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# DIPLOMARBEIT

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"Biological properties of essential oils – an update"

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Auf diesem Weg möchte ich mich bei allen bedanken, die mir bei der Anfertigung dieser Arbeit Unterstützung boten.

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## ABSTRACT

In the last years more and more studies on the biological properties of essential oils have been published that it seemd wortwhile 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 antinociceptive, anticancer, antiinflammatory, penetration enhancing, insect repellent, antiviral and antioxidative properties of essential oils. Many essential oils are used since long ago in the folkmedicine. The biological properties of various essential oils were 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. Non the less, more studies are necessary to analyze the biological properties of other essential oils or to prove their mechanism of action.

# ZUSAMMENFASSUNG

In den letzten Jahren sind immer mehr Studien über die Wirksamkeit von ätherischen Ölen publiziert worden, sodass es sinnvoll erschien, die neuesten Arbeiten und Studien der Jahre 2009 und 2008 sowie mit der zweiten Hälfte von 2007 zusammenzufassen, gerade nachdem erst vor kurzem so eine Zusammenfassung wissenschaftlicher Arbeiten der Jahre 2000 bis 2007 zu diesem Thema erschien.

Die Schwerpunkte dieser Übersicht liegen auf der antinozizeptiven, antikanzerogenen, entzündungshemmenden, penetrationsfördernden, insektenabweisenden, antiviralen und antioxidativen Wirkung von ätherischen Ölen. Zahlreiche ätherische Öle werden schon seit langem aus Erfahrung in der Volksmedizin verwendet. Für einige dieser Öle konnte die Wirksamkeit in diversen Studien bewiesen werden und ihre Anwendung in der Therapie von Schmerzen, Entzündungen, Virenerkrankungen oder Krebs, sowie ihre Wirkung als Insektenschutz oder ihre Anwendung zur Verbesserung der Eindringtiefe anderer Pharmaka wurde in vielen Fällen bestätigt. Dennoch besteht der Bedarf an weiterführenden Studien, um die Wirkung anderer ätherischen Öle zu untersuchen oder um diverse Wirkmechanismen zu klären.

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# 1. INTRODUCTION

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

The focus of this overview lies on the antinociceptive, anticancer, antiinflammatory, penetration enhancing, insect repellent, antiviral and antioxidative properties of EOs.

Chapter 2 deals with the antinociceptive activity of selected EOs, which is a reduction in pain sensitivity made within neurons when endorphin or a similiar opium-containing substance combines with a receptor.
In fact that cancer belongs to a hugh class of diseases, which cause more than 10% of all human deaths, Chapter 3 deals with the anticancer activity of EOs.
The antiinflammatory properties of EOs are described in Chapter 4. The chronic inflammation leads to a number of diseases and is needed to be treated by 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 to decrease the barrier resistance. In Chapter 5, 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 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 (Chapter 6).

A virus is a small infectious particles (20-300nm), 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 know about 5.000 viruses in detail. Chapter 7 deals with the antiviral activity of selected EOs.
Chapter 8 is about the antioxidant activity of EOs. Antioxidants like 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.

## 2. ANTINOCICEPTIVE EFFECT

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

Sousa et al. analyzed the antinociceptive and anti-inflammatory effects of the essential oil from *Eremanthus erythropappus* (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. The essential oil inhibited in the formalin-induced nociception test the paw licking of the mice 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 with mice the essential oil lead to a significant increase of the reaction time after 30, 60 and 90 min of treatment, at doses of 200mg/kg and 400 mg/kg. The same doses lead to an inhibition of carrageenan-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 intrapleural injection of carrageenan. The study

demonstrates clearly the analgesic, the anti-inflammatory and the antinociceptive effect of *E. erythropappus* oil. [4]

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

Liapi et al. studied the antinociceptive properties of 1,8-cineole and β-pinene, two monoterpenes, from the essential oil of *Eucalyptus camaldulensis* (Myrtaceae) leaves, in rodents (mice and rats) using the tail-flick and hot-plate tests, reflecting the spinal and supraspinal levels. Morphine and naloxone were used for comparison. 1,8-Cineole showed in both algesic stimuli an antinociceptive 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. It leads to supraspinal antinociceptive actions in rats only and reversed the antinociceptive 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* (Boraginaceae). The study showed a clear antinociceptive effect of the essential oil [7] as well as the one by Kamatou et al.. He reported on the biological activities and phytochemistry of South African *Salvia* species, that belongs to the Lamiaceae. The genus *Salvia* encompasses 900 species worldwide. *Salvia* is known for its use to treat microbial infections, cancer, malaria, inflammation etc. The major compounds of the essential oil are monoterpene hydrocarbons, oxygenated mono-terpenes and sesquiterpenes. [8]

Takaki et al. investigated the anti-inflammatory and antinociceptive effects of *Rosmarinus officinalis* L. 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, antirheumatic, carminative effects. The tests of the antinociceptive effects were carried out using the acetic acid-induced writhing and the hot-plate test in mice. 500mg/kg REO lead 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 carrageenan-induced edema 1-4 hours after injection of the phlogistic agent, is caused by REO at doses of 250, 500 and 750 mg/kg. The administration of REO showed in the hot-plate test unremarkable effects on response latency, whereas control injection of meperidine induced clear antinociceptive effects. At doses of 70, 125 and 250 mg/kg REO showed a remarkable antinociceptive effect in the acetic acid-induced abdominal writhing test compared with control animals. The conclusion of this study is that REO possesses peripheral antinociceptive activity. [9] Also Martinez et al. reported on the antinociceptive effect of this Lamiacean essential oil using a rat model of arthritic pain. A dose-dependent antinociceptive effect is produced by the essential oil, manifested as a significant reduction of the disfunction in the pain-induced functional impairment model in the rat (PIFIR model), mainly at high doses. The major compounds, analyzed 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 antinociceptive 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 nonselective opioid receptor antagonist). In both cases an inhibition of the antinociceptive response was demonstrated. An involvement, at least in part, of endogenous opioids and the serotonergic system via 5-HT(1A) in the antinociceptive effect of *Rosmarinus officinalis* essential oil in the PIFIR model is possible. [10]

Sakurada et al. studied the capsaicin-induced antinociceptive activities of bergamot (*Citrus bergamia*, Risso) essential oil, Rutaceae, (BEO) by intraplantar injection into the mouse hindpaw. An intense and short-lived licking or biting response toward the injected hindpaw is produced by an intraplanar injection of capsaicin. After the intraplanar 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 showed as well the antinociceptive effect of *Salvia sclarea*, linalool

chemotype of *Thymus vulgaris*, *Lavandula angustifolia* and *Lavandula hybrida* Reydovan on the capsaicin-induced nociceptive response, while testing the essential oil of *Citrus sinensis* was without effect. Another result of this study is the pharmacological activity of linalool, showing besides the antinociceptive one also an antihyperalgesic, anticonvulsant and antiinflammatory effect. The study confirms the importance of linalool or linalyl acetate in BEO or these compounds as constituents of other essential oils in the antinociceptive therapy. [11]

The antinociceptive effects of the essential oil of *Mentha x villosa*, Lamiaceae, (EOMV) leaves and its major constituent piperitenone oxide in mice were investigated by Sousa et al. (2009). By the fact that the essential oil of this herb possesses many pharmacological acivities, such as antispasmodic effects, the antinociceptive activity of the oil and its major constituent piperitenone oxide (PO) were assumed. After an oral administration of 200mg/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. EOMV caused as well 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 +/- 2.7 s (N = 12) and 3.0 +/- 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 antinociceptive activity, what is probably a so-called indirect anti-inflammatory effect, which does not involve the central nervous system. [12]

