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Biological properties of essential oils: an updated review

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ABSTRACT: In the last few years more and more studies on the biological properties of essential oils have been published and it seemed worthwhile to compile the studies of 2009, 2008 and the second part of 2007. Such an overview covering the scientific literature mainly from 2000 onwards, up to the first half of the year 2007, has been published recently. The focus of this overview lies on the anti-nociceptive, anticancer, anti-inflammatory, penetration-enhancing, insect repellent, antiviral and antioxidative properties of essential oils. Many essential oils have been used for centuries in folk medicine and in recent years the biological properties of various essential oils have been proved by a number of studies. Their use in the treatment of pain, inflammation, viral diseases and cancer and their potential to enhance the penetration of other drugs, their insect repellent activity and their antioxidative effects were confirmed. Nonetheless, more studies are necessary to analyse the biological properties of other essential oils or to prove their mechanism of action. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: essential oils; anti-nociceptive effect; anticancer effects; antiphlogistic activity; penetration enhancement; insect repellent activity; antiviral activity; antioxidative activity

Introduction

Essential oils (EOs), also known as volatile oils, are concentrated natural plant products which contain volatile aroma compounds. These mixtures of volatile compounds (mainly mono- and sesquiterpenoids, benzoids, phenylpropanoids, etc.) exert different biological actions on humans, animals, and other plants.[1] EOs are extracted by distillation and expression, and are popular as ingredients of perfumes, cosmetics and household cleaning products, as well as being used for flavouring food and drink. But EOs are as well very useful in the treatment of different diseases and their medicinal application has become very popular and this is also valid with many of their constituents as single-fragrance compounds.

The focus of this overview, which compiles only substantial publications out of the huge mass of papers found in the literature (poor ones have not been considered), is directed to the anti-nociceptive, anticancer, anti-inflammatory, penetration enhancing, insect repellent, antiviral and antioxidative properties of EOs, and these aspects are dealt with in the following seven sections of this review.

The next section deals with the anti-nociceptive activity of selected EOs, which is a reduction in pain sensitivity made within neurons when endorphin or a similar opium-containing substance combines with a receptor.

Cancer belongs to a huge class of diseases, which cause more than 10% of all human deaths, and the third section (p. 410) deals with the anticancer activity of EOs.

The anti-inflammatory properties of EOs are described in the fourth section (p. 412). Chronic inflammation leads to a number of diseases and needs to be treated by using anti-inflammatory drugs.

Many essential oils have the potential to improve transdermal drug delivery. They are known as penetration enhancers, sorption promoters or accelerants. These oils are able to penetrate into the skin and decrease the barrier resistance. In the fifth section (p. 415), the penetration-enhancing effect of some essential oils will be discussed.

Some facts show that the use of synthetic chemicals to control insects and arthropods raises several obvious concerns related to the environment and human health. So, there is a growing demand for alternative repellents or natural products. These products possess good efficacy and are environmentally friendly. Essential oils from plants belonging to several species have been extensively tested to assess their repellent properties as a valuable natural resource, as discussed in the sixth section (p. 416).

A virus is a small infectious particle (20–300 nm), which is able to infect cells of another living organism, in which it can replicate itself. Viruses can lead to infections, which provoke an immune response that usually eliminates the infecting virus. Nowadays, we have detailed information concerning about 5000 viruses. The penultimate section (p. 419) deals with the antiviral activity of selected EOs.

The last section (p. 421) deals with the antioxidant activity of EOs. Antioxidants such as vitamins, enzymes or minerals are able to neutralize free radicals. They have a health-enhancing effect on our organism because they protect cells from oxidant damage. This section deals with the antioxidant activity of EOs with mono- and sesquiterpene compounds as well as with phenolic components, such as eugenol, carvacrol, thymol, etc. However, it was the aim of this overview to emphasize mono- and

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sesquiterpene constituents and not to compare the activity between phenolic and non-phenolic EO constituents, which should be the topic of a future paper.

Anti-nociceptive Effect

A nociceptor is a sensory receptor that responds to potentially damaging stimuli by sending nerve signals to the spinal cord and brain. The anti-nociceptive effect is a reduction in pain sensitivity made within neurons when endorphin or a similar opium-containing substance combines with a receptor.[2,3] In this section, the anti-nociceptive effects of some essential oils and/or single-fragrance compounds will be reported.

Sousa *et al*. analysed the anti-nociceptive and anti-inflammatory effects of the essential oil from *Eremanthus erythropappus* (DC.) McLeish (Asteraceae) leaves. β-Pinene (23.2%), β-caryophyllene (22.9%), β-myrcene (10.0%) and germacrene D (9.4%) are the main compounds of the essential oil. About 11% and 27% of acetic acid-induced writhing in mice is inhibited by doses of 200 and 400 mg/kg. In the formalin-induced nociception test using mice the essential oil inhibited paw licking by 29% (400 mg/kg) in the first phase and by 33% (200 mg/kg) and 38% (400 mg/kg). In the second phase, in the hot-plate test using mice the essential oil led to a significant increase of the reaction time after 30, 60 and 90 min of treatment, at doses of 200 mg/kg and 400 mg/kg. The same doses led to an inhibition of carrageen-induced paw oedema in rats by 15% and 37%. A significant reduction of the exudate volume (by 20% and 49%) and leucocyte mobilization (by 6% and 17%) is caused by doses of 200 mg/kg and 400 mg/kg administered 4 h before intra-pleural injection of carrageen. The study clearly demonstrates the analgesic, anti-inflammatory and anti-nociceptive effect of *E. erythropappus* oil.[4]

In 2008 the anti-nociceptive activity of the volatile oils of *Hyptis pectinata* L. Poit. (Lamiae) genotypes was analysed by Aragon-Blank *et al*. *H. pectinata* is very common in Brazilian folk medicine, where it is used to treat inflammations, bacterial infections and ache. The analysis is based upon abdominal writhe models induced by acetic acid and the hot-plate test. Six genotypes of the volatile oil were investigated. The main compounds of all genotypes are sesquiterpenes. In both models all the genotypes showed an anti-nociceptive effect. The major inhibitory effect at a dose of 100 mg/kg body weight exerted the genotype SAM002. The outcome of the study was that the volatile oil of *H. pectinata* shows peripheral and central anti-nociceptive effects.[5]

Liapi *et al*. studied the anti-nociceptive properties of 1,8-cineole and β-pinene, two monoterpenes, from the essential oil of *Eucalyptus camaldulensis* Dehnh. (Myrtaceae) leaves, in rodents (mice and rats) using the tail-flick and hot-plate tests, reflecting the spinal and supra-spinal levels. Morphine and naloxone were used for comparison. In both algesic stimuli 1,8-cineole showed an anti-nociceptive activity compared to morphine, but naloxone did not antagonize 1,8-cineole. From this it follows that there is a significant synergism between 1,8-cineole and morphine. β-Pinene is supposed to be a partial agonist of the μ-opioid receptors; however, as one of the authors stated, this activity as a morphine analogue is very weak. It leads to supra-spinal anti-nociceptive actions in rats only and reversed the anti-nociceptive effect of morphine and naloxone.[6]

The pharmacokinetics and tissue distribution of the sesquiterpene α-humulene in mice was investigated in 2008 by Chaves *et al*. α-Humulene is the main active constituent isolated from the plant *Cordia verbenacea* DC. (Boraginaceae). The study showed a clear anti-nociceptive effect of the essential oil.[7] A study by Kamatou *et al*. showed similar results. In an overview these authors reported on the biological activities and phytochemistry of about 20 South African *Salvia* species (among them, for example, *S. officinalis* L., *S. africane-lutea* L., *S. africana-caerulea* L., *S. albicaulis* Benth., *S. namaensis* Schinz, *S. verbena* L., etc.), which belong to the Lamiaceae. The genus *Salvia* encompasses 900 species worldwide. These species are known for their use to treat microbial infections, cancer, malaria, inflammation, etc. The major compounds of the essential oil are monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpenes.[8]

Takaki *et al*. investigated the anti-inflammatory and anti-nociceptive effects of *Rosmarinus officinalis* L. (Lamiaceae) essential oil (REO) in experimental animal models. An inducement to this study is the common use of REO in folk medicine because of its antispasmodic, analgesic, anti-rheumatic and carminative effects. The tests of the anti-nociceptive effects were carried out using the acetic acid-induced writhing test and the hot-plate test in mice. REO at a dose of 500 mg/kg led to a significant reduction of the volume of pleural exudate and slightly decreased the number of cells that had migrated compared with these of the control animals. A noticeable inhibition of carrageen-induced oedema 1–4 h after injection of the phlogistic agent, is caused by REO at doses of 250, 500 and 750 mg/kg. In the hot-plate test the administration of REO showed unremarkable effects on response latency, whereas a control injection of meperidine induced clear anti-nociceptive effects. At doses of 70, 125 and 250 mg/kg REO showed a remarkable anti-nociceptive effect in the acetic acid-induced abdominal writhing test compared with control animals. The conclusion of this study is that REO possesses peripheral anti-nociceptive activity.[9] Also, Martinez *et al*. reported on the anti-nociceptive effect of this Lamiacean essential oil using a rat model of arthritic pain. A dose-dependent anti-nociceptive effect is produced by the essential oil, manifested as a significant reduction of the dysfunction in the pain-induced functional impairment model in the rat (PIFIR model), mainly at high doses. The major compounds, analysed by gas chromatography–mass spectrometry, are α-pinene (14.1%), camphene (11.5%), β-pinene (12.0%), myrcene (3.3%), α-phellandrene (7.9%), eucalyptol (8.6%), 2-bornanone (3.4%), camphor (8.8%), isoborneol (3.5%), borneol (4.9%) and bornyl acetate (6.5%). The analysis of the anti-nociceptive effect was made in combination with 0.12 mg/kg WAY100635®, s.c. (an antagonist of 5-HT_{1A} receptors) or 1 mg/kg naloxone, i.p. (a non-selective opioid receptor antagonist). In both cases an inhibition of the anti-nociceptive response was demonstrated. An involvement, at least in part, of endogenous opioids and the serotonergic system via 5-HT_{1A} in the anti-nociceptive effect of *R. officinalis* essential oil in the PIFIR model is possible. Moreover, the properties of camphor as a TRP modulator should be mentioned.[10]

Sakurada *et al*. studied the capsaicin-induced anti-nociceptive activities of bergamot (*Citrus bergamia*, Risso) essential oil, Rutaceae, (BEO) by intra-plantar injection into the mouse hind paw. An intense and short-lived licking or biting response toward the injected hind paw is produced by an intra-plantar injection of capsaicin. After the intra-plantar injection of BEO the capsaicin-induced nociceptive response was reduced significantly. The main compounds of BEO are monoterpene hydrocarbons, such as limonene, γ-terpinene, β-pinene and oxygenated derivatives, linalool and linalyl acetate. The studies also showed the anti-nociceptive effect of *Salvia sclarea* L., linalool chemotype of

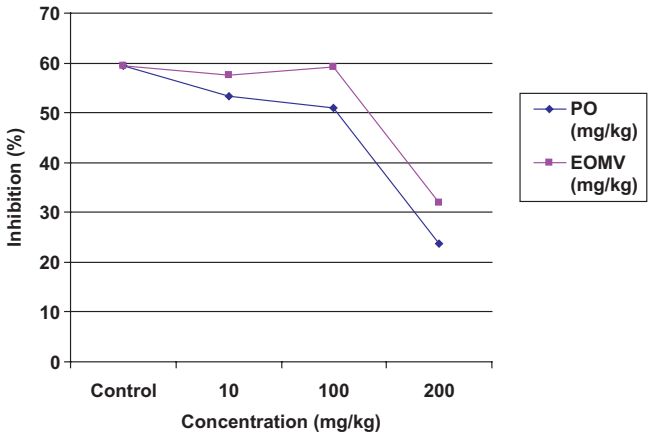

Figure 1. Inhibitory effect of piperitenone oxide (PO) and of the essential oil of *Mentha x villosa* (EOMV) on the nociceptive reaction to intraperitoneal acetic acid injection in mice. (Newly drawn according to Sousa *et al*.[12])

Thymus vulgaris L., *Lavandula angustifolia* Mill. and *Lavandula hybrida* Reydovan on the capsaicin-induced nociceptive response, while testing the essential oil of *Citrus sinensis* L. (Osbeck) was without effect. Another result of this study is the pharmacological activity of linalool, which, in addition to an anti-nociceptive effect, also shows anti-hyperalgesic, anticonvulsant and anti-inflammatory effects. The study confirms the importance of linalool as a TRPA1 agonist or linalyl acetate in BEO or these compounds as constituents of other essential oils in anti-nociceptive therapy.[11]

The anti-nociceptive effects of the essential oil of *Mentha x villosa* L., Lamiaceae, (EOMV) leaves and its major constituent, piperitenone oxide, in mice were investigated by Sousa *et al*. in 2009. Because the essential oil of this herb possesses many pharmacological activities, such as antispasmodic effects, the anti-nociceptive activity of the oil and its major constituent, piperitenone oxide (PO), were assumed. After an oral administration of 200 mg/kg of EOMV and PO, a significant reduction of the writhings induced by acetic acid was observed (Figure 1). At lower doses (10 and 100 mg/kg body weight) any significant changes in the number of writhings were not induced. In addition, EOMV caused a reduction in the paw licking time for the second phase of the formalin test, when administered at higher doses, e.g. 100 and 200 mg/kg. At 100 and 200 mg/kg, PO reduced this second phase to 8.3 $\pm$ 2.7 s ($N = 12$) and 3.0 $\pm$ 1.2 s ($N = 10$), respectively (Figure 2). Naloxone is not able to reverse this effect of EOMV and PO. Additionally, EOMV and PO had no significant effect on the first phase of the formalin test. The interpretation of the hot-plate and tail immersion test proved that EOMV and PO, at doses up to 200 mg/kg, showed no analgesic activity. The results of this study illustrate that EOMV and PO possess anti-nociceptive activity, what is probably a so-called indirect anti-inflammatory effect, which does not involve the central nervous system.[12]

The phytochemistry and biological activities of about 30 *Phlomis* species (among them, for example, *Ph. integrifolia* Hub.-Mor., *Ph. linearis* Boiss.&Bal. and *Ph. viscose* Poiret) were reported in an overview by Limen-Ben Amor. The genus *Phlomis* L., which includes 100 species, is part of the Lamiaceae family and is used to treat various conditions such as diabetes, gastric ulcer, haemorrhoids, inflammation and wounds. This review aims to sum up recent research on the phytochemistry and pharmacological properties of the genus *Phlomis*. The major constituents of the essential oils are monoterpenes (α-pinene, limonene and linalool), sesquiterpenes (germacrene D and β-caryophyllene, a CB2 agonist), aliphatic compounds (e.g. 9,12,15-octadecatrienoic acid methyl ester) and fatty acids. The study comes to the conclusion that *Phlomis* species have, *inter alia*, anti-nociceptive, anti-diabetic, anti-inflammatory, anticancer and antioxidant properties.[13]

