instructions, which depended on the three groups. The first group – the 'negative-expectancy group' – received instructions that Spanish sage oil would impair their memory in the second test when compared to the first (odourless) one. The second group – the 'positive-expectancy group' – was misled to believe that Spanish sage oil would have a positive influence on their memory. Thus, they would be expected to have a better performance measurement in the second memory test. The control group was not informed about any benefits of Spanish sage oil in order to have a basis point. After the test, participants were instructed they had to do the second memory task which was similar to the first one. As expected, the 'negative-expectancy group' had fewer scores in the second memory test than in the first memory test. Likewise as expected, the 'positive-expectancy group' performed better in the second memory test than in the first. Astonishingly, contrary to prediction, participants in the control group who did not have a verbal suggestion did not remember an increased number of words in the second memory task. These results reconfirm that the actions of aromatherapy were partly based on psychological phenomena, in particular expectancies, which could be seen specifically in matters of manipulation of expectations.<sup>[59]</sup>

'Indoor air quality in school' was the topic of an investigation by Asakura.<sup>[60]</sup> An interesting finding was that the mixture of volatile organic chemicals from teaching materials and room cleaning products could lead to a malfunction of memory. As a remedy in this case, the odour of essential oil of cypress could improve cognition power.<sup>[58]</sup> A similar review with 27 references concerning the positive influence of the EO of coriander seeds (*Coriandrum sativum* L., Apiaceae) on memory was made by Dai *et al.*<sup>[61]</sup>

Absinthe is a well-known alcoholic liquor that contains extracts from the wormwood plant (*Artemisia absinthium* L., Asteraceae). The monoterpene ketone  $\beta$ -thujone is frequently discussed as possible toxic ingredient; however, the dose–response relationship is not often mentioned and the association that the psychotropic effects of absinthe are due to the content of thujone is not scientifically proven. Thujone itself is an ingredient in modern commercial absinthes as well as in

historic absinthes before it was forbidden, but the quantities in both are so low that a pharmacological effect can be excluded. The effects of absinthe can be explained by chronic alcohol abuse in accordance with today's standards of information.<sup>[61]</sup> Thirteen samples of authentic absinthe – before it was forbidden (prior to 1915) – were tested by Lachenmeier *et al.*<sup>[62]</sup> in order to investigate the parameters that were supposed to contribute to the toxicity of absinthe. The authors assumed that the concentration of thujone in pre-ban absinthe was overestimated in the past because the average content of thujone was  $25.4 \pm 20.3$  mg/l. Post-ban (1915–1988) and modern commercial absinthes (2003–2006) were analysed as well. The researches showed that the concentrations of thujone were similar compared to those before the prohibition. Altogether, nothing except ethanol was found in the absinthes that could explicate the syndrome 'absinthism'.<sup>[62]</sup>

In a certain respect it is a matter of course that in this section experiments with human subjects are reported. Indeed, this is the case with the one exception (see Faixova and Faix<sup>[56]</sup>) where, among other activities of the EOs of rosemary and peppermint, the activity level and alertness of mice and dogs were discussed. Here, also the possible mechanism of these activities (e.g. inhibition of acetyl cholinesterase through synergic interaction by 1,8-cineole) has been mentioned. In nearly all other papers positive effects of various EOs on learning, memory and attention were reported and evidenced by a placebo control. A comparison of the effects of EOs with those of their major constituents has been reported only one time (see Shimizu *et al.*<sup>[54]</sup>). Special attention should be called to the paper by Robbins and Broughan<sup>[59]</sup> who investigated the 'expectation problem' in such studies and clearly showed that a person's expectation plays an important part for the outcome of such experiments. Special care in performing such assessments is necessary to avoid a manipulation of the subjects. Finally, another paper is worth extra mention (see Lachenmeier *et al.*<sup>[63]</sup>) where the 'absinthe–thujone problem' has been reported.

## Action on Relaxation, Sedation and Sleep

Relaxation is an emotional state which is characterized by the absence of arousal caused by rage, anxiety or worry. Therefore it is a condition of low tension.<sup>[64]</sup> Sedation means a medical procedure to calm down the functions of the central nervous system with the aid of sedative drugs.<sup>[65]</sup> Sleep is defined as a natural repetitive state without consciousness in which sensory and motor activities are temporarily inactive. The ability to react to stimuli is decreased but it is easier to wake up compared to coma.<sup>[66]</sup> To achieve a state of relaxation or sedation or to induce sleep, benzodiazepines are often used as sedatives or hypnotics. This means that one medicine causes different effects. It should be mentioned that there is a certain overlap between the terms 'sedative' and 'hypnotic'.<sup>[67]</sup> The use of EOs additionally or instead of conventional medication will be argued in this section.