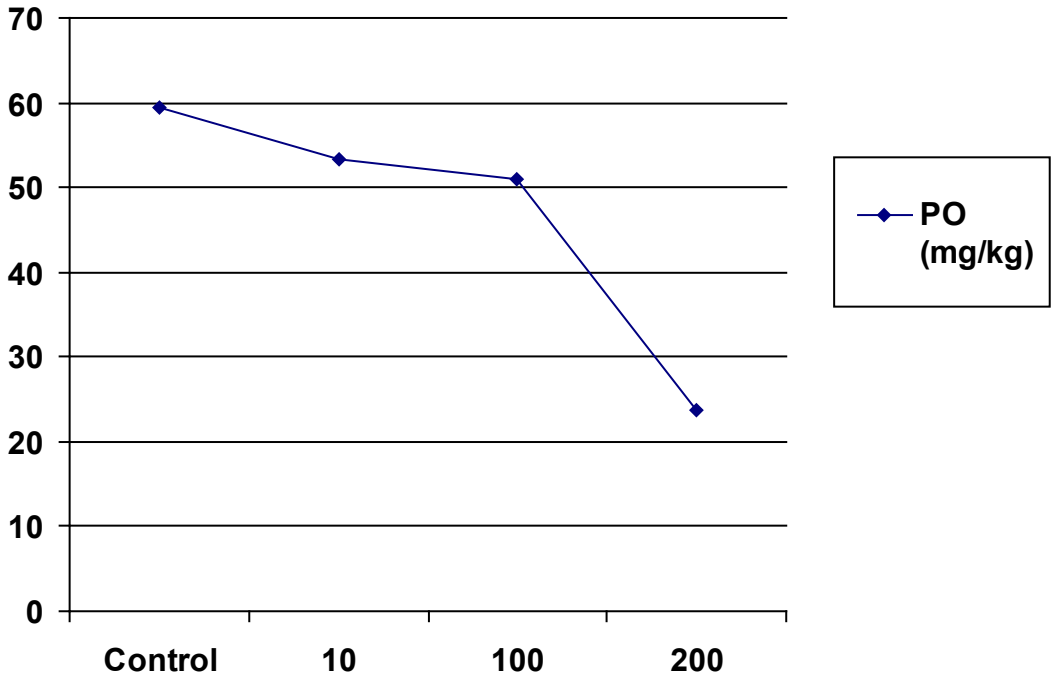


Figure 1: Inhibitory effect of piperitenone oxide (PO) on the nociceptive reaction to intraperitoneal acetic acid injection in mice. [a]

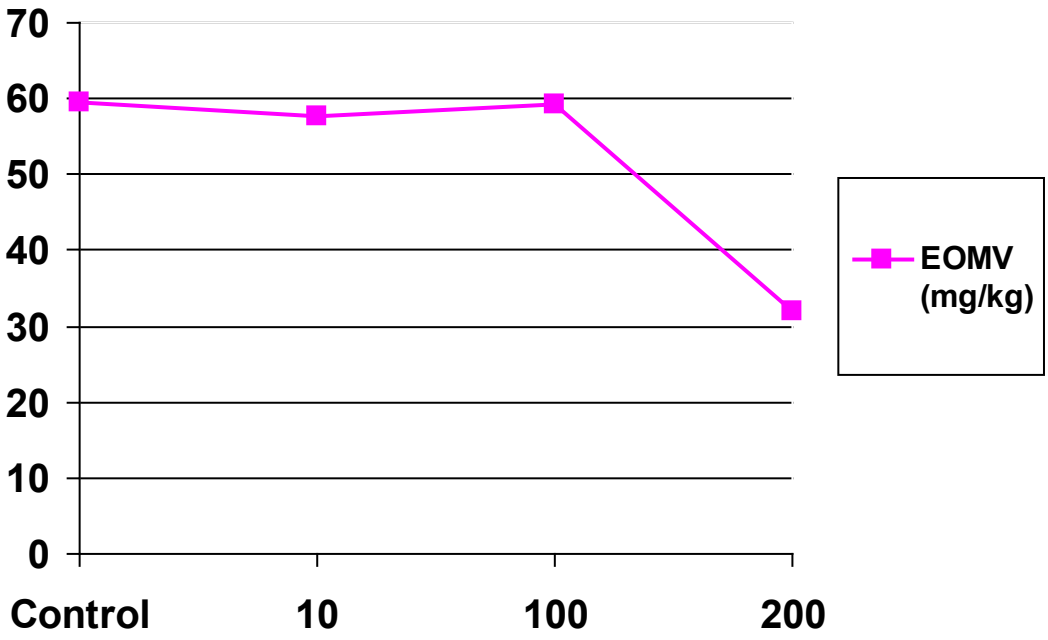


Figure 2: Inhibitory effect of the essential oil of *Mentha x villosa* (EOMV) on the nociceptive reaction to intraperitoneal acetic acid injection in mice. [b]

The phytochemistry and biological activities of *Phlomis* species were studied by Limen-Ben Amor. The genus *Phlomis* L. is part of the Lamiaceae familiy and includes 100 species and is used to treat various conditions such as diabetes, gastric ulcer, hemorrhoids, 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 oil are monoterpenes (α-pinene, limonene and linalool), sesquiterpenes (germacrene D and β-caryophyllene), aliphatic compounds (e.g. 9,12,15-octadecatrienoic acid methyl ester), fatty acids (e.g. hexadecanoic acid) and other components (e.g. trans-phytol, 9,12,15-octadecatrien-1-ol). The study comes to the conclusion that *Phlomis* species have *inter alia* antinociceptive, antidiabetic, anti-inflammatory, anticancer and antioxidant properties. [13]

Amorim et al. analyzed the antinociceptive 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, hypoglycemic and diuretic complains and is also applied in the cosmetics industry. The present study is about the chemical composition, the antinociceptive and hypothermic profile of the essential oil of pitangueira leaves. The main constituent, analyzed by GC-MS, is a mixture of atractylone and 3-furanoeudesmene. After an oral administration of the essential oil it came to a significant inhibition of the acetic acid-induced abdominal constrictions, additional to an increase of the latency time in hot-plate test and a hypothermic effect. The isolated furanosesquiterpenes are discussed to be responsible for the antinociceptive and hypothermic effect. [14]

In the paper of De Lima et al. the antinociceptive activity of 1-nitro-2-phenylethane, the main component of *Aniba canelilla* essential oil (ACEO) was described. *Aniba canelilla,* Lauraceae*,* is known for its use in the Amazon folk medicine as antispasmodic, antidiarrhoeic, 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 lead 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 formaline 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 an analgesic activity, probably of peripheral origin. The physical mechanism is not completely understood. An involvement of the opioid receptors in the antinociceptive action observed to 1-nitro-2-phenylethane is assumed. [15]

In another study the antinociceptive potency of the rhizome essential oil of *Zingiber zerumbet*, 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. Doses of 30, 100 and 300 mg/kg lead after the intraperitoneal administration of the EOZZ 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 formaline-induced paw licking test, while the EOZZ significantly reduced the painful stimulus in both neurogenic and inflammatory phase of the test. Additionally, in these two tests the antinociceptive effect of the EOZZ could be reversed by naloxone, a nonselective opioid receptor antagonist. This is an 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 antinociceptive activities and the folk medicinal use of the EOZZ to relieve some pain conditions is justified. [16]

## 3. ANTICANCER ACTIVITY

The medical term for cancer is malignant neoplasm. Cancer belongs to a hugh class of diseases, which cause more than 10% of all human deaths. Affected are humans as well as animals 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]

2007 Legault et al. 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 tumor 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, anticarcinogenic and local anaesthetic activities. Administration of β-caryophyllene at non-cytotoxic concentrations lead to a clear increase of the anticancer activity of α-humulene and isocaryophyllene on MCF-7 cells. About 50% and 69% of 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 the ten-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 lead to the suggestion that β-caryophyllene stimulates the drug accumulation by different mechanism of action and that β-caryophyllene helps paclitaxel to pass through the membrane and potentiates on this way its anticancer activity.[18]