Amorim *et al*. analysed the anti-nociceptive and hypothermic evaluation of the leaf essential oil and isolated terpenoids from *Eugenia uniflora* L., Myrtaceae, (Brazilian Pitanga), which is also called Brazilian cherry tree and is used in folk medicine to cure inflammations, rheumatic pain, fever, hypo-glycaemic and diuretic complaints and is also applied in the cosmetics industry. The present study concerns the chemical composition, the anti-nociceptive and hypothermic profile of the essential oil of *E. pitangueira* leaves. The main constituent, analysed by GC-MS, is a mixture of atractylone and 3-furanoeudesmene. Oral administration of the essential oil significantly inhibited acetic acid-induced abdominal constrictions, increased the latency time in the hot-plate test, and had a hypothermic effect. The isolated furano-sesquiterpenes are considered to be responsible for the anti-nociceptive and hypothermic effect. Furthermore, the morphine-like activity of furano sesquiterpenoids has to be mentioned.[14]

The anti-nociceptive activity of 1-nitro-2-phenylethane, the main component of *Aniba canelilla* (Kunth.) essential oil (ACEO), was described in the paper by De Lima *et al*.[15] *A. canelilla*, Lauraceae, is known for its use in the Amazon folk medicine as an antispasmodic, anti-diarrhoeal, carminative, as a tonic agent and stimulant of the digestive and central nervous system. The analgesic activity of ACEO in mice was shown in the preliminary study. 1-Nitro-2-phenylethane, the main component of ACEO, was dosed at 15, 25 and 50 mg/kg in the writhing test and led to a reduction of the abdominal writhes in a significant manner. In the hot-plate test no alterations in the latency time, compared to the control, could be observed. 1-Nitro-2-phenylethane was assayed at 50, 100 and 200 mg/kg. In the formalin test the second phase of the algesic stimulus decreased significantly by doses at 50 and 25 mg/kg of this naturally occurring nitro compound. The conclusion of this study is that 1-nitro-2-phenylethane exerts analgesic activity, probably of peripheral origin. The physical mechanism is not completely understood. Involvement of the opioid receptors in the anti-nociceptive action observed for 1-nitro-2-phenylethane is assumed.[15]

In another study the anti-nociceptive potency of the rhizome essential oil of *Zingiber zerumbet* Mill., Zingiberaceae, (EOZZ) was investigated using chemical and thermal models of nociception, namely the acetic acid-induced abdominal writhing test, the hot-plate test and the formalin-induced paw licking test. After intra-peritoneal administration of EOZZ, doses of 30, 100 and 300 mg/kg led to a significant dose-dependent inhibition of acetic acid-induced abdominal writhing, similar to the effect of acetylsalicylic acid (100 mg/kg). Analogous promising results were obtained in the hot-plate test and in the formalin-induced paw licking test, while EOZZ significantly reduced the painful stimulus in both the neurogenic and inflammatory phases of the test. Additionally, in these two tests the anti-nociceptive effect of EOZZ could be reversed by naloxone, a non-selective opioid receptor antagonist. This is evidence that the opioid system is involved in the analgesic mechanism of action. On the basis of these data, EOZZ possesses both central and peripheral anti-

nociceptive activities and the folk medicinal use of EOZZ to relieve some pain conditions is justified.[16]

Coming to a conclusion about these reports on anti-nociceptive properties of EOs and/or their constituents it is noticeable that only four of the cited studies, namely Kamatou *et al*.,[6] Martínez *et al*.,[10] Sousa *et al*.[12] and Sulaiman *et al*.,[16] tried to shed more light on the molecular mechanism of this effect by using the non-selective opioid receptor antagonist naloxone. Important for assessing the activity of such a 'medicament' is also the determination of its effective dose which has been reported in various papers.[4,9,12,15,16] Finally, another aspect of quality is the fact that different tests in these assessment procedures have been carried out to ascertain the results and this was the case in most of the studies cited. All three quality parameters could be found only in the papers by Sousa *et al*.[12] and Sulaiman *et al*.[16] Still remarkable is the study by De Lima *et al*. where the occurrence of 1-nitro-2-phenylethan as an EO constituent is reported.[15] This rather unusual main compound in an EO is responsible for the anti-spasmodic and anti-nociceptive activity which has been assessed in various doses and using at least two test systems; however, the detailed interaction with opioid receptors has not been ascertained yet.

Anticancer Activity

The medical term for cancer is malignant neoplasm. Cancer belongs to a huge class of diseases, which cause more than 10% of all human deaths. Humans as well as animals are affected at all ages. Cancer is characterized by uncontrolled growth of cells disregarding the normal limits, invasion and, in the worst case, metastasis, the expansion of the disease to another non-nearby organ via lymph or blood.[17]

In 2007, Legault and Pichette investigated the potentiating effect of β-caryophyllene on the anticancer activity of α-humulene, isocaryophyllene and paclitaxel against MCF-7, DLD-1 and L-929 human tumour cell lines. β-Caryophyllene is a widely distributed sesquiterpene, which is found in the essential oils of various plants and known for its anti-inflammatory, antibiotic, antioxidant, anti-carcinogenic and local anaesthetic activities. Administration of β-caryophyllene at non-cytotoxic concentrations led to a clear increase of the anticancer activity of α-humulene and isocaryophyllene on MCF-7 cells. About 50% and 69% inhibition of cell growth is achieved by α-humulene or isocaryophyllene when they are administered alone at doses of 32 µg/ml, but when they are combined with 10 µg/ml β-caryophyllene, the inhibition of the cell growth amounts to 75% and 90% (Figure 3). Furthermore, β-caryophyllene is also able to potentiate the anticancer effects of paclitaxel on MCF-7, DLD-1 and L-929 cell lines. The combination of paclitaxel and 10 µg/ml β-caryophyllene achieved the best effect in DLD-1 cells, enhancing the paclitaxel activity to about 10-fold. Moreover, β-caryophyllene, at doses ranging from 2.5 to 40 µg/ml, has the potential to increase the intracellular accumulation of paclitaxel–oregon green and of calcein but not of verapamil. This led to the suggestion that β-caryophyllene stimulates drug accumulation by a different mechanism of action and that β-caryophyllene helps paclitaxel to pass through the membrane and in this way potentiates its anticancer activity.[18]

After many studies showing the potential of chemo-preventive phytochemicals, especially, to increase the sensitivity of cancer cells to conventional anticancer drugs, in 2008 Ravizza *et al*. investigated linalool, a plant-derived monoterpene alcohol that

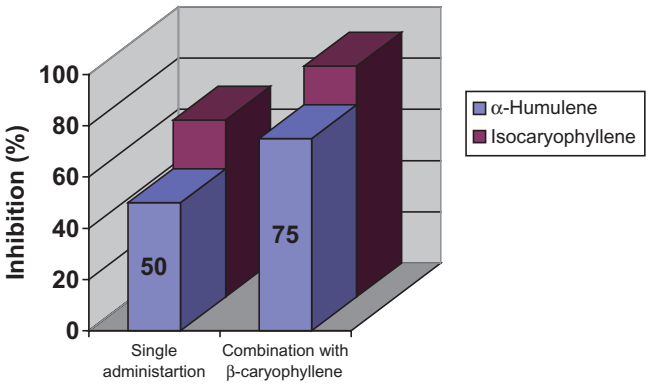

Figure 2. The inhibition of cell growth achieved by α-humulene and isocaryophyllene. (Newly drawn according to Legault and Pichette[18])

is found in the essential oils from many aromatic plants. These authors found that linalool is able to reverse doxorubicin resistance in human breast adenocarcinoma cells. The focus of this study was two human breast adenocarcinoma cell lines, MCF7 WT and multi-drug resistant MCF7 AdrR, both as a single agent and in combination with doxorubicin (DOX). Linalool only sparsely inhibited cell proliferation, but in sub-toxic concentrations it led to a higher DOX-induced cytotoxicity and pro-apoptotic effects in both cell lines. In MCF7 AdrR cells a promising synergism was noticed, which may be related to the capacity of linalool to enhance DOX accumulation and the induction of a decrease in Bcl-xL levels. In summary, this study showed that linalool furnished an improvement of the therapeutic index of anthracyclines in the treatment of breast cancer, especially in multi-drug resistant (MDR) tumours.[19]

Rezvanfar *et al*. investigated the protection of cyclophosphamide-induced toxicity in reproductive tract histology, sperm characteristics, and DNA damage by a herbal source, as evidence for the role of free-radical toxic stress. Cyclophosphamide (CP) is an anticancer alkylating agent that shows toxic effects on the male reproductive tract. The focus of this study was the essential oil of the Lamiaceae *Satureja khuzestanica* Jamzad (SKEO), an established herbal antioxidant. To show that SKEO has a protective effect, similar to the toxicity of cyclophosphamide, on the reproductive system of rats, the total antioxidant power (TAP) and lipid peroxidation (LPO) in testis and plasma, blood levels of sex hormones, sperm characteristics, DNA integrity, chromatin quality and fertility in male rats were tested. For the evaluation of spermatogenic disorders, histo-pathological analysis of testis and epididymides and staining of mast cells were carried out. Within the framework of the study the administration by gavage of cyclophosphamide (6 mg/kg/day) and SKEO (225 mg/kg/day), alone or in combination, was arranged for 28 days. The rats who were exposed to cyclophosphamide showed an increase of testicular and plasma LPO, a decrease in TAP and plasma testosterone and both spermatogenesis and fertility were impaired. This impairment is caused by a decrease in sperm quality, which was associated with increased DNA damage and decreased chromatin quality. The administration of cyclophosphamide and SKEO significantly improved CP-induced changes in plasma testosterone, sperm quality, spermatogenesis and fertility, toxic stress, and DNA damage. The conclusion of this study is that the toxic effects by cyclophosphamide on androgenesis and spermatogenesis is arranged by free radicals. Through its antioxidant potential and androgenic activity, the essential oil of

S. khuzestanica protects the reproductive system from toxicity of cyclophosphamide.[20]

Verma *et al*. analysed the induction of mitochondrial-dependent apoptosis by an essential oil from *Tanacetum gracile* Hook., (Asteraceae), (TGEO), an alpine aromatic herb that contains about 40 constituents. The main compounds are lavandulol (21.5%), lavandulyl acetate (1.7%), α-pinene (11.2%), 1,8-cineole (15.2%), *cis*-β-ocimene (6.9%), borneol (6.1%), limonene (5.1%) and chamazulene (3.7%).

TGEO led to an inhibition of HL-60 cell proliferation with an IC50 (the half maximal inhibitory concentraion) of 27 µg/ml and to an induction of apoptosis in human leukaemia HL-60 cells. This effect was measured by several biological end points. Furthermore, TGEO led to an improvement of annexin V–fluorescein isothiocyanate (FITC) binding of the cells, an increase of the sub-G0 DNA fraction, a decrease of mitochondrial membrane potential, a liberation of cytochrome *c* from mitochondria, activating caspase-9 and caspase-3, and an increase of cleavage of poly(ADP-ribose)-polymerase (PARP) in HL-60 cells. This study showed the anticancer activity of the essential oil of *T. gracile*, with the conclusion that the oil exerts an induction of the apoptosis through the mitochondrial-dependent pathway in HL-60 cells.[21]

Another study reported on the chemo-typical variation of essential oils in the medicinal plant *Anemopsis californica* (Nutt.) Hook., Saururaceae. In the framework of the study the steam-distilled oil from roots/rhizomes of *A. californica* (ACEO) was used to screen its anticancer bioactivity. The focus was the growth inhibitory activity against several human cancer lines: A549 (lung), MCF7 (breast), PC3 (prostate) and HCT116 (colon). However, no activity against these cell cultures was found. On account of this ethno-botanical use of ACEO to treat uterine cancer this essential oil was analysed against AN3CA (uterine) and HeLa (cervical) human cancer cell lines. The result was an anti-proliferative activity against AN3CA and HeLa cells *in vitro*. The IC50 values for the root oil were 0.056% and 0.052% (v/v) for the AN3CA and HeLa cells. The three main compounds, thymol, piperitone and methyleugenol, were tested independently for growth inhibitory activity against AN3CA and HeLa cells. They also inhibited cell growth. The IC50 values for these three compounds against each cell line was determined and compared with the concentration of these compounds in the root oil of *A. californica*. The inhibition may be the result of a synergistic relationship between the combined abundant compounds, piperitone and methyleugenol, or also with a minor component in the oil. In conclusion, the study showed the specific bioactivity against uterine and cervical cancer cell lines of steam-distilled oil of *Anemopsis* root tissue, thus supporting the traditional and cultural use of ACEO to treat uterine cancer.[22]

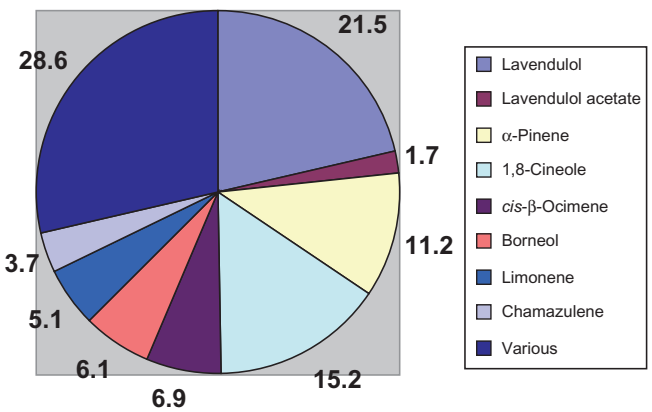

Figure 3. The composition (%) of the essential oil of *Tanacetum gracile*. (Newly drawn according to Verma *et al*.[21])

Because some *Eucalypus* species (Myrtaceae) possess antimicrobial and anti-tumour properties, Ashour analysed the antibacterial, anti-fungal, and anticancer activities of volatile oils and extracts from stems, leaves and flowers of *Eucalyptus sideroxylon* A. Cunn ex Woolls and *Eucalyptus torquata* grown in Egypt. To analyse the anticancer activity a sulforhodamine B assay was used, an evaluation of cell density which is based on the measurement of cellular protein content. The *in vitro* cytotoxic activities of the essential oils and extracts were tested against a human hepatocellular carcinoma cell line (HEPG2) and a human breast adenocarcinoma cell line (MCF7). The results showed that the oils of *E. torquata* leaves and stems and of *E. sideroxylon* leaves exert cytotoxic effect activity against MCF7 cells, but none have any effect on HEPG2 cells.[23]

In 2009 Sharma *et al*. investigated the anticancer activity of the essential oil from a lemon grass variety of *Cymbopogon flexuosus* (Nees ex Steud), Poaceae. The *in vitro* cytotoxicity against 12 human cancer cell lines and the mechanism of cell death, relating to the morphological chances in tumour cells, were analysed. The *in vitro* cytotoxicity studies showed auspicious results: the essential oil led to a dose-dependent high cytotoxicity with IC50 values of 4.2 and 79 µg/ml, relative to various human cancer cell lines. IC50 values of 4.2 and 4.7 µg/ml were shown using the 502713 (colon) and IMR-32 (neuroblastoma) cell lines. The *in vivo* anticancer activity of this essential oil was tested with the solid and ascitic Ehrlich and sarcoma-180 tumour models in mice, which were both clearly inhibited by an intra-peritoneal (i.p.) administration of the essential oil. More precisely, at 200 mg/kg i.p. the oil caused growth inhibition of 97% and 58% in both solid and ascitic tumour forms of Ehrlich ascites carcinoma. In sarcoma-180 cells, the oil caused growth inhibition of 94% and 37% at an equal dose. Morphological studies of the essential oil showed a distinct loss of surface projections, chromatin condensation and apoptosis in HL-60 cells, whilst in sarcoma-180 solid tumour cells the oil led to a condensation and fragmentation of nuclei typical of apoptosis. Typical changes for apoptosis were also found when treating the ascites cells from animals with the essential oil from *C. flexuosus*. The study indicates that this essential oil shows interesting results in anticancer activity by activating the apoptotic process, thus reducing tumour cell viability.[24]