The relaxing effects of rose oil (*Rosa damascena* Mill., Rosaceae) on human beings were investigated by Hongratanaworakit.<sup>[68]</sup> The EO was applied on the skin and after trans-dermal absorption autonomic and emotional parameters were assessed in 40 healthy test persons. The test persons had to wear breathing masks to avoid inhalation of the aroma and possible consequent olfactory stimulation. Blood pressure, breathing rate, blood oxygen saturation, pulse rate and skin temperature were measured to

estimate the autonomic parameters. Rating scales were used to assess the emotional responses. The rose oil induced a significant decrease in some autonomic measurements, such as breathing rate, blood oxygen saturation and blood pressure compared to placebo, thus indicating the reduction of autonomic arousal. Concerning the emotional parameters, the subjects in the odour group felt more relaxed and calm but less vigilant in comparison to the subjects in the non-odour group. These results convinced the author of the relaxing effects of rose oil and justified its use in aromatherapy, even to treat depression or stress in humans.<sup>[68]</sup>

The multiply applied lavender EO even has the reputation to be supportive to relaxation, but the empirical literature to this effect is very conflicting. According to Howard and Hughes<sup>[69]</sup> several previous studies could have been influenced by expectancy. So, these authors designed a study to determine whether lavender (*Lavandula angustifolia* Mill., Lamiaceae) odour or expectancy or both have an effect on post-stress relaxation. Ninety-six healthy female students participated in the double-blind, placebo-controlled study. The probands were exposed to lavender aroma, placebo or no aroma when they relaxed after an exciting cognitive test. When aroma was presented, an instruction manipulated the students' expectancies and made them think that the aroma would have an influence on their ability to relax. As a result, aroma did not affect any galvanic skin responses during the relaxation. However, the authors imagined that previous associations of lavender aromatherapy which assisted to relaxation possibly were influenced by expectancies; they are easy to manipulate.<sup>[69]</sup>

In a further study, De Sousa *et al.*<sup>[70]</sup> reported on the pharmacological effect-depressant effect in the phenobarbital-induced test, thus indicating a sedative ability of these monoterpenes (ten monoterpenes with a similar structure) on the CNS. The sedative actions of agarwood oil (*Aquilaria* species, in Japanese 'Jinkoh') and spikenard extract ('Kanshokoh') were assessed in Japanese research by Takemoto *et al.*<sup>[71]</sup> Both are traditionally used in incenses and sachets. Prior to all experiments, trials with sedative lavender oil were done to ascertain the validity of the experimental system. Two different types of agarwood oil (the first from a Hong Kong market, and the second from Vietnam) showed a sedative effect on mice in the open field test when inhaled; therefore a spontaneous vapour administration system was used. Furthermore, qualitative GC and GS/MS analysis were carried out to identify the principal components of the volatile oil. In agarwood number 1, benzylacetone, and in agarwood number 2,  $\alpha$ -gurjunene and (+)-calarene, were found as main compounds. The inhaled hexane extract of spikenard induced sedation in mice; its principal component was calarene. In order to reproduce the results of the corresponding oil or extract, the single fragrances calarene, benzylacetone and  $\alpha$ -gurjunene were administered to mice. The most efficient dose was lower than their original content in the oil or extract. A second time sedative activity was observed. So, the effectiveness of this experimental system to assess the sedative properties of EOs was proved.<sup>[71]</sup> *Kaempferia galanga* L. (Zingiberaceae) is an aromatic plant which is traditionally used as medicine in tropics and subtropics of Asia (e.g. in China, Japan or Indochina). Its rhizome is used as carminative, diuretic, stomachal, insecticidal, to cure pain and cough as well as incense and food spice. It is also applied to treat stress, anxiety and to induce sleep. The hexane extract of this plant and its active compounds were focus of a research

by Huang *et al.*<sup>[2]</sup> Therefore, mice had to inhale the hexane extract and the two principal ant components, ethyl *trans-p*-methoxycinnamate and ethyl cinnamate. The results showed that inhalation of the hexane extract in different doses (1.5 and 10 mg) had a reductive effect on locomotor activity, indicating sedative and relaxant properties. The two components had strong sedative effects as well (0.0014 and 0.0012 mg). Therefore the sedative property of *K. galanga* L. was demonstrated and the application of this plant and its constituents in aromatherapy can be recommended.<sup>[2]</sup>

