9 Biological Activities of Essential Oils

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9.1 INTRODUCTION

The term “biological” in this context comprises all properties, from for example “abdomen” to “zymase,” which any natural product may possess. And these can be attributed to the whole animated nature, to all living organisms, namely plants, animals, and especially humans. However, only the effects of essential oils (EOs) on human beings is the topic of the present chapter. Excluded therefore, are all “botanical” activities, for example, plant care and interplant communication such as the prevention of germination of seeds of a potentially rivalry plant by emitting an EO, or the “cry for help” of a plant when it is attacked by pests and the “victim” volatilizes a fragrance which itself attracts enemies of these varmints. Neither are covered animal messengers, so-called pheromones, as well as EOs as veterinary therapeutics in animal care and feed. All these properties go beyond the scope and frame of this treatise and would rather fill a separate volume.

Therefore, the subject matter of this chapter is the therapeutic uses of EOs and/or single fragrance compounds in human medicine and care. However, even this field seems to be too extensive, so that cosmetic uses and repellents are excluded, too. Since a chapter on the pharmacological properties of EOs is already contained in this volume (see Chapter 10), only those pharmacological activities
which are of secondary (but not less important!) interest to the traditional pharmacologist will be discussed in this chapter. In particular, these are properties which do not directly aim at the central or autonomic nervous systems and for which the molecular mechanisms are only of minor relevance, for example, antioxidative effects, anticancer properties, penetration enhancing activities, and so on.

In general, the prominent literature databases have been searched and the literature mainly back to the year 2000 is discussed in this chapter. Readers interested in earlier studies are referred to three reviews by the author, which were published some years ago (Buchbauer, 2002, 2004, 2007). However, where it seemed necessary also earlier investigations have been included here and topics are discussed shortly which were not dealt with in these earlier compilations. Nevertheless, in a few cases some overlapping with related chapters may occur in the present book.

9.1.1 Anticancer Properties*

A very promising field of treatment with EOs is their application against tumors. Especially since the 1990s the anticancer properties of EOs and/or their main constituents and/or metabolites have gained more and more interest, inasmuch as such a “natural” therapy is accepted all over the world by the patients. One of the most prominent compounds in that sense is either \(d\)-limonene, the main constituent of the EO of sweet orange peel oil (\(Citrus\) \(sinensis\), Rutaceae) as well as of other citrus fruit peel oils, or perillyl alcohol, the most important metabolite of this monoterpene hydrocarbon. Perillyl alcohol has been developed as a clinical candidate at the National Cancer Institute because of its greater potency than limonene, which may enable potentially effective systemic concentrations of the active principles to be achieved at considerably lower doses (Phillips et al., 1995). Perillyl alcohol is effective as an inhibitor of farnesyl transferase. In the early developmental stages of mouse lung carcinogenesis the \(ras\)-protein undergoes a series of modifications, and farnesylation at the cysteine is one of these, which leads to the anchoring of \(ras\)-p-21-gen to the plasma membrane in its biologically active state. Perillyl alcohol administered to test mice showed a 22% reduction in tumor incidence and a 58% reduction in tumor multiplicity (Lantry et al., 1997). Perillyl alcohol reduced the growth of hamster pancreatic tumors (>50% of the controls), or even led to a complete regression (16%). Thus, perillyl alcohol may be an effective chemotherapeutic agent for human pancreatic cancer (Löw-Baselli et al., 2000; Stark et al., 1995). Perillyl alcohol also inhibited significantly the incidence (percentage of animals with tumors) and multiplicity (tumor/animals) of invasive adenocarcinomas of the colon and exhibited increased apoptosis of the tumor cells. Scientists from the Purdue University report that the rate of apoptosis is over sixfold higher in perillyl alcohol-treated pancreatic adenocarcinoma cells than in untreated cells, and that the effect of perillyl alcohol on pancreatic tumor cells is significantly greater than its effect on nonmalignant pancreatic ductal cells (Stayrock et al., 1997). Moreover, this monoterpene alcohol-induced increase in apoptosis in all of the pancreatic tumor cells is associated with a 2–8-fold increase in the expression of a proapoptotic protein which preferentially stimulates the apoptosis in malignant cells. Perillyl alcohol is also effective in reducing liver tumor growth. Two weeks after diethyl nitrosoamine exposure was discontinued, the animals were divided into perillyl alcohol-treated and untreated groups. The mean liver tumor weight for the perillyl alcohol-treated rats of perillyl alcohol treatment was 10-fold less than that for the untreated animals (Mills et al., 1995). A newer study found that this monoterpene alcohol potentially attenuates ferric-nitrilo-acetate-induced oxidative damage and tumor promotional events (Jahangir et al., 2007). Monoterpenes such as \(d\)-limonene and perillyl alcohol, as well as other terpene alcohols, such as geraniol, carveol, farnesol, nerolidol, \(\beta\)-citronellol, linalool, and menthol, showed inhibitory activities on induced neoplasia of the large bowl and duodenum. Nerolidol, especially, has an impact on the protein prenylation and is able to reduce the adenomas in rats fed with these compounds to an extent of about 82% compared to the

controls (Wattenberg, 1991). Geraniol prevents the growth of cultured tumor cells, especially those of rat hepatomas and melanomas (Yu et al., 1995). Dietary geraniol increased the 50% survival time of mice significantly and even 20% of the animals remained free of tumors when fed a geraniol-containing diet 14 days before an intraperitoneal transfer of the tumor cells (Shoff et al., 1991). Similar studies indicate that the colon tumors of animals fed with perillyl alcohol exhibited increased apoptosis as compared to those fed the control diet (Reddy et al., 1997). Therefore, consumption of diets containing fruits and vegetables rich in monoterpenes, such as \( d \)-limonene, reduces the risk of developing cancer of the colon, mammary gland, liver, pancreas, and lung (Crowell, 1999).

In the following, the anticancer activity of some EOs published since 2000 up to now will be discussed. In these papers several different cell lines were used to determine the anticancer activity of the EOs tested: A-549 (human lung carcinoma cell line), B16F10 (mouse cell line), CO25 (N-ras transformed mouse myoblast cell line), DLD-1 (human colon adenocarcinoma cell line), Hep-2 (human laryngeal cancer cell line), HL-60 (human promyelocytic leukemia cell line), J774 (mouse monocytic cell line), K562 (human erythroleucemic cell line), nuclear-factor-\( \kappa \)-B (human mouth epidermal carcinoma cell line), M14 WT (human melanoma cell line), Neuro-2a (mouse neuroblastoma cell line), P388 (murine leukemia cell line), SP2/0 (mouse plasmocytoma cell line), and then Caco-2, K562, MCF-7, PC-3, M4BEU, ACHN, Bel-7402, Hep G2, HeLa, and CT-26 (different human cancer cell lines).

El Tantawy et al. (2000) investigated the EO of \textit{Senecio mikanioides} O. (cape ivy, Asteraceae), grown in Egypt. The main components of the oil of the plant’s aerial parts were \( \alpha \)-pinene (23%) and \( \beta \)-myrcene (11.3%), whereas dehydroaromadendrene (31.8%) and camphene (19.7%) were the major compounds in the underground organs, analyzed by gas chromatography/mass spectrometry (GC-MS). Both EOs had a potent cytotoxic activity against the growth of certain human cell lines \textit{in vitro}. Another study dealt with the EO of \textit{Nigella sativa} (black cumin, Ranunculaceae) seeds and its main constituent, thymoquinone (Badary et al., 2000). This substance was tested against fibrosarcomas induced by 20-methylcholanthrene (MC) in Swiss albino mice \textit{in vivo} and \textit{in vitro}. The mice got 0.01% thymoquinone in drinking water 1 week before and thereafter MC treatment. At the end of the experiment there was a significant inhibition of MC-induced fibrosarcoma compared to MC alone (tumor incidence 43%, less MC-induced mortality). In comparison to the control group, in the liver of MC-induced tumor-bearing mice a reduction in hepatic lipid peroxides, an increase in glutathione content and enzyme activities of glutathione S-transferase (GST) and quinone transferring (QT) are observed. Furthermore, the \textit{in vitro} tests showed an inhibition of the survival of the tumor cells. These data indicate that thymoquinone could be a powerful chemopreventive agent against fibrosarcomas induced by 20-methylcholanthrene (MC) in Swiss albino mice \textit{in vivo} and \textit{in vitro}. The mice got 0.01% thymoquinone in drinking water 1 week before and thereafter MC treatment. At the end of the experiment there was a significant inhibition of MC-induced fibrosarcoma compared to MC alone (tumor incidence 43%, less MC-induced mortality). In comparison to the control group, in the liver of MC-induced tumor-bearing mice a reduction in hepatic lipid peroxides, an increase in glutathione content and enzyme activities of glutathione S-transferase (GST) and quinone transferring (QT) are observed. Furthermore, the \textit{in vitro} tests showed an inhibition of the survival of the tumor cells. These data indicate that thymoquinone could be a powerful chemopreventive agent against MC-induced fibrosarcoma tumors, probably because of its interference with DNA synthesis. Some years later, Ali and Blunden (2003) published a review about the seeds of black cumin, which is used in folk medicine. The EO and its major constituent thymoquinone were found to have anti-neoplastic activity and to be protective against nephrotoxicity and hepatotoxicity induced by diseases or chemicals.

The anticancerogenic effect of the EO of the \textit{Melissa officinalis} L. (Lamiaceae) was investigated by Allahverdiyev et al. (2001) using cell cultures of Hep-2 cells derived from human laryngeal cancer. The activity of the EO was examined by morphologic changes and by flow cytometry, compared to methotrexate (MTX, an antagonist of folic acid) and etoposid, a partial synthetically obtainable glycoside of podophyllotoxin. The EO was able to terminate the cells of the G1 and S phases, whereas MTX was active in the S and G2 phases and etoposid in the G1 phase. Their findings showed that the essential balm oil possesses anticancerogenic effects because MTX blocks the transfer of one-carbon fragments by its affinity to dihydrofolic acid reductase, which leads to an obstruction of the nucleic acid synthesis. De Sousa et al. (2004) found in an \textit{in vitro} assay that the EO of this plant was very effective against various human cancer cell lines (A549, MCF-7, Caco-2, HL-60, K562) and mouse cell lines (B16F10).

Legault et al. (2003) performed a study about the antitumor activity of balsam fir oil (\textit{Abies balsamea}, Pinaceae) using the tumor cell lines MCF-7, PC-3, A-549, DLD-1, M4BEU, and CT-26.
Balsam fir oil was active against all tumor cell lines with GI₅₀ values ranging from 0.76 to 1.7 mg/mL. After GC-MS analysis among the monoterpenes found (about 96%) α-humulene proved itself to be responsible for cytotoxicity (GI₅₀ 55 μM). Both the EO and α-humulene induced a dose- and time-dependent decrease in cellular glutathione (GSH) content and an increase in reactive oxygen species (ROS) production.

Zeytinoglu et al. (2003) studied the effects of carvacrol, one of the main compounds in the EO of oregano (obtained from the Lamiales Origanum onites L.) on the DNA synthesis of N-ras-transformed mouse myoblast cells CO25. This monoterpenic phenol was able to inhibit the DNA synthesis in the growth medium and ras-activating medium, which contained dexamethasone. The authors concluded that carvacrol may find application in cancer therapy because of its growth inhibition of myoblast cells even after activation of mutated N-ras-oncogene. Also, Ipek et al. (2003) investigated the thymol-isomer carvacrol using the in vitro sister-chromatid-exchange (SCE) assay on human peripheral blood lymphocytes. The inhibitory effect of carvacrol was checked in the presence of mitomycin C (MMC) in the same assay. The formation of SCE was not increased by any dose of carvacrol, while it decreased the rate of SCE induced by MMC. These findings demonstrate that carvacrol shows a significant antigenotoxic activity in mammalian cells, indicating its usage as an antigenotoxic agent.

The fresh leaf the EO of the Moraceae Streblus asper Lour. comprising the major compounds phytol (45.1%), α-farnesene (6.4%), trans-farnesyl acetate (5.8%), caryophyllene (4.9%), and trans,trans-α-farnesene (2.0%) was tested against mouse lymphocytic leukemia cells (P388) and showed a significant anticancer activity (ED₅₀ 30 μg/mL) (Phutdhawong et al., 2004). Also another EO, this time from the fruits of the Anonaceae Xylopia aethiopica (Ethiopian pepper), a plant grown in Nigeria, showed in a concentration of 5 mg/mL a cytotoxic effect in the carcinoma cell line (Hep-2) (Asekun and Adeniyi, 2004). Last but not least, also terpinen-4-ol, the major component of the tea tree oil (TTO) (Melaleuca alternifolia, Myrtaceae), was investigated by Calcabrini et al. (2004) as to its anticancer effects in human melanoma M14 WT cells and their drug-resistant counterparts, M14 adriamycin-resistant cells. TTO as well as terpinen-4-ol were able to impair the growth of human M14 melanoma cells, whereupon the effect was stronger on their resistant variants, which express high levels of P-glycoprotein in the plasma membrane, overcoming resistance to caspase-dependent apoptosis exerted by P-glycoprotein-positive tumor cells.

Some other EOs from “prominent” plants were investigated if they could be used in cancer therapy. One of these plants is the Asteraceae Chrysanthemum boreale Makino whose EO was studied on the apoptosis of KB cells by Cha et al. (2005). Different cytotoxic effects hallmarking apoptosis (DNA fragmentation, apoptotic body formation, and sub-G1 DNA content) proceeded dose dependently. The caspase-3 activity was induced rapidly and transiently by treatment with an apoptosis-inducing concentration of the EO. Eugenol isolated from clove oil (Eugenia caryophyllata, Myrtaceae) was investigated by Yoo et al. (2005) using human promyelocytic leukemia cells (HL-60) and might be a potent agent in cancer therapy. After treatment with eugenol the HL-60 cells showed hallmarks of apoptosis such as DNA fragmentation and formation of DNA ladders in agarose gel electrophoresis. Apoptotic cell death was induced via generation of ROS, inducing a mitochondrial permeability transition, reducing antiapoptotic protein bcl-2 level and inducing cytochrome C release to the cytosol. In traditional medicine very prominent is the bog myrtle Myrica gale L. (Myricaceae), a native plant in Canada as well as in Scotland. GC-MS analysis of the leaf EO revealed 53 components with myrcene, limonene, α-phellandrene, and β-caryophyllene as the major compounds. In the 60-min fraction of this oil the caryophyllene oxide content was higher (9.9%) than in the 30-min fraction (3.5%). The anticancer activity of these extracts was tested in human lung carcinoma cell line A-549 and human colon adenocarcinoma cell line DLD-1. The 60-min fraction showed a higher anticancer activity against both cell types than the 30-min fraction. The higher cell growth inhibition induced by the 60-min fraction could be caused by the accumulation of sesquiterpenes (Sylvestre et al., 2005).
Some Thai medicinal plants were checked for their antiproliferative activity on human mouth epidermal carcinoma (KB) and murine leukemia (P388) cell lines by Manosroi et al. (2006) using the MTX assay. From 17 Thai plants the Myrtaceae *Psidium guajava* L. (common guava) leaf oil showed the highest antiproliferative activity in KB cell line, which is 4.37 times more potent than vincristine, a well-known mitosis inhibitor. In P388 cells the Lamiaceae *Ocimum basilicum* L. (basil) oil had the highest effect, which is 12.7 times less potent than the thymin antagonist 5-fluorouracil.

In another study, the EO of the leaves of the Euphorbiaceae *Croton flavens* L. (yellow balsam) from Guadeloupe, a native plant from the Caribbean area, was analyzed by Sylvestre et al. (2006) and as main components viridiflorene (12.2%), germacrone (5.3%), (E)-γ-bisabolene (5.3%), and β-caryophyllene (4.9%) ascertained. The EO was found to be active against human lung carcinoma cell line A-549 and human colon adenocarcinoma cell line DLD-1. Three of the 47 components of the EO, namely α-cadinol, β-elemene, and α-humulene, showed also a cytotoxic activity against tumor cell lines. Yu et al. (2007) tested the EO of the rhizome of the Aristolochiaceae *Aristolochia mollissima* for its cytotoxicity on four human cancer cell lines (ACHN, Bel-7402, Hep G2, HeLa). The rhizome oil possessed a significantly greater cytotoxic effect on these cell lines than the oil from the aerial plant.

The success of chemotherapeutic agents is often hindered by the development of drug resistance, with multidrug-resistant phenotypes reported in a number of tumors. In a recent study of an Italian research team, the effects of the monoterpene alcohol linalool on the growth of two human breast adenocarcinoma cell lines were investigated, both as a single agent and in combination with doxorubicin. Linalool inhibited only moderately cell proliferation; however, in subtoxic concentrations potentiates doxorubicin-induced cytotoxicity and proapoptotic effects in both cell lines, MCF7 WT and MCF7 AdrR. The results of the Italian author group suggest that linalool improves the therapeutic index in the management of breast cancer, especially multidrug resistance (MDR) tumors (Ravizza et al., 2008).

The EO of *Cyperus rotundus* (Cyperaceae) contains cyperene, α-cyperone, isolongifolen-5-one, rotundene, and cyperorotundene as principal constituents. An *in vitro* cytotoxicity assay indicated that this oil was very effective against L1210 leukemia cells, which correlates with significantly increased apoptotic DNA fragmentation (Kilani et al., 2008).

Finally, Yan et al. (2008) reported on the cytotoxic activity of the EO and extracts of *Lynderia strychnifolia* (Lauraceae), a plant which is widely used in traditional Chinese medicine. Three human cancer cell lines (A549, HeLa, and Hep G2) were examined by *in vitro* assays. The strongest cytotoxicity on the cancer cells showed the leaf oil with 50% inhibitory concentration (IC₅₀) values ranging between 22 and 24 μg/mL after 24 h of treatment. The EO of the leaves and also of the roots exhibited greater cytotoxicity than ethanol extracts.

### 9.1.2 Antinociceptive Effects*

Although it has been mentioned in the introduction that in this book the comprehensive term “biological properties” does not include activities affecting the central and the peripheral nervous system, nevertheless for this subchapter a small exception had to be made, because the antinociceptive system belongs to the central nervous system. The function of antinociception is to aggravate the forwarding of pain impulses, which alleviates the sensation of pain. It is assumed that the so-called nociceptors are nerve endings responsible for nociception. They are sensory receptors that send signals, which cause the perception of pain in response to a potentially damaging stimulus. When the nociceptors are activated, they can trigger a reflex. Due to this system it can be explained why pains in a stress situation (e.g., caused by an injury after a traffic accident) are not noted in the first instance, but later after the decay of the tension. To test this pain-relieving capacity,

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experimentally generated pains in animal experiments were performed (Hunnius, 2007). The animal experiments that were mostly used are as follows:

1. **Formalin test**: A small volume of formalin is injected in the hind paw of a rat or a mouse and the pain-related behavior (paw lifting, paw licking) is observed. There are two phases of the test. In the first phase (10 min) the immediate reaction, that reflects the activation of peripheral nociceptors, is measured. The second phase (60 min) reflects a spinal hypersensitization (Pharmacon, 2007).
2. **Acetic acid-induced writhing test**: Acetic acid is administered intraperitoneally to the test animals and the number of writhings is registered.
3. **Tail-flick test**: The tail is irritated by a thermal stimulus and the movement of the tail is monitored.
4. **Hot-plate test**: The test animal is put on a heated surface (“a hot plate”) and the thermal pain reflexes are recorded.
5. **Carrageenin edema test**: The test animals get carrageenin injected and the volume of the paw is measured.
6. **Dextran edema test**: The test animals get dextran injected and the paw volume is measured.
7. **PBQ-induced abdominal constriction test**: The test animals get injected intraperitoneally a solution of p-benzoquinone and the number of abdominal contractions is recorded.