Finally, Lukas *et al*. investigated the composition of essential oil compounds from different Syrian populations of *Origanum syriacum* L. (Lamiaceae). The main compounds are carvacrol and/or thymol, depending on the populations. Thymoquinone is also an important compound of *O. syriacum* L. which shows very promising anticancer activity. Thymoquinone was found in extracts in a wide range between 0.04% and 23.7%. This high concentration of thymoquinone has the potential for use in anticancer therapy but further studies are necessary.[25]

Apart from cardiovascular attacks cancer causes the most deaths in humans and often results in long and painful sickness. Therefore, the search also for natural anticancer compounds is very important in as much EOs are cheap compared to synthetic medicaments. The anticancer activities reported afore in this section can be attributed to either monoterpene alcohols or sesquiterpene hydrocarbons. These data, with one exception, are the results of studies on different human tumour cell lines and

upon determination of the most effective dose. The anticancer activity caused by apoptosis of the tumour cells are reported in high quality papers, namely Ravizza *et al*.,[19] Verma *et al*.[21] and Sharma *et al*.[24]

Antiphlogistic Activity

A human organism reacts to harmful stimuli, like pathogenes, damaged cells or irritants with an inflammation, a protective attempt to remove the injury or infection and to start the healing process. There are a number of inflammatory mediators, such as tumour necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-8, IL-10 and prostaglandin E_2 (PGE_2). Without inflammation, wounds and infections would not heal and the progressive destruction of the tissue would continue. We can differentiate between two kinds of inflammation: (1) acute inflammation, an initial response of the body to harmful stimuli; and (2) chronic inflammation, which leads to a number of diseases and needs to be treated by anti-inflammatory drugs.[26,27]

In 2007 Tekeoglu *et al*. analysed the anti-inflammatory effects of thymoquinone, an ingredient of the volatile oil of *Nigella sativa* L. (Ranunculaceae), on rheumatoid arthritis in rat models. The arthritis was induced by Freund's incomplete adjuvant. The rats were assigned to five groups: group 1, controls 0.9% NaCl ($n = 7$); group 2, 2.5 mg/kg thymoquinone ($n = 7$); group 3, 5 mg/kg thymoquinone ($n = 7$); group 4, bacilli Chalmette–Guerin (BCG) 6×10^5 CFU ($n = 7$); and group 5, methotrexate 0.3 mg/kg ($n = 7$). Signs of inflammation on the claw and radiological signs were searched for and TNF-α and IL-1β were measured. The results, compared to the control group, showed that thymoquinone suppressed adjuvant-induced arthritis in rats.[28]

In another study Juhas *et al*. dealt with the anti-inflammatory effect of thymoquinone and borneol on trinitrobenzene sulfonic acid (TNBS)-induced colitis in mice. Thymoquinone is the active ingredient of the volatile oil of *N. sativa* seeds, and borneol is the active ingredient of *Salvia officinalis* essential oil, Lamiaceae. The administration of thymoquinone at a concentration of 0.05% and of borneol at a concentration of 0.09% or 0.18% was carried out 5 days before the induction of TNBS colitis. Seven days after the administration of TNBS, macroscopic and histological scores were evaluated. The results showed no significant changes between the experimental and control groups, but a promising decrease in pro-inflammatory cytokine (IL-1β and IL-6) mRNA expression in colon tissue in the 0.09% and 0.18% borneol-treated groups in mice was obtained. Based on these data, it was not possible to confirm the anti-inflammatory effects of thymoquinone in TNBS colitis. However, borneol is able to significantly suppress the pro-inflammatory cytokine mRNA expression.[29] The same test was used with a combination of thyme oil (*Thymus vulgaris*, Lamiaceae) and oregano essential oil (*Origanum vulgare*, Lamiaceae) on TNBS-induced colitis in mice by Bukovská *et al*.[30] Three concentrations were tested: (1) 0.4% thyme and 0.2% oregano oils; (2) 0.2% thyme and 0.1% oregano oils; and (3) 0.1% thyme and 0.05% oregano oils. After the administration of the oil – especially at the medium dose – a decrease of the mRNA levels of pro-inflammatory cytokines IL-1β, IL-6, GC-CSF and TNF-α was obtained. Furthermore, the medium dose led to a promising decrease of the amount of IL-1β and IL-6 proteins as well as of the mortality rate and the macroscopic damage of the colon tissue and furnished an increase of the body weight gain recovery. The results showed that the combination of thyme and oregano oil is able to reduce the production of pro-inflammatory cytokines.[30]

Fernandes *et al*. tested the anti-inflammatory properties of two sesquiterpenes isolated from essential oil of *Cordia verbenacea* (DC.) (Boraginaceae), α-humulene and (–)-*trans*-caryophyllene. The oral administration of both compounds led to an effective reduction of mouse paw oedema induced by either platelet activating factor or bradykinin or ovalbumin. Moreover, α-humulene and (–)-*trans*-caryophyllene led to promising inhibitory effects on the mouse and rat carrageen-induced paw oedema. After oral administration, α-humulene is able to diminish oedema formation caused by histamine injection, but a systemic treatment prevented the generation of both TNF-α and IL-1β in carrageen-injected rats. (–)-*trans*-Caryophyllene is only able to diminish TNF-α release. Additionally, both compounds led to a reduction of the production of PGE_2, as well as inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) expression, induced by the intra-plantar injection of carrageen in rats. The anti-inflammatory properties of both compounds were comparable to those observed in dexamethasone-treated animals, used as positive control drug. This study showed that α-humulene and (–)-*trans*-caryophyllene represent important tools for the management and/or treatment of inflammatory diseases.[31] These two sesquiterpenes from the essential oil of *C. verbenacea* were the focus of a study by Medeiros *et al*.[32] The biological activities were investigated in a model of acute inflammation in rat paw, induced by lipopolysaccharide (LPS) and characterized by paw oedema, neutrophil recruitment, cytokine production, activation of mitogen-activated protein (MAP) kinases and nuclear factor kappa B (NF-κB) and up-regulated expression of kinin B1 receptors. Both compounds are able to reduce neutrophil migration and activation of NF-κB induced by LPS in the rat paw. The single administration of α-humulene significantly reduced the increase in TNF-α and IL-1β levels, paw oedema and the up-regulation of B1 receptors following treatment with LPS. Moreover, neither compound was able to interfere with the activation of the MAP kinases extracellular-signal regulated kinase (ERK), p38 and c-Jun N-terminal kinase (JNK). The results of this study are in agreement with those of the aforementioned paper.[31,32]

In 2008 the common household plant *Rosmarinus officinalis* L., Lamiaceae, popularly named rosemary, was analysed by Takaki *et al*.[9] As *R. officinalis* is used in folk medicine in many parts of the world because of its antispasmodic, analgesic, anti-rheumatic, carminative, cholagogue, diuretic, expectorant and anti-epileptic effects, rosemary essential oil (REO) was evaluated. To analyse the anti-inflammatory activity of REO, the inflammatory exudate volume and also the leucocyte migration in carrageen-induced pleurisy and carrageen-induced paw oedema tests in rats were used. An administration of REO at doses of 500 mg/kg led to a significant reduction of the volume of pleural exudate and to slight decrease of the number of cells that had migrated compared with the control animals. A promising inhibition of carrageen-induced oedema 1–4 h after injection of the phlogistic agent were obtained by 250, 500 and 750 mg/kg of REO. This study showed that REO possesses promising anti-inflammatory and peripheral anti-nociceptive activity, evaluated by using the acetic acid-induced writhing and hot-plate test in mice.

Kim *et al*. investigated the anti-inflammatory activities of the hydro-distilled essential oil from *Farfugium japonicum* (L.) Kitam, Asteraceae, (FJEO), for the first time. The main components, analysed by GC-MS, are 1-undecene (22.4%), 1-nonene (19.8%), β-caryophyllene (12.3%), α-copaene (3.7%), γ-curcumene (2.9%), germacrene D (2.7%), and 1-decene (2.1%). The evaluation showed that FJEO is an effective inhibitor of LPS-induced NO

and PGE_2 production in RAW 264.7 cells (a mouse macrophage-like cell line). Furthermore, FJEO led to dose-dependent decreases in iNOS and COX-2 mRNA expression. To assure the safety of the dermal application of FJEO the cytotoxicity was tested by colorimetric MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays in human dermal fibroblast and keratinocyte HaCaT cells. The results showed that the essential oil possesses a low cytotoxicity at 100 μg/ml and that FJEO is a promising medicament for the treatment of inflammation as well as for topical application, but further studies will be necessary.[33]

Lin *et al*. tested the anti-inflammatory activity of fruit essential oil from *Cinnamomum insularimontanum* Hayata (CIEO), Lauraceae. The main compounds, citral (35.9%), citronellal (24.6%), citronellol (16.8%), α-pinene (9.5%), β-pinene (4.3%), limonene (1.8%) and camphene (1.7%), were analysed by GC-MS. CIEO led to a significant inhibition of NO production and presented IC50 values of 18.68 and 13.18 μg/ml. The protein expression assay showed that CIEO led to a decrease in the expression of IκB-kinase (IKK), iNOS and nuclear NF-κB and to an increase of IκBα in dose-dependent manners. Furthermore, the anti-inflammatory mechanism of citral, the major constituent of CIEO, was blocked via the NF-κB pathway, but it could not efficiently suppress the activity of COX-2. Moreover, 0.1 and 0.3 mg of citral showed promising anti-inflammatory activity in the assay of croton oil-induced mouse ear oedema. The inflammation reduced to 22% and 83%. Based on these results, CIEO and its major constituent citral may be considered as a potential anti-inflammatory medicine in the future.[34]

The inhibition of COX-2 by the volatile oil from dried roots of *Lithospermum erythrorhizon* Siebold. & Zucc., Boraginaceae, (LEEO was analysed by Kawata *et al*. The main components have been investigated by GC-MS. More than 50 components of the oil were found. The major constituents were 2-methylbutanoic acid (21.5%), 3-methylbutanoic acid (12.6%), 2-methylpropanoic acid (9.0%), methyl linoleate (8.8%), methyl oleate (6.3%), methyl palmitate (6.1%), and 2-methyl-2-butenoic acid (5.7%). The anti-inflammatory activity of LEEO was evaluated by studying the *in vitro* inhibition of ovine COX-1 and COX-2 activity. The results showed selective COX-2 inhibition. At doses of 50 μg/ml, LEEO led to a 39% inhibition of the COX-2 activity.[35]

A study by Martins *et al*. dealt with the anti-inflammatory and antioxidant activities of the volatile oil from the fruit peel of *Garcinia brasiliensis* Mart., Clusiaceae, (GBEO). The main components were analysed by GC-MS. More than 35 components were found and identified, including oxygenated sesquiterpenes (43%), γ-muurolene (10.3%), spathulenol (8.7%), δ-cadinene (8.3%), torreyol (8.0%), α-cadinol (7.0%), cadalene (6.3%), and γ-cadinene (5.3%). To analyse the anti-inflammatory activity, GBEO was evaluated by using the rat-paw oedema model induced by carrageen. The results showed an inhibition of the inflammatory process 3 h after carrageen administration. Moreover, GBEO possesses poor antioxidant activity.[36]

The anti-inflammatory properties of *Ocotea quixos* Lam. (Lauraceae) essential oil (OQEO) *in vitro* and *in vivo* were analysed by Ballabeni *et al*. in 2009. The anti-inflammatory effects of the main components of the essential oil, *trans*-cinnamaldehyde and methyl cinnamate, were tested as well. The results show that OQEO and *trans*-cinnamaldehyde are able to significantly reduce LPS-induced NO release from J774 macrophages at non-toxic concentrations. They also inhibited the LPS-induced COX-2 expression and increased forskolin-induced cAMP production, whilst methyl cinnamate shows no effects in these tests. Furthermore, the essential oil (30–100 mg/kg, p.o.) and *trans*-cinnamaldehyde (10 mg/kg, p.o.) showed anti-inflammatory effects in carrageen-induced rat paw oedema without damaging gastric mucosa. The conclusion of this study is that *O. quixos* Lam. essential oil has striking anti-inflammatory gastro-sparing activity.[37]

A similar author group to that above[33] analysed the chemical composition, antioxidant, anti-elastase and anti-inflammatory activities of *Illicium anisatum* L. essential oil (IAEO). The main component of IAEO, belonging to the family of Illiciaceae, is eucalyptol (21.8%), analysed by GC-MS. A focus of this study was to identify the mechanism of the anti-inflammatory activity. The results showed that the IAEO led to a significant inhibition of the production of LPS-induced NO and PGE_2 in RAW 264.7 cells. Furthermore, these essential oils led to a dose-dependent decrease of the expression of iNOS and COX-2 proteins and iNOS and COX-2 mRNA. Moreover, the study dealt with the cytotoxic effects of the essential oil to prove its safety. The oil showed low cytotoxicity at 100 μg/ml, tested by MTT assays in human dermal fibroblast and keratinocyte HaCaT cells. The MTT assay is a standard colorimetric test which is used to determine cytotoxicity. The conclusion of this study is that IAEO shows anti-inflammatory potential, but additional *in vitro* and *in vivo* tests are necessary as proof of its safety and efficacy.[38]

Ashour *et al*. investigated the chemical composition and biological activity of the essential oil obtained from *Bupleurum marginatum* Wall. Ex DC., Apiaceae (BMEO). The focus of the study, besides the chemical composition, was the antioxidant, anti-inflammatory, anti-microbial and *in vitro* cytotoxic activity. The main components, analysed by capillary gas chromatography (GLC-FID) and gas chromatography–mass spectrometry (GLC-MS), are tridecane (13.2%), undecane (10.4%), pentadecane (8.7%), β-caryophyllene (5.5%) and β-caryophyllene oxide (5.3%). The anti-inflammatory activity was evaluated by the inhibition of both PGE_2 production and lipoxygenase. The results showed an IC50 value of 63.64 μg/ml for lipoxgenase and an inhibition of 26.04% of PGE_2 at doses of 25 μg/ml. The conclusion of this study is that BMEO has promising anti-inflammatory effects but, again, further studies are necessary.[39]

The effects of lemongrass essential oil [*Cymbopogon citratus*, (DC.) Stapf, (Poaceae)] on IL-1β and IL-6 production by macrophages was analysed by Sforcin *et al*., because the oil is known for its insecticidal, anti-microbial and therapeutic properties, but knowledge of the effects on the immune system is uncertain. To analyse the oil's anti-inflammatory properties, the *in vivo* and *in vitro* effects of water extracts of lemongrass were tested on pro-inflammatory cytokine (IL-1β and IL-6) production by macrophages of BALB/c mice. A BALB/c mouse is an albino, laboratory-bred strain of the house mouse. The results showed an inhibition of the production of IL-1β by macrophages, but the water extract induces IL-6 production. Furthermore, the essential oil of lemongrass led to an inhibition of the cytokine production *in vitro*. The major components of lemongrass water extracts are linalool oxide and epoxy-linalool oxide. The main compounds of the essential oil are neral and geranial. Based on these data, the authors suggest an anti-inflammatory activity of lemongrass.[40]