The sedative and hypnotic properties of the volatile oil from fresh leaves of *Eucalyptus urophylla* S. T. Blake (EOEU, Myrtaceae) and *Eucalyptus brassiana* S. T. Blake (EOEB, Myrtaceae) were investigated by Teixeira *et al.*<sup>[72]</sup> Either 200 or 400 mg/kg of the EOs were orally applied on mice before several studies, such as pentobarbital sleeping time, open-field test or pentylene tetrazole (PTZ)-induced seizure test were conducted. Both EOEU and EOEB had the ability to prolong the sleeping time and, moreover, they reduced the activity in the open-field test. In the PTZ-induced seizure test it could be observed that only EOEU was capable of saving more mice from death. For this reason the authors accorded to the ethnopharmacological application of *Eucalyptus* species, and, after supplementing toxicological studies, they acclaimed future researches to evaluate their use as sedative remedies.<sup>[72]</sup> Brazilian folk medicine uses the aromatic plant rosewood (*Aniba rosaea* Ducke, Lauraceae), which occurs in the Amazon region, due to its sedative effects. Almeida *et al.*<sup>[73]</sup> reported on the sedative effects of rosewood oil in mice. Therefore, the time to loss of righting reflex (LORR) was measured. The time from the application of the drug to LORR was considered as latency and the time between LORR and recovery was considered as duration of sleep. It was observed that rosewood at doses of 200 and 300 mg/kg could significantly prolong the sleeping time and reduced the sleep latency. Pentobarbital provoked similar latency as rosewood oil at a dose of 100 mg/kg. Both injected together caused a significant potentiation – thus a much longer sleeping time – than the two substances alone. The components of the EO were analysed via GC and GC/MS; high concentrations of linalool (87.7%), but also  $\alpha$ -terpineol (3.1%), geraniol (1.2%), linalool oxide (1.5%) and some oxygenated sesquiterpenes (4.7%) in lower concentrations were found. Moreover, the influence on the isolated nerve using the single sucrose-gap techniques were assessed. The compound action potential amplitude was assessed to notice changes in excitability of the isolated nerve. By increasing the rosewood oil concentration, the amplitude decreased in a dose-dependent manner. Together, all these results showed that linalool-rich rosewood oil possesses sedative properties, which could be explained by a decrease in the action potential amplitude and consequently a reduction in neuronal excitability.<sup>[73]</sup>

As anxiolytic and sedative effects of sweet orange (*Citrus sinensis* [L.] Osbeck, Rutaceae) oil have already been observed, Fewell *et al.*<sup>[74]</sup> conducted a study to determine if (+)-limonene, the main compound of this citrus oil is detectable in blood after aromatherapy massage with sweet orange oil. First, the authors carried out a retro-perspective analysis of experiences of patients who had received an aromatherapy some years ago. Sedative effects were reported. Afterwards the authors started the real study. Thirty-nine subjects were randomly divided into two treatment groups and were massaged with sweet orange oil diluted in coconut oil (2.5%). Twenty test persons had to

wear a breathing apparatus to exclude inhaled oil vapour during the aroma massage, other 16 human beings were without an olfactory occlusion while they were massaged and three subjects were massaged with oil at a higher concentration (4%). Blood samples were analysed via GC/MS and (+)-limonene was detectable (about 59 nmol/l) in the first 10 min of massage with sweet orange oil, even if probands had worn the breathing masks. The levels of (+)-limonene were higher after massages with the higher concentrated oil (89 nmol/l). So, there was a dermal uptake of (+)-limonene during the massages with sweet orange oil in therapeutic concentrations. Yet, the concentrations of (+)-limonene in blood were very low, probably because it was metabolized to perillal alcohol and derivatives. Even when dermal uptake and olfactory uptake were combined the concentration was not very high; this indicated that the (+)-limonene uptake was less than 1%. Due to these findings the authors assumed that sedative effects normally attributed to (+)-limonene were unlikely to be a direct systemic action of the oil. A more likely explanation would be olfactory and/or cognitive influences.<sup>[74]</sup>

Mubassara *et al.*<sup>[75]</sup> described the effects of EOs from cinnamon (*Cinnamomum verum* Presl, Lauraceae), coriander (*Coriandrum sativum* L., Apiaceae), clove (*Syzygium aromaticum* Merrill & Perry, Myrtaceae) and mixed ones on the response of GABA<sub>A</sub> receptors and sleeping time in mice. cRNAs of GABA<sub>A</sub> were injected in *Xenopus* in order to express the ionotropic GABA<sub>A</sub> receptors in their oocytes. The EOs could potentiate the response of GABA<sub>A</sub> receptors caused by the ligand GABA in the experiments. To investigate the influences of the volatile oils on sleeping time, the EOs were i.p. injected or inhaled prior to the GABA agonist pentobarbital. A co-administration of the EOs showed a prolongation in sleeping time. The authors presumed that the tested oils could help recipients feel more relaxed when an oil was inhaled and modulate mood through binding to GABA<sub>A</sub> receptors.<sup>[75]</sup> A similar study by a similar author team was conducted 1 year later. The influence of volatile oils on the response of GABA<sub>A</sub> receptors, sleeping time in mice and additionally on the plasma adrenocorticotrophic hormone (ACTH) levels of rats were the emphasis of a research by Aoshima *et al.*<sup>[76]</sup> The ionotropic GABA-receptors were expressed in *Xenopus* oocytes by injecting their cRNAs, thus the influence of 17 EOs on these receptors could be evaluated. All but five of these 17 examined oils could clearly potentiate the response of the GABA<sub>A</sub> receptors induced by their ligand GABA. A prolongation of the pentobarbital-induced sleeping time was observed when EOs with a potentiating effect were co-administered. Single fragrance compounds such as terpinen-4-ol or 1-octen-3-ol reduced significantly the ACTH concentrations in rats under stress. These findings were interpreted as indicating mood modulation through acting on GABA<sub>A</sub> receptors and relaxing, sleeping, and anti-stress effects by EOs.<sup>[76]</sup>