The antinociceptive effects of *N. sativa* (black cumin, Ranunculaceae) oil and its major component thymoquinone were investigated by Abdel-Fattah et al. (2000). After oral administration of doses ranging from 50 to 400 mg/kg the nociceptive response was suppressed in the hot-plate test, tail-pinch test, acetic acid-induced writhing test, and in the early phase of formalin test. There was also an inhibition of nociceptive response in the late phase of the formalin test after systemic administration and i.p. injection of thymoquinone. The s.c. injection of naloxone (1 mg/kg) significantly blocked the antinociceptive effect of *N. sativa* oil and thymoquinone in the early phase of the formalin test. In *N. sativa* oil- and thymoquinone-tolerant mice the antinociceptive effect of morphine was significantly reduced, but not vice versa. These findings indicate that *N. sativa* oil as well as thymoquinone induce an antinociceptive effect by means of an indirect activation of μ1- and μ-opioid receptor subtypes.

The antinociceptive effects of *Satureja hortensis* L. (summer savory, Lamiaceae) extracts and EO, a medicinal plant used in Iranian folk medicine as stomachic, muscle, and bone pain deliver was assessed by Hajhashemi et al. (2002). The hydroalcoholic extract as well as the polyphenolic fraction and EO of the aerial parts of the herb were screened for their antinociceptive activity in the light tail-flick test, as well as in the formalin and also in the acetic acid-induced writhing test. While
there was no significant result in the light tail-flick test the EO decreased the number of writhings induced by acetic acid compared to the control at the highest doses given. In the formalin test the hydroalcoholic extract, then the polyphenolic fraction and the EO showed an antinociceptive activity, which could not be reversed by pretreatment with naloxone or caffeine. These findings demonstrate that this effect caused by *Satureja hortensis* L. is not mediated by opioidergic or adenosine receptors.

Another *Satureja* species of the Lamiaceae family, namely *Satureja thymbra* L., was investigated by Karabay-Yavasoglu et al. (2006). The antinociceptive activity of the EO was assessed in mice by the formalin test and in rats by the light tail-flick test and the hot-plate test. An antinociceptive effect could only be detected in the hot-plate test during the early phase and the late phase. In the tail-flick test the EO did not produce any significant antinociceptive effect. Nevertheless the authors concluded that the EO of *Satureja thymbra* may have an analgesic activity in mice and rats.

A screening of the leaf EO of the Lauraceae *Laurus nobilis* L. (sweet bay) for antinociception in mice and rats was made by Sayyah et al. (2003). They reported a significant analgesic effect in tail flick and formalin tests, which was comparable to reference analgesics such as morphine and piroxicam.

In another study the antinociceptive effects of *Teucrium polium* L., a wild-growing Iranian plant belonging to the Lamiaceae, were investigated by Abdollahi et al. (2003). The total extract and the EO significantly inhibited pain-related behavior in the acetic acid-induced writhing test compared to the control.

The hydroalcoholic extract, the polyphenolic fraction, and the EO of the Lamiaceae *Zataria multiflora* Boiss. (*Zataria*), a plant used in traditional medicine for pain therapy and several gastrointestinal diseases, were checked by Jaffary et al. (2004) using writhing, tail flick, and formalin test in mice and rats. As main components in the EO linalool, linalyl acetate, and *p*-cymene could be detected. In the writhing test the EO and the hydroalcoholic extract were able to decrease the pain reflexes significantly (*p* < .05, *n* = 6). Both EO and hydroalcoholic extract were effective in tail-flick test (*p* < .05, *p* < .01, *n* = 6), whereas oral administration did not show any effect, which indicates an inactivation or extensively metabolism in liver or in gastrointestinal sections (see Figure 9.1). Furthermore, antinociception was indicated in both phases of formalin test (*p* < .01, *n* = 6). Due to the overall activity in these tests scientists concluded that *Zataria multiflora* has a clear central and peripheral antinociceptive activity.

The antinociceptive effects of *Lavandula hybrida* Reverchon “Grosso” (Lamiaceae) EO and its main components linalool and linalyl acetate were examined by Barocelli et al. (2004). The number of acetic acid-induced writhings was significantly decreased after oral administration of 100 mg/kg or

![FIGURE 9.1](image-url)  
*FIGURE 9.1* Antinociceptive activity of *Zataria multiflora* in tail-flick test in rats.
Inhalation of lavender EO for 60 min. After pretreatment with naloxone, atropine, and mecamylamine the postinhalative analgesia in hot-plate test was suppressed, which indicates an involvement both of the opioidergic and of the cholinergic system.

In another study the involvement of adenosine A1 and A2A receptors in (−)-linalool-induced antinociception was found by Peana et al. (2006b). The authors also mentioned that they have already shown the antinociceptive effect of (−)-linalool in recent studies in different animal models. The antinociceptive and antihyperalgesic effects were ascribed to the stimulation of opioidergic, cholinergic, and dopaminergic systems as well as to the interaction with K-channels, the local anesthetic activity, the negative modulation of glutamate transmission, and the blockade of N-methyl-D-aspartic acid (NMDA) receptors (Peana et al., 2006a). In the present study the authors used 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), a selective A1 receptor antagonist, and 3,7-dimethyl-1-propargylxanthine (DMPX), a selective A2A receptor antagonist, to measure the depression of the antinociceptive effect of (−)-linalool in the hot-plate test in mice. The decrease of the antinociceptive effect of linalool was significantly for both DPCPX (0.1 mg/kg i.p.) and DMPX (0.1 mg/kg i.p.) at the highest doses tested. These results indicate that the antinociceptive effects of (−)-linalool, the natural occurring enantiomer in the EOs of lavender, are, at least partially, mediated by adenosine A1 and A2A. The authors also examined the role of nitric oxide (NO) and prostaglandin E2 (PGE2) using lipopolysaccharide (LPS)-induced responses in macrophage cell line J774.A1. The nitrite accumulation in the culture medium was significantly inhibited after exposure of LPS-stimulated cells to (−)-linalool, whereas the LPS-stimulated increase of inducible nitric oxide synthetase (iNOS) expression was not inhibited at all. On the other hand, (−)-linalool had no effect on the release of PGE2 and on the increase of inducible cyclooxygenase-2 (COX-2) expression. These findings demonstrate that the reduction of NO production is, at least partially, responsible for the molecular mechanism of (−)-linalool antinociceptive effect, supposably through cholinergic and glutamatergic activities. Coming back to an earlier study of this author group (Peana et al., 2003), the influence of opioidergic and cholinergic systems in (−)-linalool-induced antinociception was examined. In acid-induced writhing test a significant reduction of writhings could be shown at doses ranging from 25 to 75 mg/kg. Whereupon the effect was completely inverted by naloxone, an opioid receptor antagonist, and by atropine, an unselective muscarinic receptor antagonist. In hot-plate test only the dose of 100 mg/kg was of significance. Moreover (−)-linalool showed a dose-dependent increase of motility effects, which excludes the participation of any sedative effect. The conclusion of this study is that opioidergic and cholinergic system play an important role in (−)-linalool-induced antinociception.

In a recent published study, the contribution of the glutamergic system in the antinociception elicited by (−)-linalool in mice was investigated (Batista et al., 2008). This monoterpane alcohol administered intraperitoneally, or orally, or intrathecally inhibited dose dependently glutamate-induced nociception in mice. Furthermore, (−)-linalool reduced significantly the biting response caused by intrathecal injection of glutamate when this alcohol was given i.p. This antinociception is possible due to mechanisms operated by ionotrophic glutamate receptors, namely AMPA, NMDA, and kainite.

In a further study De Araujo et al. (2005) screened the EO of Alpinia zerumbet (Pers.) Burtt. et Smith (shell ginger, Zingiberaceae), an aromatic plant native to the tropical and subtropical regions of the world and used in folk medicine for various diseases, including hypertension. In the acetic acid-induced writhing test the oral administration was effective, in the hot-plate test the EO increased the remedy time and also paw licking could be reduced significantly in the second phase of formalin test at 100 mg/kg. At 300 mg/kg a decrease was noticed in both phases of the test. After pretreatment with naloxone i.p. the analgesia was reversed significantly, completely for the first phase and partially for the second phase of the test. Therefore, also this EO shows a dose-dependent antinociceptive effect, which supposably includes the participation of opiate receptors.
Lino et al. (2005) used the EO of *Ocimum micranthum* Willd. (Lamiaceae) from Northeastern Brazil to study its antinociceptive activity in the hot plate and in the acetic acid-induced writhing test. Upon administration of low doses the EO could inhibit the number of writhings up to 79%. The antinociceptive effect was not influenced by pretreatment with naloxone. In the formalin test paw-licking time decreased to 61%. Also in this case the pretreatment with naloxone could not reverse the antinociceptive effect, confirming that there is no involvement of the opioid system. However, an involvement of the NO system was suggested because of the reverse of the antinociception by L-arginine in the second phase of the formalin test.

Santos et al. (2005) tested the antinociceptive activity of leaf EO of the Euphorbiaceae *Croton sonderianus* in mice using chemical and thermal methods. After i.p. injection of acetic acid, formalin, and capsaicin the EO could provoke an inhibition of nociception. On the other hand, there was no evidence for effectiveness against thermal nociception in the hot-plate test; however, the acetic acid-induced writhing and the capsaicin-induced hind-paw licking could be reduced more effectively. The antinociceptive effect in both capsaicin and formalin test was significantly antagonized by glibenclamide. These findings indicate that glibenclamide-sensitive KATP+ channels are involved in the antinociceptive effect of *Croton sonderianus* EO.

The same author studied also the antinociceptive properties in animal experiments after oral administration of 1,8-cineole, a terpenoid oxide in many EOs. By pretreatment of mice with naloxone the antinociceptive effect of this bicyclic ether was not inverted in the formalin test (Santos et al., 2000).

Methyleugenol, a prominent fragrance substance because of its allergenic potential, was isolated from *Asiasari radix* (Aristolochiaceae) and its antinociceptive effect on formalin-induced hyperalgesia in mice investigated by Yano et al. (2006). The oral administration of methyleugenol suppressed the duration of licking and biting in the second phase of the test in the same way as diclofenac, a nonsteroidal anti-inflammatory drug. Furthermore, the substance could decrease pain-related behaviors induced by intrathecal injection of NMDA, whereas diclofenac did not influence this behavior. All these antinociceptive effects of methyleugenol were depressed by bicuculline, a γ-amino butyric acid (A) antagonist, whereas COX-1 and -2 activity was not affected. The conclusion of this study was that methyleugenol is a potent inhibitor of NMDA-receptor-mediated hyperalgesia via GABA(A) receptors.

Iscan et al. (2006) analyzed the EO of the Asteraceae *Achillea schischkinii* Sosn. and *Achillea aleppica* DC. ssp. *aleppica* by GC and GC-MS and found as main component in both oils 1,8-cineole (32.5% and 26.1%). For testing the antinociceptive effect, male Swiss albino mice were used for the p-benzoquinone-induced abdominal constriction test. The number of abdominal contractions (writhing moments) was counted for 15 min, whereupon the antinociceptive activity was illustrated as percentage change from writhing controls. As reference drug Aspirin® at doses of 100 and 200 mg/kg was used. The EO of *Achillea aleppica* ssp. *aleppica*, which was also rich in bisabolol and its derivates, could induce a significant antinociception by reducing the number of writhes. In comparison with acetylsalicylic acid the active component of *Achillea aleppica* ssp. *aleppica* was not as potent as the drug. There are a number of isolated components from both EOs, which cause an antinociceptive effect. In particular, (−)-linalool has been closely investigated. The molecular mechanisms of the antinociceptive effect are different. They can be mediated by adenosine A1 and A2A or NMDA receptors, or the reduction of the NO production can play an important role. Also some can be glibenclamid-sensitive KATP+ -channel dependent or influenced by the opioidergic or cholinergic system.

Finally, also the antinociceptive and anti-inflammatory effects of the EO from *Eremanthus erythropappus* (Asteraceae) leaves were reported by a Brazilian author group. The EO proved to be significantly antinociceptive in the acetic acid-induced writhing test in mice, as well in the formalin test, and also in both phases of the paw-licking test, and in the hot-plate test. The exudate volume after intrapleural injection of carrageenan was significantly reduced as well as the leukocyte mobilization by administration of this oil 4 h before the start of the study (Sousa et al., 2008).
9.1.3 **Antiviral Activities**

Besides the manifold, well-documented, and, in human and veterinary medicine, very often used antimicrobial and antifungal properties of nearly all EOs (see Chapter 12), this group of natural compounds also possesses distinct antiviral properties. Viruses are submicroscopic particles (ranging from 20 to 300 nm) that can infect cells of a biological organism. They replicate themselves only by infecting a host cell and cannot reproduce on their own. Unlike living organisms, viruses do not respond to changes in their environment (Hunnius, 2007). EOs are able to suppress the viruses in different ways. They can inhibit their replication or they can prevent their spread from cell to cell. In the following the antiviral activity of some EOs against different viruses such as Herpes simplex virus type 1 and type 2 (HSV-1 and HSV-2), pseudorabies virus (PrV), influenza virus A3, Junin virus (JUNV), and dengue virus type 2 (DEN-2) will be discussed. In 1999, Benencia et al. (1999) published their results on the antiviral activity of sandalwood oil (*Santalum album*, Santalaceae) against Herpes simplex virus type 1 and type 2. The authors found that the EO inhibited the replication of the viruses. HSV-1 was more influenced than HSV-2 dose dependently.

De Logu et al. (2000) investigated the inactivation of HSV-1 and HSV-2 and the prevention of cell-to-cell virus spread by the EO of the Asteraceae *Santolina insularis*. The plaque-reduction assay showed an IC$_{50}$ values of 0.88 µg/mL for HSV-1 and 0.7 µg/mL for HSV-2, respectively, whereas another test on Vero cells showed a cytotoxic concentration (CC$_{50}$) of 112 µg/mL, which leads to a CC$_{50}$/IC$_{50}$ ratio of 127 for HSV-1 and 160 for HSV-2. These findings indicate that the antiviral effect of the EO was caused by direct virucidal effects. There was no antiviral activity detected in a postattachment assay. Due to attachment assays it was shown that virus adsorption was not reduced. Additionally, the reduction of plaque formation assay indicated that the EO reduced cell-to-cell transmission of both HSV-1 and HSV-2.

Another study was made on the antiviral activity of Australian TTO and eucalyptus oil against Herpes simplex virus in cell culture by Schnitzler et al. (2001). The authors used a standard neutral red dye uptake assay to evaluate the cytotoxic effects of TTO and eucalyptus oil and found a moderate toxicity for RC-37 cells of both oils approaching 50% (TC$_{50}$) at very low concentrations. In the plaque-reduction assay an IC$_{50}$ for plaque formation of 0.0009% HSV-1 and 0.0008% HSV-2 for TTO and of 0.009% (HSV-1) and 0.008% (HSV-2) for eucalyptus oil was determined. In a viral suspension test a very strong virucidal activity against HSV-1 and HSV-2 could be shown. TTO reduced plaque formation by 98.2% for HSV-1 and by 93.0% for HSV-2 at noncytotoxic concentrations, respectively, whereas eucalyptus oil reduced virus titers by 57.9% for HSV-1 and 75.4% for HSV-2 at noncytotoxic concentrations. Additionally, the authors investigated the mode of antiviral action of both EOs. After pretreatment of the virus prior to adsorption plaque formation was clearly inhibited. The findings show that both oils affect the virus before or during adsorption, but not after penetration into the host cell. The authors suggest the application of both oils as antiviral agents in recurrent herpes infections, although the active components are yet unknown.

Farag et al. (2004) examined the chemical and biological properties of the EOs of different *Melaleuca* species (teatree, TTO, Myrtaceae). The authors used the EOs of the fresh leaves of *Melaleuca ericifolia*, *Melaleuca leucadendron* (weepin teatree), *Melaleuca armillaris*, and *Melaleuca styphelioides*. Methyl eugenol (96.8%) was the main compound of the EO of *Melaleuca ericifolia*, whereas 1,8-cineole (64.3%) was the major constituent of *Melaleuca leucadendron*. The EO of *Melaleuca armillaris* was rich in 1,8-cineole (33.9%) and of terpinen-4-ol (18.8%). The main constituents of *Melaleuca styphelioides* were caryophyllene oxide (43.8%) followed by (−)spathulenol (9.6%). The highest virucidal effect of the EOs against HSV-1 in African green monkey kidney cells (Vero) by plaque reduction was caused by the volatile oil of *Melaleuca armillaris* (up to 99%), followed by that of *Melaleuca leucadendron* (92%) and *Melaleuca ericifolia* (91.5%).

Primo et al. (2001) examined *in vitro* the antiviral activity of the EO from the Lamiaceae *Minthostachys verticillata* (Griseb.) Epling against HSV-1 and PrV using the viral plaque-reduction assay. The EO influences HSV-1 and PrV multiplication, an activity which is attributed to the main constituents of the EO, namely menthone (39.5%) and especially pulegone (44.6%). The therapeutic index values attained 10.0 and 9.5 for HSV-1 and PrV, respectively.

In a chicken embryo hemagglutination valence-reduction test, Yan et al. (2002) investigated the inhibition of influenza virus A3 by the Lamiaceae *Mosla chinensis* EO. The authors assessed the activity of the EO for treatment against pneumonia in experiments with mice and found that the cytopathic effect (CPE) caused by influenza virus A3 was reduced in Vero cells by this EO. The hemagglutination valence was reduced from 1:1280 to 1:20 and 1:160 at concentrations of 500, 250, and 50 mg/mL in 9-day-old chicken, respectively. At a dosage of 100 µg/g/d, the therapeutic treatment of mice against pneumonia was successful.

The virucidal effect of the EO of the Lamiaceae *Mentha piperita* against HSV-1 and HSV-2 was tested *in vitro* on RC-37 cells using a plaque-reduction assay (Schuhmacher et al., 2003). The EO showed high virucidal effects against HSV-1 and HSV-2. At concentrations that did not produce a cytotoxic effect plaque formation was significantly inhibited by 82% for HSV-1 and 92% for HSV-2, respectively. A reduction of more than 90% for both herpes viruses could be achieved at higher concentrations of peppermint oil. The authors also demonstrated that the antiviral effect depended on time. After 3 h of incubation of HSV with the EO an antiviral activity of about 99% was shown. To investigate the mechanism of antiviral action, peppermint oil was added at different times to the cells or viruses during infection. When HSV was pretreated with the EO before adsorption, both HSV types were significantly reduced. These findings demonstrate that *Mentha piperita* oil influences the virus before adsorption, but not after penetration into the host cell. The EO also reduces plaque formation of an acyclovir-resistant strain of HSV-1 significantly by 99%. This oil might be useful for topical application as a virucidal agent in recurrent infection, considering its lipophilic properties, which enables it to penetrate the skin.

Another study as to the inhibitory effect of some EOs on HSV-1 replication *in vitro* was carried out by Minami et al. (2003). The best results were achieved by lemongrass, which inhibited the viral replication completely even at a concentration of 0.1%.