(–)-α-Bisabolol is an optimal active sesquiterpene alcohol that is found in plants such as *Vanillosmopsis erythropappa* (DC.) Sch.Bip. and *Matricaria chamomilla* L. The compound is known for its antiseptic and anti-inflammatory activity and especially because of its gastro-protection on acute gastric mucosal lesions. The focus of the study by Moura Rocha *et al*. was to investigate

the gastro-protective action of (–)-α-bisabolol on ethanol- and indomethacin-induced ulcer models in mice. (–)-α-Bisabolol (100 and 200 mg/kg) has the potential to protect the gastric mucosa from ethanol (0.2 ml/animal p.o.) and indomethacin-induced ulcer (20 mg/kg p.o.). The gastro-protective effects of (–)-α-bisabolol could not be reversed by the administration of l-NAME (10 mg/kg i.p.), glibenclamide (10 mg/kg i.p.) or indomethacin (10 mg/kg p.o.). Ethanol and indomethacin are able to reduce the amount of non-protein sulfhydryl (NP-SH) groups, while (–)-α-bisabolol has the potential to significantly decrease the reduction of these levels on ulcer-induced mice, but not in mice without ulcer. In conclusion, (–)-α-bisabolol led to a protection of the gastric mucosa from ethanol- and indomethacin-induced ulcer. This effect is associated with an increase in the bioavailability of gastric sulfhydryl groups, which leads to a reduction of gastric oxidative injury induced by ethanol and indomethacin.[41] A study by Al-Howiriny *et al*. had the same objective, using *Origanum majorana* ('marjoram'), Lamiaceae, on various models of gastric mucosal injury in rats. The anti-ulcerogenic activity of the ethanol extract, even if this is not an essential oil (!) of *O. majorana* L., was evaluated in ulcers induced by hypothermic restraint stress, indomethacin and necrotizing agents (i.e. 80% ethanol, 25% NaCl and 0.2 M NaOH). Furthermore, the basal gastric acid secretion using pylorus-ligated Shay rat model was tested. The administration of marjoram at doses of 250 and 500 mg/kg of body weight leads to a significant decrease of the incidence of ulcers, basal gastric secretion and acid output. *O. majoranum* L. also has the potential to regenerate ethanol-induced depleted gastric wall mucus and non-protein sulfhydryl contents. Moreover, *O. majoranum* L. is able to lower the increase in the concentration of malondialdehyde (MDA). A histo-pathological assessment demonstrated the ulcer preventing potential as well. Additionally, the acute toxicity was analysed to prove the safety of the extract in mice.[42]

Loizzo *et al*. investigated the *in vitro* biological activity of *Salvia leriifolia* Benth. (Lamiaceae) essential oil (SLEO). The main compounds of SLEO are camphor (10.5%), 1,8-cineole (8.6%), camphene (6.2%) and α-pinene (4.7%). The study showed a promising antioxidant activity and cholinesterase inhibitory activity. Furthermore, SLEO inhibited lipopolysaccharide-induced NO production with an IC50 value of 165 μg/ml. By using the MTT assay the absence of cytotoxicity at 1000 μg/ml was evaluated in 142BR cells.[43]

Because carvacrol is the most important component of thyme oil, it was investigated by Hotta *et al*. Carvacrol is able to activate peroxisome proliferator-activated receptor-α and -γ (PPAR). These receptors are ligand-dependent transcription factors and are involved in the control of COX-2 expression, which plays an important role in inflammation. The biological properties of carvacrol were investigated especially in thyme oil. PPAR-γ-dependent suppression of COX-2 promoter activity was observed in response to carvacrol treatment. Carvacrol suppressed LPS-induced COX-2 mRNA and protein expression in human macrophage-like U937 cells. This led to the result that carvacrol regulates COX-2 expression through its agonistic effect on PPAR-γ.[44] Furthermore, the essential oils of clove (*Syzygium aromaticum* (L.) Merril & Perry, Myrtaceae), rose (*Rosa sp*., Rosaceae), eucalyptus (*Eucalyptus sp*., Myrtaceae), fennel (*Foeniculum vulgaris* Mill., Apiaceae) and bergamot (*Citrus limon* L., Rutaceae) were investigated. The study showed that also these oils led to a suppression of COX-2 promotor activity in cell-based transfection assays using bovine arterial endothelial cells.

Yoon *et al*. tested the biological activities of *Cryptomeria japonica* (Thumb. Ex L.F.) D.Don essential oil, Cupressaceae (CJEO). The major components, analysed by gas chromatography, are kaurene (17.2%), elemol (10.9%), γ-eudesmol (9.4%), and sabinene (8.9%). The anti-inflammatory activity of CJEO was tested on CJEO on nitric oxide (NO), PGE_2, TNF-α, IL-1β, and IL-6 production in LPS-activated RAW 264.7 macrophages. Pro-inflammatory cytokine and mediator tests showed the excellent dose-dependent anti-inflammatory activity of CJE. Furthermore, to analyse the antibacterial properties of CJEO the disk diffusion method and minimum inhibitory concentration values were used. CJEO showed excellent antibacterial activities against *Propionibacterium acnes* and *Staphylococcus epidermidis*, which are both acne-causing bacteria. The study proved that CJEO is a promising acne-mitigating candidate for skin health,[45] as well as the essential oil of *Abies koreana* Mill., (Pinaceae). This oil was investigated by the same author as to its anti-inflammatory and anti-bacterial versus skin pathogenic effect. The focus of this study was the treatment of acne vulgaris, which is a combined result of a bacterial infection and the inflammatory response to that infection. AKEO showed excellent antibacterial activities against drug-susceptible and drug-resistant *P. acnes* and *S. epidermidis*, both acne-causing bacteria. Furthermore, AKEO led to a reduction of the LPS-induced secretion of TNF-α, IL-1β, IL-6, NO and PGE_2 in RAW 264.7 cells. The results of this study showed that AKEO is a promising treatment for acne vulgaris.[46]

Because the fruits of *Heracleum persicum* L. (Apiaceae) are used as a pain killer in Iranian folklore medicine, Hajhashemi *et al*. investigated the anti-inflammatory and analgesic properties of *H. persicum* essential oil, (HPEO), and a hydro-alcoholic extract (HPHE) in animal models. The major components of HPEO are hexyl butyrate (56.5%), octyl acetate (16.5%), hexyl 2-methylbutanoate (5.2%) and hexyl isobutyrate (3.4%). The acetic acid-induced writhing response and formalin test were used in male mice to analyse the analgesic activity. After the administration of HPEO at doses of 50–200 mg/kg and HPHE at doses of 250 and 500 mg/kg, a significant reduction of acid-induced abdominal constrictions were obtained. HPEO and HPHE also led to a reduction of the pain response in the second phase of the formalin test. For evaluation of the anti-inflammatory effect, carrageen-induced rat paw oedema was used. At doses of 100 and 200 mg/kg of HPEO and at doses of 400 mg/kg of HPHE a significant reduction of paw oedema could be observed. The study clearly shows that HPEO and HPHE have significant analgesic and anti-inflammatory effects.[47]

Moraes *et al*. investigated the effects of the essential oil of *Citrus aurantium* L. (CAEO), Rutaceae, and its main compound, the monoterpene limonene, on gastric mucosa. CAEO is known for its wide use as a flavouring agent, which is found in many common food items, as well as for its medicinal use throughout the world to treat gastritis and gastric disorders. A dose of 250 mg/kg p.o. of CAEO and 245 mg/kg p.o. of limonene provided very effective (99%) gastro-protection against injuries induced by absolute ethanol or non-steroidal anti-inflammatory drugs (NSAIDs) in rats. It is important to notice that neither CAEO nor limonene interfere with gastric H^+ secretion, serum gastrin or glutathione levels in gastric mucosa. The gastro-protective action of CAEO and limonene occurs due to an increase in the gastric mucus production induced by conserving the basal PGE_2 levels after challenge by agents harmful to the gastric mucosa.[48]

A Fabaceae oil, namely from *Pterodon emarginatus* Vogel seeds (PEEO) was investigated by Dutra *et al*. in order to assess its anti-ulcerogenic and anti-inflammatory activities using different methods such as inducing ulcers with ethanol, indometacin and HCl/ethanol or inducing pleurisy by carrageen in Swiss albino rats. After oral administration of 100, 300 and 500 mg/kg of PEEO significant protection against such ulcers was obtained. Moreover, PEEO led to a promising reduction in the exudate volume and inhibited leucocyte and neutrophil influx in carrageen-induced pleurisy. Furthermore, PEEO led to a significant decrease of nitric oxide (NO) and IL-1 levels, without affecting TNF-α production. The results of this study showed that PEEO is a promising new therapeutic option to treat gastric ulcers and inflammatory diseases.[49]

The anti-inflammatory effect of the essential oil of the *Cleistocalyx operculatus* (Roxb.) Merr. & Perry buds, Myrtaceae (COEO) was the research topic of Dung *et al*. The buds of this plant are widely used in folk medicine to treat gastric diseases as well as an antiseptic agent in China and other parts of the world. This study showed that COEO is able to inhibit LPS-induced secretion of pro-inflammatory cytokines, including TNF-α and IL-1β, in RAW 264.7 cells. Moreover, it was possible to suppress the mRNA expression of TNF-α and IL-6ββ by treatment with COEO in LPS-stimulated RAW 264.7 cells. COEO is able to block LPS-induced transcriptional activation of NF-κB in RAW 264.7 as well as suppress the nuclear translocation of the p65 subunit. Furthermore, COEO led to an inhibition of phorbol ester-induced increase in ear swelling and skin water content in BALB/c mice. The results of this study showed that COEO possesses an anti-inflammatory effect because it suppresses the expression of pro-inflammatory cytokines, which is mediated, at least in part, by blocking NF-κB activation.[50]

Thematically not too far away from the studies about anti-nociception are the papers dealing with the antiphlogistic activities of EOs or single EO constituents. On account of either inflammation mediators, namely the various factors, such as TNF-α, PGE_2, the whole palette of interleukins, etc., and as well as expressed enzymes, such as iNOS or COX-2, and also the activation of certain kinases, the mechanism of antiphlogistic activity is more or less ascertained. The studies focus either on a decrease of the cited factors, or the suppression of, for example, COX-2 expression. All the papers cited report the effects of either EOs or even single compounds on the aforementioned parameters. In the studies by Ballabeni *et al*.[37] and Hotta *et al*.[44] the action of the EO as well as one or two of its main constituents was the topic of the research. The majority of the studies used animal experiments; lesser results were obtained upon *in vitro* tests with cell systems, among which the research with human cell lines[33] or macrophage-like U937 cells[44] are worth special mention. It is remarkable that – especially as Kim *et al*.[33] and Ashour *et al*.[39] analysed – medium-sized alkanes in combination with sesquiterpene hydrocarbons (β-caryophyllene, α-humulene) exert antiphlogistic activity. That this property can also attributed to α-bisabolol[41] – one of the most prominent sesquiterpene alcohols – is already known. An alcoholic extract of majoram,[42] although not an EO, showed remarkable potential in preventing the formation of gastric ulcers, as could be shown in a histopathological assessment of the gastric wall mucus of mice. Therefore, in conclusion, to prevent or alleviate inflammation, EOs or their major constituents, are very important 'natural' medicaments for the first steps in self-medications.

Table 1. The potential of various essential oils to increase the enhancive permeation

Essential oil tested	Increase of the enhancive permeation
Fructus *Evodia*	3.46
Radix *Saposhnikoviae*	3.00
Rhizoma *Atractylodes lancea*, Radix *Aucklandiae*	2.36
Radix *Curcuma wenyujin*	2.32
Rhizoma *Notopterygii*	2.28
Radix *Notopterygii*	2.01
Lignum *Aquilariae Resinatum*	1.37
Herba *Schizonepetae*	1.29

Penetration Enhancement

Many essential oils have the potential to improve transdermal drug delivery. They are known as penetration enhancers, sorption promoters or accelerants. These oils are able to penetrate into the skin and to decrease the barrier resistance. A number of potential mechanisms of action have been identified for skin penetration enhancers; for example, the interaction of essential oils with liquid crystals of skin lipids.[51]

In 2007, Long *et al*. investigated the skin toxicology and penetration enhancement of skin absorption of volatile oil extracted from tender branches of *Camellia oleifera* L., Theaceae (COEO). The potential of COEO as an penetration enhancer was tested on nitrendipine (a pyridine calcium channel blocker), baicalin (a flavonoid that affects the GABA receptors) and nimesulide (an NSAID with analgesic and antipyretic properties) for percutaneous absorption. The effects of different concentrations of their volatile oil in mice treated with nitrendipine or baicalin or nimesulide were assessed *in vitro*. The results showed that COEO led to powerful enhancement effects.[52]

The effect of eight different volatile oils of Chinese Material Medica on the percutaneous absorption of ibuprofen *in vitro* were compared by Luo *et al*. Focus of this study are the volatile oils of Fructus *Evodia* (*E. hupehensis* Dode, Rutaceae), Radix *Saposhnikoviae* (*S. divariata* (Turcz.) Schischk., Apiaceae), Rhizoma *Atractylodes lancea* Thumb., Asteraceae, Radix *Aucklandiae* (*Saussurea costus* (Falc.) Lipsch, Asteraceae), Radix *Curcuma wenyujin* Y.H.Chen et Ling, (Zingiberaceae), Rhizoma and Radix *Notopterygii* (*N. incisum*, Ting ex H.T. Chang, Apiaceae), Lignum *Aquilariae resinatum* Lam., Thymelaeaceae, and Herba *Schizonepetae* (*S. tenuifolia* Briq., Lamiaceae). To analyse their potential to enhance the penetration of ibuprofen, a penetration experiment apparatus *in vitro* was used. The cumulative amount of ibuprofen was determined by HPLC. All the eight mentioned volatile oils led to an enhancement of the penetration of ibuprofen in different degrees (Table 1). The results of this study showed that all the tested volatile oils have the potential to enhance the percutaneous absorption of ibuprofen *in vitro*, whilst the volatile oil of Fructus *Evodia* and Radix *Saposhnikoviae* showed a clearly better penetration enhancing effect.[53]

The focus of a study by Bai *et al*. was the effects of the volatile oils of *Rhizoma zingiberis* (Zingiberaceae), Rhizoma *Acori tatarinowii* Schott (Araceae, RAT), Semen *Myristicae* (*M. fragrances* Houtt., Myristicaceae, SM) and Pericarpium *Citri reticulatae* Blanco (Rutaceae, PCR) on the percutaneous penetration of bullatine A,

Table 2. The activation energy for labetalol hydrochloride in water, in vehicle *per se*, and in the presence of 5% w/v basil oil

Medium	Activation energy (kcal/mol)
Water	23.16
Vehicle *per se*	18.71
Presence of 5% w/v basil oil	10.98

a reference standard, via hairless mouse skin *in vitro*. The effects of these oils were tested with an improved Franz diffusion test and compared with Azone. The increasing amount of bullatine A in the plasma of the mice was determined by HPLC. In fact the penetration enhancement of bullatine A with 7% volatile oil of RAT and SM, 5% volatile oil of PCR and 3% Azone were clearly noticeable. The conclusion of this study is that the volatile oil of Rhizoma *A. tatarinowii*, Semen *Myristicae* and Pericarpium *C. reticulatae* have the potential to enhance the permeation of bullatine A.[54]