The dried roots of *Nardostachys chinensis* Batal., which belong to Valerianaceae, are called spikenard. Takemoto *et al.*<sup>[77]</sup> carried out a study to investigate its volatile components valerenen-4,7(11)-diene and  $\beta$ -maaliene. Their sedative properties on mice when inhaled were observed. Both diminished the locomotor activity of the rodents in a dose-dependent manner, but even at lower concentrations, and both sedated mice within 10 min. The strongest effect of  $\beta$ -maaliene was observed at a dose of 0.14%, whereas the strongest effect of valerenen-4,7(11)-diene was observed at a dose of 0.06%, therefore the last one had a stronger sedative activity. Mice pre-treated with caffeine could be calmed through the application of valerenen-4,7(11)-diene.

The sleeping time of pentobarbital pre-treated mice could be prolonged about 2.7 times with valerenen-4,7(11)-diene. In conclusion, the vapour inhalation with the tested EOs was a non-harmful method which could also be applied to children and older people to achieve sedative effects and treat insomnia and may also even be suitable for the treatment of ADHD.<sup>[77]</sup> Huang *et al.*<sup>[78]</sup> described the chemical composition and hypnotic properties of the EO of a further Valerianaceae plant, namely *Valeriana officinalis* VAR. *latifolia* Vol. The hypnotic properties were assessed in mice pre-treated with pentobarbital. The sleep latency was significantly shorter whereas the sleeping time was prolonged (500 mg and 750 mg/kg were applied i.p., a dose-dependent effect was noticed).<sup>[79]</sup> The volatile oil of another Valerianaceae, namely *Valeriana amurensis* P. A. Smirn. ex Kom., improved the rate of falling asleep and prolonged the total sleeping time of mice; the oil had a synergistic action with pentobarbital. A prolongation of sleeping phases and REM phases were noticed as well.<sup>[79]</sup> Furthermore, sedative, sleep-inducing and hypotensive effects were noticed from the EO of *Casimiroa edulis* Llave & Lex. (Rutaceae) leaves, commonly known as white sapote, traditionally used in Costa Rica. The oil was obtained by hydrodistillation and afterwards analysed via GC/MS.<sup>[80]</sup>

The influence of odourants on respiration during sleep was the focus of another research. Thirty-six subjects participated in the study and were observed for one night. Either the pleasant lavender, vanillin, vetiver oil (*Vetiveria zizanioides* Nash, Poaceae), and the unpleasant ammonium sulfide were presented via an olfactometer during the night every 9, 12 or 15 min. Between 21 and 37 scent presentations were provided per night and each subject was tested with one of the four scents. Sleep was assessed via polysomnography and nasal and oral respiration was measured. The fragrances did not enhance the rate of arousals or wake up, but had an influence on the respiration. It could be observed that all four odourants transiently reduced inhalation and enhanced exhalation for up to six breaths following odour presentation. This effect was neither modified by the valence nor by stage of sleep. These findings showed that odourants had an influence on respiratory patterns in sleep.<sup>[81]</sup>

Coming to a conclusion, the treatment of insomnia, or at least difficulties falling asleep, is a main domain of aromatherapy. Thus, placebo controlled studies with human subjects have been carried out.<sup>[68,69,74,81]</sup> Additionally, other papers report on the results obtained in animal experiments. Here, dose-dependent assays with either EOs or single EO constituents were performed,<sup>[72,73,75]</sup> in some cases even comparisons between EOs and single fragrance compounds drawn<sup>[2,71,76]</sup> and explanations as to the mechanism given.<sup>[75,76]</sup> Nearly all of the cited papers report on the beneficial effects of the investigated EOs in view of their relaxing, sedative or sleep inducing efficiency. Only the results of a study conducted with sweet orange oil and also with (+)-limonene contradicted the before cited papers and lead to the conclusion that a systemic action is unlikely and the observed sedative effects are rather due to cognitive and/or olfactory influences.<sup>[74]</sup> Chiming in with Hongratanaworakit<sup>[59]</sup> is also the report by Howard and Hughes<sup>[69]</sup> that the relaxation caused by lavender aromatherapy is possibly influenced by expectation. The studies by Huang, *et al.*<sup>[2]</sup> and Takemoto *et al.*<sup>[71]</sup> use extracts and not EOs, and Takemoto *et al.*<sup>[71]</sup> additionally use the very expensive EO of agarwood. Finally, Arzi *et al.*<sup>[81]</sup> deal with respiration frequency, an indicator of relaxation and sleep depth.