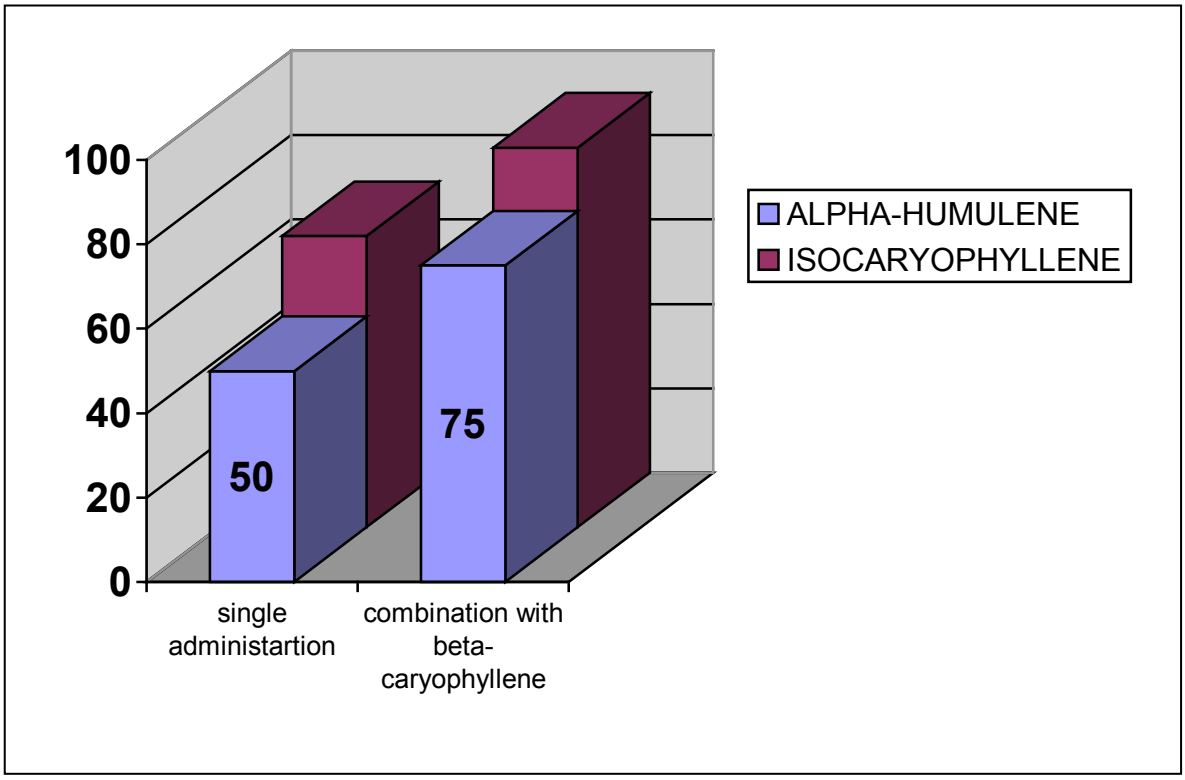


Figure 3: The inhibition of cell growth achieved by α-humulene and isocaryophyllene. [c]

After many studies showing the potential of chemopreventive phytochemicals especially to increase the sensitivity of cancer cells to conventional anticancer drugs, 2008 Ravizza et al. investigated linalool, a plant-derived monoterpene alcohol, that is found in the essential oils from many aromatic plants and is able to reverse the doxorubicin resistance in human breast adenocarcinoma cells. The focus of this study were two human breast adenocarcinoma cell lines, MCF7 WT and multidrug resistant MCF7 AdrR, both as a single agent and in combination with doxorubicin (DOX). Linalool only sparsely inhibited cell proliferation, but in subtoxic concentrations it lead to a higher DOX-induced cytotoxicity and pro-apoptotic effects in both cell lines. In MCF7 AdrR cells a promising synergism is noticed, which may be justified 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 MDR (multidrug resistance) tumors. [19]

Rezvanfar et al. investigated the protection of cyclophosphamide-induced toxicity in reproductive tract histology, sperm characteristics, and DNA damage by an herbal source, as an 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* (SKEO), an established herbal antioxidant. To show that SKEO has a protective effect, similiar 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, histopathological 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 (6mg/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 conlusion 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 *Satureja khuzestanica* protects the reproducive system from toxicity of cyclophosphamide. [20]

Verma et al. analyzed the induction of mitochondrial-dependent apoptosis by an essential oil from *Tanacetum gracile* (Asteraceae), (TGEO), an alpine aromatic herb, that contains about 40 constituents. The main compounds are lavendulol (21.5 %), lavendulol 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 %).

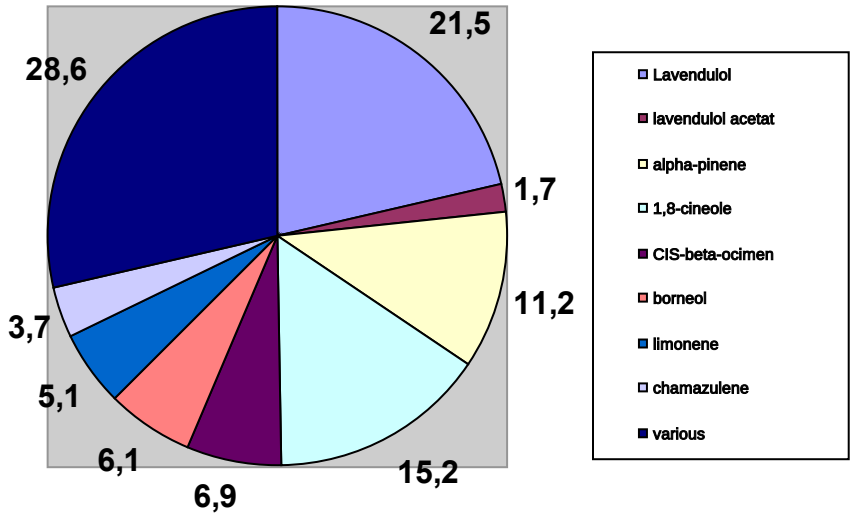


Figure 4: The composition of the essential oil of *Tanacetum gracile*. [d]

TGEO lead to an inhibition of HL-60 cell proliferation with an IC(50) of 27 µg/ml and to an induction of apoptosis in human leukemia HL-60 cells. This effect was measured by several biological end points. Furthermore, TGEO lead to an improvement of annexin V-FITC binding of the cells, an increase of sub-G (0) DNA fraction, a drop of mitochondrial membrane potential, a liberation of cytochrome C from mitochondria, activating caspase-9 and caspase-3, and an increase of cleavage of PARP in HL-60 cells. This study showed the anticancer activity of the essential oil of *Tanacetum*

*gracile*, with the conclusion that the oil exerts an induction of the apoptosis through the miochondrial dependent pathway in HL-60 cells. [21]

Another study reported on the chemotypic variation of essential oils in the medicinal plant, *Anemopsis californica*, Saururaceae. In the framework of the study the steam distilled oil from roots/rhizomes of *Anemopsis 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). But no activity against these cell cultures was found. On account of this ethnobotanical use of ACEO to treat uterine cancer, this essential oil was analyzed against AN3CA (uterine) and HeLa (cervical) human cancer cell lines. The result was an antiproliferative activity against AN3CA and HeLa cells *in vitro*. The IC(50) 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 inhibited as well cell growth. The IC(50) values for these three compounds against each cell line was determined and compared with the concentration of these compounds in the root oil of *Anemopsis californica*. The inhibition is maybe 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]

By the fact that some *Eucalypus* species (Myrtaceae) possess antimicrobial and antitumor properties, Ashour analyzed the antibacterial, antifungal, and anticancer activities of volatile oils and extracts from stems, leaves, and flowers of *Eucalyptus sideroxylon* and *Eucalyptus torquata* grown in Egypt. To analyze the anticancer activity a sulphorhodamine 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 human hepatocellular carcinoma cell line (HEPG2) and human breast adenocarcinoma cell line (MCF7). The results showed that the oils of *Eucalyptus torquata* leaves and stems and of *Eucalyptus sideroxylon* leaves exert a cytotoxic effect activity against MCF7 cells, but none effect on HEPG2 cells. [23]