Another Chinese research group analysed the effect of *Atractylodes* rhizome oil (Asteraceae) and other volatile oils on percutaneous absorption of baicalin, a flavonoid that affects GABA receptors. The focus of this study are the *atractylodes* rhizome oil, patchouli oil (*Pogostemon patchouli* DESF., Lamiaceae) and angelica volatile oil (*Angelica archangelica* L., Apiaceae). To test their potential to enhance the penetration of baicalin, the modified Valia–Chien diffusion cells (a horizontal glass diffusion cells) were used. It was possible to show with saline isotonic solution as receptor fluid and different concentrations of the three volatile oils as enhancers, that the penetration of baicalin through the skin is improved. The best effect was achieved by *atractylodes* rhizome. The results of this study showed that *atractylodes* rhizome oil, patchouli oil and angelica volatile oil improve the skin penetration of baicalin.[55]

The potential of basil oil (*Ocimum basilicum* L., Lamiaceae), a volatile oil containing terpene alcohols, to enhance the skin penetration of labetalol hydrochloride was investigated by Jain *et al*. Labetalol hydrochloride is an alpha and beta blocker that is used in the treatment of hypertension. The reference substances were camphor, geraniol, thymol and clove oil. The saturation solubility of labetalol hydrochloride was identified in water, vehicle (ethanol : water, 60:40 v/v) and vehicle containing 5% w/v terpene alcohols. Similar saturation solubilities were identified suggesting an insignificant increase in labetalol hydrochloride flux across rat skin on account of thermodynamic activity. By performing *in vitro* abdominal skin permeation studies using a side-by-side glass diffusion cell, the permeation of labetalol hydrochloride in vehicle and in the presence of 5% w/v enhancer was analysed. A number of parameters, such as steady-state flux, permeability coefficient, lag time, partition coefficient, diffusion coefficient, and enhancement ratios (ERs) were calculated from the permeation data. The maximum enhancement was achieved by basil oil (ER = 46.5). The fact that the activation energies for labetalol hydrochloride are the lowest in the presence of basil oil, suggests the creation of new polar pathways in the skin for enhanced permeation of labetalol hydrochloride (Table 2). The results of this study show that basil oil is a promising penetration enhancer for improved transdermal drug delivery of labetalol.[56]

A somewhat neglected and/or underestimated research field as to the biological properties of EOs is their penetration-enhancing properties. To administer smaller amounts of drugs but with more or the less equal effectiveness sounds very promising. Unfortunately, no studies on human patients are reported here; there are only two animal studies (see Luo *et al*.[54] and Jain *et al*.[56]) and four *in vitro* experiments using diffusion cell systems (see Long *et al*.,[52] Luo *et al*.[53,55] and Jain *et al*.[56]). A more detailed study as to the mechanism of this penetration enhancement activity has been mentioned already.[1] More serious research would be very helpful for sick patients on account of fewer unwanted side effects, which is particularly necessary for cancer patients.

Insect Repellent Activity

Some facts indicate that the use of synthetic chemicals to control insects and arthropods raises several obvious concerns related to the environment and human health. So, there is a growing demand for alternative repellents or natural products. These products possess good efficacy and are environmentally friendly. Essential oils from plants belonging to several species have been extensively tested to assess their repellent and even insecticidal properties as a valuable natural resource.[57] In the following, the insect repellent activity of some essential oils will be discussed, as well as their uses against insect pests – lice, fleas, beetles, mites etc. – and also insects that destroy stored products and crops.

In 2007 Rajkumar *et al*. investigated the repellent effect of selected plant essential oils against the malaria fever mosquito *Anopheles stephensi* in mosquito cages. The five tested oils were *Centella asiatica* (L.) Urb., Apiaceae, *Ipomoea cairica* (L.) Sweet, Convolvulaceae, *Momordica charantia* L., Cucurbitaceae, *Psidium guajava* L., Myrtaceae, and *Tridax procumbens* L., Asteraceae. The oils were tested at three concentrations: 2, 4 and 6%. In general, a dose-dependent effect was noticed. The highest concentration (6%) led to the highest repellency effect. The results showed a high repellency effect at a concentration of 6% of *I. cairica, M. charantia* and *T. procumbens*, which lasted for more than 300 min. *C. asiatica* and *P. guajava* exhibited a lower repellency effect at the same concentration, which lasted less than 150 min. Ethanol, which was used as a control, showed only 8 min repellency. Based on these data, *I. cairica, M. charantia* and *T. procumbens* are promising repellents.[58]

The repellent activity of seven other essential oils against the three cockroach species *Periplaneta americana*, *Blattella germanica* and *Neostylopyga rhombifolia* were analysed by Thavara *et al*. under laboratory conditions. The seven tested essential oils were *Boesenbergia rotunda* (L.) Mansf.A., Zingiberaceae, *Citrus hystrix* DC., Rutaceae, *Curcuma longa* L., Zingiberaceae, *Litsea cubeba* (Lour.) Pers., Lauraceae, *Piper nigrum* L., Piperaceae, *Psidium guajava* L., Myrtaceae, and *Zingiber officinale* Roscoe., Zingiberaceae. Naphthalene was used as a control. The results showed that the best repellency was exhibited by *C. hystrix*, which led to complete repellency (100%) against *Periplaneta americana* and *Blattella germanica*, under laboratory conditions. Moreover, *C. hystrix* exhibited as well the highest repellency (among the tested oils) of about 88% against *Neostylopyga rhombifolia*. Furthermore, *C. hystrix*, formulated as 20% active ingredient in ethanol, exhibited also in the field the highest repellency of about 86% reduction in cockroaches. The best effect was found against *Periplaneta americana* and *Neostylopyga rhombifolia*, which lasted a week after treatment. In conclusion, this study showed that *C. hystrix* essential oil has a remarkable potential for use as a potent repellent.[59]

In 2008, Noosidum *et al*. investigated the effects of the essential oils of *Melaleuca leucadendron* L., Myrtaceae, *Litsea cubeba* (Lour.) Pers., Lauraceae, and *Litsea salicifolia* (Roxb. Ex Nees) Hook, Lauraceae against *Aedes aegypti* females by using an excito-repellency test chamber. The focus of this study was to evaluate the mortality of *Aedes aegypti* females following a 24-h holding period post-contact and non-contact trials. Mosquitoes which escaped after direct contact with essential oils of *M. leucadendron* and *L. salicifolia* did not die, but those who had a direct contact with *L. cubeba* showed only low mortality (2.3–20.4%). Furthermore, in all non-contact trials, no mortality was observed in escaped females no matter which essential oil was used. However, there was low mortality in non-escaped mosquitoes that were exposed to *L. cubeba* (0–14.3%) and *L. salicifolia* (0–17.1%). Independent of the test concentration, *Aedes aegypti* exhibited a higher escape rate from contact chambers when it was treated with *M. leucadendron* and *L. cubeba* compared to *L. salicifolia*. The highest non-contact repellent response was exhibited by *L. salicifolia*. Based on these data, the three essential oils possess promising irritant and repellent properties against *Aedes aegypti*, but further studies will be necessary.[60]

Moharramipour *et al*. reported on the repellent and fumigant toxicity of the essential oil from *Thymus persicus* L., Lamiaceae, against two stored-product beetles *Tribolium castaneum* and *Callosobruchus maculatus*. The evaluation was executed under the following terms and conditions: the repellent and fumigant toxicity were evaluated against 1- to 7-day-old adult beetles at 27 $\pm$ 1°C and 65 $\pm$ 5% RH (rate of humidity) in the dark. At the highest concentration (2 µl/ml acetone) *Tribolium castaneum* and *Callosobruchus maculatus* showed a repellency of 70.4% and 82.4%, respectively. Moreover, the fumigation bioassays exhibited *Callosobruchus maculatus* adults that were significantly more fragile to the essential oil than *Tribolium castaneum* adults. This is proved by the LC50 values. *Callosobruchus maculatus* adults possess a LC50 value of 2.39 µl/l air and *Tribolium castaneum* possess a LC50 value of 234.42 µl/l air. The strong repellency, fumigant toxicity and the safety suggest that *T. persicum* is a promising candidate for use in the management of stored-product pests.[61]

An investigation dealing with the repellent effects of catmint, *Nepeta cataria* L., Lamiaceae, oil formulations against black flies (*Simulium decorum* Walker) and mosquitoes (primarily *Aedes intrudens* Dyar) in the field in Maine and Florida was carried out in 2008 by Spero *et al*. The essential oil was hydrogenated to enrich the dihydronepetalactone diastereomers. The results of the evaluation in Maine showed that protection from black flies lasted for 6 h or more with all formulations. Liquid formulations at 15 wt% active ingredient conferred complete protection for 7.5 h. Moreover, the results showed that all formulations led to protection against mosquitoes for more than 4 h. The best result was obtained with more than 8 h complete protection. The results of the evaluation in Florida showed that all formulations led to protection for more than 4 h from a mixed population of mosquitoes. The 15 wt% lotion conferred complete protection from bites for more than 6 h.[62]

In fact, the use of whole plants and their products as insect repellents is very common in north-eastern Tanzania, as Kweka *et al*. reported in an ethno-botanical study. The study took place at Moshi in the Kilimanjaro region. To investigate which species are used by the local population to prevent biting insects, interviews and bioassays were made. The bioassays helped to evaluate the protective potential of selected plants extracts. The most popular plants were *Ocimum suave* Willd., Lamiaceae, *Ocimum kilimandscharicum* L., Lamiaceae, *Azadirachta indica* A.Jun., Meliaceae, *Eucalyptus globulus* (Labill.), Myrtaceae, and *Lantana camara* L., Verbenaceae. These plants are used fresh or by burning the leaves. *O. suave* and *O. kilimandscharicum* were used by 67% out of 120 households interviewed. Furthermore, the bioassay, comparing *O. suave* and *O. kilimandscharicum* with citronella and DEET (*N*,*N*-diethyl-*meta*-toluamide) was designed to analyse the repellence and feeding inhibition of untreated and treated arms of volunteers. To investigate the knockdown effects and mortality of *Anopheles arabiensis*, *Anopheles gambiae* and *Culex quinqefasciatus*, filter papers impregnated with *Ocimum*-extracts were used. The results showed a high biting protection (83% to 91%) and feeding inhibition (71% and 92%) against the three mosquito species. Moreover, *Ocimum*-extracts led to a longer induction of KD90 (the time, in minutes, needed to knock down 90% of mosquitoes) in mosquitoes than citronella. A dose of 30 mg/m^2 of *O. suave* and *O. kilimandscharicum* on filter papers led, after 24 h, to a mortality of 57% and 47%, while the mortality was 68% for citronella. Therefore, these plants are really very promising repellents as well.[63]

Müller *et al*. investigated the repellent ability of essential-oil candles against biting insects. The tested oils were geraniol, linalool and citronella. The vapours of the oils were analysed outside, where these products are normally used. Citronella candles were able to reduce the number of female mosquitoes by 35% and sand flies by 15% at a distance of 1.0 m. Better results were obtained for linalool, which led to a reduction of female mosquitoes by 65% and sand flies by 49%. Nevertheless, the best results showed geraniol candles caused a reduction of female mosquitoes by 82% and sand flies by 70%. The repellency dropped significantly by increasing the distance to 2 m and 3 m. Furthermore, another focus of this study was to compare the degree of personal protection. Geraniol, as the best performing candle, was tested under conditions of high and low biting pressure. In a high biting environment, geraniol was able to reduce the mosquito pressure by an average of 56% and the sand fly pressure by 62% (1 m distance). In the low biting pressure environment, geraniol led to a reduction of the mosquito pressure by an average of 62%. At this site, no sand flies were present.[64] Another study by this author group reported this time on the indoor protection of citronella, linalool and geraniol candles against mosquito and sand fly bites. The evaluation was conducted in a high biting pressure environment in Israel. Five per cent of citronella candles exhibited a repellency rate of 29% against mosquitoes, 5% linalool exhibited 71%, and 5% geraniol candles exhibited 86%. The results showed that geraniol candles are about twice as effective as linalool candles and about five times as effective as citronella candles. Moreover, 5% citronella candles exhibited a repellency rate of 25% against sand flies, 5% linalool exhibited 55%, and 5% geraniol candles exhibited 80%. The results showed that geraniol candles are about five times as effective as citronella candles and about twice as effective as linalool candles.[65]

Pushpanathan *et al*. investigated the essential oil of *Zingiber officinalis* Roscoe. (Zingiberaceae) as a mosquito larvicidal and repellent agent against the filarial vector *Culex quinquefasciatus*. The larval mortality was found after 24 h treatment for the late third instar. The LC50 value was 50.78 ppm. The skin repellent test at different concentrations (1.0, 2.0, 3.0 and 4.0 mg/cm^2) of *Z. officinalis* exhibited 100% protection up to 120 min. Not only does the essential oil of *Z. officinalis* possess an agreeable odour but, based on these data, it is also a promising candidate as a repellent agent against filarial vector *Culex quinquefasciatus*.[66]

Abdel-Sattar *et al*. studied the chemical composition of fruit and leaf essential oils of *Schinus molle* L., Anacardiaceae, and its insecticidal and insect repellent activity against *Trogoderma granarium* and *Tribolium castaneum*. The main components were analysed by GC-MS and more than 60 components were identified. The main constituent in both oils is *p*-cymene. The results of this study showed that, in fact, the high yield and efficacy of *S. molle* is a promising lead for active insecticidal agents.[67] Another investigation by Benzi *et al*. laid emphasis on the repellent and toxic activities of the essential oils extracted from leaves of *Aloysia polystachya* Griseb. & Moldenke and *Aloysia citriodora* Palau, Verbenaceae, and from leaves and fruits of *S. molle var. areira* against adults of *Rhizopertha dominica*. To evaluate the contact toxicity topical application and the filter paper assay were used. The filter paper impregnation was also used for fumigant and repellent assays. *A. polystachya* was as effective as *S. molle* leaves in topical tests. Based on the class scale, *Aloysia citriodora* was the most effective oil in the case of repellent assays. *A. polystachya* was the most toxic plant on contact toxicity by the filter paper assay (LC50, 26.6 mg/cm^2). Moreover, the fumigant toxicity was only investigated with the fruits and leaves of *S. molle*, but there were no great differences between them.[68]

The essential oils of different Australian native plants in 5% v/v formulations were evaluated by Maguranyi *et al*. as to their repellency against *Aedes aegypti*, *Culex quinquefasciatus* and *Culex annulirostris* under laboratory conditions. The three most effective oils were *Leptospermum petersonii* J.R.Forster & G.Forster, Myrtaceae, *Prostanthera melissifolia* F. Muell., Lamiaceae, and *Melaleuca alternifolia* Maiden & Betche ex Cheel, Myrtaceae. These oils were compared with DEET (*N*,*N*-diethyl-3-methylbenzamide), a topical insect repellent containing synthetic active ingredients and a commercially available botanical insect repellent. The longest protection time (110 min) of the essential oils was against *P. melissifolia* against *Cx. quinquefasciatus*. The mean protection times against *Aedes aegypti* were lower than those for the *Culex* spp. Nevertheless, the longest protection time of all the compared substances was afforded by DEET against *Aedes aegypti*. Based on these data, these essential oils from Australian native plants offer only limited protection against biting mosquitoes. Moreover, it was indicated that a blend of essential oils offer commercial potential as a short-period repellent. Nevertheless, DEET-based repellents are necessary in areas with a high risk of mosquito-borne disease.[69]

Lee *et al*. investigated the acaricidal activities of major constituents from the oil of *Juniperus chinensis* L., Cupressaceae, (JCEO) leaves against house-dust and stored-food mites, compared with those of DEET. For the analysis, the impregnated fabric disk bioassay against *Dermatophagoides* spp. and *Tyrophagus putrescentiae* was used. The toxicity differs with the chemical composition as well as the doses. The LD50 values of JCEO against *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus* and *Tyrophagus putrescentiae* were 21.60, 19.89 and 38.10 µg/cm^2. By using GC-MS, bornyl acetate was identified as the principal acaricidal component. The LD50 of bornyl acetate (2.94 µg/cm^2) against *Dermatophagoides farinae* was significantly lower than those of DEET (37.13 µg/cm^2). Similar results were obtained when bornyl acetate was tested against these two mites. This study showed that bornyl acetate has the potential to be used as a control agent against house-dust and stored-food mites.[70]

Louses and flies belong to those animals which are either pests or spread infections. This is the reason why Khater *et al*. investigated, for the first time, the lousicidal, ovicidal and repellent efficacy of some essential oils against the buffalo louse, *Haematopinus tuberculatus*, and flies infesting water buffaloes in Egypt. *Cinnamomum camphora* L., Lauraceae, *Allium cepa* L., Amaryllidaceae, *Mentha piperita* L., Lamiaceae, *Matricaria chamomilla* L., Asteraceae, and *Rosmarinus officinalis* L., Lamiaceae were tested. For the *in vitro* studies, filter paper contact bioassays were used to test the oils and their lethal activities were compared with that of D-phenothrin. Four minutes after the treatment, the LC50 values (the concentration of a chemical which kills 50% of a sample population) and the lethal time (LT50) values after treatment with 7.5% *C. camphora*, *A. cepa*, *M. piperita*, *M. chamomilla*, *R. officinalis* and D-phenothrin were as listed in Table 3.