## Anticonvulsive Action and Treatment of Epilepsy

Epilepsy is a chronic, recurrent disorder and its symptoms can appear paroxysmally, caused by excessive neuronal activity and a reduction of the seizure threshold in the central nervous system.<sup>[82]</sup> One discussed mechanism is that neurons are able to release the excitatory neurotransmitter glutamate in huge concentrations which can bind on the glutamatergic neurons and thereby cause an excessive calcium release in the post-synaptic cells. Another possible theory is that mutations lead to ineffective GABA (an inhibitory neurotransmitter) action. Epilepsy is classified into more than 40 different types and each of them shows a unique combination of seizure type, typical age of onset, EEG finding, treatment and prognosis. It is estimated, that about 50 million people worldwide suffer from epilepsy. In general, epilepsy can be controlled but not healed with medication; however, 30% of patients have uncontrolled seizures even if they take the best available medicines.<sup>[83]</sup> In this section, the influences of EOs on the treatment of epilepsy as an alternative therapy will be discussed.

Pathan *et al.*<sup>[84]</sup> concerned themselves with the anticonvulsant activity of safranal in status epilepticus (SE) in mice. SE is special type of epilepsy and characterized by constant seizure activity more than 30 min or discontinuous seizure activity and unconsciousness for more than 30 min, also associated with mortality. As synthetic remedies often have many undesirable side effects, natural bioactive substances are in demand. Safranal, a monoterpene aldehyde, is the active component of the extract of *Crocus sativus* L. stigmas (Iridaceae) and responsible for the saffron odour. To this volatile oil are attributed anticonvulsive effects because of its agonistic activity on the GABA<sub>A</sub> receptors. For an experiment, SE was induced in mice by i.p. administered phenytoin (50 mg/kg) and 2 h later s.c. applied pentylene tetrazole (PTZ, 100 mg/kg). Thirty-six animals were allocated to one of six groups. The first group received normal saline as a normal control, the second received diazepam as a positive control, the third, fourth and fifth groups were administered safranal in different doses (0.1, 0.2 or 0.4 ml/kg) and the last group was treated with pure sesame oil as vehicle control. Only the standard reference drug diazepam showed total protection against the PTZ-induced SE, whereas normal saline and sesame oil did not show any activity. Safranal at lower doses did not show anticonvulsive activity either, but a significant protection was observed at higher doses of safranal. Due to these findings the authors presumed that safranal caused dose-dependent protection against PTZ-induced SE and mortality in rodents. For this reason, safranal could be a potential future supplementary therapy in the treatment of SE.<sup>[84]</sup>

The monoterpenoid alcohol terpinen-4-ol is constituent in many EOs of several plants. Similar monoterpenoid alcohols have already attested their anticonvulsant activity in animal models, as reported in a study by de Sousa *et al.*<sup>[85]</sup> They treated mice with terpinen-4-ol and observed a remarkable reduction in the spontaneous motor activity, an increase in the waiting period of seizures evoked by PTZ and the convulsions caused by picrotoxin, and a reduction of the number of tonic hind paws convulsions.<sup>[85]</sup>

Wahab and his team<sup>[86]</sup> analysed the anticonvulsant potency of the volatile oil of nutmeg (*Myristica fragrans* Houtt., Myristicaceae), using popular seizure models for animals such

as the maximal electroshock model or the PTZ, strychnine and bicuculline test. The action of nutmeg oil started rapidly but the anticonvulsive effect did not last very long, however, it was significantly anticonvulsant effective in the MES model. It even possessed dose dependent anticonvulsive activity when jerks were induced by PTZ. As the experiments showed, nutmeg EO could delay the start of strychnine-induced seizures in the hind limb extensor. Although the results suggested an anticonvulsive potency at lower doses, weak pro-convulsant activity against PTZ and bicuculline was observed at higher doses. However, nutmeg oil had a wide therapeutic index, because it did not impair locomotor activity when doses up to 600 µl/kg were applied in the neurotoxicity test. Moreover, the LD<sub>50</sub> value (2150 µl/kg) was much higher as the doses needed for the anticonvulsive effects (50–300 µl/kg). On the basis of these data the authors presumed that nutmeg oil might have the potential to treat grand mal and partial seizures because it prohibited convulsions in conventional animal models. It must be pointed out that – due to slight potentiation of clonic seizure activity – nutmeg volatile oil is not qualified for the treatment of myoclonic and absence seizures.<sup>[86]</sup>