Furthermore, Garcia et al. (2003) studied the virucidal activity against HSV-1, JUNV, and DEN-2 of eight different EOs obtained from plants of San Luis Province, Argentina. The EOs of *Lippia junelliana* and *Lippia turbinata* (Verbenaceae) exhibited the highest virucidal effect against JUNV at virucidal concentrations (VC50) values from 14 to 20 ppm, whereas the EOs of *Aloysia gratissima* (whitebrush, Verbenaceae), *Heterotheca latifolia* (camphorweed, Asteraceae), and *Tessaria absinthioides* (tessaria, Asteraceae) reduced JUNV from 52 to 90 ppm. The virucidal activity depended on time and temperature. The EOs of *Aloysia gratissima*, *Artemisia douglasiana* (mugwort, Asteraceae), *Eupatorium patens* (Asteraceae), and *Tessaria absinthioides* inhibited HSV-1 in the range of 65–125 ppm. A discernible effect on DEN-2 infectivity could only be produced by *Artemisia douglasiana* and *Eupatorium patens* with VC50 values of 60 and 150 ppm, respectively.

The antiviral activity of the EO of the Lamiaceae *Melissa officinalis* L. against HSV-2 was examined by Allahverdiyev et al. (2004). The effect of the essential oil on HSV-2 replication in Hep-2 cells was tested in five different concentrations (25, 50, 100, 150, and 200 µg/mL). Up to a concentration of 100 µg/mL *Melissa officinalis* oil did not cause any toxic effect to Hep-2 cells, but it was slightly toxic at concentrations over 100 µg/mL. At nontoxic concentrations the replication of HSV-2 was reduced. Recently, Schnitzler et al. (2008) confirmed these findings: The lipophilic nature of the EO of lemon balm helps to affect the virus before adsorption thus exerting a direct antiviral effect. After the penetration of the herpes virus into the host cell there was no affection recorded anymore.

Yang et al. (2005) studied the anti-influenza virus activities of the volatile oil from the roots of the Asclepiadaceae *Cynanchum stauntonii* and found that the volatile oil caused an antiviral effect against influenza virus *in vitro* and also in *in vivo* experiments and was able to prevent the number of deaths induced by the virus in a dose-dependent manner.
Another study was carried out on the liposomal incorporation of *Artemisia arborescens* L. (powis castle, Asteraceae) EO and its *in vitro* antiviral activity by Sinico et al. (2005). The antiviral effect was tested against HSV-1 by a quantitative tetrazolium-based colorimetric method. The authors found that the EO can be incorporated in good amounts in vesicular dispersions and that these vesicle dispersions were stable for at least 6 months. During this period neither oil leakage nor vesicle size alteration occurred and even after a year of storage oil retention was still good, but vesicle fusion was present. The best antiviral results were observed when vesicles were made with P90H (=hydrogenated (P90H) soy phosphatidyl-choline).

An evaluation of antiviral properties of various EOs from South American plants was carried out by Duschatzky et al. (2005). The authors assessed the cytotoxicity and *in vitro* inhibitory activity of the EOs against HSV-1, DENV-2, and JUNV by a virucidal test. The best results were observed with the EOs of *Heterothalamus alienus* (Asteraceae) and *Buddleja cordobensis* (Scrophulariaceae) against JUNV, with virucidal concentration 50% (VC50) values of 44.2 and 39.0 ppm and therapeutic indices (cytotoxicity to virucidal action ratio) of 3.3 and 4.0, respectively. The oils caused the inhibitory effect interacting directly with the virions.

Reichling et al. (2005) investigated the virucidal activity of a β-triketone-rich EO of the Myrtaceae *Leptospermum scoparium* (manuka oil) against HSV-1 and HSV-2 *in vitro* on RC-37 cells (monkey kidney cells) using a plaque-reduction assay. The addition of the oil to the cells or viruses at different times during the infection cycle made it possible to determine the mode of the antiviral action. After pretreatment with manuka oil 1 h before cell infection both virus types were significantly inhibited. At concentrations that were not cytotoxic, the plaque formation reduction reached levels of 99.5% for HSV-1 and 98.9% for HSV-2. The IC50 of the EO for virus plaque formation was 0.0001% V/V (=0.96 μg/mL) for HSV-1 and 0.00006% V/V (=0.58 μg/mL) for HSV-2. When the host cells were pretreated before viral infection, plaque formation could not be influenced. After the virus penetrated the host cells only the replication of HSV-1 particle was significantly reduced to about 41% by manuka oil.

A phytochemical analysis and *in vitro* evaluation of the biological activity against HSV-1 of *Cedrus libani* A. Rich. (cedar of libanon, Pinaceae) was made by Loizzo et al. (2008a). The authors identified the active constituents for the *in vitro* antiviral activity against HSV-1 and evaluated the cytotoxic effects in Vero cells. The IC50 values of cones and leaves extract were 0.50 and 0.66 mg/mL, respectively, without provoking a cytotoxic effect, whereas the EO showed a comparable activity with an IC50 value of 0.44 mg/mL. In another study the author group found that the EO of *Laurus nobilis* (Lauraceae) and *Thuja orientalis* (Cupressaceae) were very effective against SARS-coronavirus (IC50: 120 ± 1.2 μg/mL, resp. 130 ± 0.4 μg/mL) and against Herpes simplex virus type 1 (IC50: 60.0 ± 0.5 μg/mL, resp. > 1000 μg/mL) (Loizzo et al., 2008b).

Ryabchenko et al. (2007, 2008) presented a study that dealt with antitumor, antiviral, and cytotoxic effects of some single fragrance compounds. The antiviral properties were investigated in an *in vitro* plaque formation test in 3T6 cells against mouse polyoma virus. Natural and synthetic nerolidol showed the highest inhibitory activity, followed by trans,trans-farnesol and longifolene.

### 9.1.4 Antiphlogistic Activity*

Processes by which the body reacts to injuries or infections are called inflammations. There are several inflammatory mediators such as the tumor necrosis factor-α (TNF-α); interleukin (IL)-1β, IL-8, IL-10; and the PGE2. In the following the inhibitory effects of some EOs on the expression of these inflammatory mediators and on other reasons for inflammations will be shown. Shinde et al. (1999) performed studies on the anti-inflammatory and analgesic activity of the Pinaceae *Cedrus deodara* (Roxb.) Loud. (deodar cedar, Pinaceae) wood oil. They examined the volatile oil obtained by steam distillation of the wood of this *Cedrus* species for its anti-inflammatory and analgesic

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effect at doses of 50 and 100 mg/kg body weight and observed a significant inhibition of carrageenin-induced rat paw edema. At doses of 100 mg/kg body weight both exudative–proliferative and chronic phases of inflammation in adjuvant arthritic rats were reduced. In acetic acid-induced writhing and also in hot-plate test both tested doses exhibited an analgesic effect in mice.

The anti-inflammatory-related activity of EOs from the leaves and resin of species of Protium (Burseraceae), which are commonly used in folk medicine, was evaluated by Siani et al. (1999). The resin oil contains mainly monoterpenes and phenylpropanoids: α-terpinolene (22%), p-cymene (11%), p-cimen-8-ol (11%), limonene (5%), and dillapiol (16%), whereas the leaves dominantly comprise sesquiterpenes. The authors tested the resin of Protium heptaphyllum (PHP) and the leaves of Protium strumosum (PS), Protium grandifolium (PG), Protium lewellyni (PL), and Protium hebetatum (PHT) for their anti-inflammatory effect using a mouse pleurisy model induced by zymosan and LPS. In addition, they screened the plants for their NO production from stimulated macrophages and for the proliferation of neoplastic cell lines: Neuro-2a (mouse neuroblastoma), SP2/0 (mouse plasmocytoma), and J774 (mouse monocytic cell line). After administration of 100 mg/kg p.o. 1 h before stimulation with zymosan, an inhibition of protein extravasation could be observed with the oils from PHP, PS, and PL, whereas total or different leukocyte counts could not be reduced. Also the neutrophilic accumulation could be decreased by the oils from PG, PL, and PHT, while PHP and especially PL lead to a reduction of LPS-induced eosinophilic accumulation in mouse pleural cavity. PHT also showed the ability to inhibit the mononuclear accumulation.

The NO production from stimulated mouse macrophages could be changed by in vitro treatment with the EOs. A reduction of the LPS-induced NO production of 74% was achieved by PHP and of 46% by PS. On the contrary, PL caused an increase of 49% in NO production. Concerning the cell-line proliferation, Neuro-2a was affected in the range of 60–100%, SP2/0 of 65–95%, and J774 of 70–90%. As to the suggestion of the authors, these EOs could be used as efficient pharmacological tools.

Another study was made on the anti-inflammatory effect in rodents of the EO of the Euphorbiaceae Croton cajucara Benth. (Sacaca, Euphorbiaceae) by Bighetti et al. (1999). At a dose of 100 mg/kg the EO exerted an anti-inflammatory effect in animal models of acute (carrageenin-induced paw edema in mice) and chronic (cotton pellet granuloma) inflammation. Compared with the negative control a dose-dependent reduction of carrageenin-induced edema was achieved. This EO also reduced chronic inflammation by 38%, whereas diclofenac only achieved an inhibition of 36%. The migration of neutrophils into the peritoneal cavity could not be inhibited by the EO. The anti-inflammatory effect seemed to be related to the inhibition of COX.

Santos et al. (2000) investigated the anti-inflammatory activity of 1,8-cineole, a terpenoid oxide present in many plant EOs. Inflammation could be reduced in some animal models, that is, paw edema induced by carrageenin and cotton pellet-induced granuloma. This effect was caused at an oral dose range of 100–400 mg/kg. The authors suggest a potentially beneficial use in therapy as an anti-inflammatory and analgesic agent.

The anti-inflammatory effect of the EO of the Myrtaceae Melaleuca alternifolia (TTO) was evaluated by Hart et al. (2000). The authors tested the ability of TTO to inhibit the production of inflammatory mediators such as the TNF-α, IL-1β, IL-8, IL-10, and the PGE2 by LPS-activated human peripheral blood monocytes. A toxic effect on monocytes was achieved at a concentration of 0.016% vol/vol by TTO emulsified by sonication in a glass tube into culture medium containing 10% fetal calf serum (FCS). In addition, a significant suppression of LPS-induced production of TNF-α, IL-1β and IL-10 (by approximately 50%), and PGE2 (by approximately 30%) after 40 h could be observed with the water-soluble components of TTO at a concentration equivalent to 0.125%. The main constituents of this EO were terpinen-4-ol (42%), α-terpineol (3%), and 1,8-cineole (2%). When tested individually, only terpinen-4-ol inhibited the production of the inflammatory mediators after 40 h.

The mechanisms involved in the anti-inflammatory action of inhaled TTO in mice were investigated by Golab et al. (2007). The authors used sexually mature, 6–8-week-old, C57BI10 × CBA/H
(F1) male mice and divided them into two groups. One group was injected i.p. with zymosan to induce peritoneal inflammation and the other simultaneously with antalarmin, a CRH-1 receptor antagonist, to block hypothalamic–pituitary–adrenal (HPA) axis function. After 24 h of injection the mice were killed by CO₂ asphyxia, and the peritoneal leukocytes (PTLs) isolated and counted. Additionally, the levels of ROS and COX activity were detected in PTLs by fluorometric and colorimetric assays, respectively. The result was that TTO inhalation led to a strong anti-inflammatory effect on the immune system stimulated by zymosan injection, whereas PTL number, ROS level, and COX activity in mice without inflammation were not affected. The HPA axis was shown to play an important role in the anti-inflammatory effect of TTO and antalarmin was observed to abolish the influence of inhaled TTO on PTL number and their ROS expression in mice with experimental peritonitis. In mice without inflammation these parameters were not affected.

A further study was made on the anti-inflammatory activity of linalool and linalyl acetate constituents of many EOs by Peana et al. (2002). The authors evaluated the anti-inflammatory effect of (−)-linalool, that is, the naturally occurring enantiomer, and its racemate form, present in various amounts in distilled or extracted EOs. Due to the fact that in linalool-containing oils there is also linalyl acetate present, this monoterpene ester was also tested for its anti-inflammatory activity. Both the pure enantiomer and its racemate caused a reduction of edema after systemic administration in carrageenin-induced rat paw edema test. Better results could be observed with the pure enantiomer, which elicited a delayed and more prolonged effect at a dose of 25 mg/kg, whereas the efficiency of the racemate form lasted only for 1 h after carrageenin administration. At higher doses, there were no differences between the (−) enantiomer and the racemate and there could be achieved no increase of the effect with increasing the dose. Equimolar doses of linalyl acetate on local edema did not provoke the same effect as the corresponding alcohol. These results demonstrate a typical prodrug behavior of linalyl acetate.

Salasia et al. (2002) examined the anti-inflammatory effect of cinnamyl tiglate contained in the volatile oil of kunyit (Curcuma domestica Val.; Zingiberaceae) on carrageenin-induced inflammation in Wistar albino rats (Rattus norvegicus). Cinnamyl tiglate was found in the second fraction of the volatile oil at a concentration of 63.6%. After induction of an inflammation in rats by injection of 1% carrageenin and administration of cinnamyl tiglate orally at various doses (control group treated with aspirin) and the EO a plethysmograph measured the degree of inflammation: At a dose of 17.6% of the volatile oil of kunyit/kg body weight the highest anti-inflammatory effect could be observed ($p \leq 0.01$), followed by the effect of a dose of 4.4%/kg body weight ($p \leq 0.05$). At lower doses the inflammation could not be reduced ($p \geq 0.05$).

The in vitro anti-inflammatory activity of the EO from the Caryophyllaceae Ligularia fischeri var. spiciformis (ligularia) in murine macrophage RAW 264.7 cells was evaluated by Kim et al. (2002). They examined the effects of the EOs isolated from various plants on LPS-induced release of NO, PGE₂, and TNF-α by the macrophage RAW 264.7 cells. The EO of Ligularia fischeri var. spiciformis achieved the best results among the tested oils inhibiting significantly the LPS-induced generation of NO, PGE₂, and TNF-α in RAW 264.7 cells. Additionally, the EO reduced the expression of iNOS and COX-2 enzyme in a dose-dependent manner. Therefore, the mechanism of the anti-inflammatory effect of this EO is the suppression of the release of iNOS, COX-2 expression, and TNF-α.

The in vitro anti-inflammatory activity of the EO of the Asteraceae Chrysanthemum sibiricum in murine macrophage RAW 264.7 cells was evaluated by Lee et al. (2003). The aim was to study the effect not only on the formation of NO, PGE₂, and TNF-α, but also on iNOS and COX-2 in LPS-induced murine macrophage RAW 264.7 cells. The EO had a similar effect on both enzymes and the inhibitory effects were concentration dependent. Additionally, the volatile oil also furnished a reduction of the formation of TNF-α.

An evaluation of the anti-inflammatory activity of the EOs from the Asteraceae Porophyllum ruderale (PR) (yerba porosa) and Conyza bonariensis (CB) (asthmaweed) in a mouse model of pleurisy induced by zymosan and LPS was made by Souza et al. (2003). The activity of the main compounds of each oil, β-mycrene (in PR), and limonene (in CB) in the LPS-induced pleurisy
model as well as the immunoregulatory activity was examined by measurement of the inhibition of NO and production of the cytokines, \( \gamma \)-interferon, and IL-4. After oral administration of the oils, a reduction of the LPS-induced inflammation including cell migration could be observed. A similar effect could be provoked with the use of limonene alone. Pure \( \beta \)-myrcene and limonene were also able to reduce the production of NO at not cytotoxic doses. In addition, \( \beta \)-myrcene and limonene also inhibited significantly \( \gamma \)-interferon.

The anti-inflammatory effect of the leaf EO of the Lauraceae \textit{Laurus nobilis} Linn. (sweet bay) in mice and rats was investigated by Sayyah et al. (2003). A dose-dependent anti-inflammatory effect could be observed in the formalin-induced edema test, which could be compared with the effect of nonsteroid anti-inflammatory drugs such as piroxicam.

Silva et al. (2003) examined the anti-inflammatory effect of the EO of three species of the Myrtaceae \textit{Eucalyptus citriodora} (EC, lemon eucalyptus), \textit{Eucalyptus tereticornis} (ET, forest red gum), and \textit{Eucalyptus globulus} (EG, blue gum eucalyptus), from which many species are used in Brazilian folk medicine to treat various diseases such as cold, flu, fever, and bronchial infections. An inhibition of rat paw edema induced by carrageenan and dextran, neutrophil migration into rat peritoneal cavities induced by carrageenan and vascular permeability induced by carrageenan and histamine could be observed. But there were no consistent results obtained for parameters as activity and dose–response relationship, which demonstrates the complex nature of the oil and the assays used. However, these findings provide support for the traditional use of \textit{Eucalyptus} in Brazilian folk medicine and further investigations should be made in order to develop possibly new classes of anti-inflammatory drugs from components of the EOs of the \textit{Eucalyptus} species.

The anti-inflammatory activity of the EOs of \textit{Ocimum gratissimum} (African brasil, Lamiaceae), \textit{Eucalyptus citriodora} (lemon eucalyptus, Myrtaceae), and \textit{Cymbopogon giganteus} (Poaceae) was evaluated by Sahouo et al. (2003). The authors tested the inhibitory effect of the three plants \textit{in vitro} on soybean lipooxygenase L-1 and COX function of prostaglandin H synthetase. The two enzymes play an important role in the production of inflammatory mediators. The EO of \textit{Eucalyptus citriodora} evidently suppressed L-1 with an IC\(_{50}\) value of 72 \( \mu \)g/mL. Both enzymes were inhibited by only one EO that of \textit{Ocimum gratissimum} with IC\(_{50}\) values of 125 \( \mu \)g/mL for COX function of PGHS and 144 \( \mu \)g/mL for L-1, respectively, whereas the oils of \textit{Eucalyptus citriodora} and \textit{Cymbopogon giganteus} did not affect the COX.

Lourens et al. (2004) evaluated the \textit{in vitro} biological activity and chemical composition of the EOs of four indigenous South African \textit{Helichrysum} species (Asteraceae), such as \textit{Helichrysum dasyanthum}, \textit{Helichrysum felinum} (strawberry everlasting), \textit{Helichrysum excisum}, and \textit{Helichrysum petiolare} (licorice plant). An interesting anti-inflammatory effect could be observed in the lipooxygenase-5 assay at doses between 25 and 32 \( \mu \)g/mL. Analysis of the chemical composition showed that the EOs comprise mainly monoterpenes such as \( \alpha \)-pinene, 1,8-cineole, and \( p \)-cymene, only the oil of \textit{Helichrysum felinum} was dominated by sesquiterpenes in low concentrations with \( \beta \)-caryophyllene as main compound on top.

The composition and \textit{in vitro} anti-inflammatory activity of the EO of South African \textit{Vitex} species (Verbenaceae), such as \textit{Vitex poora} (waterberg poora-berry), \textit{Vitex rehmannii} (pipe-stem tree), \textit{Vitex obovata} ssp. \textit{obovata} (hairy fingerleaf), \textit{V. obovata} ssp. \textit{wilmsii}, and \textit{Vitex zeyheri} (silver pipe-stem tree), were analyzed by Nyiligira et al. (2004). After determination of the composition of the EOs by GC-MS their \textit{in vitro} anti-inflammatory activity was investigated in a 5-lipooxygenase assay. All EOs effectively suppressed 5-lipooxygenase, which plays an important role in the inflammatory cascade. The best results were achieved by \textit{V. poora} with an IC\(_{50}\) value of 25 ppm. The use of the EO data matrix presents chemotaxonomic evidence, which supports infrageneric placement of \textit{V. poora} in subgenus \textit{Vitex}, whereas the other four species are placed in subgenus \textit{Holmskioldiopsis}.