Table 3. LC50 and LT50 values of various essential oils and D-phenothrin against louses and flies

Oil or D-phenothrin	LC50 value (%)	LT50 value (min)
Cinnamomum camphora	2.74	0.89
Allium cepa	7.28	2.75
Mentha piperita	12.35	15.39
Matricaria chamomilla	18.67	21.32
Rosmarinus officinalis	22.79	11.60
D-Phenothrin	1.17	1.94

All the oils except *R. officinalis* were ovicidal to the eggs of *H. tuberculatus*. In contrast to the *in vitro* assays, the *in vivo* treatments showed that the pediculicidal activity of the oils was more potent in comparison with D-phenothrin. All treated lice were killed after 0.5–2 min, whereas with D-phenothrin, 100% mortality was reached only after 120 min. Additionally, a reduction of the number of lice infesting buffaloes a few days after treatment with the oils, except *R. officinalis*, and D-phenothrin was obtained. Furthermore, all tested substances are able to repel flies significantly, namely *Musca domestica*, *Stomoxys calcitrans*, *Haematobia irritans* and *Hippobosca equina*, for 6 and 3 days post-treatment. Based on these data, the study showed that some Egyptian essential oils have the potential for the development of new, rapid and secure lousicides and insect repellents for controlling lice and flies which infest water buffaloes.[71]

Sfara *et al*. carried out a study with the aim of evaluating the fumigant and repellent activity of five essential oils (from eucalyptus, geranium, lavender, mint and orange) and seven monoterpenes (eucalyptol, geraniol, limonene, linalool, menthone, linalyl acetate and menthyl acetate) on first-instar nymphs of the bloodsucking bug *Rhodnius prolixus* Stahl, a vector of Chagas disease in several Latin American countries. To evaluate the fumigant activity, exposing the nymphs to the vapours emitted by 100 µl of essential oil or monoterpene was tested in a close vessel. The knockdown time 50% (KT50) for eucalyptus essential oil was 216 min. This is seven times less toxic than dichlorvos, a volatile organophosphorus insecticide, which was used as a positive control. The other essential oils showed only poor fumigant activity. Less than 50% of the nymphs were knocked down after more than 500 min of exposure. The KT50 values for the monoterpenes were 117 min for eucalyptol, 409 min for linalool, 474 min for menthone, and 484 min for limonene. Eucalyptol was 3.5 times less toxic than dichlorvos. After 540 min of exposure to geraniol, linalyl acetate or menthyl acetate, no affected nymphs were observed. Additionally, the repellency was tested by a video tracking system. Two different concentrations of essential oil or

monoterpenes were studied: 40 and 400 μg/cm^2. The results showed that mint and lavender essential oil and menhone only produced a light repellent effect at 400 μg/cm^2. Geraniol and menthyl acetate showed a repellent effect at both tested concentrations. Nevertheless, the repellent effect of all tested substances was less than that produced by DEET.[72]

The effects of thymol from the essential oil of *Tachyspermum ammi* (L.) Sprague, Apiaceae against *Anopheles stephensi* was investigated by Pandey *et al*. The larvicidal, oviposition-deterrent, vapour toxicity, and repellent activity against the malarial vector were evaluated. Thymol showed an LD50 value of 48.88 toward fourth-instar larvae of *A. stephensi*. So it was 1.6-fold more toxic than the oil, which showed an LD50 value of 80.77 μg/ml. After treatment with vapours of thymol the egg laying by female adults of this fly was significantly more reduced compared to the treatment with the essential oil. The evaluation of the egg hatching and larval survival showed similar results. The vapour toxicity assay exhibited an LC50 value of 185.4 mg/mat for the crude oil against adults of *A. stephensi*, whereas thymol showed an LC50 value of 79.5 mg/mat. After 1 h, the treatment of adult flies with 25.0 mg/mat of thymol demonstrated complete repellency. The same degree of repellency was obtained by the oil of *T. ammi* at the dose of 55.0 mg/mat. This indicates that thymol possesses two-fold activity.[73]

The focus of another study was to prove whether plant-derived products can be used as *Dermanyssus gallinae* repellents. Thus George *et al*. investigated the repellence of plant essential oils against *Dermanyssus gallinae* and the toxicity to the non-target invertebrate *Tenebrio moilitor*. The poultry red mite *Dermanyssus gallinae* causes losses in egg production, anaemia and even death of hens. Moreover, it is necessary that these essential oils show a minimal impact on non-target organisms. The tested oils were manuka, thyme, palmarosa, caraway, spearmint, black pepper and juniper leaf. The evaluation showed that all these oils repel these mites at 0.14 mg oil/cm^3 during the first 2 days of study. Most effective was thyme oil, which was a repellent until the end of the study period (13 days). Additionally, the toxicity of these oils was also tested against mealworm beetles (*Tenebrio molitor*). The results showed that the toxicity to *Tenebrio molitor* differed at the same concentration. For example, the essential oil of palmarosa and manuka were not more toxic than the control. Moreover, there was neither a significant association between the rank toxicity and repellence of oils to the mites, nor the toxicity of oils to the mites and mealworm beetles.[74]

Eamsobhana *et al*. investigated the repellent effects of 13 aromatic essential oils against *Leptotrombidim imphalum* chiggers, a vector of scrub typhus, which is a rickettsial disease and endemic in many parts of Asia. An efficient *in vitro* test method was used by exposing the sand flea for up to 5 min. Only four of the 13 tested oils exhibited promising repellent effects. At 5% concentration, *Syzygium aromaticum* (L.) Merril & Perry oil (Myrtaceae) led to 100% repellency. *Melaleuca alternifolia* Maiden & Betche ex Cheel oil (Myrtaceae) exhibited 100% repellency at 40% concentration. *Zingiber cassamunar* Roxb., Zingiberaceae, and *Eucalyptus globulus* Labill., Myrtaceae, led undiluted to 100% repellency. Furthermore, only 100% of *Pelargonium graveolens* L'Herit ex Aix, Geraniaceae, led to more than 50% repellency. *Styrax tonkinensis* L., Styraceae, oil did not show any repellency. Based on these data, this study showed that several essential oils have the potential as sand flea repellents. In particular, *S. aromaticum* oil may be safer and more economical as a sand flea repellent than commercially available chemicals.[75]

As already mentioned in the introduction to this section the main goal of all research in this field is to prevent pathogenic reactions in humans and/or domestic animals after bites of such pest insects (or damage of stored products). In any case the first step is the repellence of the insects; insecticidal or knock-down effects are further steps. The majority of the papers cited report on the repellent activity of EOs and also of some of their constituents, e.g. geraniol,[64,65] which in many studies proved to be the most effective natural compound but every time less effective compared with the synthetic substance DEET. Hence, it is unreal for people not to expect to be bitten during indoor and outdoor activities for a whole day and night, as well as not to be bitten during sleep after administering or using only EOs. Either the mean duration of such a natural repellent is only about 240 min (see Spero *et al*.[62]) or the distance for which 86% repellency is guaranteed is only 1 m[64] at the moment without consideration of the repellents leave-on the skin. Thus, up to now, either synthetic repellents such as DEET[69,73] or a combination of it with, for example, the monoterpene alcohol geraniol, offer the best solution to the aforementioned human expectations. Pure EOs or single EO constituents are too volatile to promise a longer-lasting effect.[69]

Antiviral Activity

A virus is a small infectious particle (20–300 nm) that is able to infect cells of another living organism, in which it can replicate itself. Viruses cannot reproduce on their own: a virus is composed of genes and a protein coat and some have an envelope of fat that surrounds them. Viruses can lead to infections, which provoke an immune response that usually eliminates the infecting virus. Nowadays, we know about 5000 viruses in detail.[76]

In 2007, Saddi *et al*. investigated the activities of the essential oil from *Artemisia arborescens* L., Asteraceae, against herpes simplex virus 1 and 2 (HSV-1 and HSV-2) because new prophylactic and therapeutic tools are needed. The result of this study showed that the IC50 values were 2.4 and 4.1 μg/ml for HSV-1 and HSV-2, respectively. These values were tested with a plaque reduction assay. This method allows the number of plaques formed by a virus sample to be counted, from which the actual virus concentration can be determined. Moreover, the MTT reduction assay was used. This is a quantitative colorimetric method that measures the activity of enzymes that reduce MTT to formazan, giving a purple colour. By using the MTT reduction method the determination of the cytotoxicity assay against Vero cells showed a CC50 value of 132 μg/ml, indicating a CC50/IC50 ratio of 55 for HSV-1 and 32.2 for HSV-2. Furthermore, the study showed that the antiviral activity of the essential oil is principally due to direct virucidal effects. By using a yield reduction assay, it was possible to observe poor activity against HSV-1 at higher concentrations when added to cultures of infected cells. Moreover, there was no inhibition observed by attachment assay, penetration assay and post-attachment virus neutralization assay. Additionally, the essential oil was able to inhibit the lateral diffusion of both HSV-1 and HSV-2. Based upon these data, this study showed that the essential oil from *A. arborescens* possesses antiviral activity against HSV-1 and HSV-2 because of its ability to inactivate the virus and to inhibit the cell-to-cell virus diffusion.[77]

HSV-1 and HSV-2 were also the goal of assessment in the next two studies dealing with the essential oils of *Eugenia caryophyllus* (L.) Merril & Perry, Myrtaceae, and of *Cedrus libani* A.Rich.,

Pinaceae. In the first study the antiviral activity this Myrtaceae and of eugenol against standard HSV-1(F), standard HSV-2(G) and ten HSV isolates was investigated by Tragoolpua *et al*. The results of this study showed that *E. caryophyllus* was able to inhibit HSV-1(F), HSV-2(G), two HSV-1 isolates (2, 30) and four HSV-2 isolates (1, 2, 3, 21) in the plaque reduction assay. Eugenol was only able to inhibit HSV-1 isolates 1 and 30. Additionally, *E. caryophyllus* and eugenol led to an inactivation of particles of HSV standard strains. After the treatment with *E. caryophyllus* and eugenol, the total virus yield of HSV standard strains and isolates declined after 30 h. Moreover, extracts of *E. caryophyllus* showed higher antiviral replication on HSV-2(G) than on HSV-1(F). The inhibition of the viral yield of HSV-1 isolates was significantly higher than standard HSV-1(F) and standard HSV-2(G) was also inhibited more than most of the HSV-2 isolates.[78] In the second paper an *in vitro* evaluation of the biological activity against herpes simplex virus type 1 (HSV-1) of *C. libani* was carried out. This cedrus plant is widely used as traditional medicine in Lebanon to treat different infection diseases. The main constituents of wood essential oil, analysed by GC-MS, were himachalol (22.5%), β-himachalene (21.9%), and α-himachalene (10.5%), while the leaves ethanol extract was characterized by a high content of germacrene D (29.4%). The main constituents of the cones ethanol extract were α-pinene (51.0%) and β-myrcene (13.0%). Cytotoxicity was evaluated by the MTT assay in Vero cells. The results of this study showed that the ethanol extracts of cones and leaves possess an interesting activity with IC50 values of 0.50 and 0.66 mg/ml at non-cytotoxic concentration. The essential oil showed a similar activity with an IC50 value of 0.44 mg/ml.[79]

Another study dealt with the *in vitro* antiviral activities against SARS-CoV and HSV-1 of the essential oils of seven Lebanon species: *Laurus nobilis* L., Lauraceae, *Juniperus oxycedrus ssp. oxycedrus* L., Cupressaceae, *Thuja orientalis* (L.) Franco, Cupressaceae, *Cupressus sempervirens ssp. pyramidalis* L., Cupressaceae, *Pistacia palaestina* Boiss., Anacardiaceae, *Salvia officinalis* L., Lamiaceae, and *Satureja thymbra* L., Lamiaceae. The focus of this study was to evaluate the oils inhibitory activity against SARS-CoV and HSV-1 replication *in vitro* by visually scoring the virus-induced cytopathogenic effect post-infecto. The most promising oil with the highest activity against SARS-CoV was *L. nobilis* oil with an IC50 value of 120 µg/ml and a selectivity index (SI) of 4.16. The major constituents of this oil, determined by GC-MS analysis, were β-ocimene, 1,8-cineole, α-pinene, and β-pinene. The most promising oil with the highest activity against HSV-1 was *J. oxycedrus* oil with an IC50 value of 200 µg/ml and a SI of 5.0. The major constituents of this oil are α-pinene and β-myrcene.[80]

The essential oil of another Myrtaceaen species, namely *Eucalyptus globulus* Labill. was investigated by Cermelli *et al*. and its effect on respiratory bacteria and viruses assessed. The activity of *E. globulus* essential oil was determined for 120 isolates of *Streptococcus pyogenes*, 20 isolates of *S. pneumoniae*, 40 isolates of *S. agalactiae*, 20 isolates of *S. aureus*, 40 isolates of *Haemophilus influenzae*, 30 isolates of *H. parainfluenzae*, 10 isolates of *Klebsiella pneumoniae*, 10 isolates of *Stenotrophomonas maltophilia* and two viruses, a strain of adenovirus and a strain of mumps virus. The antibacterial activity was tested by the Kirby–Bauer paper method, minimum bactericidal concentration and minimum inhibitory concentration. The Kirby–Bauer paper method, also called the agar diffusion test, is used for measuring the effect of an anti-microbial agent against bacteria grown in culture. By using the MTT test the cytotoxicity was evaluated on VERO cells. The most influenced were *H. influenzae*, *H. parainfluenzae*, *S. maltophilia* and *S. pneumoniae*. Moreover, only a mild activity on mumps virus was found.[81]