2009 investigated Sharma et al. the anticancer activity of the essential oil from a lemon grass variety of *Cymbopogon flexuosus*, Poaceae. The *in vitro* cytotoxicity against twelve human cancer cell lines and the mechanism of cell death, relating to the morphological chances in tumor cells, were analyzed. Auspicious results showed the *in vitro* cytotoxicity studies: The essenial oil lead to a dose-dependent high cytotoxicity with an IC(50) value, the half maximal inhibitory concentration, of 4.2 and 79 µg/ml, relative to various human cancer cell lines. An IC(50) value 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 tumor models in mice, which were both clearly inhibited by an intra-peritoneal administration of the essential oil. More precisely, at 200 mg/kg intra-peritoneal the oil lead to a growth inhibition of 97% and 58% in both solid and ascitic tumor forms of Ehrlich Ascites carcinoma. In case of Sarcoma-180, the oil furnished a growth inhibition of 94% and 37% at an equal dose. The 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 tumor cells the oil lead to a condensation and fragmentation of nuclei typical of apoptosis. Typical changes for apoptosis were as well found treating the ascites cells from animals with the essenial oil from *Cymbopogon flexuosus*. The study indicates that this essenial oil shows interesting results in the anticancer acivity by activating the apoptotic process, thus reducing the tumor cell viability. [24]

Agrawal studied the potential of Curcumin and various synthetic analogues as anticancer agents. Curcumin, a natural phytochemical and a major constituent of *Curcuma longa,* Zingiberaceae, has been much explored in the last decade. Curcumin is able to interfere with multiple cell signalling pathways, including apoptosis, proliferation (HER-2, EGFR, and AP-1), angiogenesis (VEGF), and inflammation (NF-κB, TNF, IL-6, IL-1, COX-2, and 5-LOX). The study obtaines more than 700 curcumin analogues, which also show anticancer activity in various models and various cell lines. [25]

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 as well an important compound of *Origanum syriacum* L. which shows a very

promising anticancer activity. Thymoquinone was found in the extracts in a wide range between 0.04% and 23.7%. This high concentration of thymoquinone offers its use in the anticancer therapy but further studies are necessary. [26]

## 4. ANTIPHLOGISTIC ACTIVITY

A human organism reacts on 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 like the tumor necrosis factor-α (TNF- α), interleukin (IL)-1β, IL-8, IL-10 and the prostaglandine E2 (PGE2). Without inflammations wounds and infections would not heal and the progressive destruction of the tissue would continue. We can differ two kinds of inflammations: the acute inflammation, as an initial response of the body to harmful stimuli, and the chronic inflammation, which leads to a number of diseases and is needed to be treated by anti-inflammatory drugs. [27] [28]

2007 Tekeoglu et al. analyzed the anti-inflammatory effects of thymoquinone, as an ingredient of the volatile oil of *Nigella sativa* (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 x 105 CFU (n = 7); 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. [29]

Another study by 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 *Nigella sative* 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 five days before the induction of

TNBS colitis. Seven days after the donation of TNBS, macroscopic and histological scores were evaluated. The results showed no significant changes between experimental and control group. 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. But borneol is able to supress significantly the proinflammatory cytokine mRNA expression. [30] 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..[31] Three concentrations were tested: 0.4% thyme and 0.2% oregano oils; 0.2% thyme and 0.1% oregano oils; 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 lead 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 colonic 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. [31]

Fernandes at el. tested the anti-inflammatory properties of two sesquiterpenes isolated from *Cordia verbenacea´*s essential oil (Boraginaceae), -humulene and (-)-trans-caryophyllene. The oral administration of both compounds lead to an effective reduction of platelet activating factor-, bradykinin- and ovoalbumin-induced mouse paw edema. Moreover, α-humulene and (-)-trans-caryophyllene lead to promising inhibitory effects on the mouse and rat carrageenan-induced paw edema. After an oral donation, α-humulene is able to diminish the edema formation caused by histamine injection, but a systemic treatment prevented both tumor necrosis factor-α (TNFα) and interleukine-1β (IL-1β) generation in carrageenan-injected rats. (-)-trans-Caryophyllene is only able to diminish TNFα release. Additionally, both compounds lead to a reduction of the production of prostaglandine E(2) (PGE(2)), as well as inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) expression, induced by the intraplantar injection of carrageenan 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. [32] These two sesquiterpenes from the essential oil of *Cordia verbenacea* were focos of another study by Medeiros et al.. [33] The biological activities were investigated in a model of of acute inflammation in rat paw, induced by lipopolysaccharid (LPS) and characterized by paw edema, neutrophil recruitment, cytokine production, activation of MAP kinases (mitogen-activated protein) and NF-κB and up-regulated expression of kinin B(1) 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 edema and the up-regulation of B(1) receptors following treatment with LPS. Moreover, both compounds were not able to interfere with the activation of the MAP kinases, ERK (extracellular-signal regulated kinase), p38 and JNK (c-Jun N-terminal kinase). The results of this study are in agreement with those of the afore mentioned paper[32]. [33]

2008 the common household plant *Rosmarinus officinalis*, Lamiaceae, popularly named rosemary, was analyzed by Takaki et al.. [9] By the fact that *Rosmarinus officinalis* is used in folk medicine in many parts of the world because of its antispasmodic, analgesic, antirheumatic, carminative, cholagogue, diuretic, expectorant and antiepileptic effects, rosemary essential oil (REO) was evaluated. To analyze the anti-inflammatory activity of REO, the inflammatory exudate volume and also the leukocyte migration in carrageenan-induced pleurisy and carrageenan-induced paw edema tests in rats were used. An administration of REO at doses of 500 mg/kg lead to a significant reduction of the volume of pleural exudate and to a slightly decrease of the number of cells that had migrated compared with the control animals. A promising inhibition of carrageenan-induced edema 1-4 hours after the 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 antinociceptive 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 hydrodistilled essential oil from *Farfugium japonicum* (FJEO), Asteraceae, for the first time. The main components, analyzed 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. Furthermore, FJEO lead to dose-dependent decreases in the iNOS and COX-2 mRNA expression. To assure the safety of the dermal application of FJEO the cytotoxicity was tested by colorimetric MTT 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 treatment of inflammations as well as for topical application, but further studies will be necessary. [34]

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 analyzed by GC-MS. CIEO lead to a significant inhibition of NO production and presented an IC(50) value of 18.68 and 13.18 μg/ml. The protein expression assay showed that CIEO lead to a decrease of the expression of IKK (iκB-kinase), 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 an COX-2. Moreover, 0.1 and 0.3 mg of citral showed a promising anti-inflammatory activity in the assay of croton oil-induced mice ear edema. 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. [35]

The Cyclooxygenase-2 (COX-2) inhibitory effects of the volatile oil from dried roots of *Lithospermum erythrorhizon* (LEEO), Boraginaceae, were analyzed by Kawata et al. The main components have been investigated by GC-MS. More than fifty 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 studiing the in-vitro inhibition of ovine COX-1 and COX-2 activity. The results showed a selective COX-2 inhibition. At doses of 50 μg/ml, LEEO lead to an inhibition of the COX-2 activity of 39%. [36]

Another study by Martins et al. dealt with the anti-inflammatory and antioxidant activities of the volatile oil from the fruit peel of *Garcinia brasiliensis* (GBEO), Clusiaceae. The main components were analyzed 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 analyze the anti-inflammatory activity, GBEO was evaluated by using the rat-paw edema model induced by carrageenan. The results showed an inhibition of the inflammatory process 3 hours after carrageenan administration. Moreover, GBEO possesses a poor antioxidant acivity. [37]

The anti-inflammatory properties of *Ocotea quixos* Lam. (Lauraceae) essential oil (OQEO) *in vitro* and *in vivo* were analyzed by Ballabeni V. et al.. 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 reduce significantly LPS-induced NO release from J774 macrophages at non-toxic concentrations. They inhibited as well 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 (30-100mg/kg p.o.) and trans-cinnamaldehyde (10mg/kg p.o.) showed anti-inflammatory effects in carrageenan-induced rat paw edema without damaging gastric mucosa. The conclusion of this study is that *Ocotea quixos* Lam. essential oil has striking anti-inflammatory gastro-sparing activity. [38]