Ganapaty et al. (2004) examined the composition and anti-inflammatory activity of the Geraniaceae \textit{Pelargonium graveolens} (rose geranium) EO. Citronellol, geranyl acetate, geraniol, citronellyl formate, and linalool were identified in the leaf oil by GC-MS. A significant anti-inflammatory effect could be observed in the carrageenan-induced rat paw edema test.
Another study was made on the in vitro anti-inflammatory activity of paenol from the EO of the Paoniaceae *Paeonia moutan* (tree peony) and its derivative methylpaenol by Park et al. (2005). The authors isolated paenol (2-hydroxy-5-methoxyacetophenone) by silica gel column chromatography and methylated it by dimethylsulfate to yield methylpaenol (2,5-di-O-methylacetophenone). A suppression of the NO formation in LPS-induced macrophage RAW 264.7 cells was observed with both compounds in nitrite assay. Additionally, a reduction of iNOS-synthase and COX-2 formation was achieved in the Western blotting assay. These findings demonstrate that paenol is partly responsible for the anti-inflammatory effect of *Paeonia moutan* and that synthesized derivates are promising candidates for new anti-inflammatory agents.

The anti-inflammatory effect of the EO from the leaves of indigenous *Cinnamomum osmophloeum Kaneh.* (camphor tree, Lauraceae) was studied by Chao et al. (2005). Twenty-one components, among which the monoterpens 1,8-cineole (17%) and santolina triene (14.2%) and the sesquiterpenes spathulenol (15.7%) and caryophyllene oxide (11.2%), were analyzed as main constituents. In the anti-inflammatory assay it was found that the EO exerted a high capacity to suppress pro-IL-1β protein expression induced by LPS-treated J774A.1 murine macrophage at dosages of 60 μg/mL. Additionally, IL-1β and IL-6 production was reduced at the same dose. The TNF-α production could not be influenced by this dose of the EO.

A further study was carried out on the anti-inflammatory activity of the EO from *Casaeria sylvestris* Sw. (wild coffee, Flacourtiaeaceae) by Esteves et al. (2005). The EO having a total yield of 2.5% showed a LD₅₀ of 1100 mg/kg in mouse. Its composition was analyzed by GC and mainly sesquiterpenes, such as caryophyllene, thujopsene, α-humulene, β-acoradiene, germacrene-D, bicyclogermacrene, calamenene, germacrene B, spathulenol, and globulol identified as main compounds. After oral administration of this EO to rats, a reduction by 36% in carrageenan-induced edema was achieved in the rat assay (p < 0.05, Student’s t-test). In rat paw edema dextran-induced and vascular permeability assay using histamine, no significant result could be observed. Additionally, the writhing test using acetic acid demonstrated an inhibition of writhes with the EO by 58% and with indomethacin by 56%.

Ramos et al. (2006) investigated the anti-inflammatory activity of EOs from five different Myrtaceae species, *Eugenia brasiliensis* (grumichama), *Eugenia involucrate*, *Eugenia jambolana*, *Psidium guajava* (guava), and *Psidium widgrenianum*. The oils were obtained by steam distillation and analyzed by GC-MS and the correlation of retention indexes. In *Eugenia brasiliensis*, *Eugenia involucrate*, and *Psidium guajava* mainly sesquiterpenes could be identified, whereas monoterpenes dominated in *Psidium widgrenianum* and *Eugenia jambolana*. Afterwards the volatile compounds in zymosan and LPS-induced inflammatory models were tested. In zymosan-induced pleurisy, no reduction of leukocyte accumulation or protein leakage could be observed after p.o. administration of up to 100 mg/kg. *Eugenia jambolana* suppressed the total leukocyte (up to 56%) and eosinophil (up to 74%) migration in LPS-induced pleurisy, but the response did not correlate with the dose. *Psidium widgrenianum* only inhibited eosinophil migration (up to 70%) and both *Eugenia jambolana* and *Psidium widgrenianum* were also tested in vitro for their inhibitory effect on the production of NO. A potent suppression was achieved by *Eugenia jambolana* (up to 100%) in a dose-dependent manner, whereas only a moderate effect could be observed with *Psidium widgrenianum* (51%) test at concentrations below cytotoxic activity (25 μg/well).

The anti-inflammatory activity of the Myrtaceae *Eugenia caryophyllata* (clove) EO was evaluated in an animal model by Oztürk et al. (2005). The chemical composition of this EO by GC yielded β-caryophyllene (44.7%), eugenol (44.2%), α-humulene (3.5%), eugenyl acetate (1.3%), and α-copaene (1.0%) as main constituents. Then the volumes of the right hind paws of rats were measured with a plethysmometer in eight groups: physiological serum, ethylalcohol, indomethacin (3 mg/kg), etodolac (50 mg/kg), cardamom (0.05 ml/kg), EC-I (0.025 ml/kg), EC-II (0.050 ml/kg), EC-III (0.100 ml/kg), and EC-IV (0.200 ml/kg). Afterwards the drugs were injected i.p. and γ-carrageenan s.c. into the plantar regions and the difference of the volumes determined after 3 h. A reduction of the inflammation by 95.7% could be observed with indomethacin, to a lesser extent...
by the other seven groups (Table 9.1). The result of the study was that the EO of *Eugenia caryophyllata* exerted a remarkable anti-inflammatory effect. Alitonou et al. (2006) investigated the EO of Poaceae *Cymbopogon giganteus*, widely used in traditional medicine against several diseases, from Benin for its potential use as an anti-inflammatory agent. The analysis of this EO furnished *trans*-p-1(7),8-menthadien-2-ol (22.3%), *cis*-p-1(7),8-menthadien-2-ol (19.9%), *trans*-p-2,8-menthadien-1-ol (14.3%), and *cis*-p-2,8-menthadien-1-ol (10.1%) as main components. Additionally, it was found that the leaf EO suppressed the 5-lipoxygenase *in vitro*. Moreover, the antiradical scavenging activity was measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method (as to this property see Section 9.1.6).

A further study was carried out on the anti-inflammatory effect of the EO of Iranian black cumin seeds (BCS) (*N. sativa* L.; Ranunculaceae) by Hajhashemi et al. (2004). *p*-Cymene (37.3%) and thymoquinone (13.7%) were found to be the main compounds. For the detection of the anti-inflammatory activity, carrageenan-induced paw edema test in rats was used and also the croton oil-induced ear edema in mice. After oral administration of this EO at various doses no significant anti-inflammatory effect could be observed in the carrageenan test, whereas i.p. injection of the same doses significantly reduced carrageenan-induced paw edema. At doses of 10 and 20 µL/ear, BCS-EO also caused a reduction of a croton oil-induced edema. An anti-inflammatory effect could be observed after both systemic and local administration and thymoquinone seemed to play an important role in this pharmacological effect.

The downregulation of the leukotriene biosynthesis by thymoquinone, the active compound of the EO of the Ranunculaceae *N. sativa*, and its influence on airway inflammation in a mouse model was examined by El Gazzar et al. (2006). Bronchial asthma is often caused by chronic airway inflammation and leukotrienes are potent inflammatory mediators. Their levels arose in the air passages when an allergen challenge has been going on. The authors sensitize mice and challenged them with ovalbumin (OVA) antigen, which led to an increase of leukotrien B4 and C4, Th2 cytokines, and eosinophils in bronchoalveolar lavage (BAL) fluid. Additionally, lung tissue eosinophilia and nose goblet cells were remarkably elevated. After administration of thymoquinone before OVA challenge, 5-lipoxygenase expression by lung cells was suppressed and therefore the levels of LTB4 and LTC4 were reduced. A reduction of Th2 cytokines and BAL fluid and lung tissue eosinophilia, all parameters of airway inflammation, could also be observed. These findings demonstrate the anti-inflammatory activity of thymoquinone in experimental asthma.

Also El Mezayen et al. (2006) studied the effect of thymoquinone, the major constituent of the EO of *N. sativa* seeds, on COX expression and prostaglandin production in a mouse model of allergic airway inflammation. Prostaglandins play an important role in modulating the inflammatory responses in a number of conditions, including allergic airway inflammation. They are formed through arachidonic acid metabolism by COX-1 and -2 in response to various stimuli. The authors sensitized mice and challenged them through the air passage with OVA, which caused a significant

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**TABLE 9.1**

Reduction of Inflammation by the EO of *Eugenia caryophyllata* (EC), Indomethacin, and Etodolac in Animal Model

<table>
<thead>
<tr>
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<th>Reduction of Inflammation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indomethacin (3 mg/kg)</td>
<td>95.7</td>
</tr>
<tr>
<td>Etodolac (50 mg/kg)</td>
<td>43.4</td>
</tr>
<tr>
<td>EC-I (0.025 mL/kg)</td>
<td>46.5</td>
</tr>
<tr>
<td>EC-II (0.050 mL/kg)</td>
<td>90.2</td>
</tr>
<tr>
<td>EC-III (0.100 mL/kg)</td>
<td>66.9</td>
</tr>
<tr>
<td>EC-IV (0.200 mL/kg)</td>
<td>82.8</td>
</tr>
</tbody>
</table>
elevation of the PGD2 and PGE2 expression in the airways. Additionally, inflammatory nose cells and Th2 cytokine levels in the BAL fluid, lung airway eosinophilia, and goblet cell hyperplasia were raised and the COX-2-protein expression in the lung was induced. After i.p. injection of thymoquinone for 5 days before the first OVA challenge a significant decrease in Th2 cytokines, lung eosinophilia, and goblet cell hyperplasia could be observed, which was caused by the suppression of COX-2 protein expression and a reduction of the PGD2 production. Thymoquinone also slightly inhibited the COX-1 expression and the production of PGE2. This time the results demonstrated the anti-inflammatory effect of thymoquinone during the allergic response in the lung caused by the suppression of PGD2 synthesis and Th2-driven immune response.

The effect of thymoquinone from the volatile oil of black cumin on rheumatoid arthritis in rat models was investigated by Tekeoglu et al. (2006). Arthritis was induced in rats by Freund’s incomplete adjuvant and the rats were divided into five groups: controls 0.9% NaCl \( (n = 7) \), 2.5 mg/kg thymoquinone \( (n = 7) \), Bacilli Chalmette Guerin (BCG) \( 6 \times 10^5 \) CFU \( (n = 7) \), and MTX 0.3 mg/kg \( (n = 7) \). The level of inflammation was characterized by radiological and visual signs on the claw and by TNF-\( \alpha \) and IL-1\( \beta \) expression and the results of the different groups were compared. Thymoquinone reduced adjuvant-induced arthritis in rats, which was confirmed clinically and radiologically.

Biochemical and histopathological evidences for beneficial effects of the EO of the Lamiaceae *Satureja khuzestanica* Jamzad, an endemic Iranian plant, on the mouse model of inflammatory bowel diseases were found by Ghazanfari et al. (2006). The EO was tested on the experimental mouse model of inflammatory bowel disease, which is acetic acid-induced colitis and used prednisolone as control. For best results, also biochemical, macro- and microscopic examinations of the colon were performed. In acetic acid-treated mice a significant increase of lipid peroxidation could be observed compared to the control group, which was significantly restored by treatment with the EO and prednisolone. The EO decreased the lipid peroxidation up to 42.8% dose dependently, whereas prednisolone caused a decrease of 33.3%. Also a significant increase of the myeloperoxidase activity could be observed compared to the control group in acetic acid-treated mice, which was also significantly restored by treatment with the EO and prednisolone. The EO lowered the myeloperoxidase activity by 25% and 50% on average, whereas that of the control group was decreased by 53%. In addition, the EO- and prednisolone-treated groups exhibited significantly lower score values of macro- and microscopic characters after comparison to the acetic acid-treated group. These findings demonstrate that the beneficial effect of *Satureja khuzestanica* Jamzad EO could be compared to that of prednisolone. The antioxidant, antimicrobial, anti-inflammatory, and antispasmodic properties of the EO may be responsible for the protection of animals against experimentally induced inflammatory bowel diseases.

The biological activity and the composition of the EOs of 17 indigenous *Agathosma* (Rutaceae) species were examined by Viljoen et al. (2006a) in order to validate their traditional use. The analysis of the EOs furnished 322 different components. The anti-inflammatory activity was detected with the 5-lipoxygenase assay and all oils inhibited inflammations \textit{in vitro} with *Agathosma collina* achieving the best results \( (IC_{50} \text{ value of about } 25 \mu g/mL) \). These results show that the EOs of the different *Agathosma* species strongly suppress the 5-lipoxygenase.

The chemical composition and biological activity of the EOs of four related *Salvia* species (Lamiaceae) also indigenous to South Africa was evaluated by Kamatou et al. (2006). The authors isolated the EOs from fresh aerial parts by hydrodistillation and analyzed the chemical composition by GC-MS. The differences between the different species were rather quantitative. Forty-three components were identified accounting for 78% of *Salvia africana-caerulea*, 78% of *Salvia africana-lutea*, 96% of *Salvia chamelaeagnea*, and 81% of *Salvia lanceolata* total EO. *Salvia africana-caerulea* and *Salvia lanceolata* mainly contained oxygenated sesquiterpenes (59% and 48%, respectively), whereas *Salvia chamelaeagnea* was dominated by oxygen-containing monoterpenes (43%) and *Salvia africana-lutea* by monoterpane hydrocarbons (36%). The anti-inflammatory effect was tested with the 5-lipoxygenase method.
Viljoen et al. (2006b) studied the chemical composition and in vitro biological activities of seven Namibian species of *Eriocephalus L.* (Asteraceae). The EOs of *Eriocephalus ericoides* ssp. *ericoides* (samples 1 and 2), *Eriocephalus merxmuelleri*, *Eriocephalus scariosus*, *Eriocephalus dinteri*, *Eriocephalus luederitzianus*, *Eriocephalus klinghardtensis*, and *Eriocephalus pinnatus* were analyzed by GC-MS. *Eriocephalus ericoides* ssp. *ericoides* (sample 1), *Eriocephalus merxmuelleri*, and *Eriocephalus scariosus* contained high levels of 1,8-cineole and camphor and *Eriocephalus scariosus* was also rich in santolina alcohol (14.8%). Most camphor was found in *Eriocephalus dinteri* (38.4%), whereas the major compound of *Eriocephalus ericoides* ssp. *ericoides* (sample 2) was linalool (10.4%). The composition of *Eriocephalus luederitzianus* and *Eriocephalus klinghardtensis* was similar, both containing high levels of α-pinene, β-pinene, p-cymene, and γ-terpinene. *Eriocephalus luederitzianus* additionally was rich in α-longipinene (10.3%) and β-carophyllene (13.3%). *Eriocephalus pinnatus* was different from the other taxa containing mainly isoamyl 2-methylbutyrate (7.9%) and isoamyl valerate (6.5%). The anti-inflammatory activity was evaluated using the 5-lipoxygenase enzyme and *Eriocephalus dinteri* achieved best results (IC₅₀: 35 μg/mL).

Wang and Zhu (2006) studied the anti-inflammatory effect of ginger oil (*Zingiber officinale*, Zingiberaceae) with the model of mouse auricle edema induced by xylene and rat paw edema induced by egg white for acute inflammation and the granuloma hyperplasia model in mouse caused by filter paper for chronic inflammation. Additionally, the influence of ginger oil on delayed-type hypersensitivity (DHT) induced by 2,4-dinitrochlorobenzene (DNCB) in mice was observed. The authors found that ginger oil significantly inhibited both mouse auricle edema and rat paw edema and it also reduced the mouse granuloma hyperplasia and DHT.

The anti-inflammatory activity of *Carlina acanthifolia* (acanthus-leaved thistle, Asteraceae) root EO was evaluated by Dordevic et al. (2007). In traditional medicine the root of the plant is used for the treatment of a variety of diseases concerning stomach and skin. The anti-inflammatory activity was tested in the carrageenan-induced rat paw edema assay and the oil inhibited the edema in all applied concentrations. The effect could be compared to indomethacin, which was used as control.

Another study was made on the anti-inflammatory effect of the EO and the active compounds of the Boraginaceae *Cordia verbenacea* (black sage) by Passos et al. (2007). It was found that the carrageenan-induced rat paw edema, the myeloperoxidase activity, and the mouse edema elicited by carrageenan, bradykinin, substance P, histamine, and the platelet-activating factor could be inhibited after systemic (p.o.) administration of 300–600 mg/kg EO. It also suppressed carrageenan-evoked exudation, the neutrophil influx to the rat pleura and the neutrophil migration into carrageenan-stimulated mouse air pouches. Additionally, a reduction of edema caused by *Apis mellifera* venomous OVA in sensitized rats and OVA-evoked allergic pleurisy could be observed. TNF-α was significantly inhibited in carrageenan-treated rat paws by the EO, whereas the IL-1β expression was not influenced. No affection was caused of neither the PGE2 formation after intrapleural injection of carrageenan, nor of the COX-1 or COX-2 activities in vitro. Both sesquiterpenes, α-humulene and trans-caryophyllene (50 mg/kg p.o.) obtained from the EO, lead to a remarkable reduction of the carrageenan-induced mouse paw edema. All in all, this study demonstrated the anti-inflammatory effect of the EO of *Cordia verbenacea* and its active compounds. The possible mechanism of this effect might be caused by the interaction with the TNF-α production. The authors suggest that *Cordia verbenacea* EO could represent new therapeutic options for the treatment of inflammatory diseases.

The topical anti-inflammatory effect of the leaf EO of the Verbenaceae *Lippia sidoides* Cham. was studied by Monteiro et al. (2007). In northwestern Brazil, the plant is widely used in the social medicine program “Live Pharmacies” as a general antiseptic because of its strong activity against many microorganisms. After topical application of 1 and 10 mg/ear, in 45.9% and 35.3%, a significant reduction (p < 0.05) of the acute ear edema induced by 12-tetradecanoylphorbol 13-acetate (TPA) could be observed.

The anti-inflammatory effects of the EO from *Eremanthus erythropappus* leaves (Asteraceae) have already been discussed in the chapter dealing with antinociception (Sousa et al., 2008). An interesting study concerning the healing of *Helicobacter pylori*-associated gastritis by the
volatile oil of “Amomum” (several Amomum species, which are used in similar manner to cardamon (Elettaria cardamomum), Zingiberaceae) was published recently by a Chinese author group. The effects of this EO on the expressions of mastocarcinoma-related peptide and platelet-activating factor was assessed and its potential mechanism discussed. The mechanism of this volatile oil for its anti-gastritis activity could be the influence on the decrease of the expression of the platelet-activating factor and thus regulating the hydrophobicity of the gastric membrane. About 88% of the patients with a proven *Helicobacter pylori* infection were treated and showed a higher healing rate compared to the control group having received a traditional “Western” tertiary medicinal treatment (Huang et al., 2008).