There are many reports concerning anise (*Pimpinella anisum* L., Apiaceae) and its biological properties (e.g. its antiepileptic potency). Up to now, though, the specific cellular mechanism has not yet been described in detail. Janahmadia *et al.*<sup>[87]</sup> investigated whether the volatile oil of anise had any influence on the bioelectrical activity in the neurons of snails in a control condition or after an epileptic seizure had been caused by PTZ. To measure intracellular activity, current clamp technique was used and thus the influence of anise oil in concentrations of 0.01% or 0.05% alone or combined with PTZ on firing pattern, action potential configuration and post-spike potentials was estimated. As a result, the fruit oil of *P. anisum* L. caused neuronal hyper-excitability in snails, partially because it reduced the after-hyperpolarization. Therefore, the authors pointed out that anise oil should be used very carefully when patients suffer from epilepsy.<sup>[87]</sup>

Even the common household spice sweet basil (*Ocimum basilicum* L., Lamiaceae) and other species of the same genus are used to treat several diseases of the central nervous system. The CNS depressant and anticonvulsant properties of *O. basilicum* L. leaf EO in several experiments were investigated by Oliveira and her working team.<sup>[88]</sup> The principal components, among them 1,8-cineole, linalool and geraniol, showed in a general pharmacological screening that the EO was a CNS depressant at all doses, including reduction of spontaneous activity, ptosis, ataxia and sedation. In addition, all doses caused also a remarkable prolongation in sleeping time and a decrease in the sleep latency. In the PTZ and picrotoxin seizure tests, *O. basilicum* L. oil enhanced the latency for development of convulsions. In the PTZ test the effects evoked by the EO could be reversed with the aid of flumazenil. However, the EO did not interfere with strychnine-induced epileptic seizures. These findings revealed that the EO of sweet basil had a depressant influence on the CNS and anticonvulsant qualities, probably due to an interaction with central GABAergic receptors.<sup>[88]</sup>

A chemical and pharmacological study of halfbar (*Cymbopogon proximus* chst. ex A. Rich., Poaceae) volatile oil was made by El Tahir *et al.*<sup>[89]</sup> In Egypt, this plant is used in folk medicine as an antispasmodic and diuretic drug. The EO was obtained by hydro-distillation and afterwards a GC/MS analysis was carried out. The chromatogram revealed eight peaks

according to the eight principal components, among them the major component piperitone (72.4%). The EO was applied orally as well as i.p. to rats and mice, and later on several parameters were determined: the oral absorption was 80–90%. Volatile oil administered i.p. led to a decrease of arterial blood pressure in anaesthetized rats in a dose-dependent manner. Thereby, the changes in the heart rate were not significant except in the largest dose, where a decrease of 16% was noticed. These decreases could not be antagonized by atropine or mepyramine but could be reduced with indomethacin. Significant changes in the electrocardiography (ECG) were not observed. In the seizure tests the EO was i.p. applied (1.2 ml/kg) to mice, followed by an induction of convulsions with electric shock, PTZ, picrotoxin or strychnine. A complete protection was only observed against electrically caused convulsions. Furthermore, the oil administered to pigeons in doses of 0.4 and 0.8 ml/kg could prevent ouabain-induced vomiting. In conclusion, the essential oil showed cardiovascular depressant effects as well as antiemetic and anticonvulsant activity.<sup>[89]</sup>

Sakurada *et al.*<sup>[90]</sup> dealt with the biological activities of bergamot (*Citrus bergamia* sso, Rutaceae) essential oil. The pharmacological properties of linalool, one of the main compounds, were investigated and it was evidenced that linalool exerted anticonvulsant effects in animal experiments.<sup>[90]</sup>

In this section all results were obtained in animal experiments, normally using rodents (mice), but also snails<sup>[87]</sup> and pigeons.<sup>[89]</sup> The cited papers report on the reliable possibility of the investigated EOs being used as an alternative therapy. However, nutmeg oil, on account of its potentiation of clonic seizures, should not administered on patients suffering of myoclonic and absence seizures<sup>[86]</sup> and anis oil should only be used very carefully because it exerted a neuronal hyper-excitability in the test animals (snails). Alas, sound experiments with human subjects were not reported in the last two and half years.

## Action on Dementia and Alzheimer's Disease

Alzheimer's disease (AD), the most common form of neurodegenerative disorders, is characterized by a deficit in cholinergic mediated neurotransmission which is jointly responsible for the impairment of cognitive functions and the progressive loss of memory. For symptomatic management of AD, acetyl cholinesterase inhibitors (AChEIs) like tacrine, donepezil, rivastigmine or galanthamine play an important role. They reversibly bind to the active site of the enzyme acetyl cholinesterase (AChE), which hydrolyses the important neurotransmitter acetylcholine (ACh). As a result, the concentration of ACh in the synaptic cleft increases and consequently the symptoms of AD are improved. Only one inhibitor, namely rivastigmine, inhibits enzyme butyryl cholinesterase (BuChE) which hydrolyses ACh in the periphery, but this can cause peripheral cholinergic related side effects.<sup>[91]</sup>