Nearly the same authorgroup as above[34] analyzed the chemical composition, antioxidant, anti-elastase, and anti-inflammatory activities of *Illicium anisatum* essential oil (IAEO). The main component of IAEO, belonging to the family of Illiciaceae, is eucalyptol (21.8%), analyzed by GC-MS. One of the focus of this study was to identify the mechanism of the anti-inflammatory activity. The results showed that the IAEO lead to a significant inhibition of the production of LPS-induced NO and PGE2 in RAW 264.7 cells. Furthermore, this essential oils lead 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 proof 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 (3-(4,5-dimethylthiazol-2-yl)-

2,5-diphenyltetrazolium bromide) is a standard colorimetric tests which is used to determine cytotoxicity. The conclusion of this study is that IAEO shows an anti-inflammatory potential, but additional *in vitro* and *in vivo* tests are necessary as proof of IAEO´s safety and efficacy. [39]

Ashour et al. investigated the chemical composition and biological activity of the essential oil obtained from *Bupleurum marginatum*, Apiaceae (BMEO). Focus of the study was besides the chemical composition, the antioxidant, anti-inflammatory, antimicrobial and *in-vitro* cytotoxic activity. The main components, analyzed 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 prostaglandine E2 (PGE2) production and lipoxygenase. The results show an IC(50) value of 63.64 µg/ml for lipoxgenase and an inhibition of 26.04% of PGE2 at doses of 25 µg/ml. The conclusion of this study is that BMEO has promising anti-inflammatory effects, but also here further studies are necessary. [40]

The effects of lemongrass essential oil (*Cymbopogon citratus,* Poaceae) on IL-1β and IL-6 production by macrophages was analyzed by Sforcin et al., because the oil is known for its insecticidal, antimicrobial and therapeutic properties, but the knowledge about the effects on the immune system is uncertain. To analyze the oils 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 mice 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 lead 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. [41]

(-)-α-Bisabolol is an optimal active sesquiterpene alcohol, who is found in plants such as *Vanillosmopsis erythropappa* and *Matricaria chamomilla*. The compound is known for its anti-septic and anti-inflammatory activity and especially because of its

gastroprotection on acute gastric mucosal lesions. The focus of this study by Moura Rocha et al. was to investigate the gastroprotective action of (-)-α-bisabolol on ethanol and indomethacin-induced ulcer models in mice. (-)-α-bisabolol (100 and 200mg/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 gastroprotective effects of (-)-α-bisabolol could not be reverted 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 decrease significantly the reduction of these levels on ulcer-induced mice, but not in mice without ulcer. In conclusion, (-)-α-bisabolol lead to a protection of the gastric mucosa from ethanol and indomethacin-induced ulcer. This effect is associated with the increase of gastric sulfhydryl groups bioavailability, what leads to a reduction of gastric oxidative injury induced by ethanol and indomethacin. [42] Another 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 antiulcerogenic activity of the ethanol extract even if this is not an essential oil (!) of *Origanum majorana* L. was evaluated in hypothermic restraint stress-, indomethacin-, necrotizing agents- (80% ethanol, 25% NaCl and 0.2 M NaOH) induced ulcers. 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. *Origanum majoranum* L. has as well the potential to regenerate the ethanol-induced depelted gastric wall mucus and nonprotein sulfhydryl contents. Moreover, *Origanum majoranum* L. is able to lower the increase in the concentration of malondialdehyde (MDA). A histopathological assessment demonstrated the ulcer preventing potential as well. Additionally, the acute toxicity was analyzed to prove the safety of the extract in mice. [43]

The anti-inflammatory properties of curcumin, were the topic of a research project by Jurenka. This major constituent of *Curcuma longa* (Zingiberaceae) has a long history of use in Ayurveda medicine to treat inflammations. The main components are curcumin, demethoxycurcumin, bisdemethoxycurcumin, as well as constituents of the volatile oil, such as turmerone, atlantone and zingiberone. Curcumin is known for its effects on cancer, antioxidant and antimicrobial properties. Curcumin is a highly pleiotropic

molecule, which is able to interact with a lot of molecular targets involved in inflammation. Curcumin shows the potential to be indicated as a therapeutic agent in diseases like pancreatitis, arthritis and chronic anterior uveitis as well as certain kinds of cancer. These effects were tested in cell cultures, animal research and clinical trials. An important disadvantage is curcumin´s rapid plasma clearance and conjugation, what limits its therapeutic usefulness. [44]

Loizzo et al. investigated the *in vitro* biological acivity of *Salvia leriifolia* (Lamiaceae) Benth. 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 cholineserase inhibitory activity. Furthermore, SLEO inhibited lipopolysaccharide-induced NO production with an IC(50) value of 165 μg/ml. By the MTT assay the absence of cytotoxicity at 1000μg/ml was evaluated in 142BR cells. [45]

Due to its most important component of thyme oil, carvacrol 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 cyclooxygenase-2 (COX-2) expression, which plays an important role in inflammation. The biological properties of carvacrol were investigated especially in thyme oil. PPARγ-dependent suppression of COX-2 promoter activity was observed in response to carvacrol treatment. Carvacrol suppressed lipopolysaccharide (LPS)-induced COX-2 mRNA and protein expression in human macrophage-like U937 cells. This lead to the result that carvacrol regulates COX-2 expression through its agonistic effect on PPARγ. [46] Furthermore, also the essential oils of clove (*Syzygium aromaticum*, Myrtaceae), rose (*Rosa sp*., Rosaceae), eucalyptus (*Eucalyptus sp*., Myrtaceae), fennel (*Foeniculum vulgaris*, Apiaceae) and bergamot (*Citrus limon*, Rutaceae) were investigated. The study showed that also these oils lead 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* essential oil, Cupressaceae (CJEO). The major components, analyzed 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), prostaglandin E2 (PGE2), tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 production in

lipopolysaccharide (LPS)-activated RAW 264.7 macrophages. Pro-inflammatory cytokine and mediator tests showed the excellent dose-dependent anti-inflammatory activity of CJE. Furthermore, to analyze 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,[47] as well as the essential oil of *Abies koreana* (Pinaceae). This oil was investigated by the same author as to its anti-inflammatory and antibacterial against skin pathogenes 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 -resistant Propionibacterium acnes and Staphylococcus epidermidis, both acne-causing bacteria. Furthermore, AKEO lead to a reduction of the lipopolysaccharide (LPS)-induced secretion of tumor necrosis factor α (TNF-α), interleukine-1β (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 of acne vulgaris. [48]

By the fact that the fruits of *Heracleum persicum* (Apiaceae) are used as pain killer in Iranian folkloric medicine, Hajhashemi et al. investigated the anti-inflammatory and analgesic properties of *Heracleum persicum* essential oil, (HPEO), and hydroalcoholic 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 analyze 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 lead to a reduction of the pain response of the second phase of formalin test. For evaluation of the anti-inflammatory effect, carrageenan-induced rat paw edema was used. At doses of 100 and 200 mg/kg of HPEO and at doses of 400mg/kg of HPHE a significant reduction of paw edema could be observed. The study clearly shows that HPEO and HPHE have significant analgesic and anti-inflammatory effects. [49]

Moraes et al. investigated the effects of the essential oil of *Citrus aurantium* (CAEO), Rutaceae, and its main compound, the monoterpene limonene, on gastric mucosa. CAEO is known for its widely use as a flavouring agent, which is found in many common food items, as well as for its medicinally use throughout the world to treat gastritis and gastric disorders. 250mg/kg p.o. of CAEO and 245mg/kg p.o. of limonene provided very effective (99%) gastroprotection against injuries induced through absolute ethanol or NSAID (non-steroidal anti-inflammatory drug) in rats. It is important to notice that neither CAEO nor limonene interfere with gastric H(+) secretion, serum gastrin or glutathione level in gastric mucosa. The gastroprotective 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. [50]