### 9.1.5 Penetration Enhancement*

A number of EOs are able to improve the penetration of various drugs through living membranes, for example, the skin. The improvement of the penetration can be achieved by the interaction of the EOs with liquid crystals of skin lipids. In the following, the penetration enhancing effect of some EOs will be discussed. For determination of the penetration enhancing effect different experimental setups were used: Valia–Chien horizontal diffusion cells, Keshary–Chien diffusion cells, and Franz diffusion cells. Furthermore, the scientists using polarizing microscopy, differential scanning calorimetry (DSC), x-ray diffraction, and high performance liquid chromatography (HPLC) to detect the penetration through the skin.

The interaction of eucalyptus oil with liquid crystals of skin lipids was proved by Abdullah et al. (1999) using polarizing microscopy, DSC, and x-ray diffraction. Crystal 1 (matrix 1) consisted of five fatty acids of stratum corneum, crystal 2 (matrix 2) consisted of cholesterol together with five fatty acids. Dispersion and swelling of the lamellar structure were observed after application of small amounts of eucalyptus oil, whereas large amounts resulted in their breakage and disappearance. The EO did not promote the formation of any other structures. This interaction seems to be the explanation for the increase of permeation of drugs through stratum corneum in the presence of eucalyptus oil and similar penetration enhancers.

Li et al. (2001) investigated the effects of eucalyptus oil on percutaneous penetration and absorption of a clobetasol propionate cream using vertical diffusion cells. The *in vitro* penetration of the cream containing 0.05% clobetasol propionate through mouse abdominal skin was detected at 2, 4, 6, 8, 10, and 24 h (cumulative amount $Q$, $\mu g/g$) and at steady state ($J$, $\mu g/cm^2/h$). The quantity of clobetasol propionate within the whole stria of skin after 24 h ($D$, $\mu g/g$) was measured too. Eucalyptus oil was able to increase $Q$ and $J$, whereas $D$ was not influenced in that way, which indicates that eucalyptus oil would increase clobetasol propionate percutaneous absorption and cause unwanted side effects.

Cinnamon oil, eugenia oil, and galangal oil have been studied for their potency as percutaneous penetration enhancers for benzoic acid (Shen et al., 2001). Valia–Chien horizontal diffusion cell and HPLC were used to detect benzoic acid penetration through skin.

Skin penetration of benzoic acid was significantly enhanced by all three volatile oils. In combination with ethanol and propylene glycol the amount of benzoic acid was increased, but the permeability coefficients were decreased. In conclusion, cinnamon oil, eugenia oil, and galangal oil might be used as percutaneous penetration enhancers for benzoic acid.

Monti et al. (2002) tried to examine the effects of six terpene-containing EOs on permeation of estradiol through hairless mouse skin. Therefore, *in vitro* tests with cajeput, cardamom, melissa, myrte, niaouli, and orange oil (all 10% wt/wt concentration in propylene glycol) have been carried out. Niaouli oil was found as the best permeation promoter for estradiol. Tests with its single main components 1,8-cineole, $\alpha$-pinene, $\alpha$-terpineol, and $d$-limonene (all 10% wt/wt concentration in propylene glycol) showed that the whole niaouli oil was a better activity promoter than the single compounds. These data demonstrate complex terpene mixtures to be potent transdermal penetration enhancers for moderately lipophilic drugs like estradiol.

Different terpene-containing EOs have been investigated for their enhancing effect in the percutaneous absorption of trazodone hydrochloride through mouse epidermis (Das et al., 2006). Fennel oil, eucalyptus oil, citronella oil, and mentha oil were applied on the skin membrane in the transdermal device, as a pretreatment or both using Keshary–Chien diffusion cells and constantly stirring saline phosphate buffer of pH 7.4 at 37 ± 1°C as receptor phase. Pretreatment of the skin with EOs increased the flux values of trazodone hydrochloride compared with the values obtained when the same EOs were included in the transdermal devices. The percutaneous penetration flux was increased with skin permeation by 10% EOs in the following order: fennel oil > eucalyptus oil > citronella oil > mentha oil. The quantity of trazodone hydrochloride kept in the skin was very similar for all EOs and much higher than in control group.

An in vitro study about Australian TTO was made by Reichling et al. (2006). The aim of the study was to investigate the penetration enhancement of terpinen-4-ol, the main compound of TTO, using Franz diffusion cells with heat separated human epidermis and infinite dosing conditions. The three semisolid preparations with 5% TTO showed the following flux values: semisolid oil-in-water (O/W) emulsion (0.067 μL/cm²/h) > white petrolatum (0.051 μL/cm²/h) > ambiphilic cream (0.022 μL/cm²/h). Because of the lower content of terpinen-4-ol, the flux values were significantly reduced compared to native TTO (0.26 μL/cm²/h). The papp values for native TTO (1.62 ± 0.12 × 10⁻⁷ cm/s) and ambiphilic cream were comparable (2.74 ± 0.06 × 10⁻⁷ cm/s), whereas with white petrolatum (6.36 ± 0.21 × 10⁻⁷ cm/s) and semisolid O/W emulsion (8.41 ± 0.15 × 10⁻⁷ cm/s) higher values indicated a penetration enhancement. Between permeation and liberation there was no relationship observed (Table 9.2). The stratum corneum absorption and retention of linalool and terpinen-4-ol was investigated by Cal and Krzyzaniak (2006). Both monoterpens were applied to eight human subjects as oily solution or as carbomeric hydrogel. The stratum corneum absorption after application of carbomeric hydrogel was better than that achieved with an oily solution. Two mechanisms of elimination from the stratum corneum were observed: evaporation from the outer layer and drainage of the stratum corneum reservoir via penetration into dermis. The retention of the monoterpens in the stratum corneum during the elimination phase was a steady state between 6 and 13 μg/cm².

Also linalool alone, one of the most prominent monoterpene alcohols, is used in many dermal preparations as penetration enhancer. In a series of in vitro studies it was shown that linalool enhanced its own penetration (Cal and Sznitowska, 2003) as well as the absorption of other therapeutics, such as haloperidol (Vaddi et al., 2002a, 2002b), metoperidol (Komuru et al., 1999), propa- nolol hydrochloride (Kunta et al., 1997), and transcutol (Ceschel et al., 2000). Cal (2005) showed in another in vitro study the influence of linalool on the absorption and elimination kinetics and was able to prove that this monoterpene alcohol furnished the highest absorption ratio compared to an oily solution or an O/W emulsion.

Wang, L.H. et al. (2008) reported on enhancer effects on human skin penetration of aminophylline from cream formulations and investigated for this report four EOs, namely rosemary, ylang, lilac, and peppermint oil compared with three plant oils, the liquid wax jojoba oil, and the fatty oils.

<table>
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<th>Flux Values (μL/cm²/h)</th>
<th>Papp Values (cm/s)</th>
</tr>
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<tbody>
<tr>
<td>Native TTO</td>
<td>0.26</td>
<td>1.62 ± 0.12 × 10⁻⁷</td>
</tr>
<tr>
<td>Ambiphilic cream</td>
<td>0.022</td>
<td>2.74 ± 0.06 × 10⁻⁷</td>
</tr>
<tr>
<td>Semisolid O/W emulsion</td>
<td>0.067</td>
<td>8.41 ± 0.15 × 10⁻⁷</td>
</tr>
<tr>
<td>White petrolatum</td>
<td>0.051</td>
<td>6.36 ± 0.21 × 10⁻⁷</td>
</tr>
</tbody>
</table>
of corn germs and olives. In this study the EOs were less effective in their penetration enhancement than the three plant oils. The effect of penetration enhancers on permeation kinetics of nitrendipine through two different skin models was evaluated by Mittal et al. (2008) and also this author group found that the used EOs (thyme oil, palmarosa oil, petit grain oil, and basil oil) were inferior in their penetration enhancement effects to oleic acid but superior to a lot of other common permeation enhancers, such as sodium lauryl sulfate, myristic acid, lauric acid, Tween 80, or Span 80. In contrast to these two reports Jain et al. (2008) found that basil oil is a promising penetration enhancer for improved drug delivery of labetolol. The effect of clove oil on the transdermal delivery of ibuprofen in the rabbit in vitro and in vivo methods was investigated by Shen et al. (2007). The in vitro results indicated a significant penetration enhancement effect of the clove oil whereas the in vivo results showed a weaker enhancement. The good transdermal delivery of ibuprofen from the essential clove oil could be attributed to the principal constituents eugenol and acetyl eugenol.

9.1.6 Antioxidative Properties*

Free radicals are aggressive, unstable, and highly reactive atoms or compounds because of their single electron. They attack other molecules to reach a steady stage, thereby changing their properties and making disorders inside possible. Free radicals result from products from different metabolic activities. A large number occur because of smog, nitrogen oxides, ozone, cigarette smoke, and toxic heavy metal. Also chemicals such as organic solvents, halogenated hydrocarbons, pesticides, and cytostatic drugs cause a high number of free radicals. If a lot of energy is built or has to be supplied, such as sporty high-performance, extreme endurance sports, sunbathing, solarium, exposure, x-rays, UV radiation, pyrexia, or infections, they are also massively produced (Schehl and Schroth, 2004). Preferred for attack are nucleic acids of the DNA and RNA, proteins, and especially polyunsaturated fatty acids of the membrane lipids. To protect the body’s own structure from damages, all aerobe living cells use enzymatic and nonenzymatic mechanisms. Scavengers are able to yield electrons and so they dispose free radicals. Also, enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase are very important in protection mechanisms (Eckert et al., 2006). Oxidative and antioxidative processes should keep the balance. If the balance is in benefit for the oxidative processes, it is called “oxidative stress.”

9.1.6.1 Reactive Oxygen Species

Basically, oxygen radicals (Table 9.3) are built by one-electron reduction in the context of autoxidation of cell-mediated compounds. The superoxide radical arises from autoxidation of hydroquinone, flavine, hemoglobin, glutathione, and other mercaptans and also from UV light, x-rays, or gamma rays. Two-electron reduction yields hydrogen peroxide. The very reactive hydroxyl radical is built by three-electron reduction, which is mostly catalyzed by metal ion (Löffler and Petrides, 1998). A lot of other oxygen radicals are accumulated by secondary reactions because they damage the biomolecules, ROS participate in different diseases, mainly cancer, aging, diabetes mellitus, and atherosclerosis.

9.1.6.2 Antioxidants

Antioxidants are substances that are able to protect organisms from oxidative stress. A distinction is drawn between three types of antioxidants: enzymatic antioxidants, nonenzymatic antioxidants, and repair enzymes.

Well-known naturally occurring antioxidants are vitamin C (ascorbic acid), which are contained in many citrus fruits, or on the other hand members of vitamin E family, which appear for example in nuts and sunflower seeds. Also β-carotene and lycopine, which also belong to the family of carotenoides, are further examples of natural antioxidants. On the other hand, there are many synthetic

substances such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and the water-soluble vitamin E derivative troxol (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) noted for their antioxidative activity. Different studies suspect the synthetic antioxidants to cause different diseases, so there has been much interest in the antioxidative activity of naturally occurring substances (Salehi et al., 2005).

To the group of enzymatic antioxidants belongs the manganese- or zinc-containing SOD, the selenium-containing glutathione peroxidase, and the iron-containing catalase. Their capacity is addicted to adequate trace elements and minerals (Schehl et al., 2004). Nonenzymatic antioxidants have to be admitted with food or substitution, for example, \( \alpha \)-tocopherol, l-ascorbic acid, \( \beta \)-carotene, and secondary ingredients of plants. Repair enzymes delete damaged molecules and substitute them.

### 9.1.7 Test Methods

#### 9.1.7.1 Free Radical Scavenging Assay

This spectrophotometric assay uses the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent (Yadegarinia et al., 2006). The model of scavenging stable DPPH-free radicals can be used to evaluate the antioxidative activities in a relatively short time (Conforti et al., 2006). The samples are able to reduce the stable free DPPH radical to 1,1-diphenyl-2-picrylhydrazyl that is yellow colored. The hydrogen or electron donation abilities of the samples are measured by means of the decrease of the absorbance resulting in a color change from purple to yellow (Gutierrez et al., 2006). Another procedure can be applied for an EO. A dilution of the EO in toluene is applied onto a thin-layer chromatography (TLC) plate and toluene-ethyl-acetate is used as a developer (Sökmen et al., 2004a). The plates are sprayed with 0.4 mM DPPH in methanol. The active compounds were detected as yellow spots on a purple background. Only those compounds, which changed the color within 30 min, are taken as a positive result.

#### 9.1.7.2 \( \beta \)-Carotene Bleaching Test

The lipid peroxidation inhibitory activities of EOs are assessed by the \( \beta \)-carotene bleaching tests (Yadegarinia et al., 2006). In this method, the ability to minimize the coupled oxidation of \( \beta \)-carotene and linoleic acid is measured with a photospectrometer. The reaction with radicals shows a change in this orange color. The \( \beta \)-carotene bleaching test shows better results than the DPPH assay because it is more specialized in lipophilic compounds. The test is important in the food industry because the test medium is an emulsion, which is near to the situation in food, therefore allowable alternatives to synthetic antioxidants can be found. An only qualitative assertion uses the TLC procedure. A sample of the EOs is applied onto a TLC plate and is sprayed with \( \beta \)-carotene and linoleic acid. Afterwards, the plate is abandoned to the daylight for 45 min. Zones with constant yellow colors show an antioxidative activity of the component (Guerrini et al., 2006).

<table>
<thead>
<tr>
<th>TABLE 9.3</th>
<th>Reactive Oxygen Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Name</td>
</tr>
<tr>
<td>( \text{O}_2^- )</td>
<td>Superoxide radical</td>
</tr>
<tr>
<td>( \text{HO}_2^- )</td>
<td>Perhydroxyl</td>
</tr>
<tr>
<td>( \text{H}_2\text{O}_2 )</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>( \text{HO}^+ )</td>
<td>Hydroxyl radical</td>
</tr>
<tr>
<td>( \text{RO}^- )</td>
<td>R-oxyl radical</td>
</tr>
<tr>
<td>( \text{ROO}^- )</td>
<td>R-dioxy radical</td>
</tr>
</tbody>
</table>

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9.1.7.3 Deoxyribose Assay
This assay is used for determining the scavenging activity on the hydroxyl radical. The pure EO is applied in different concentrations (Dordević et al., 2006). The competition between deoxyribose and the sample about hydroxyl radicals that are engendered by an Fe$^{3+}$/EDTA/H$_2$O$_2$ system is measured. The radicals were formed to attack the deoxyribose and they are detected by their ability to degrade 2-deoxy-2-ribose into fragments. These degradation products generate with 2-thiobarbituric acid (TBA) at a low pH and upon heating pink chromogens. The TBA-reactive substances could be determined spectrophotometrically at 532 nm. So the damage of 2-deoxy-2-ribose by the radicals is detected with the aid of the TBA assay.

9.1.7.4 TBA Test
This assay is basically used to appoint the lipid oxidation. It is an older test for receiving the oxidation status of fats spectrophotometrically. Thereby the aldehydes, which are generated by the autoxidation of unsaturated fatty acids, are converted into red or yellow colorimeters with TBA. But it is also used for the determination of the potency of antioxidants with thiobarbituric acid reactive substances (TBARS). Thereby the antioxidant activity is measured by the inhibition of the lipid oxidation. It concerns the spectrophotometric detection of malonic aldehyde, one of the secondary lipid peroxidation products, which generates a pink pigment with TBA (Ruberto et al., 2000; Varda-Ünlü et al., 2003).

9.1.7.5 Xanthine–Xanthine Oxidase Assay
Superoxide radicals are produced by a xanthine–xanthine oxidase system. Xanthine is able to generate O$_2^•$ and H$_2$O$_2$ by using xanthine oxidase as a substrate. The superoxide radicals are able to reduce the yellow colored nitro triazolium blue (NTB) to the blue formazan, which is used for monitoring the reaction. The superoxide anions are measured spectrophotometrically. This test method was developed to explore the reaction between antioxidants and O$_2^•$. So the inhibition of the superoxide reductase is a mark for the ability of antioxidative activity.

9.1.7.6 Linoleic Acid Assay
This test system was developed to determine the ability of substances to inhibit the generation of hydroxy peroxides at the early stages of the oxidation of linoleic acid, as well as for its inhibitory potential after the formation of secondary oxidized products such as aldehydes, ketones, or hydrocarbons (Jirovetz et al., 2006). Either the oxidation of linoleic acid is monitored by measuring the values of conjugated dienes or TBARS spectrophotometrically or hydroperoxy-octadeca-dienoic acid isomers (HOPES) generated during the oxidation are measured (Marongiu et al., 2004).

The biological features of *Mentha piperita* L. (peppermint, Lamiaceae) oil and *Myrtus communis* L. (myrtle, Myrtaceae) oil from Iran were studied by Yadegarinia et al. (2006). One of the main constituents of the EO of *Myrtus communis* is 1,8-cineole (also called eucalyptol), which showed the most powerful DPPH radical scavenging activity. Myrtle oil contains about 18% 1,8-cineole and showed a scavenging activity of 3.5% upon reduction of the DPPH radical to the neutral DPPH-H form. Better results yielded the β-carotene bleaching test. Also the EO of *Mentha aquatica* (water mint) contains up to 14% 1,8-cineole and proved itself in the DPPH assay to be an acceptably radical scavenger and the most active compound in the oil. With *Mentha communis* oil comprising 21.5% limonene, an antioxidative activity was assessed with about 43% in contrast to the reference oil of *Thymus porlock* (Lamiaceae) having an efficiency of 77% (Mimica-Dukic et al., 2003). Also α-terpinene, a constituent of the EO of *Mentha piperita* (about 20%), showed in both test procedures a scavenging activity lower than that of the standard found with the EO of *Thymus porlock*, but similar to the efficiency of the synthetic antioxidant trolox (23.5% versus 28.3%). Another main compound of this oil is β-caryophyllene with about 8%. *Mentha piperita* oil attained a scavenging power of 23.5% and is also able to inhibit the lipid oxidation, which was determined in the β-carotene bleaching test. The results of the standard and the EO were correlated with the results from the
DPPH assay. The peppermint oil shows a lipid peroxidation inhibition of 50% compared with 77% of the standard.

*Sesuvium portulacastrum* (sea purslane, Ficoidaceae) was collected in the northern, western, and central part of Zimbabwe to determine the chemical activity of the essential leaf oils, which showed besides an antibacterial and antifungal also a significant antioxidative activity (Magwa et al., 2006). Sea purslane is used by the traditional healers in Southern Africa to treat various infections and kidney problems. The secondary metabolites from this plant species have a great potential as substitutes for synthetic raw materials in food, perfumery, cosmetic, and pharmaceutical industries. Thus, the composition and the biological activities of the EO of this Ficoidaceae were studied. One of the major chemical compounds is 1,8-cineole (6.8%). The antioxidative testing was carried out by a modified β-carotene bleaching test. The background of these test methods is that β-carotene is a yellow antioxidant, which becomes colorless when it encounters light or oxygen. With the attendance of another antioxidant, which is not so sensitive to light or oxygen, the yellow color of the β-carotene could continue for some time. Retention of the yellow areas around the test compounds shows an antioxidative activity. In comparison to the positive control (ascorbic acid) with a diameter of 27 mm, the EO showed a 15.9-mm zone of color retention. Mainly responsible to the antioxidative activity besides this bicyclic ether is the content of 2-β-pinene (13.6%), α-pinene (14%), limonene (6.4%), and ocimene, α-terpinolene, and camphene, which also belong to the group of main compounds of the EO of *Sesuvium portulacastrum*.