As the alkaloid galanthamine was originally isolated from the bulbs of plants of the Amaryllidaceae family (e.g. snowdrops and daffodils), Okello *et al.*<sup>[91]</sup> investigated the *in vitro* inhibition of human acetyl- and butyryl cholinesterase by *Narcissus poeticus* L. flower absolute, a member of Amaryllidaceae as well. The analysis of the flower absolute, by means of GC/MS furnished three major components, namely benzyl alcohol (11.0%), phenylethyl alcohol (17.5%) and benzyl benzoate (19.0%). Geranial, neral, linalool, cymene and 1,8-cineole were found in much lower doses, and these single fragrances can inhibit the cholinesterase. Before the tests were started, the absolute was

diluted into 80% ethanol, and then several dilutions were prepared. The new rapid colorimetric determination method of Ellman was used to assess the inhibition of cholinesterase, adapted for 96-well micro-titre plates. AChE from human erythrocytes, BuChE from human serum, the dilutions and some additives were mixed in the micro-titer plates, incubated and finally the reaction was started. The change in an absorbance kinetic mode was measured at 405 nm, and two controls were assessed as well. As a result, both AChE and BuChE were inhibited dose-dependently; 39.1% inhibition was observed at the highest dose of 0.1 mg/ml. The authors concluded that it was uncertain whether the therapeutic application of *N. poeticus* L. would be reasonable. Generally, extracts of *Narcissus* species are rarely used in aromatherapy, but are used in the perfume industry. As reported, this absolute had primarily behavioural effects; therefore *N. poeticus* L. absolute could have a place in more cognitive domains. Future studies are needed.<sup>[91]</sup>

Agitation is the most ordinary symptom in people who are suffering from severe dementia. It is defined by restlessness and aggressive behaviour which can even be dangerous for the family members or the nursing staff. Mostly, neuroleptic drugs are administered, although the efficacy is not satisfying and many side effects exist. Therefore, the potential of EOs from *Melissa officinalis* L. and *Lavandula angustifolia* Mill., both Lamiaceae in the treatment of agitation in people with severe dementia was investigated by Elliott *et al.*<sup>[92]</sup> It is already proved that both possess calming and sedative properties and can enhance memory. Furthermore, a radioligand binding was conducted in order to determine the receptor binding properties. The results showed that lavender as well as balm oil (EO of *M. officinalis*) significantly inhibited the binding of radioligands to the muscarinic M<sub>1</sub>, 5-HT<sub>2A</sub>, histamine H<sub>3</sub> receptors and GABA<sub>A</sub> receptor channel site. Melissa oil showed a wider receptor binding profile than lavender oil and even could affect the binding to 5-HT<sub>1A</sub> receptors and the agonist site of the GABA<sub>A</sub> receptor. Thus it could be demonstrated via radioligand binding that lavender and balm oil interact with several receptors of neurotransmitters and therefore can be used to reduce the symptoms of agitation. However, balm oil additionally is able to increase the time patients were involved in constructive activities and thus reduce the time they were socially withdrawn.<sup>[92]</sup> A dual radioligand binding and electrophysiological trial with focus on ligand-gated ion channels was performed by Huang *et al.*<sup>[93]</sup> to assess the pharmacological profile of EOs from the plants just discussed above. As mentioned before, both have clinical benefits in the treatment of agitations. Lavender oil inhibited TBPS (*t*-butylbicyclophosphorothionate) binding to GABA<sub>A</sub> receptor channel of the rat forebrain, but no effects on NMDA, AMPA and nicotinic acetylcholine receptors were observed. A combination of both EOs (50:50) was able to inhibit flunitrazepam binding but, in contrast, single oils had no effect. The electrophysiological tests with rat cortical cultures revealed that lavender oil reversibly inhibited GABA-induced currents in dose-dependent manner, but no inhibition of NMDA- or AMPA-induced currents was noticed. Lavender EO showed a depressant effect on neurotransmission.<sup>[93]</sup> The EO of *Melissa officinalis* L. (Lamiaceae) inhibited the binding of TBPS to GABA<sub>A</sub> receptor channel, but exerted – similar to lavender aroma – no effects on NMDA, AMPA or nicotinic acetylcholine receptors. Electrophysiological tests showed that balm oil could reversibly inhibit GABA-induced currents dose-dependently, but no inhibition of NMDA- or AMPA-

induced currents was observed. Herewith the pharmacological profile of these EOs was explained.<sup>[94]</sup>

The potency of *L. angustifolia* ill. on agitated behaviours in patients with dementia was also topic of an investigation by Lin *et al.*<sup>[95]</sup> Seventy Chinese elderly adults suffering either from AD, vascular dementia or other dementia participated in the cross-over randomized clinical trial. They were divided into two groups. First, the aroma group had to inhale lavender EO for 3 weeks; afterwards they changed into the control group which received an odourless sunflower preparation to inhale for another 3 weeks; the other group did the diametrically opposite. The Chinese version of the Cohen–Mansfield Agitation Inventory (CCMAI) and Neuropsychiatric Inventory (CNPI) were applied to assess the clinical response. As a result, the mean CCMAI and the mean CNPI scores declined after the aromatherapy with lavender. Summarizing, the lavender therapy was well tolerated during the study and demonstrated a significant improvement in agitations of dementia. Therefore aromatherapy with lavender could be an important addition or alternative to psychotropic medications.<sup>[95]</sup>