A Fabaceae-oil, namely from *Pterodon emarginatus* seeds (PEEO) was investigated by Dutra et al. in order to assess its antiulcerogenic and anti-inflammatory activities using different methods such as inducing ulcers with ethanol, indometacin and HCL/ethanol or inducing pleurisy by carrageenan in Swiss albino rats. After an oral administration of 100, 300 and 500 mg/kg of PEEO a significant protection against such ulcers was obtained. Moreover, PEEO lead to a promising reduction in the exudate volume and inhibited leucocyte and neutrophil influx in carrageenan-induced pleurisy. Furthermore, PEEO lead 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. [51]

The anti-inflammatory effect of the essential oil of the *Cleistocalyx operculatus* 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 inhibite lipopolysaccharide (LPS) –induced secretion of pro-inflammatory cytokines, including TNF-α and IL-1β, in RAW 264.7 cells (a mouse macrophage-like cell line). Moreover, it was possible to suppress the mRNA expression of TNF-α and IL-6β by treatment with COEO in LPS-stimulated RAW 264.7 cells. COEO is able to block LPS-induced transcriptional activation of of NF-κB in RAW 264.7 as well as it suppresses the nuclear translocation of p65 subunit. Furthermore, COEO lead 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. [52]

## 5. 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. [53]

2007 Long et al. investigated the skin toxicology and penetration enhancement of skin absorption of volatile oil extracted from tender branchers of *Camellia oleifera*, 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 (a NSAID with analgesic and antipyretic properties) for percutaneous absorption. The effects of different concentrations of their volatile oil in nitrendipine-, or baicalin-, or nimesulide-treated mice were assessed *in vitro*. The results showed that the COEO lead to powerful enhancement effects. [54]

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, Radix Saposhnikoviae, Rhizoma *Atractylodes lancea,* Radix Aucklandiae, Radix *Curcuma wenyujin*, Rhizoma and Radix Notopterygii, Lignum *Aquilariae Resinatum* and Herba Schizonepetae. To analyze 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 lead to an enhancing of the penetration of ibuprofen in different degrees. 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. [55]

| Tested essential oil | 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 |

Table 1 : The potential of *Fructus Evodia, Radix Saposhnikoviae, Rhizoma Atractylodes lancea Radix Aucklandiae, Radix Curcuma wenyujin, Rhizoma and Radix Notopterygii, Lignum Aquilariae Resinatum* and *Herba Schizonepetae* to increase the enhancive permeation. [A]

The focus of a study of Bai et al. were the effects of the volatile oils of *Rhizoma zingiberis* (Zingiberaceae), Rhizoma *Acori Tatarinowii* (RAT), Semen *Myristicae* (SM) and Pericarpium *Citri Reticulatae* (PCR) on the percutaneous penetration of bullatine A, 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 *Acori Tatarinowii*, Semen Myristicae and

Pericarpium *Citri Reticulatae* have the potential to enhance the permeation of bullatine A.[56]

Another Chinese research group analyzed the effect of *Atractylodes* rhizome oil and other volatile oils on percutaneous absorption of baicalin, a flavonoid that affects the GABA receptors. The focus of this study are the *atractylodes* rhizome oil, patchouli oil and angelica volatile oil. 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 concentrationes of the three volatile oils as enhancers, that the penetration of baicalin through the skin is getting better. 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. [57]

The potential of basil oil, a volatile oil containing terpene alcohols, to enhance the skin penetration of labetalol hydrochloride (LHCl) was investigated by Jain et al. LHCl is an α- and β-blocker that is used in the treatment of hypertension. The reference substances were camphor, geraniol, thymol and clove oil. Saturation solubilities of labetalol hydrochloride were identified in water, vehicle (ethanol:water, 60:40 v/v) and vehicle containing 5% w/v terpene alcohols. Similiar saturation solubilities were identified suggesting an insignificant increase in LHCl 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 LHCl in the vehicle and in presence of 5% w/v enhancer was analyzed. A number of parameters like steady state flux, permeability coefficient, lag time, partition coefficient, diffusion coefficient, and enhancement ratios (ER) were calculated from the permeation data. The maximum enhancement was achieved by basil oil (ER= 46.5). The fact that the activation energies for LHCl are the lowest in presence of basil oil, suggests creation of new polar pathways in the skin for enhanced permeation of LHCl. The results of this study show that basil oil is a promising penetration enhancer for improved transdermal drug delievery of labetalol. [58]

| | Activation energy for LHCl |
|---|---|
| In water | 23.16 kcal/mole |
| In vehicle per se | 18.71 kcal/mole |
| In presence of 5% w/v basil oil | 10.98 kcal/mole |

Table 2: The activation energy for LHCl in water, in vehicle per se and in presence of 5% w/v basil oil. [B]

## 6. INSECT REPELLENT ACTIVITY

Some facts show that the use of synthetic chemicals to control insects and arthropods raises several obvious concerns related to 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. [59] In the following, the insect repellent activity of some essential oils will be discussed, as well as their uses against pests insects, lice, fleas, beetles, mites etc. and also insects which destroy store products and crops.

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* (Apiaceae), *Ipomoea cairica* (Convolvulaceae), *Momordica charantia* (Cucurbitaceae), *Psidium guajava* (Myrtaceae) and *Tridax procumbens* (Asteraceae). The oils were tested at three concentrations: 2, 4 and 6%. In general, a dose-dependent effect was noticed. The highest concentration (6%) lead to the highest repellency effect. The results showed a high repellency effect at a concentration of 6% of *Ipomoea cairica, Momordica charantia* and *Tridax procumbens*, which lasted for more than 300 minutes. *Centella asiatica* and *Psidium guajava* exhibited a lower repellency effect at the same concentration, which lasted only less than 150 minutes. Ethanol, which was used as a control, showed only 8 minutes

repellency. Based on these data, *Ipomoea cairica, Momordica charantia* and *Tridax procumbens* are promising repellents. [60]

The repellent activity of seven other essential oils against the three cockroach species Periplaneta americana, Blattella germanica and Neostylopyga rhombifolia were analyzed by Thavara et al. under laboratory conditions. The seven tested essential oils were *Boesenbergia rotunda* (Zingiberaceae), *Citrus hystrix* (Rutaceae), *Curcuma longa* (Zingiberaceae), *Litsea cubeba* (Lauraceae.), *Piper nigrum* (Piperaceae), *Psidium guajava* (Myrtaceae) and *Zingiber officinale* (Zingiberaceae). Naphthalene was used as a control. The results showed that the best repellency was exhibited by *Citrus hystrix,* which lead to a complete repellency (100%) against Periplaneta americana and Blattella germanica, under laboratory conditions. Moreover, *Citrus hystrix* exhibited as well the highest repellency (among the tested oils) of about 88% against Neostylopyga rhombifolia. Furthermore, *Citrus 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 *Citrus hystrix* essential oil has a remarkable potential to be used as a potent repellent. [61]

2008 investigated Noosidum et al. the effects of the essential oils of *Melaleuca leucadendron* (Myrtaceae), *Litsea cubeba* (Lauraceae) and *Litsea salicifolia* (Lauraceae) against Aedes aegypti females by using an excito-repellency test chamber. Focus of this study was to evaluate the mortality of Aedes aegypti females following 24 h holding period post-contact and non-contact trials. Mosquitos which escaped after direct contact with essential oils of *Melaleuca leucadendron* and *Litsea salicifolia* did not die, but those who had a direct contact to *Litsea cubeba* showed only low mortality (2.3-20.4%). Furthermore, in all non-contact trials, there was no mortality observed in escaped females no matter which essential oil was used. But there was a low mortality in non-escaped mosquitos who were exposed to *Litsea cubeba* (0-14.3%) and *Litsea salicifolia* (0-17.1%). Independent from the test concentration, Aedes aegypti exhibited a higher escape rate from contact chambers when it was treated with *Melaleuca leucadendron* and *Litsea cubeba* compared to *Litsea salicifolia.* The highest non-contact repellent response exhibited *Litsea salicifolia.* Based on these data, the three essential oils

possess promising irritant and repellent properties against Aedes aegypti, but further studies will be necessary. [62]