*Thymus* (thyme, Lamiaceae) is a very big genus, to which more than 300 evergreen species belong. All of them are well-known aromatic and medical plants and also the oil of different species are used against various diseases. So, many species of this genus were tested for their biological activities inclusive the antioxidative properties of the EOs and their constituents. The EOs of *Thymus caespitiius* (Azoricus thyme, Lamiaceae), *Thymus camphoratus* (camphor thyme), and *Thymus masticina* (mastic thyme) show in different investigations an antioxidative activity, which is comparable to the action of α-tocopherol, a well-known naturally occurring antioxidant (Miguel et al., 2003). One reason for this capacity could be that the EO of all three species contains a high concentration of 1,8-cineole. Because species of the genus *Thymus* (thyme) are widely used as medicinal plants and spices, the antioxidative activity of the EO of *Thymus pectinatus* comprising up to 16% γ-terpinene was determined by multifarious test systems. To detect the hydroxyl scavenging activity a deoxyribose assay was used, a DPPH assay was arranged, and the inhibition of lipid peroxidation formation by a TBA assay was measured. Fifty percent of the free DPPH radicals were scavenged by the EO, which is a stronger antioxidative activity than that of the used standards (BHT, curcumin, and ascorbic acid). Furthermore, the EO showed an inhibitory effect in the deoxyribose assay, and comparable results emanated from the TBA assay, where the oil had an IC₅₀ value similar to the activity of BHT. Finally, also the EO of *Thymus zygis* (sauce thyme) which contains among other monoterpenes p-cymene showed a dose-dependent antioxidative activity (Dorman and Deans, 2004). Furthermore, the above described species, *Thymus pectinatus*, is characterized by a very high content of the phenolic compound thymol. Therefore, two fractions of this EO, characterized by a high content of thymol (95.5% and 80.7%, respectively), were studied using four different test methods to assess their antioxidative activity. Fifty percent of the free radicals in the test with DPPH were scavenged by 0.36 ± 0.10 μg/mL of the EO. This is a stronger inhibition as the controls afford. Then the deoxyribose assay was used to identify the inhibition of the degradation of hydroxyl radicals by the EO and its main compounds. The investigation showed that thymol has an IC₅₀ value of 0.90 ± 0.05 μg/mL and the EO an IC₅₀ value of 1.40 ± 0.03 μg/mL. To prove the inhibition of the lipid peroxidation, also the TBA method was used and afforded an IC₅₀ value of 9.50 ± 0.02 μg/mL for the EO. Also in this case the EO showed a better protection than the control substances BHT and curcumin, so to say the two major compounds, thymol and carvacrol, show a very strong antioxidative activity. Then the EO of another thyme species with a thymol content of about 31%, namely *Thymus eigii*, was studied by the DPPH and the β-carotene bleaching test (Tepe et al., 2004). For the rapid screening DPPH on TLC was applied. Compendiously after the plate had been sprayed
three spots appeared, which were identified as thymol, carvacrol, and α-terpineol. Thus, an antioxidative activity of this EO was established as well. Also in the volatile extract of *Thymus vulgaris* (common thyme), thymol dominates with 72%. In a test system, where the inhibition of hexane oxidation is used to determine the antioxidative activity, thymol shows one of the strongest activities. In a concentration of 10 μg/mL, the capacity to decrease the oxidation is equal to the activity of BHT and α-tocopherol. These two, well-known, antioxidants inhibit the hexane oxidation up to 89% and 99% in a concentration of 5 μg/mL over 30 days. Also carvacrol, an isomer of thymol and existing in a concentration of about 6% in this oil, is able to hinder the oxidation of hexane by 95–99% at 5 μg/mL over a period of 30 days. This is comparable with the activity of BHT and α-tocopherol (Randonic et al., 2003; Sacchetti et al., 2004; Faleiro et al., 2005). These findings could be confirmed by a very recent study of Chizzola et al. (2008). The antioxidative activity depends on the concentration of the phenolic constituents thymol and/or carvacrol.

Thymol as well as carvacrol protects low-density lipoprotein (LDL) from oxidation (Pearson et al., 1997), and this antioxidative activity is dependent on the concentration: below 1.25 μM no effect was detected, whereas at a concentration between 2.5 and 5.0 μM thymol offers a very strong antioxidative capacity. Finally, the EOs of 15 different wild grown *Thymus* species show the ability to delay lard becoming rancid, because of the inhibition of lipid oxidation. The value of generated peroxides was measured to detect the antioxidative activity. Therefore, variable concentrations of the EO were added to lard and each mixture stored at 60ºC. In regular time intervals samples were taken and the peroxide amount was determined. As standards BHT, BHA, and thymol were used. A “thymol- and a thymol/carvacrol group” were able to keep the peroxide value low for a period of 7 days. The EOs of *Thymus serpyllus* (creeping thyme) and *Thymus sspathulifolius* comprise high contents of carvacrol (58% and 30% respectively) and exhibit both an antioxidative activity near to BHT. Another species is *Thymus capitata* (Corido thyme), with an amount of 79% carvacrol, and shows an antioxidative activity assessed by the TBA assay in variable concentrations. There is no difference at the concentration of 1000 mg/L between the capacity of the oil and the control BHT, but the antioxidative properties are better than BHA and α-tocopherol. In a micellar model system, where the decrease of just generated conjugated dienes is used to determine the antioxidative ability, *Thymus capitata* oil showed an antioxidative index of 96% and proved a good protective activity in the primary lipid oxidation. The oil is better effective against lipid oxidation and in higher concentrations better than BHA and α-tocopherol. The addition of a radical inducer reduced the antioxidative activity of the EO. Carvacrol is capable to prevent up to 96% the primary step of lipid oxidation. Also *Thymus pectinatus* EO contains carvacrol as one of the main compound and shows a strong antioxidative activity. Fifty percent inhibition of lipid peroxidation (detected by the TBA assay) was attained at a concentration of 5.2 μg/mL carvacrol (Vardar-Ünlü et al., 2003; Miguel et al., 2003; Hazzit et al., 2006).

The EO of *Ziziphus clinopodioides* ssp. *rigida* (blue mint bush) was isolated by hydrodistillation of the dried aerial parts, which was collected during the anthesis. The main compounds are thymol and 1,8-cineole with a content of 8% and 2.7%, respectively. Different extracts were tested by the DPPH assay to determine the antioxidative activity and showed that the free radical scavenging activity of the menthol extract was superior to all other extracts. Polar extracts exhibited stronger antioxidant activity than nonpolar extracts (Salehi et al., 2005).

Many species of the genus *Artemisia* (wormwood, Asteraceae) are used as spices, for alcoholic drinks and also in the folk and traditional medicine. The chemical compounds and the antioxidative activity of the EOs isolated from the aerial parts of *Artemisia absinthium* (vermouth), *Artemisia santonicum* (sea wormwood), and *Artemisia spicigera* (sluggish wormwood) were investigated (Kordali et al., 2005). The analysis of the EO of *Artemisia santonicum* and *Artemisia spicigera* showed two main components, namely 1,8-cineole and camphor. In addition, it is noticed that the EO of these two species contain no thujone derivates in contrast to *Artemisia absinthium*. Earlier studies have also shown that 1,8-cineole and camphor are main components of the EO of some *Artemisia* species. The antioxidative activity of the EO of *Artemisia santonicum* and
Artemisia spicigera was analyzed by the thiocyanate method and a high antioxidative activity was found. With the thiocyanate test and the DPPH radical scavenging assay, the high antioxidative activity of Artemisia santonicum EO is ensured. On account of their adequate antioxidative activity, Artemisia santonicum and Artemisia spicigera could possibly be used in the liqueur-making industry, because they do not include thujone derivates. The antioxidative activity of the EO from another wormwood species, namely Artemisia molinieri (mugwort), was investigated by chemoluminescence. The main compounds of this EO are α-terpinene with 36.4% and then 1,4-cineole (<17%), germacrene D (up to 15%), p-cymene, and ascaridol. In the used test method the EO or α-tocopherol, the reference compound, are added to a solution of AAPH (2,2-azobis (2-amidinopropane)-dihydrochloride) and luminol. Chemoluminescence intensities of both blank and assay were monitored and the percentage of inhibition was calculated. The EO showed a high antioxidative activity that is similar to the activity of α-tocopherol (Masotti et al., 2003). Also Artemisia abyssinica and Artemisia afra (African wormwood)—due to the content of α-terpineol—showed an inhibition in the deoxyribose assay (Burits et al., 2001). A Canadian author team investigated seven wild collected Artemisia species from Western Canada, determined the composition of the EOs from their aerial parts, and found—interestingly—that these oils exerted only weak antioxidant activities in the β-carotene/linoleate model and DPPH test (Lopes-Lutz et al., 2008).

The GC-MS analysis of the EO of the herbal parts of Achillea biebersteinii Afan (yarrow, Asteraceae), which were collected during the anthesis, furnished a high number of oxygenated monoterpenes, such as piperitone (34.9%), 1,8-cineole (13.0%), and camphor (8.8%) as the main compounds. The antioxidative activity has been assessed by four different methods: free radical scavenging assay with DPPH, hydroxyl radical scavenging with the deoxyribose assay, inhibition of the lipid peroxidation with the TBA test, and inhibition of the superoxide radicals xanthine–xanthine assay. Curcumin, ascorbic acid, and BHT were taken as positive control. Difficulties arising from the water-insoluble parts of the extracts render the spectroscopic measuring negatively. On this account, only the water-soluble parts of the extract could be determined for antioxidative action. The results of the tests showed that the EO possesses a better capacity in the donation of the hydrogen atoms or electrons than curcumin and BHT, but there was no difference in the action to ascorbic acid (Sökmen et al., 2004). Also hydroxyl radical scavenging and the lipid peroxidation inhibition were more effective than curcumin. Due to these results the EO of Achillea biebersteinii could be a valuable raw material for natural antioxidant additives. Also the EO of Achillea millefolium ssp. millefolium (common yarrow) in which 1,8-cineole and α-terpineol are major constituents shows a strong antioxidative activity. This was assessed by the deoxyribose assay, the xanthine–xanthine assay, and the DPPH test (Candan et al., 2003). Also the inhibition of the lipid peroxide generation was measured. The results of these tests were compared with the antioxidative activity of three well-known and often used antioxidants, namely BHT, curcumin, and ascorbic acid. On account of the scavenging activity for the stable free radical DPPH with an IC₅₀ value of 1.56 μg/ml, this oil showed a better antioxidative capacity than the just aforementioned well-known antioxidants. Other Achillea species wild samples of Achillea ligustica (Ligurian yarrow), collected in different parts of Sardinia, are also characterized by the content of 1,8-cineole as one of the main compounds of the EO with an average value of about 2.9%. In the DPPH assay the EO shows an interesting antioxidative activity that can be used for applying such oils as nutraceutical products or as additives in food industry (Tuberoso et al., 2005).

Helichrysum (strawflower, Asteraceae) species are traditionally used for the treatment of wounds, infections, and respiratory conditions (Lourens et al., 2004). In the inflammatory process free radicals adsorb on the phagocyte cells, so the antioxidative activity of the extract and the EOs of different Helichrysum species were examined. Therefore, the plant material of Helichrysum dasyanthum, Helichrysum excisum, and Helichrysum petiolare were collected and the EO obtained by hydrodistillation. The GC-MS analysis showed that 1,8-cineole (20–34%), p-cymene (6–10%), and α-pinene (3–17%) are principal components of the EO. The EOs of all three different species show an antioxidative activity in the DPPH assay, but it has to be noticed that the extract, which was obtained by a cold
extraction, had a better activity in the assay than the EOs. These test scores are an important argument for the use of some plants of this genus in the traditional medicine (Grassmann et al., 2000).

Also the EO of the fresh fruits by *Xylopia aethiopica* (Meleguetta pepper, Negro pepper, and African pepper tree, Anonaceae) is characterized by the occurrence of 1,8-cineole and β-pinene. But also EOs from the leaves (containing about 17% β-pinene and even 24.5% germacrene D), barks, stems, and roots are known and examined for their composition and antioxidative activities. The antioxidative activity of the EO from different parts of the plant was determined by the free radical scavenging assay with DPPH and the xanthine–xanthine assay. All tested samples were found to interact with the stable free radical DPPH in a time-dependent manner (Karioti et al., 2004). The highest radical scavenging in the DPPH assay was measured with the EO of the fresh fruits (comprising about 9% germacrene D) with an interaction of 85.6%. Generally, all the EOs of the different parts possess the ability to scavenge free radicals. Also the xanthine–xanthine assay showed that the capability to reduce the superoxide radical is given.

Plants of the genus *Melaleuca* (Tea tree, Myrtaceae) are rich in volatile oils. On this account, different species were investigated for their biological activity as well as for their antioxidative capacity. A GC-MS analysis revealed that 1,8-cineole is the major compound of the EO extracted from *Melaleuca armillaris* (33.9%). For the study 50 male albino rats were treated differently. Besides the control group the rats received multiple doses of the EO 3 times a week for 1 month. To evaluate the antioxidative activity the following estimations were carried out: SOD, vitamin C, catalase, glutathione, and lipid peroxides (Farag et al., 2004). An alternation of the antioxidant status induced by CCl₄, as free radical inducer, before and after the administration of the EO was studied as well. The EO of *Melaleuca armillaris* increased the value of vitamin E and vitamin C. The same effect was given by the level of SOD and lipid peroxide compared to the control group. Only the level of catalase was decreased by the treatment of *Melaleuca* oil. TTO from *Melaleuca alternifolia* (Australian tea tree) is known for its wide spectrum of biological activities, therefore the antioxidative properties of TTO was assessed by various methods, such as the DPPH radical scavenging or the hexanal/hexanoic acid assay (Hyun-Jin et al., 2004). The crude EOs, as well as the most active fractions (fractions 5 and 6 after silica gel open column chromatography and C₁₈-HPLC), were used for the investigation. The three major compounds in these fractions were γ-terpinene (20.6%), α-terpinene (9.6%), and α-terpineol. A correlation between the scavenging activity and the concentration was emerged in the DPPH assay. At a concentration of 10 mM, the activity of α-terpinene with more than 80% was near to the scavenging capacity of BHT. Eighty-two percent activity was determined for a concentration of 180 mM which is also close to the synthetic antioxidant (85%). It was shown that α-terpinene had the strongest scavenging effect in this test system compared to the other two compounds. The second test system, which was performed, was the hexanal/hexanoic acid assay. In lower concentrations (30 and 90 mM) α-terpinene exhibited a strong inhibitory activity at first but it decreased extremely fast. The inhibitory effect increased with the arising concentration. α-Terpinene (180 mM) as well as γ-terpinene had a blocking action of 65% over the 30 days. It should be noticed that the antioxidative activity of BHT was stronger compared to any isolated compound of the TTO at lower concentrations.

Vitamin E is a natural antioxidant, which occurs in the plasma red cells and tissues, disarms the free radicals, and anticipates the peroxidation of polyunsaturated fatty acids and phospholipids. Also vitamin C is one of the naturally occurring antioxidants, which actually increase the efficiency of vitamin E to avoid the lipid peroxidation. SOD protects the cells of hydrogen peroxide anion free radicals, furnishes the decrease of the catalase which decomposes H₂O₂, thus yielding a reduction of the oxidative process. This research shows that the EO of *Melaleuca armillaris*, which has 1,8-cineole as the major chemical compound, can be used as a suppressor for free radicals and is able to avoid damages caused by oxidative stress generated by chemical or physical factors.

Myrtol standardized and eucalyptus oil, which both contain about 70–80% 1,8-cineole, were examined in a Fenton system which is a very sensitive indicator for ROS such as α-keto-γ-methiolbutyric acid (KMB) or 1-aminocyclopropane-1-carboxylic acid (ACC). OH radicals are generated by
the reaction between hydrogen peroxide and Fe$^{2+}$ (Grassmann et al., 2000). The KMB is transformed into ethene, carbon dioxide, formiate, and dimethyldisulphide. The ethene can be detected in very low quantities by GC. Both oils prevent KMB before destruction and so no ethene can be detected.

The EOs of citrus fruits (Rutaceae)—obtained upon pressing the peels—are also called “agrumen oils” and are characterized normally by a high content of the monoterpenic hydrocarbon d-limonene which even after boiling the fruits remains in the peel in a substantial quantity. So, citrus fruits are a very interesting source for the occurrence of antioxidants. Twenty-six citrus fruits and their flavor compounds were investigated for an antioxidative activity by the thiocyanate test. The EO of *Citrus sinensis* Osbeck var. Sanguinea Tanaka form a *Taroceco* (Tarocco orange) which contains as major component limonene (84.5%) shows a very high antioxidative activity of about more than 90%. Also the oil of *Citrus aurantium* Linn. var. *cyathifera* Y. Tanaka (Dadai) exerts an antioxidative activity up to 19% but it was slightly weaker than that of the standard troxol, a watersoluble vitamin E derivative. Also all the citrus EOs show an antioxidative activity against linoleic acid peroxidation (Song et al., 2001). The scavenging activity of the authentic compounds was evaluated in the DPPH test and the activity from limonene was assessed from 8.8% to 16.5%, whereas γ-terpinene showed that in this test a noticeable radical scavenging effect for γ-terpinene was 84.7%, which is 3.5 times stronger as that of trolox. The results of the thiocyanate test reveal that the EOs and their flavor components can be used in the food industry to protect aliments of oxidation and to avoid lipid peroxidation. Correlating results are afforded by another investigation of 24 different citrus species and their authentic compounds. γ-Terpinene showed also in the linoleic acid assay a very strong antioxidative activity, which manifests itself in a better peroxide value than trolox. The EOs obtained from *Citrus yuko* Hort. ex Tanaka (Yuko) and *Citrus limon* Brum. f. cv. *Eureka* (Lisbon lemon) contain an appreciable content of γ-terpinene with 18.6% and 8.8%, respectively. Both citrus species offered a strong antioxidative activity of more than 90% due to the high amount of γ-terpinene in the oils (Choi et al., 2000). Ao et al. (2008) studied whether EOs from Rutaceae can effectively scavenge singlet oxygen upon irradiation, using electron spin resonance and found that the investigated 12 oils (eight of them obtained by conventional expression, four by steam distillation) enhanced singlet oxygen production, whereby the content of limonene is made responsible for this result. However, two expressed oils and three oils obtained by steam distillation showed singlet oxygen scavenging activity.

The EO of *Cuminum cyminum* (cumin, Apiaceae) consists of 21.5% limonene which is able to abate the concentration of the DPPH free radical, although its efficiency is a bit lower than that of trolox. Since the EO is also able to decrease the lipid peroxidation, a β-carotene bleaching test was arranged. This assay furnished better results than the DPPH free radical scavenging test. The possibility for that could be the higher specificity of this test method for lipophilic compounds. α-Pinene is another major constituent of this oil and contributes as well to the antioxidative activity. The implication of these investigations is the fact that the EO of cumin is competent enough to neutralize free radicals and to protect unsaturated fatty acids of oxidation (Gachkar et al., 2007).