The next six studies on antiviral activities of essential oils were published by the research team of Reichling. At first Koch *et al*. investigated the inhibitory effect of essential oils against HSV-2. Essential oils from anise (*Pimpinella anisum* L., Apiaceae), hyssop (*Hyssopus officinalis* L., Lamiaceae), thyme (*Thymus vulgaris*, L., Lamiaceae), ginger (*Zingiber officinalis*, Roscoe, Zingiberaceae), chamomile (*Matricaria recutita* L., Asteraceae) and sandalwood (*Santalalum album* R.Br., Santalaceae) were screened for their inhibitory effect against herpes simplex virus type 2 (HSV-2) *in vitro* on RC-37 cells using a plaque reduction assay. The results showed IC50 values at 0.016%, 0.0075%, 0.007%, 0.004%, 0.003% and 0.0015% for anise oil, hyssop oil, thyme oil, ginger oil, chamomile oil and sandalwood oil. All tested oils led to a dose-dependent virucidal activity against HSV-2. The essential oils were added at different stages during the viral infection cycle, to analyse their mechanism of work. When HSV-2 was pre-incubated with hyssop oil, thyme oil or ginger oil, the plaque formation was significantly reduced by more than 90%. Moreover, there was no inhibitory effect when the essential oils were added to the cells prior to infection with HSV-2 or after the adsorption period. Maybe the essential oils interact with the viral envelope. The most promising oil was chamomile, which showed a high selectivity index.[82] Furthermore, this group investigated the efficacy of anise oil (*Pimpinella anisum* L., Apiaceae), dwarf-pine oil (*Pinus pumila* (Pall.) Regel, Pinaceae) and chamomile oil (*Matricaria recutita* L., Asteraceae) against different thymidine-kinase-positive (aciclovir-sensitive) and thymidine-kinase-negative (aciclovir-resistant) herpes simplex virus type 1 (HSV-1) strains. Clinical HSV-1 isolates, which contain frame-shift mutations in the thymidine kinase (TK) gene (an insertion or a deletion), yield a non-functional thymidine kinase enzyme resulting in phenotypical resistance against aciclovir. The *in vitro* tests, using a plaque reduction assay, showed that all essential oils possess a high capacity of antiviral activity against the aciclovir-sensitive HSV strain KOS, aciclovir-resistant clinical HSV isolates and the aciclovir-resistant strain Angelotti. Moreover, the oils led to a significant reduction of plaque formation by 96.6–99.9% at maximum concentrations. These results were obtained when herpes viruses were pre-incubated with drugs before attachment to host cells. However, when adding these compounds during the replicant phase, there was no significant effect on viral infectivity. This leads to the conclusion that anise oil, dwarf-pine oil and chamomile oil affected the virus by interrupting adsorption of herpes viruses, while aciclovir is effective after attachment inside the infected cells.[83] Then, some essential oils from the family Myrtaceae, like cajeput (*Melaleuca leucadendron* L.), clove (*Syzygium aromaticum* (L.) Merril & Perry, kanuka (*Kunzea ericoides* (A.Rich.) Joy Thomps, and manuka (*Leptospermum scoparium* J.R. Forster & G. Forster) were analysed by Schnitzler *et al*. A focus of this study was to evaluate the oils' cytotoxicity in a standard neutral red assay (NRU). This is a cell survival assay based on the ability of viable cells to incorporate and bind neutral red (NR). Maximum non-cytotoxic concentrations for *M. leucadendron* oil and *S. aromaticum* oil were determined at 0.006%, *K. ericoides* oil and *L. scoparium* oil were more cytotoxic with a maximum non-cytotoxic concentration of 0.001%. Moreover, the results of this study showed that manuka essential oil possesses a high capacity of virucidal activity against HSV-1 as well as against drug-resistant HSV-1 isolates in viral suspension tests.[84] Also, the antiviral activity of lemon balm oil, the essential oil of *Melissa*

officinalis L., Lamiaceae, against enveloped herpes viruses was investigated. The major constituents of *M. officinalis*, analysed by GC-MS, were the monoterpene aldehydes citral a, citral b and citronellal. The inhibitory activity against HSV-1 and HSV-2 was evaluated *in vitro* on monkey kidney cells using a plaque reduction assay. The results of this study showed that the IC50 value of balm oil for HSV plaque formation was determined at high dilutions of 0.0004% and 0.00008% for HSV-1 and HSV-2. Moreover, lemon balm oil, at non-cytotoxic concentrations, led to a significant reduction of the plaque formation by 98.8% for HSV-1 and 97.2% for HSV-2. Another focus of this study was to analyse the mode of antiviral action by using a time-on-addition assays. This is a multi-well assay for identifying a compound inhibiting the replication cycle of a micro-organism. HSV-1 and HSV-2 were significantly inhibited by pretreatment with balm oil prior to the infection of cells. Based upon these data, it become obvious that lemon balm oil affected the virus before adsorption, but not after penetration into the host cell.[85] In another paper the focus was the essential oil of star anise (*Illicium verum* Hook F., Illiaceae), some phenylpropanoids, and sesquiterpenes, such as *trans*-anethole, eugenol, β-eudesmol, farnesol, β-caryophyllene and β-caryophyllene oxide, which are present in many essential oils. Their antiviral activity against HSV-1 *in vitro* was examined by plaque reduction assays. The results showed that star anise essential oil led to a reduction of viral infectivity by 99%, while phenylpropanoids reduced the HSV infectivity by 60–80%. Sesquiterpenes inhibited the infectivity by 40–98%. Furthermore, star anise oil and all isolated compounds showed anti-HSV-1 activity because they led to a direct inactivation of free virus particles in viral suspensions assays. Additionally, star anise oil, which is rich in *trans*-anethole, exhibited a high selectivity index of 160 against HSV. Among the isolated compounds only β-caryophyllene showed a similar high selectivity index of 140. In conclusion, star anise essential oil, phenylpropanoids and sesquiterpenes are promising candidates as antiviral agents against HSV-1.[86] Finally, another study reported on the examination of the antiviral activity of selected monoterpenes and of the essential oils from eucalyptus (*Eucalyptus* sp., Myrtaceae), tea tree (*M. alternifolia*, Myrtaceae) and thyme (*Thymus* sp., Lamiaceae) and of their major monoterpene compounds α-terpinene, γ-terpinene, α-pinene, *p*-cymene, terpinen-4-ol, α-terpineol, thymol, citral and 1,8-cineole against HSV-1 *in vitro*. The results showed that these essential oils led to a reduction of viral infectivity by more than 96%, while these monoterpenes are only able to reduce the infectivity by about 80%. Furthermore, an analysis of the mode of antiviral action was accomplished. The essential oils and monoterpenes revealed only moderate antiviral effects when they were added to host cells prior to infection or after entry of HSV into cells. All the tested drugs led to an interaction in a dose-dependent manner with HSV particles. They inactivated viral infection. Furthermore, the study showed that among the analysed compounds monoterpene hydrocarbons were slightly superior to monoterpene alcohols in their antiviral activity. The highest selectivity index was shown by α-pinene and α-terpineol. Moreover, the mixtures of different monoterpenes, which are present in natural tea tree essential oil, revealed a 10-fold higher selectivity index and a lower toxicity than its isolated single monoterpenes.[87]

Garazzo *et al*. investigated the *in vitro* antiviral activity of *M. alternifolia* essential oil (tea tree oil, TTO), Myrtaceae, and of its main components, terpinen-4-ol, α-terpinene, γ-terpinene, *p*-cymene, terpinolene and α-terpineol. The antiviral activity was tested against polio type 1, ECHO 9, Coxsackie B1, adeno type 2, HSV-1 and HSV-2 by 50% plaque reduction assay. The anti-influenza virus assay was based on the inhibition of the virus-induced cytopathogenicity. The results showed that TTO and some of its compounds, e.g. terpinen-4-ol, terpinolene and α-terpineol, possess an inhibitory effect on influenza A/PR/8 virus subtype H1N1 replication at non-cytotoxic concentrations. Furthermore, TTO showed an ID50 value of 0.0006% (v/v), which was much lower than its CD50 value with 0.025% (v/v). All the compounds showed no virucidal activity against polio 1, adeno 2, ECHO 9, Coxsackie B1, HSV-1 and HSV-2, while TTO exhibited a slight virucidal effect against HSV-1 and HSV-2. The results of this study showed that TTO is a promising drug in the treatment of influenza virus infection.[88]

The inhibitory effect of essential oils of *Lippia alba* Mill., Verbenaceae, *Lippia origanoides* Kunth., Verbenaceae, *Oreganum vulgare* L., Lamiaceae, and *Artemisia vulgaris* L., Asteraceae, on yellow fever virus (YFV) replication was investigated by Meneses *et al*. The cytotoxicity on Vero cells was evaluated by the MTT reduction method. The results showed CC50 values less than 100 µg/ml and minimal inhibitory concentration (MIC) of 3.7 and 11.1 µg/ml. The CC50/MIC ratio was 22.9, 26.4, 26.5 and 8.8 for *L. alba*, *L. origanoides*, *O. vulgare* and *A. vulgaris*. Moreover, 11.1 µg/ml of *L. origanoides* oil led to a 100% reduction of virus yield. The same results were observed with 100 µg/ml of *L. alba*, *O. vulgare* and *A. vulgaris* oils. Furthermore, when Vero cells were treated with essential oil before the adsorption of untreated virus, no reduction of virus yield was observed. In conclusion, the tested oils showed antiviral activity against YFV through direct virus inactivation.[89]

To combat viral infections simply by EOs or their constituents seems to be a promising medication considering the fact that many viral infections cannot be counteracted so easily by administering only a lozenge. Either vaccinations or avoiding becoming infected (e.g. by more frequent hand washing), or confinement to bed, etc. are the usual strategies to keep well or to regain health. However, using these natural compounds is not sufficient because some virus types remain quasi sleeping in the body (e.g. HSV), or the virucidal compared to the virustatic effect is rather mediocre (for example, see Saddi *et al*.[77] and Astani *et al*.[87]) post-infection. Most of the cited papers report excellent-to-good effects when test cells (e.g. Vero cells[77]) are treated before infection. Most research has been carried out with herpes simplex virus, although four studies (Loizzo *et al*.,[80] Cermelli *et al*.,[81] Garozzo *et al*.[88] and Meneses *et al*.[89]) deal with other virus strains. Remarkable are the effects against SARS-CoV post-infection upon administration of *L. nobilis* oil,[80] or of TTO against H1N1 virus replication,[88] or at least of *A. arborescens* oil at higher concentration, but showing only a poor activity against HSV-1 infected cells.[77] In many cases the whole EOs are more effective than their constituents, as well as EOs with a higher concentration of mono- and/or sesquiterpene hydrocarbons than oxygenated terpenes (see, for example, Astani *et al*.[86]). Summarizing these results, there is still much do in order to detect really effective EOs against viral infections.

Antioxidant Activity

Antioxidants, such as vitamins, enzymes or Fe^{2+}, etc. are able to neutralize free radicals. They exert a health-enhancing effect on the human organism because they protect cells from oxidant damage.

In 2007 Sharififar *et al*. investigated the antioxidant and free-radical scavenging activities of the essential oils from flowers and fruits of *Otostegia persica* (Burm.) Boiss., Lamiaceae. By using GC/MS analysis about 30 components were identified in each oil. The major constituents of the essential oil flowers (EOFLs) were α-pinene (17.2%), 1-octen-3-ol (13.4%) and cubenol (7.3%) and most prominent in the essential oil of the fruits (EOFR) was hexadecanoic acid (11.1%). Another focus of this study was to screen the oils for their possible antioxidant activity. Two complementary test systems were used: (1) the 2,2-diphenyl-1-1-picrylhydrazyl (DPPH) free-radical scavenging test; and (2) ammonium thiocyanate. The results showed that EOFLs possess greater antioxidant and radical scavenging activity in both tests. In the DPPH free-radical scavenging, EOFLs showed antioxidant activity with an IC50 value of 19.8 $\pm$ 1.8 µg/ml. In the second test system, EOFLs exhibited an inhibition rate of oxidation of linoleic acid of 93.5 $\pm$ 2.8. The high amount of oxygenated monoterpenes in EOFLs may be the reason for the higher antioxidant activity.[90]

Chaieb *et al*. investigated the antioxidant properties of the essential oil of clove (*Eugenia caryophyllata* (L.) Merril & Perry, Myrtaceae). The major components, as analysed by GC/MS, were eugenol (88.6%), eugenyl acetate (5.6%), β-caryophyllene (1.4%) and 2-heptanone (0.9%). The antioxidant activity was evaluated by the DPPH free-radical scavenging test and the anti-radical dose required to cause 50% inhibition (IC50) was recorded. The results exhibited that the oil showed a very strong radical scavenging activity with an IC50 value of 0.2 µg/ml. A comparison was made with the synthetic antioxidant *tert*-butylated hydroxytoluene which exhibited an IC50 value of 11.5 µg/ml. Furthermore, the oil showed promising anti-fungal effects.[91]

Da Silva *et al*. investigated the antioxidant activity of essential oil and methanol extract of *Aniba canelilla* Kunth., Lauraceae. 1-Nitro-2-phenylethane was identified as the main volatile component (70.2–92.1%). The oils exhibited a DPPH scavenging activity (EC50) of 198.17 $\pm$ 1.95 µg/ml. This is low in comparison with the EC50 value of wood methanol extracts (4.41 $\pm$ 0.12 µg/ml), which was equivalent to that of trolox, used as an antioxidant standard. The high antioxidant activity of this species may be based upon the high amount of total phenolics (710.53 $\pm$ 23.16 mg of GAE/g).[92] Furthermore, the chemical composition and the antioxidant capacity of the essential oil of *Lippia schomburgkiana* Kunth., Verbenaceae) was investigated. The major constituents were identified as 1,8-cineole (64.1%) and α-terpineol (12.0%). Furthermore, the antioxidant activity was evaluated by the DPPH free-radical scavenging test. The methanol extract of *L. schomburgkiana* led to an inhibition of the DPPH radical, showing an EC50 value of 16.1 $\pm$ 0.7 µg/ml. This EC50 value is only three times lower than that of trolox with 4.7 $\pm$ 0.4 µg/ml. This leads to the conclusion that *L. schomburgkiana* possesses high antioxidant activity. Moreover, the brine shrimp bioassay was used to measure the LD50 values. It is possible to evaluate the *in vivo* lethality in a simple zoological organism (the brine shrimp). But the brine shrimp bioassay, which was carried out on the oil, showed high toxicity (49.6 $\pm$ 0.4 µg/ml).[93]