Moharramipour et al. reported on the repellent and fumigant toxicity of the essential oil from *Thymus persicus* (Lamiaceae) against two stored-product beetles Tribolium castaneum and Callosobruchus maculatus. The evaluation was executed under following terms and conditions: The repellent and fumigant toxicity were evaluated against 1-7 days old adult beetles at 27 +/- 1 degrees C and 65 +/- 5% RH (rate of humidity) in dark condition. Tribolium castaneum and Callosobruchus maculatus showed at highest concentration (2µl/ml acetone) a repellency of 70.4% and 82.4%. Moreover, the fumigation bioassays exhibited that Callosobruchus maculatus adults were significantly more fragile to the essential oil than Tribolium castaneum adults. This is proved by the LC(50) values. Callosobruchus maculatus adults possess a LC(50) value of 2.39 µl/l air and Tribolium castaneum possess a LC(50) value of 234.42 µl/l air. The strong repellency, fumigant toxicity and the safety suggest that *Thymus persicum* is a promising candidate to be used in the management of stored-product pests. [63]

An investigation dealing with the repellent effects of catmint, *Nepeta cataria* (Lamiaceae), oil formulations against black flies (Simulium decorum Walker) and mosquitos (primarily Aedes intrudens Dyar) in the field in Maine and Florida was carried out 2008 by Spero et al. The essential oil was hydrogenated to enrich the dihydronepetalactone diastereomers. The results of the evaluation in Maine showed that the protection from black flies lasted for 6 h or more with all formulations. Liquid formulations at 15 wt% active ingredient conferred a complete protection for 7.5 h. Moreover, the results showed that all formulations lead to a protection against mosquitos 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 lead to a protection for more than 4 h from a mixed population of mosquitos. The 15 wt% lotion conferred a complete protection from bites for more than 6 h. [64]

In fact the use of whole plants and their products as insect repellents is very common among north-eastern Tanzania, as Kweka et al. reported in an ethnobotanical study. The study took place at Moshi in Kilimanjaro region. To investigate which species are used

by the locals 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* (Lamiaceae), *Ocimum kilimandscharicum* (Lamiaceae), *Azadirachta indica* (Meliaceae) *Eucalyptus globulus* (Myrtaceae) and *Lantana camara* (Verbenaceae). These plants are used fresh or by burning the leaves. *Ocimum suave* and *Ocimum kilimandscharicum* are used by 67% out of 120 interviewed households. Furthermore, the bioassay, comparing *Ocimum suave* and *Ocimum kilimandscharicum* with citronella and DEET (N,N-Diethyl-meta-toluamide) was made to analyze 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 mosquitoes species. Moreover, ocimum extracts lead to a longer induction of KD90 (the minutes needed to knock-down *90*% of mosquitoes) in mosquitoes than citronella. 30 mg/m² of *Ocimum suave* and *Ocimum kilimandscharicum* on filter papers lead after 24 h to a mortality of 57% and 47%, while the mortality was 68% for citronella. Therefore, also these plants are really very promising repellents. [65]

Müller et al. investigated the repellent ability of essential oil candles against biting insects. The tested oils were geraniol, linalool and citronella. The vapors of the oils were analyzed 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 obtaines by linalool, which lead to a reduction of female mosquitoes by 65% and sand flies by 49%. Nevertheless, the best results showed geraniol candles with 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 lead to a reduction of the mosquito pressure by an average of 62%. At this site, no sand flies were present. [66] Another study by this author group reported this time on the indoor protection of

citronella, linalool and geraniol candels against mosquitoes and sand fly bites. The evaluation was conducted in a high biting pressure environment in Israel. 5% 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 candels exhibited a repellency rate of 25% against sand flies, 5% linalool exhibited 55% and 5% geraniol candels exhibited 80%. The results showed that geraniol candles are about 5 times as effective as citronella candles and about twice as effective as linalool candles. [67]

Pushpanathan et al. investigated the essential oil of *Zingiber officinalis* Linn (Zingiberaceae) as a mosquito larvicidal and repellent agent against the filarial vector Culex quinquefasciatus. The larval mortality was found after 24 h treated for late third instar. The LC(50) value amounts 50.78 ppm. The skin repellent test at different concentration (1.0, 2.0, 3.0 and 4.0 mg/cm²) of *Zingiber officinalis* exhibited 100% protection up to 120 min. Based on these data, the essential oil of *Zingiber officinalis* possesses not only an agreable odor but is also a promising candidate as a repellent agent against filarial vector Culex quinquefasciatus. [68]

Abdel-Sattar et al. studied the chemical composition of fruit and leaf essential oils of *Schinus molle* (Anacardiaceae) and its insecticidal and insect repellent activity against Trogoderma granarium and Tribolium castaneum. By GC-MS the main components were analyzed. More than 60 components were identified. The main constituent in both oils is p-cymene. The results of this study showed, that in fact of the high yield and efficacy *Schinus molle* is a promising lead for active isecticidal agents. [69] Another investigation by Benzi et al. layed emphasis on the repellent and toxic activities of the essential oils extracted from leaves of *Aloysia polystachya* and *Aloysia citriodora* (Verbenaceae) and from leaves and fruits of *Schinus molle var. areira* (Anacardiaceae) against adults of Rhizopertha dominica. To evaluate the contact toxicity topical application and filter paper assay were used. The filter paper impregnation was also used for fumigant and repellent assays. *Aloysia polystachya* was as effective as *Schinus molle* leaves in topical tests. Based on the class scale, *Aloysia citriodora* was the most effective oil in the case of repellent assays. *Aloysia polystachya* was the most toxic plant on contact toxicity by the filter paper assay (LC50 26.6 mg/cm2). Moreover, the

fumigant toxicity was only investigated with the fruits and leaves of *Schinus molle*, but there were no big differences between them. [70]

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* (Myrtaceae), *Prostanthera melissifolia* (Lamiaceae), and *Melaleuca alternifolia* (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 possesses *Prostanthera melissifolia* against Cx. quinquefasciatus. The mean protection times against Aedes aegypti were lower than those for the Culex spp. But nevertheless, the longest protection time of all the compared substances was afforded by DEET against Aedes aegypti. Based on this 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 high risk of mosquito-borne disease. [71]

Lee et al. investigated the acaricidal activities of major constituents from the oil of *Juniperus chinensis*, Cupressaceae, (JCEO) leaves against house dust and stored food mites, compared with those of DEET. For the analysis, impregnated fabric disk bioassay against Dermatophagoides spp. and Tyrophagus putrescentiae was used. Toxicity differs with the chemical composition as well as the doses. The LD(50) of JCEO against Dermatophagoides farinae, Dermatophagoides pteronyssinus, and Tyrophagus putrescentiae were 21.60, 19.89, and 38.10 μg/cm.². By using different analysis like gas-chromatography-mass spectrometry, bornyl acetate was identified as the acaricidal component. The LD(50) of bornyl acetate (2.94 μg/cm.²) against Dermatophagoides farinae was significantly lower than those of DEET (37.13 μg/cm.²). Similiar 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.[72]

Louses and flies belong to those animals which either are 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* (Lauraceae), *Allium cepa* (Amaryllidaceae), *Mentha piperita* (Lamiaceae), *Matricaria chamomilla* (Asteraceae) and *Rosmarinus officinalis* (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 LC(50) values (Lethal Concentration 50 is the concentration of a chemical which kills 50% of a sample population) and the lethal time (LT50) values after treatment with 7.5% *Cinnamomum camphora, Allium cepa, Mentha piperita, Matricaria chamomilla, Rosmarinus officinalis* and d-phenothrin were as listed in table 3.