Limonene is also one of the major compounds of the EO of *Ocotea bofo* Kunth (Lauraceae, “anis de arbol” in Ecuador, “moena rosa” in northern Peru or “pau de quiabo” in Brazil), (~5.0%), which was tested for its antioxidative activity by three different methods. The DPPH assay furnished a scavenging activity that was higher than that of the synthetic reference trolox, but lower than the activity of the natural and commercial EO reference *Thymus vulgaris*, whereas the β-carotene bleaching test showed that the EO is comparable to standards in the inhibition of oxidation. Another method was carried out to assess the antioxidative activity using a specific test kit of photochemistry. A light emission curve was recorded over 130 s, using inhibition as the parameter to evaluate the antioxidant potential (Guerrini et al., 2006). The activity was calculated by the integral under the curve and showed that *Ocotea bofo* EO here has a comparable scavenging activity to trolox. Besides, also the aromatic monoterpenic p-cymene (about 5% in the EO) contributes to the antioxidative activity (Table 9.4).
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Salvia (belonging to the family of Lamiaceae) EO is used in folk medicine all over the world and many studies were carried out to assess its constitution and biological activity. Three different species were collected in the southern part of Africa: Salvia stenophylla (blue mountain sage), Salvia repens (creeping sage), and Salvia runcinata (African sage) (Kamatou et al., 2005). Limonene is one of the major compounds of the EO from Salvia repens with 9.8%. Interestingly, in the other two species this monoterpene was absent. The antioxidant behavior of the EO and the phenolic composition of Rosmarinus officinalis (Lamiaceae) and Salvia fruticosa M., both collected in an island of the Ionian Sea (Greece) were investigated. The principal component of the EO was 1,8-cineole and flavonoids that of the methanolic extracts. The phenolic content correlates with the antioxidant activity (Papageorgiou et al., 2008).

To determine the radical scavenging capacity, a modified DPPH test was used where the test tubes were analyzed with HPLC. Different concentrations were plated out in a 96-well plate with control wells containing dimethyl sulfoxide (DMSO). The decolorization was investigated by measuring the absorbance at 560 nm. Vitamin C provided as a positive control. The LC50 value of Salvia repens EO (comprising about 22% β-caryophyllene) rests with more than 100.0 mg/mL or Salvia multicaulis which showed an IC50 value of 17.8 μg/mL, two examples for a low scavenging activity (Erdemoglu et al., 2006). But the reason for this result could be that in this method a stable free radical is used while in other investigations unstable radicals were applied and there a better antioxidative activity was adopted.

The EO of Crithmum maritimum (=Cachrys maritima, Apiaceae, rock samphire) comprises limonene and γ -terpinene with an amount of 22.3% and 22.9%, respectively, as the major components. Two different test methods (TBA assay and a micellar model system where the antioxidative activity in different stages of the oxidative process of the lipid matrix was monitored) were used. Both assays explain the very high activity of this EO. In the TBA assay BHT and α-tocopherol were used as positive standards and the oil showed a better capacity than those substances. Comparable results were obtained by the micellar method system where the EO acts as a protector of the oxidation of linoleic acid and inhibits the formation of conjugated dienes (Ruberto et al., 2000). The modification of LDL by an oxidative process for instance can lead to atherosclerosis. Natural antioxidants such as β-carotene, ascorbic acid, α-tocopherol, EOs, and so on are able to protect LDL against this oxidative modification. γ-Terpinene proved itself to be the strongest inhibitor of all used authentic compounds for the formation of TBARS in the Cu2+-induced lipid oxidation system (Grassmann et al., 2003). So, the addition of γ-terpinene to food can possibly stop the oxidative modification of LDL and reduce the atherosclerosis risk.

α-Pinene and α-terpineol are two of the main compounds of the EO from Juniperus procera (African juniper, Cupressaceae). The oil was studied for its antioxidative activity, because the aerial parts of this plant are used in traditional medicine against different diseases and ailments, for example,

<table>
<thead>
<tr>
<th>Sample</th>
<th>DPPH Inhibition %</th>
<th>β-Carotene Bleaching Test</th>
<th>Photochemiluminescence (mmol of trolox/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocotea bofo EO</td>
<td>64.23 ± 0.03</td>
<td>75.82 ± 0.04</td>
<td>3.14 ± 0.02</td>
</tr>
<tr>
<td>Thymus vulgaris EO</td>
<td>75.64 ± 0.04</td>
<td>90.94 ± 0.05</td>
<td>0.34 ± 0.06</td>
</tr>
<tr>
<td>BHA</td>
<td>84.35 ± 0.04</td>
<td>86.74 ± 0.04</td>
<td>4.28 ± 0.5</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>94.44 ± 0.05</td>
<td>84.60 ± 0.04</td>
<td>3.94 ± 0.06</td>
</tr>
<tr>
<td>Trolox</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ulcers, headaches, stomach disorders, intestinal worms, rheumatic pains, liver diseases, as an emmenagogue, and to heal wounds. To determine the antioxidant activity in vitro test methods were used: DPPH assay, deoxyribose assay, and the assay for nonenzymatic lipid peroxidation. The EO showed an IC₅₀ value of 14.9 μL/mL in the DPPH assay, a 50% inhibition in the deoxyribose assay at 0.4 μL/mL, and an IC₅₀ value of 0.20 μL/mL by inhibition of the lipid peroxidation. On the other hand, pure α-pinene showed an IC₅₀ value of 0.78 μL/mL and 50% of the lipid peroxidation were inhibited by 0.51 μL/mL. To determine the inhibition of nonenzymatic lipid peroxidation bovine liposomes, FeCl₃, and ascorbic acid were used. The generated aldehydes form pink compounds with TBA and were detected by measuring the absorbance at 532 nm.

Eleven different EOs were investigated for their antioxidative activities. The oils extracted from Eucalyptus globulus (Eucalyptus, Myrtaceae), Pinus radiata (Monterey Pine, Pinaceae), Piper crassinervium (Piperaceae), and Psidium guajava (Guava, Myrtaceae) contain 20%, 21.9%, 10%, and 29.5% α-pinene, respectively. Three different test methods were used to evaluate the antioxidative activities of these EOs: DPPH assay, β-carotene bleaching test, and photoluminescence. In the DPPH assay the EO from Piper crassinervium expressed an activity of 43.0 ± 0.30%, which was lower than that of the reference Thymus vulgaris EO, but comparable with the activity of trolox, whereas the other oils were almost ineffective. In the β-carotene bleaching test similar data were obtained. The EOs of Eucalyptus globulus and Piper crassinervium showed results between 66% and 49% inhibition. The photoluminescence test method is very rapid on the photo-induced autoxidation inhibition of luminol by antioxidants mediated from the radical anion superoxide (Sacchetti et al., 2005). With this method only the EO of Piper crassinervium showed an antioxidative activity, whereas the other oils were almost ineffective.

One of the main compounds of the eucalyptus oil besides 1,8-cineole are the monoterpene hydrocarbons α-pinene (10–12%), p-cymene, and α-terpinene, and the monoterpen alcohol linalool. This oil is used to treat diseases of the respiratory tract in which ROS play an important role, so the antioxidative activity of eucalyptus oil was of interest. The results obtained by assessing this activity were compared with those of myrtle standardized oil of Myrtus communis (Myrtaceae), which is also used to combat infections of the respiratory tract. The antioxidative activity was determined by the Fenton test where the OH⁻-radicals are built from H₂O₂, whereas Fe²⁺ acts as the electron donator. The ROS reacts with the sensitive indicators KMB and ACC, which release a measurable signal (Grassmann et al., 2000). After an incubation time of 30 min at 37°C, the generated ethene can be quantified by GC, because KMB dissociates into ethene, carbon dioxide, formiate, and dimethylsulfide. Both, the eucalyptus oil and the myrtle standardized oil were able to inhibit the generation of ethene in dependence on the concentration of the oils. In an in vitro system the EO of Eucalyptus globulus was investigated for its property to inhibit the autoxidation of linoleic acid and compared with the activity of BHT and showed very good protection for linoleic acid with an IC₅₀ value of 7 μg. In another test the inhibition of the nonenzymatic lipid peroxidation was investigated. A solution of bovine brain extract and various concentrations of linalool were submitted to a TBA assay: Linalool caused a 50% inhibition at a concentration of 0.67 μL/mL.

The EO, obtained from the leaves of Origanum syriacum L. (Syrian oregano, Lamiaceae), is a very popular Arab spice which is used as a herbal (traditional) medicament, flavor, fragrance, and for aromatherapy in form of bath, massage, steam inhalation, and vaporization (Alma et al., 2003), was investigated with regard to the antioxidative activity of its chemical compounds, for example, γ-terpinene, p-cymene, and β-caryophyllene (about 28%, 16%, and 13% respectively) using the thiocyanate method, DPPH radical scavenging activity, and the reducing power. The latter property was determined in a concentration from 100 to 500 mg/L and compared with the reducing power of ascorbic acid. The EO was able to increase the absorbance but the reducing power is lower than that of the used standard. It should be noticed that the power of the EO increased with a higher concentration. Also in the DPPH assay the standard BHT showed a better radical scavenging than the EO. In addition, 500 mg/L EO scavenged only 17% DPPH. In comparison to that, BHT showed an activity of 82% in a concentration of 100 mg/L, again dependent on the concentration. The thiocyanate
test was carried out with concentrations of 20, 40, and 60 mg/L of the EO. Also here the activity increased with a higher concentration. The antioxidative activity was comparable to that of BHT. Even at low concentrations (20 mg/L) a high antioxidative activity was found. At least also the reducing power of the EO using potassium ferricyanide was assessed by measuring the absorbance at 700 nm; however, the EO attained only a reducing power of 0.77, compared with that of ascorbic acid with a value of 0.96. Because an increasing absorbance means a stronger reducing power, it was shown that in this case only a low reducing capacity could be recorded, as in the method with DPPH. The EO of *Origanum floribundum* comprises only 8.4% thymol, but about 30% of carvacrol. This oil was able to decrease the generation of TBARS in the TBA assay just as well as BHT, BHA, and \( \alpha \)-tocopherol, in the absence and presence of a radical inducer. The DPPH radical activity of oregano EO in a menthol extract exists, because it is able to reduce the stable free radical DPPH with values of IC\(_{50}\) ranging from 378 to 826 mg/L, however inferior to the capacity of BHA (Hazzit et al., 2006). Similar results also furnished the carvacrol-rich oil from *Origanum glandulosum* (Algerian oregano) and from *Origanum acutidens* (hops oregano). This EO was tested with the radical scavenging method using DPPH and the \( \beta \)-carotene/linoleic assay. In both test systems the oil exhibits an antioxidative activity. As shown in other investigations, the activity in the \( \beta \)-carotene/linoleic assay was higher than that in the DPPH assay and is comparable with the ability of BHT, which was used as a positive control.

Also the species oregano (*Origanum vulgare*) is characterized by a high content of thymol (33%) and carvacrol, as was shown by a GC-MS analysis of (oregano) EO. The property to inhibit the generation of malonic aldehyde in the first stage of lipid oxidation is determined by TBA test method. And the micellar model system is used to measure the decrease of conjugated dienes formed by linoleic acid spectrophotometrically at 234 nm. In a concentration of 640–800 mg/L the EO shows a protective activity, which is superior to the standard substances BHA and \( \alpha \)-tocopherol but equal to BHT. When the amount of the EO is increased to 1000 mg/L, the antioxidative capacity is better than that of BHT (Faleiro et al., 2005). The meat of poultry is very sensitive for oxidative deterioration because the meat is rich in polyunsaturated fatty acids. Turkeys are more sensitive than chicken because they cannot store \( \alpha \)-tocopherol in their tissues to the same extent. A study was carried out if a diet with the EO of *Origanum vulgare* ssp. *hirtum* (oregano) is able to degrade the susceptibility to lipid oxidation. Thirty 10-week-old female turkeys were divided into five groups. In the control group the animals were fed with a standard diet. The other turkeys were also fed with the same bush but containing different concentrations of oregano EO and oregano herbs: 5 g oregano herb/kg, 10 g oregano herb/kg, 100 mg oregano EO, and 200 mg EO. After 4 weeks all the turkeys were slaughtered and worked up. The breasts were tight, minced, and stored at 4°C over 9 days. At days 0, 3, 6, and 9 the concentration of malonic aldehyde was measured spectrophotometrically using the TBA assay. At each stage the control group showed the highest content of malonic aldehyde. On the third day the amount of malonic aldehyde increased in every group. The group with 100 mg EO addition showed a lower concentration than the control group but a higher one as the group with 200 mg EO. On the sixth day the content of malonic aldehyde also increased and similar to the previous sample the group with 200 mg EO had the lowest content. Thus, it is shown that a diet with oregano EO can delay the deterioration induced by lipid oxidation (Botsoglou et al., 2003; Florou-Paneri et al., 2005). Responsible for this result is carvacrol, which is one of the main compounds of the oil and exerts also in other studies a strong antioxidative activity. In conclusion, these results prove that the EO of *Origanum vulgare* possesses protective properties in the first and second step of lipid peroxidation and can be used to replace synthetic antioxidants in the food industry or other areas. Another species, namely *Origanum majorana* L. from Albania, showed in the DPPH test a better antiradical activity than the phenolic compound thymol. This EO exhibited a scavenging effect on the hydroxyl radical OH as well and finally was also capable of antioxidant activity in a linoleic acid emulsion system where at a concentration of 0.05% it inhibited conjugated dienes formation by 50% and the generation of linoleic acid secondary oxidized products by about 80% (Schmidt et al., 2008).
One of the main compounds with about 23% found in the volatile oil from *Trachyspermum ammi* (ajwain, ajowan, omum, Apiaceae), which is a very popular aromatic plant in India and used for flavoring food as well as in the Ayurvedic medicine, is γ-terpinene. Another main compound of the ajowan oil is *p*-cymene (about 31%), which contributes to the antispasmodic and carminative properties as well. To determine the antioxidative activity of the EO a wide range of test methods were used. To simulate the different stages of lipid peroxidation three different test methods were exerted: the determination of peroxide values, a TBA assay, and the linoleic acid assay. The antioxidative activity of the oil and of an acetone extract was compared to that of BHT, BHA, and of a control: a sample with crude linseed oil. It could be shown that the oil was a better inhibitor than the synthetic antioxidants (Singh et al., 2004). Due to the fact that all results of the different test methods correlate, it can be concluded that at a concentration of 200 ppm the inhibitor activity can be put into the following order: acetone extract > EO > BHA > BHT > control.

Terpinolene was identified as one of the main compounds (about 6%) in the EO from *Curcuma longa* (turmeric, Zingiberaceae). In the course of an investigation of 11 different EOs also turmeric oil was determined for its antioxidative activity. The EO of *Curcuma longa* showed a noteworthy scavenging activity of about 62%, an antioxidative activity twice of that of trolox but only marginally lower than that of reference oil from *Thymus vulgaris*. Also the β-carotene bleaching test furnished comparable results, namely an inhibition activity of 72% versus 91% of *Thymus vulgaris* oil and 87% of BHA. This prevention of oxidation was also shown by the photoluminescence test method that is based on the photo-induced autoxidation inhibition of luminol by antioxidants mediated from the radical anion superoxide (Sacchetti et al., 2004). The EO of *Curcuma longa* showed a noticeable inhibition activity of about 28 mmol trolox/L.

The protective effect of terpinolene with reference to the lipoproteins of human blood and compared to that of the well-known antioxidative substances, such as α-tocopherol and β-carotene, was investigated. The oxidative modification of LDL, which was obtained from the blood of healthy volunteers, can be detected with the use of the increasing absorbance at 234 nm. An elongation of the time until rapid extinction (lag-phase) exhibits an antioxidative activity. The longer the lag-phase lasts, the better is the antioxidative capacity. Similar to other test systems with other EOs, the antioxidative capacity is dependent on the concentration. In that case a higher concentration of terpinolene means that the LDL particles are better loaded with terpinolene molecules. The result of this investigation proved that the protective and thus the antioxidative activity of terpinolene is only a bit weaker than that of the most common antioxidant α-tocopherol.

In the course of the determination of the antioxidative activity of the EOs obtained from 34 different citrus species and the main compounds of the oils, among them terpinolene, were investigated for their radical scavenging activity in a DPPH test system. Terpinolene offers a scavenging power of 87%, which was much higher than the antioxidative activity of the standard trolox. Terpinolene also showed a relative lipid peroxidation rate of 18%, which is an indication of a superior antioxidative activity, because the relative lipid peroxidation rate offered by the well-known and often used antioxidant α-tocopherol was about 30%.

The leaves, flowers, and stems of *Satureja hortensis* (summer savory, Lamiaceae), a common plant widely spread in Turkey, are used as tea or as addition to foods on account of the aroma and the flavor. As a medical plant it is known for its antispasmodic, antidiarrheal, antioxidant, sedative, and antimicrobial properties. Also this EO was investigated for its antioxidative properties. The GC-MS analysis showed that besides 9% *p*-cymene, carvacrol and thymol are the main compounds of about 22 constituents of the oil. They occur at a ratio of approximately 1:1, which is representative for the genus *Satureja*, namely 29% of thymol and 27% of carvacrol. In a linoleic acid test system the EO showed an inhibition activity of 95%, this is an indicator for a strong antioxidative activity because the control BHT attained an inhibition of 96% (Güllüce et al., 2003). Thymol is one of the main components of the EO from *Satureja montana* L., ssp. *montana* (savory) and also one of the glycosidically bound volatile aglycones that were found. The EO with 45% thymol shows a very strong antioxidative capacity that was a bit lower than the standards, BHT, and α-tocopherol. The
activity of the isolated glycosides is similar to that of the EO. Identification of the volatile aglycone shows a value of 2.5% thymol (Randonic et al., 2003). To evaluate the antioxidative properties of ingredients, EO and glycosides, the β-carotene bleaching test was chosen. The DPPH assay yields a weaker result than the β-carotene bleaching test, in which the EO had an efficiency of inhibition about 95%. Compared to the inhibition from BHT of 96%, this result shows a high antioxidative activity of the EO, due to the high content of thymol and carvacrol.

Since *Nigella sativa* (black cumin, devil in the bush, fennel flower, Ranunculaceae) seeds are used for the treatment of inflammations it was reasonable to investigate the ability of the volatile oil to act as a radical scavenger (Burits and Bucar, 2000). Experiments have shown that *Nigella* oil and the main compounds are able to inhibit in liposomes the nonenzymatic lipid peroxidation. Besides carvacrol (6–12%) thymoquinone, *p*-cymene is one of the major constituents of the oil (7–15%). In the free radical DPPH test the EO showed to be a very weak scavenger for radicals (IC$_{50}$ value: 460.0 µg/ml). Totally contrary results were furnished by the TBA assay: the volatile oil of *Nigella* seeds exhibited a very strong inhibition capacity, namely at a concentration of 0.0011 µg/ml already 50% of the lipid peroxidation could be stopped.

As to the next oil a scientific confusion must be mentioned: the common name black caraway belongs to the seeds of *Nigella sativa* (Ranunculaceae) and not to the Apiaceae plant *Carum*. Therefore, *Carum nigrum* is a wrong botanical name, even if the seeds of *N. sativa* are black and resemble to the seeds of *Carum carvi*. The confusion that has been created by the authors of the articles (Singh et al., 2006) by using possibly local justified terms, but nevertheless wrong botanical names still persists.