Also, essential oils of Rutacean plants possess antioxidative properties. So, Misharina *et al*. reported on the antioxidant properties of essential oils from lemon (*Citrus limon* L., Rutaceae), pink grapefruit (*Citrus paradisi* Macfad., Rutaceae), coriander (*Coriandrum sativum* L., Apiaceae), and clove (*Caryophyllus aromaticum* = *Syzygium aromaticum* (L.) Merril & perry, Myrtaceae) buds. These oils were studied by capillary gas–liquid chromatography. The antioxidant activity was evaluated by oxidation of the aliphatic aldehyde hexanal to the carboxylic acid. The results showed that grapefruit essential oil has the lowest and clove bud essential oil the highest antioxidant activity. Moreover, mixtures containing clove bud essential oil strongly inhibited oxidation of hexanal.[94] Another Rutaceae was investigated by Kambouche *et al*. in order to evaluate the chemical composition and the antioxidant potential of *Ruta montana* L., Rutaceae, essential oil which is growing in the Oran region in the west of Algeria. The main constituents were analysed by GC-MS. About 20 compounds were identified. The major components were undecan-2-one (32.8%), nonan-2-one (29.5%), nonanol-2-acetate (18.2%) and psoralen (3.5%). Furthermore, the antioxidant activity was evaluated by the DPPH free-radical scavenging test. The results showed that *R. montana* essential oil possesses anti-radical activity in a concentration-dependent manner. It was possible to find a linear correlation between the reduction of the DPPH stable free radical and the concentration of *R. montana* essential oil.[95]

Bozin *et al*. reported on the antioxidant properties of *Achillea collina Becker ex Heimerl s.l.* and *A. pannonica Scheele* essential oils, both Asteraceae. The evaluation was made by testing the free-radical scavenging capacity towards DPPH radicals, together with effects on lipid peroxidation (LP). The essential oil of *A. pannonica* expressed higher scavenging effects on the DPPH radical with an IC50 value of 0.52 µg/ml. In the LP evaluation, essential oil of *A. collina s.l.* from Golija exhibited stronger antioxidant activity with an IC50 value of 0.75 µg/ml.[96]

Lopes-Lutz *et al*. focused their research on the Asteraceae plants and studied the chemical composition, anti-microbial and antioxidant activities of *Artemisia absinthium* L., *Artemisia biennis* Willd., *Artemisia cana* Pursh, *Artemisia dracunculus* L., *Artemisia frigida* Willd., *Artemisia longifolia* Nutt. and *Artemisia ludoviciana* Nutt., all Asteraceae. The chemical composition was evaluated by GC-MS and a total of 110 components were identified. Furthermore, the results of this study showed that the tested *Artemisia* oils showed an inhibitory effect on the growth of bacteria, yeasts, dermatophytes, *Fonsecaea pedrosoi* and *Aspergillus niger*. Moreover, the antioxidant activity was evaluated by the β-carotene/linoleate model. The determination of the DPPH radical scavenging activities exhibited only weak activities for these oils.[97]

A cluster of plant families with the most antioxidative acting species is the Lamiaceaea group. So, Chizzola *et al*. investigated the antioxidative properties of *Thymus vulgaris* L., Lamiaceae, leaves, which are rich in essential oil and antioxidative phenolic substances. In an experimental field in Austria 12 accessions were grown. The focus of this study was to analyse leaf samples from these plants as well as from a commercial thyme rich in thymol for their essential oil and their antioxidative potential. The assays for antioxidative activity were the total phenolics according to the Folin–Ciocalteu method, DPPH de-colouration and Fe^{3+} reduction (FRAP assay; the ferric reducing/antioxidant potential). The Folin–Ciocalteu method is a colorimetric assay of phenolic and polyphenolic antioxidants. It measures the amount of the substance being tested required to inhibit oxidation of the reagent. Folin–Ciocalteu reagent is a mixture of phosphomolybdate and phosphotungstate. Comparison of the results showed that the less active and the most active accession only differed by factors of 2.1 and 2.6 in total phenolics and the FRAP assay. Similar results were obtained from the DPPH assay. Moreover, the highest antioxidant activity was shown by essential oils with a high amount of the phenolic components thymol and/or carvacrol. Ethanolic extracts exhibited lower antioxidant activity.[98]

Also, the species *Salvia* is known on account of its antioxidant properties. So, Ben Farhat *et al*. studied variations in the antioxidant activity of Tunisian cultivated *Salvia officinalis* L. (Lamiaceae) essential oil, growing in different habitats. The major components, analysed by GC-MS, were α-thujone (11.6–19.2%), viridiflorol (9.9–19.5%), 1,8-cineole (8.9–15.6%), camphor (5.1–15.1%), manool (5.5–13.1%), β-caryophyllene (2.6–9.2%), α-humulene (1.9–8.9%) and β-thujone (5.5–6.2%). Based upon these data, significant differences between different collection sites were found. The antioxidant activity was assessed using post-distilled dry samples. The prevalent compounds of *S. officinalis* methanolic extracts were rosmarinic acid, carnosol, and carnosic acid. The results of this study showed that *S. officinalis* exhibited differences in the antioxidant and radical scavenging activity at different magnitudes of potency. Only the DPPH assay showed significant differences in free-radical scavenging activity among samples collected in different regions.[99]

Laouer *et al*. analysed its essential oil from the aerial part with regard to its composition and antioxidant activity. Thirty-seven compounds were identified by GC-MS. The major constituent was germacrene D (45.7%). The antioxidant activity was determined using three *in vitro* assays: (1) the scavenging effect on DPPH, (2) the ABTS test and (3) the phosphomolybdenum method. The ABTS test (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) is used to observe the reaction kinetics of specific enzymes. ABTS turns into a green end-product which can easily be identified at 420 nm with a spectrophotometer. The phosphomolybdenum method is based upon oxidation to a phosphomolybdenum blue complex by the addition of nitrite. The results of this study showed that the oil presented antioxidant activity. Furthermore, another focus of the study was the anti-microbial activity, but the essential oil showed no effects on the tested microorganisms.[100] Schmidt *et al*. investigated the chemical composition and the antioxidant effects of the essential oil from *Mentha x piperita* L., Lamiaceae. The main constituents, analysed by GC-MS, were menthol (40.7%), menthone (23.4%), (±)-menthyl acetate, 1,8-cineole, limonene, β-pinene and β-caryophyllene. The results of this study showed that *M. piperita* possessed anti-radical activity with respect to DPPH and hydroxyl radicals. Moreover, the oil exhibited a stronger antioxidant impact on the hydroxyl radical. The IC50 values were 860 µg/ml for DPPH and 0.26 µg/ml for hydroxyl radicals. Furthermore, the essential oil of *M. piperita* exhibited antioxidant activity in a linoleic acid emulsion system in terms of inhibiting conjugated diene formation by 52.4% and linoleic acid secondary oxidized products generation by 76.9% (at 0.1% concentration).[101] Also, the chemical composition and the antioxidant activity of the essential oil from cornmint (*M. canadensis*, Lamiaceae) was investigated by the same author group. The major constituents, again analysed by GC-FID and GC-MS, were menthol (41.2%) and menthone (20.4%). The anti-radical activity of cornmint oil with respect to the DPPH and hydroxyl radicals was established. The results of this study showed an IC50 value of 365.0 µg/ml for DPPH and 0.3 µg/ml for hydroxyl radicals. The antioxidant activity in terms of hydroxyl radicals was higher than that of quercetin. Furthermore, cornmint oil chelated the Fe^{3+} ions present in the solution. The oil also exhibited antioxidant activity in a linoleic acid emulsion system. At a concentration of 0.1%, the oil inhibited the formation of conjugated dienes by 57.1% and the generation of secondary oxidized products of linoleic acid by 76.1%.[102] Yang *et al*. investigated the antioxidant activities of six popular and commercially available essential oils. The tested oils were from lavender (*Lavandula angustifolia* Mill., Lamiaceae), peppermint (*M. piperita* L., Lamiaceae), rosemary (*Rosmarius officinalis* L., Lamiaceae), lemon (*Citrus limon* L., Rutaceae), grapefruit (*Citrus paradisi* Macfad. Rutaceae), and frankincense (*Boswellia carteri* (Birdw.), Burseraceae). The major components of the tested essential oils, analysed by GC-MS, were linalyl acetate (28.2%), menthol (33.4%), 1,8-cineole (46.1%), limonene (64.5% and 94.2%) and *p*-menth-2-en-ol (34.5%). The antioxidant activity of these oils was evaluated by testing free-radical scavenging capacity and lipid peroxidation in the linoleic acid system. The results of this study showed that lavender essential oil and limonene possess the highest DPPH radical scavenging activity with RC50 values of 2.1 ± 0.23% and 2.1 ± 0.04%. Furthermore, peppermint essential oil possesses the highest radical scavenging activity against the ABTS radical (1.6 ± 0.09). Lavender oil was most effective for inhibiting linoleic acid peroxidation after 10 days.[103] Finally, the antioxidant activity of the essential oils from five plants that are widely used in a Mediterranean diet were studied by Viuda-Martos. The tested oils were oregano (*Origanum vulgare* L., Lamiaceae), thyme (*Thymus vulgaris* L., Lamiaceae) rosemary (*R. officinalis* L., Lamiaceae), sage (*Salvia officinalis* L., Lamiaceae) and clove (*Syzygium aromaticum* (L.) Merril & Perry, Myrtaceae). The results of this study showed that the highest amount of total phenols (898.89 mg/l GAE) was obtained by the clove essential oil, which showed as well the highest percentage inhibition of DPPH radical (98.74%) and the highest FRAP value (1.47 TEAC; trolox equivalent antioxidant capacity). Moreover, the highest percentage inhibition of TBARS (thiobarbituric acid reactive substance) by 89.8% was exhibited by the thyme essential oil. Furthermore, this study showed that all the tested essential oils were capable of chelating iron(II), but the highest effect was achieved by the rosemary essential oil (76.1%). Additionally, the oregano essential oil showed the highest antioxidant activity index in the Rancimat test.[104]

However, not only essential oils from Lamiacean plants possess an distinct antioxidant activity: other plants were studied in order to detect 'new' natural antioxidants; for example, Martins *et al*. investigated the chemical composition and the antioxidant activity of the volatile oil from the fruit peel of *Garcinia brasiliensis* Mart., Clusiaceae. A total of 38 components were identified. The major constituents, analysed by GC-MS, were γ-muurolene (10.3%), spathulenol (8.7%), δ-cadinene (8.3%), torreyol (8.0%), α-cadinol (7.0%), cadalene (6.3%), and γ-cadinene (5.3%). The main group of compounds were oxygenated sesquiterpenes (43%), but the results of this study showed that the volatile oil possesses poor antioxidant activity.[105] Then, the essential oil composition and antioxidant activity of *Pterocarya fraxinifolia* (Lam.) Spach, Juglandaceae was investigated by Ebrahimzadeh *et al*. Sesquiterpenes and monoterpenes are the major compounds in the essential oil of the leaves. The major constituent was bisabolol oxide A (23.6%). The potential antioxidant activity of *P. fraxinifolia* bark and leaves was investigated by six *in vitro* assay systems. The DPPH free-radical scavenging assay showed an IC50 value of 3.89 ± 0.09 for leaves and 41.57 ± 1.30 µg/ml for bark. Furthermore, the leaf extract showed promising reducing power at 2.5 and 80 µg/ml, which was comparable with vitamin C ($p > 0.05$). The extracts also showed Fe^{2+} chelating ability as weak nitric oxide scavenging activity. The extracts led to an inhibition of peroxidation with values from 92% to 93% after 72 h, comparable with vitamin C activity ($p > 0.05$). Higher antioxidant activities were observed in leaf extract because of the higher total phenol and flavonoid contents.[106]

Gholivand *et al*. investigated the chemical composition and the *in vitro* antioxidant activity of the essential oil and methanol extracts of *Psammogeton canescens* (DC.), Apiaceae. The chemical composition, analysed by GC/MS, showed that the main constituents of the oil are β-bisabolene (33.4%), apiole (28.3%), α-pinene (11.9%) and dill apiole (8.2%). Furthermore, the antioxidant activities were determined by three various testing systems, namely DPPH, β-carotene/linoleic acid, and the reducing power assay. The highest radical scavenging activity in the DPPH system was executed by the polar sub-fraction of methanol extract (49.5 $\pm$ 1.21 µg/ml). Moreover, in the second case the inhibition capacity of the polar sub-fraction of 92.40% $\pm$ 0.72 was found to be the stronger one. Furthermore, in the reducing power assay, a reverse activity pattern more than in the first two systems was observed. The essential oil was a stronger radical reducer than the methanol extract in all of the tested concentrations. Based upon these data, the essential oil and methanol extracts of *P. canescens* possess significant antioxidant activities.[107] Another Myrtaceae as above was the focus of an investigation by Singh *et al*. These authors studied the chemical composition and the antioxidant activity of the essential oil from fresh and decaying leaves of *Eucalyptus tereticornis* SM., Myrtaceae. The main components of the fresh leaf oil, analysed by GC/MS, were α-pinene (28.5%) and 1,8-cineole (19.5%). The main components of the decaying leaf oil were β-citronellal (14.2%), (–)-isopulegol (13.4%), and (+)-β-citronellol (10.7%). The essential oils were evaluated for their antioxidant activity in terms of scavenging DPPH, hydroxyl radical and super-oxide anion. The results of this study showed that both essential oils possess a strong radical scavenging activity against the DPPH radical with IC50 values of 110 and 139.8 µg/ml for fresh and decaying leaf oil. Moreover, the essential oils at concentrations of 400 µg/ml also showed scavenging activity against hydroxyl radical (56–62%) and superoxide anion (65–69%). The major monoterpene constituents showed significantly less scavenging activity. In conclusion, the essential oil of fresh and decaying leaves of *E. tereticornis* are a rich source of monoterpenoids exhibiting antioxidant activity.[108] A very prominent and often-used plant was the focus of interest of Sarikurkcu *et al*. These authors investigated the chemical composition and the antioxidant activity of the essential oil and different solvent extracts (water, hexane, dichloromethane, ethyl acetate and methanol) of *Vitex agnus-castus* L., Verbenaceae, fruits from Turkey. The chemical composition was analysed by GC-MS and about 27 components were identified. The main constituents of the oil were 1,8-cineole (25%), sabinene (13.5%), α-pinene (10.6%), α-terpinyl acetate (6.7%), and (*Z*)-β-farnesene (5.4%). The evaluation of the antioxidant activities of the samples was tested by three different test systems: DPPH, β-carotene/linoleic acid and reducing power assays. The results of this study showed that the water extract exhibited excellent activity potential in all tested systems. The amount of total phenolics was very high in this extract, with 112.46 $\pm$ 1.22 µg/mg extract. Furthermore, the dichloromethane extract possesses a high amount of flavonoids. A positive correlation was observed between the antioxidant activity potential and total phenolic and flavonoid levels of the extracts.[109]

The assessment of the antioxidant activity is a relative 'simple' procedure, and because there are so many test systems it is very probable that an EO can react with such test substances. Remarkable of the reported papers is the fact that all studies focus their interest on the EO in its entirety. In only two experiments were the major single constituents also added to the assessment, namely thymol[98] and a mixture of the main monoterpenes[108] in order to establish a correlation between EO and major constituents. Nearly all cited papers use more than one test method, of which the DPPH test was the most prominent. Worth mentioning are the studies of Kambouche *et al*.,[95] where alkanones were found as the main constituents, and of da Silva *et al*.,[92] where 1-nitro-2-phenylethane was identified as the active volatile component (see also de Lima *et al*.,[15] mentioned in the second section of this review). As the main result of all these compiled studies it seems to be the fact that phenolics, e.g. thymol, carvacrol, eugenol, and the nearly non-volatile flavonoids in extracts (see da Silva *et al*.,[92] Ebrahimzadeh *et al*.[106] and Sarikurkcu *et al*.[109]) are more responsible for the antioxidant effect than mono- and sesquiterpenes.

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