The activity of lemon EO components, especially (*S*)-limonene, on dementia were assessed in a study by Zhou *et al.*<sup>[96]</sup> Thus, dementia was induced by scopolamine and the passive avoidance test (PA) as well as the open-field habituation test were applied to observe any probable anti-dementia effects. In the tests, (*S*)-limonene and its metabolite, (*S*)-perillyl alcohol, presented a strong improvement in memory. More precisely, (*S*)-perillyl alcohol could only improve associative memory in PA, but was not able to improve non-associative memory in the open-field habituation test. The neurotransmitter concentration in some cerebral regions were analysed and showed that the concentration of dopamine was lower when scopolamine was applied, but this could be reversed by (*S*)-limonene or (*S*)-perillyl alcohol administration before the scopolamine injection. With the help of the Ellman method, it even could be demonstrated that these two compounds inhibited acetyl cholinesterase activity *in vitro*.<sup>[96]</sup>

The EOs of *Marlierea racemosa* Vell. (Myrtaceae) from two different places in the Atlantic rain forest (from Cananéia and Caraguatatuba, Brazil) were analysed by Souza *et al.*<sup>[97]</sup> The special focus was on the anti-acetyl cholinesterase activity. The constituents of the volatile oil were identified with GC/MS analysis; in both samples spathulenol was found as principal component (25.1% in Cananéia and 31.9% in Caraguatatuba). In the EOs of the Cananéia plants also monoterpenes (41.2%) were found, which were not present in the other plant. The ability to inhibit AChE was investigated via colorimetric analysis. EOs from the Cananéia plants had a greater potential to inhibit the AChE (75%) compared to the other plants (35%), which can be attributed to the higher concentration in *Marlierea racemosa* Vell. collected in Cananéia.<sup>[97]</sup>

Loizzo *et al.*<sup>[98]</sup> reported on the *in vitro* biological effectiveness of *Salvia leriifolia* Benth. (Lamiaceae) in the treatment of AD. Therefore, cholinesterase inhibitory activity and anti-inflammatory properties were investigated. A GC and a GC/MS analysis revealed camphor (10.5%), 1,8-cineole (8.6%), camphene (6.2%) and  $\alpha$ -pinene (4.7%) as principal constituents. Antioxidant properties were evaluated via the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay and showed an IC<sub>50</sub> of 2.26  $\mu$ l/ml. Effects on cholinesterase inhibition were assessed via the spectrophotometric method by Ellman. A higher activity against BChE could be observed. This was an interesting finding because in the late

stage of AD BChE is predominant in the brain. The EO even inhibited the production of inflammatory mediators, probably by means of oxidative degeneration of products of phagocytes. Furthermore, monoterpenes are probably responsible for the lipopolysaccharide-induced nitrite production. Although the EO showed anticholinesterase activity, clinical data are very limited; for this reason further studies are required.<sup>[98]</sup>

Summarizing the results reported in this very important section the treatment of AD with EOs or single EO constituents still is – and will be for the next few years – only a symptomatic alleviation of the main symptoms of this ‘hostage of elderly people’, namely agitation, memory defects, aggressive behaviour and dementia. Because AD probably is caused by a deficiency of ACh in the synaptic cleft, at the moment the most promising initial step is to use acetyl cholinesterase inhibitors which help to increase the concentration of this neurotransmitter. Therefore, many *in vitro* studies aim at finding such natural inhibitors of this enzyme.<sup>[91,96–98]</sup> Besides single volatiles, also an absolute of daffodils is effective in this respect.<sup>[91]</sup> Human studies put the focus on alleviation of the symptoms<sup>[92,95,96]</sup> and showed that the EOs of lavender and balm (these two also in animal experiments) as well as the monoterpene compounds (*S*)-limonene and (*S*)-perillyl alcohol can be used in this respect.

## Conclusion

With this compilation it could be shown that in the past 3 years many new studies regarding the effects of EOs on the CNS have been published, whether to find an alternative treatment to common medications in order to reduce side effects or to justify their use in folk medicine. The components of the EOs were often analysed and the mechanisms of action were assessed. It should be mentioned that not only the EOs in their entirety are responsible for the potency, but also single fragrance compounds (e.g. linalool as the main constituent of lavender), exert many effects. The studies mentioned prove that EOs can, apart from the autonomic nervous system, also influence the CNS, even if expectancies or the mental state sometimes play an important part. Certainly, many further studies concerning the impact and properties of EOs on the CNS have to be carried out in future and thus hopefully alternative or complementary possibilities to treat diseases such as dementia and epilepsy, sleeping disorders, pain, anxiety etc. will be found.

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