The EO of *Carum nigrum* (black caraway) comprising, for example, thymol (~19%), β-caryophyllene (~8%), and germacrene D (~21%), and its oleoresin were able to scavenge free radicals in the DPPH assay with an effect of 41–71% and 50–80%, respectively. This can be compared with the efficiency of BHT and BHA. However, this activity could only be observed using a high concentration of the EO and oleoresin not in lower ones. A good antioxidative activity was assessed also by the other tests: the linoleic acid assay exhibited that the EO and oleoresin are able to decrease the rate of peroxide during the incubation time. The deoxyribose assay proved that both EO and oleoresin are able to prevent the formation of hydrogen peroxides dependently of the concentration. Also the chelating effect with iron was screened and the absorbance of the mixture determined at 485 nm (Singh et al., 2006). A mixture of this EO, the oleoresin, and crude mustard oil were studied to assess the generated peroxides as well as the TBA value was measured in order to determine secondary products of the oxidation. This mixture showed a clear antioxidative activity, better than the control, and in the linoleic system were also able to keep the amount of the peroxides, formed by the oxidation of linoleic acid, very low in comparison to the standard antioxidants BHA and BHT. Finally, the rather moderate chelating capacity could—nevertheless—be beneficial for the food industry because ferrous ions are the most effective pro-oxidants in food systems (Singh et al., 2006).

The EO of *Monarda citriodora* var. *citriodora* (lemon bee balm, Lamiaceae) contains about 10% *p*-cymene. The oil was determined for its antioxidative activity in two different in vitro test systems. In one the oxidation of lipids was induced by Fe$^{2+}$ and in the other assay AAPH was used. Oxygen in the presence of iron(II) generates superoxide anion radicals. In the aqueous phase AAPH undergoes steady-state decomposition into carbon-centered free radicals (Dorman and Deans, 2004). The oil of *Monarda citriodora* was active oil at concentrations of 50 and 100 ppm. In the test system where the radicals were built from AAPH, the oil of *Monarda citriodora* showed a concentration-dependent pattern of antioxidative activity. *Monarda citriodora* showed a better activity at 10 ppm than the EO of *Thymus zygis* and an equal inhibition power like *Origanum vulgare* but the activity was lower than that of the standards BHT and BHA. The EO obtained from the stem with leaves and the flowers of *Monarda didyma* L. (golden balm or honey balm, Lamiaceae) contains *p*-cymene, 10.5% in the stem with leaves and 9.7% in the flowers. The EO was studied by two different test methods to determine its antioxidative activity. The EO showed a good free radical scavenging
activity in the DPPH assay. Also the properties to inhibit the lipid peroxidation in the 5-lipogenase test system furnished a strong inhibition that is similar to BHT.

In some Mediterranean countries *Thymbra spicata* (spiked thyme, Lamiaceae) is applied as spice for different meals and as herbal tea. Two of the main compounds of the EO from this plant are carvacrol (86\%) and thymol (4\%). The investigation of different natural antioxidants becomes more and more interesting in order to attain more safety in the food industry. The lipid oxidation may be one of the reasons for different changes in the quality of meat and thus the addition of *Thymus spicata* EO has a positive influence on its quality. As material of examination served the Turkish meal sucuk, which is prepared of lamb, lamb tail fat, beef, salt, sugar, clean dry garlic spices, and olive oil. During ripening on the days 2, 4, 6, 8, 10, 13, and 15, a sample was taken and different parameters were determined, among them was also TBARS. They were detected spectrophotometrically by measuring the absorbance at 538 nm. During the first 8 days the TBARS increased from 0.18 to 1.14 mg/kg. The addition of *Thymus spicata* EO reduced the value of TBARS more than BHT. This result shows that the EO from *Thymus spicata* exerts a safety effect on the quality of the meat and thus can be used as a natural antioxidant in the food industry. The EO of *Thymbra capitata* (conehead thyme, Lamiaceae) collected in Portugal contains 68\% carvacrol. Both the oil and carvacrol were tested for their antioxidative activity together with sunflower oil via the assessment of liberated iodine and its titration with sodium thiosulfate solution in the presence of starch as an indicator (Miguel et al., 2003). The antioxidative capacity was determined by the peroxide value of the samples, which were taken continuously during a period when the test solution was stored at 60°C. The EO and carvacrol exhibit approximately the same antioxidative activity during a period of 36 days.

It is known that free radicals induce deterioration of food because they start the chain reaction of the oxidation of polyunsaturated fatty acids. *Zataria multiflora* (Zataria, Lamiaceae) is used for flavoring yoghurt and as a medical plant, and therefore aroused the interest to study also the biological activities of its EO. Using the ammonium thiocyanate method this oil exhibits a strong antioxidative activity, as could be shown in an experiment. To determine the inhibition of oxidation, ammonium thiocyanate and ferrous chloride were added and the absorbance was measured spectrophotometrically at 500 nm. BHT was used for the positive control. Thymol with about 38\% the main compound of this EO shows a very strong capacity to avoid the lipid peroxidation up to 80\%, which is close to the inhibition of BHT (97.8\%). The high content of thymol, carvacrol (38\%), and γ-terpinene is the reason for the excellent antioxidative activity. In the DPPH assay the results were less convincing. The EO of *Zataria multiflora* is more liable to prevent lipid peroxidation than scavenging free radicals. The value of conjugated dienes is also decreased by the oil.

Linalool is one of the main compounds (up to 15\%) of the EO obtained from *Rosmarinus officinalis* L. (rosemary, Lamiaceae). The EO was tested using two different methods: radical scavenging with the DPPH assay and the β-carotene bleaching test. The oil was able to reduce the stable free radical DPPH and showed a slightly weaker scavenging activity than the standard trolox. But the efficiency of rosemary oil was clearly weaker than that of the reference oil *Thymus porlock*. By the β-carotene bleaching test it could be shown that this EO has the ability to prevent the lipid peroxidation with a capacity close to the used standards.

The genus *Ocimum* contains various species and the EOs are used as an appendage in food, cosmetics, and toiletries. *Ocimum basilicum* (sweet basil, Lamiaceae) is used fresh or dried as a food spice nearly all over the world. The antioxidative activities of different *Ocimum* species were studied in order to assess the potential to substitute synthetic antioxidants. Linalool and eugenol (~12\%) are the main compounds in the diverse oils. In the HPLC-based xanthine–xanthine assay, the EO of *Ocimum basilicum* var. *purpurascens* (dark opal basil) contains linalool, eugenol, and β-caryophyllene as main compounds and shows a very strong antioxidative capacity with an IC₅₀ value of 1.84 μL (Salles-Trevisan et al., 2006). Linalool as a pure substance yielded the same test results. In the DPPH assay linalool showed a bit weaker activity than in the xanthine–xanthine test.
method. *Ocimum micranthum* (least basil) is original in the South and Central American tropics and is used in these territories as a culinary and medical plant. The EO comprises eugenol (up to 51%) as main compound. In the DPPH assay the EO was able to scavenge about 77% of the free DPPH radicals, which is 3 times stronger than that of trolox. The efficiency was also better than those of *Ocimum basilicum* (basil) commercial EO and only slightly weaker than those of *Thymus vulgaris* (thyme) commercial EO. These two EOs were used as a standard because of their known antioxidative activity (Sacchetti et al., 2004). The β-carotene bleaching test proved that the EO inhibited the lipid peroxidation up to 93% after an incubation time of 60 min. The antioxidative activity determined in this test is better than the activity of BHA. In a special test using the photochemoluminescence method the EO of *Ocimum micranthum* attained a 10 times better antioxidative activity as *Ocimum basilicum* and *Thymus vulgaris* commercial EO. These data are of significance because the results of this assay correlate easily with the therapeutic, nutritional, and cosmetic potential of a given antioxidant and the capability to quench $O_2^{-•}$ is useful to describe the related capacity to counteract ROS-induced damages to the body (Sacchetti et al., 2004). Furthermore, the antioxidative activities of the EOs obtained from *Thymus vulgaris* and *Ocimum basilicum* were determined by the aldehyde/carboxylic acid assay. Various amounts of the EOs were added to a dichloromethane solution of hexanal-containing undecane as a GC internal standard and the antioxidant-standard substances BHT and α-tocopherol (Lee et al., 2005). After 5 days the concentration of hexane was determined. All samples showed good antioxidative properties as well as pure eugenol furnishing an inhibition of the hexanal oxidation by 32%.

Linalool (−12%), limonene (−18%), and α-terpineol (−2%) are the main volatile compounds in an infusion prepared of the green leaves from *Illex paraguariensis* (mate, Aquifoliaceae). In order to determine the protective activity against the oxidation of lipids, the infusion was submitted to the feric thiocyanate test. It could be seen that the infusion of green mate shows similar antioxidative activity as the synthetic antioxidant BHT (Bastos-Markowicz et al., 2006).

The leaves and barks of *Cinnamomum zeylanicum* Blume syn. *Cinnamomum verum* (cinna- mon, Lauraceae) are widely used as spice, flavoring agent in foods, and in various applications in medicine (Schmidt et al., 2006). The leaf oil is very rich in eugenol (up to 75%). To investigate the antioxidative activity five different methods were used: scavenging effect on DPPH, detection of the hydroxyl radicals by deoxyribose assay, evaluation of the antioxidant activity in the linoleic acid model system, determination of conjugated dienes formation, and determination of the TBARS. In the DPPH radical scavenging assay the EO showed an inhibition of 94% at a concentration of 8.0 μg/mL. To reach a radical scavenging activity of 89% a concentration of 20.0 μg/mL was necessary, which is comparable to the efficiency of the standard compounds BHT or BHA. The EO also exhibits a very strong inhibition of the hydroxyl radicals in the deoxyribose assay. The oil prevented 90% at 0.1 μg/mL and eugenol causes a blocking of 71% at the same concentration. Quercetin, which was used in this method as a positive control, showed a weaker efficiency. In a modified deoxyribose assay, in which FeCl$_3$ is added to the sample, both the EO and eugenol showed an antioxidative activity. The EO and eugenol were able to chelate the Fe$^{3+}$ ions and so the degradation of deoxyribose was prevented. To assess the oxidation of linoleic acid the determination of the conjugated diene content and TBARS were used. Cinnamon EO is able to inhibit the generation of conjugated dienes. In a concentration of 0.01% the formation of conjugated dienes is avoided (up to 57%) similar to the efficiency of BHT (−59%). On the day 5 of linoleic acid storage, malonic aldehyde was detected with TBARS. The cinnamon oil showed an inhibitory action of 76% at a concentration of 0.01% oil compared with the 76% of BHT at the same concentration.

Another Lauraceae is *Laurus nobilis* (laurel). The EO obtained from the leaves of wild grown shrubs is characterized by a very high content of eugenol. The biological activities, especially the antioxidative properties, of the extract were studied in different *in vitro* test methods. The scavenging capacity in the DPPH assay yielded an IC$_{50}$ value of 0.113 mg/mL. Also the β-carotene bleaching test of the nonpolar fractions was able to protect the lipids from oxidation. After an incubation
time of 60 min an IC$_{50}$ value of 1 µg/mL was calculated. The last applied method to determine the antioxidative activity used liposomes obtained from bovine brain extract. This test offers a high antioxidative activity with an IC$_{50}$ value of 115.0 µg/mL. The high content of eugenol renders it possible to use Laurus nobilis leaf extract as a natural antioxidant. Similar results of the antioxidative power were found using the FRAP assay and the DPPH assay of the EO obtained from Laurus nobilis leaves collected in Dalmatia (Conforti et al., 2003). In the first test system the change in absorbance at 593 nm owing to the formation of blue-colored Fe$^{3+}$ tripyridyltriazine from a colorless Fe$^{3+}$ form by the action of electron-donating antioxidants was measured. The sample was incubated at 37ºC and over a defined period the ferric reducing antioxidant power (FRAP) values were determined. Using the DPPH assay the EO of Laurus nobilis exhibited an antioxidative activity of nearly 90% at a concentration of 20 g/L, which is near to the activity of BHT with 91%.

Many aromatic herbs are used as an aroma additive in foodstuffs and in fat-containing food systems to prevent or delay some chemical deteriorations occurring during the storage (Politeo et al., 2006). For the investigation of the antioxidative activities of the EO of Syzygium aromaticum (cloves, Myrtaceae), three variable tests methods were employed: TBA assay, radical scavenging with DPPH, and the determination of FRAP. In all three assays it was found that the cloves EO—probably on account of its high content of eugenol (up to 91%)—shows a noticeable antioxidative activity. The determination of the FRAP assay measures the aforementioned change in absorbance at 593 nm owing to the formation of blue-colored Fe$^{3+}$ tripyridyltriazine (Politeo et al., 2006). In the radical scavenging test with DPPH an inhibition capacity of 91% at 5.0 µg/mL was found for the EO. Pure eugenol, BHT, and BHA needed a concentration of 20 µg/mL to arrive at the same result. In the deoxyribose assay the EO furnished a hydroxyl radical scavenging of about 94% at 0.2 µg/mL. The inhibitory effect of eugenol was 91% at a concentration of 0.6 µg/ml. Quercetin, the positive control, showed an inhibition of 77.8% at 20.0 µg/mL. The capture of OH$^-$ by cloves oil is attributed to the hydrogen-donating ability of the phenolic compound eugenol, which is found in high concentration in the EO (Jirovetz et al., 2006). In a linoleic acid model system the inhibition of generated hydroxy peroxides in the early stages of the oxidation of linoleic acid and the secondary oxidized products were detected by two different indicators: The adoption of conjugated dienes and TBARS. At a concentration of 0.005% the cloves oil outbid the activity of BHT. The capacity of the EO was about 74% compared to 59% achieved by BHT at 0.001%. The same results were obtained by the determination of TBARS. Here the EO’s activity was also equal to BHT.

Melissa officinalis L. (lemon balm, Lamiaceae) is a well-known herb and is also used as a medicinal plant for the treatment of different diseases such as headache, gastrointestinal disorders, nervousness, and rheumatism. The EO is well known for its antibacterial and antifungal properties, so it was investigated for its antioxidative activity too (Mimica-Dukic et al., 2004). The analyses of the chemical composition of the EO showed that β-caryophyllene is one of the main compounds (~4.6%) besides geranial, neral, citronellal, and linalool. The free radical scavenging capacity was determined by the DPPH assay, the protection of lipid peroxidation was investigated with the TBA assay, and the scavenging activity of the oil for hydroxyl radicals was measured with the deoxyribose assay, also a rapid screening for the scavenging compounds was made. Lemon balm scavenge in the DPPH test 50% of the radicals at a concentration of 7.58 µg/mL compared with the standard BHT, which attained an IC$_{50}$ value of 5.37 µg/mL. At a concentration of 2.13 µg/mL the highest inhibition (about 60%) of generated hydroxyl radicals in the deoxyribose assay was found compared with BHT as a positive control (~19%). Strong antioxidative activity of the EO was also determined in the TBA test system, where the inhibition of the lipid peroxidation was determined. Sixty-seven percent inhibition was caused by 2.13 µg/mL EO, in comparison with 37% of BHT. A rapid screening for the scavenging capacity with DPPH on a TLC plate exhibited that caryophyllene is one of the most active compounds.

Croton urucurana (Euphorbiaceae) is known as “dragon’s blood” and is used in traditional medicine because of its wound and ulcer healing, antidiarrheic, anticancer, anti-inflammatory, antioxidant, and antirheumatic properties. The antioxidative fractions of the EO obtained were determined with
a rapid screening using the DPPH assay on TLC. The main compound of the most active fraction was $\alpha$-bisabolol with 38.3%. The isolated fraction exhibited a 50% radical scavenging activity at a concentration of 1.05 mg/mL. This is a lower antioxidative activity than that of BHT. The EO of *Croton urucurana* showed an activity in the DPPH assay with an IC$_{50}$ value of 3.21 mg/mL (Simionatto et al., 2007).

The EO of *Elionurus elegans* Kunth. (African pasture grass, Poaceae) was investigated for its biological activities. GC/MS analyses showed that the oil contains $\alpha$-bisabolol (1.6% in the roots and 1.2% in the aerial parts). None of the other compounds exhibited in individual test systems antioxidative activities with the exception of limonene and, which were available only in low amounts. The antioxidant activity of the EO was tested with the chemiluminescence method using a luminometer, where the chemiluminescence intensity of the reaction mixture containing the EO or a standard ($\alpha$-tocopherol), AAPH, and luminol was measured. The IC$_{50}$ value for the EO obtained from the aerial parts amounted to 30% and the 50% inhibition rate of the roots EO to 46%.

*Teucrium marum* (mint plant, Lamiaceae) is used in the traditional medicine for its antibacterial, anti-inflammatory, and antipyretic activities. The antioxidative activity of the EO, which contains about 15% $\beta$-bisabolene, was investigated in three different test systems. The inhibition of lipid peroxide formation and of superoxide radicals and the radical scavenging activity with DPPH were tested. The oil exhibited a scavenging power in the DPPH assay (IC$_{50}$ value 13.13 $\mu$g/mL), which is similar to the well-known antioxidants BHT, ascorbic acid, and trolox. Using the xanthine–xanthine assay, in which the inhibition of superoxide radicals is tested, the EO showed a better scavenging activity for the superoxide radicals than BHT (IC$_{50}$ value 0.161 $\mu$g/mL against 2.35 g/mL); however, the efficiency of ascorbic acid and trolox was higher with IC$_{50}$ values of 0.007 and 0.006 $\mu$g/mL, respectively. The inhibition of lipid peroxidation was determined with the 5-lipidooxygenase test, where the formation of the hydroxy peroxides was measured spectrophotometrically at 235 nm. The activities of the EO and trolox were similar with IC$_{50}$ values of 12.48 and 11.88 $\mu$g/mL, respectively. The inhibition power was better than ascorbic acid with an IC$_{50}$ value of 18.63 $\mu$g/mL. The antioxidative activity of BHT was higher than the activity of the EO with an IC$_{50}$ value of 3.86 $\mu$g/mL (Ricci et al., 2005).

In the course of the investigation of the antioxidative activity of EOs obtained from three different citrus species (Rutaceae), $\beta$-bisabolene was also determined for its antioxidative property. The LDL oxidation was measured spectrophotometrically at 234 nm by the formation of TBARS. The results were expressed as nmol malonic dialdehyde/mg of protein. In the test system, $\beta$-bisabolene inhibited only TBARS formation from the AAPH-induced oxidation of LDL (Takahashi et al., 2003).

Several naturally occurring EOs containing for example carvacrol, anethole, perillaldehyde, cinnamaldehyde, linalool, and $p$-cymene were investigated for their effectiveness in their antioxidant activities and simultaneously also as to their ability in reducing a decay in fruit tissues. The tested EOs show positive effects on enhancing anthocyanins and antioxidative activity of fruits (Wang, C.Y. et al., 2008). In another study, the EO from black currant buds (*Ribes nigrum* L., Grossulariaceae) was analyzed by GC-MS and GC/-O and was tested for its radical scavenging activity, which—and this was the outcome of the present study—varied within a broad range, for example, from 43% to 79% in the DPPH reaction system (Dvarenauskaite et al., 2008). The antioxidant and antimicrobial properties of the rhizome EO of four different *Hedychium* species (Zingiberaceae) were investigated by Joshi et al. (2008). The rhizome EOs from all *Hedychium* species tested exhibited moderate to good Fe$^{2+}$ chelating activity, whereas especially *Hedychium spicatum* also showed a complete different DPPH radical scavenging profile than the samples from the other species. Finally, a very strong superoxide anion scavenging and an excellent DPPH-scavenging activity besides a strong hypolipidemic property possessed a methanol fractionate of the mountain celery seed EO (*Cryptotaenia japonica* Hassk, Apiaceae). The principal constituents of this fraction after a successive gel column adsorption were $\gamma$-selinene, 2-methylpropanal, and (Z)-9-octadecenamide (Cheng et al., 2008).
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