| | LC(50) values (%) | LT(50) values (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-phenthrin | 1.17 | 1.94 |

Table 3: LC(50) values and LT(50) values of *Cinnamomum camphora*, *Allium cepa*, *Mentha piperita*, *Matricaria chamomilla*, *Rosmarinus officinalis* and D-phenothrin [C]

All the oils, except *Rosmarinus 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 *Rosmarinus officinalis*, and d-phenothrin was obtained. Furthermore, all tested substances are able to repell 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. [73]

Sfara et al. carried out a study with the aim to evaluate the fumigant and repellent activity of five essential oils (from eucalyptus, geranium, lavender, mint, and orange oil) 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, the exposing of the nymphs to the vapors emitted by 100µl of essential oil or monoterpene was tested in a close recipient. The knockdown time 50% (KT50) for eucalyptus essential oil was 216 min. This is seven times less toxic than dichlorvos, a volatile organophosphorus insecticide, that was used as a positive control. The other essential oils showed only a 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/cm². The results showed that mint and lavender essential oil and menhone only produced a light repellent effect at 400 µg/cm². Geraniol and menthyl acetate showed a repellent effect at both tested concentrations. Nevertheless, the repellent effect of all tested substances was lesser than that produced by DEET. [74]

The effects of thymol from the essential oil of *Tachyspermum ammi* (Apiaceae) against Anopheles stephensi was investigated by Pandey et al.. The larvicidal, oviposition-deterrent, vapor toxicity, and repellent activity against the malarial vector were evaluated. Thymol showed a LD(50) value of 48.88 toward fourth-instar larvae of A. stephensi. So it was 1.6-fold more toxic than the oil, that showed a LD(50) value of 80.77 µg/ml. After the treatment with vapors 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 vapor toxicity assay exhibited a LC(50) value of 185.4 mg/mat for the crude oil against

adults of A. stephensi, whereas thymol showed a LC(50) value of 79.5 mg/mat. After one hour, the treatment of adults of these flies with 25.0 mg/mat of thymol demonstrated a complete repellency. The same degree of repellency was obtained by the oil of *Tachyspermum ammi* at the dose of 55.0 mg/mat. That indicates that thymol possesses the double-fold activity.[75]

The focus of another study was to proof if 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, aenemia 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 repell these mites at 0.14 mg oil/cm³ during the first two days of study. Most effective was thyme oil, which was repellent until the end of study period (13 days). Additionally, the toxicity of these oils were as well tested against mealworm beetles (Tenebrio molitor L.). The results showed that the toxicity to Tenebrio molitir 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. [76]

Eamsobhana et al. investigated the repellent effects of thirteen 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 thirtheen tested oils exhibited promising repellent effects. At 5% concentration, *Syzygium aromaticum* oil (Myrtaceae) lead to 100% repellency. *Melaleuca alternifolia* oil (Myrtaceae) exhibited 100% repellency at 40% concentration. *Zingiber cassamunar* (Zingiberaceae) and *Eucalyptus globulus* (Myrtaceae) lead undiluted to 100% repellency. Furthermore, only 100% of *Pelargonium graveolens* (Geraniaceae) lead to more than 50% repellency. *Styrax tonkinensis* (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.

Especially, *Syzygium aromaticum* oil may be safer and more economical as a sand flea repellent than commercially available chemcials. [77]

## 7. ANTIVIRAL ACTIVITY

A virus is a small infectious particle (20-300nm), which is able to infect cells of another living organism, in which it can replicate itself. But viruses can´t reproduce on their own. A virus is composed of genes, 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 5.000 viruses in detail. [78]

2007 Saddi et al. investigated the activities of the essential oil from *Artemisia arborescens* (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 IC(50) values were 2.4 and 4.1 µg/ml for HSV-1 and HSV-2. These values were tested with plaque reduction assay. This method allows it to count the number of plaques formed by a virus sample, from which the actual virus concentration can be determined. Moreover, the MTT reduction assay was used. This is a quantitative colorimetric method measuring the activity of enzymes that reduce MTT to formazan, giving a purple color. The determination by the MTT reduction method, 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. Determinded by yield reduction assay, it was possible to observe a 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 *Artemisia 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.[79]

HSV-1 and HSV-2 virus were also the goal of assessment in the next two studies dealing with the essential oils of *Eugenia caryophyllus* (Myrtaceae) and of *Cedrus libani* (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 *Eugenia 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, *Eugenia caryophyllus* and eugenol lead to an inactivation of particles of HSV standard strains. After the treatment with *Eugenia caryophyllus* and eugenol, the total virus yield of HSV standard strains and isolates declined after 30 h. Moreover, extracts of *Eugenia 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 as well inhibited more than most of the HSV-2 isolates. [80] In the second paper an *in vitro* evaluation of the biological activity against Herpes simplex virus type1 (HSV-1) of *Cedrus libani* (Pinaceae) 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, analyzed 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 MTT assay in Vero cells. The results of this study showed that the ethanol extracts of cones and leaves possess an interesting activity with IC(50) value of 0.50 and 0.66 mg/ml at non-cytotoxic concentration. The essential oil showed a similiar activity with an IC(50) value of 0.44 mg/ml. [81]

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* (Lauraceae), *Juniperus oxycedrus ssp. oxycedrus* (Cupressaceae), *Thuja orientalis* (Cupressaceae), *Cupressus sempervirens ssp. pyramidalis* (Cupressaceae), *Pistacia palaestina* (Anacardiaceae), *Salvia officinalis* (Lamiaceae) and *Satureja thymbra* (Lamiaceae). The focus of this study was to evaluate the oil´s inhibitory activity against SARS-CoV and HSV-1 replication *in vitro* by visually scoring of the virus-induced cytopathogenic effect post-infection. The most promising oil with the highest activity against SARS-CoV was *Laura nobilis* oil with an IC(50) 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 *Juniperus oxycedrus* oil with an IC(50) value of 200 µg/ml and a SI of 5.0. The major constituents of this oil are α-pinene and β-myrcene. [82]

The essential oil of another Myrtaceaen species, namely *Eucalyptus globulus* was investigated by Cermelli et al. and its effect on respiratory bacteria and viruses assessed. The activity of Eucalyptus globulus essential oil was determined for 120 isolates of Streptococcus pyogenes, 20 isolates of S. pneumoniae, 40 isolates of S. agalactiae, 20 isolates of Staphylococcus 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 agar diffusion test, is used for measuring the effect of an antimicrobial agent against bacteria grown in culture. By the MTT test the cytotoxicity was evaluated on VERO cells. The most influenceable were H. influenzae, parainfluenzae, S. maltophilia and S. pneumoniae. Moreover, only a mild activity on mumps virus was found. [83]

The next six studies on antiviral activities of essential oils were published by the research team of Reichling. A first Koch et al. investigated the inhibitory effect of essential oils against HSV-2. Essential oils from anise (*Pimpinella anisum*, Apiaceae), hyssop (*Hyssopus officinalis*, Lamiaceae), thyme (*Thymus vulgaris*, Lamiaceae), ginger (*Zingiber officinalis*, Zingiberaceae), chamomile (*Matricaria recutita*, Asteraceae) and sandalwood (*Santalalum album,* 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 the inhibitory concentrations (IC50) 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 lead 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 preincubated 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 camomile, which showed a high selectivity index.[84] Furthermore, this group investigated the efficacy of anise oil (*Pimpinella anisum*, Apiaceae), dwarf-pine oil (*Pinus pumila*, Pinaceae) and camomile oil (*Matricaria recutita*, 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 a frameshift 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, exhibited that all essential oils possess high capacity of antiviral activity against aciclovir-sensitive HSV strain KOS, aciclovir-resistant clinical HSV isolates and aciclovir-resistant strain Angelotti. Moreover, the oils lead to a significant reduction of the plaque formation by 96.6-99.9% at maximum concentrations. These results were obtained when Herpes viruses were preincubated with drugs before attachment to host cells. But 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 camomile oil affected the virus by interrupting adsorption of Herpes viruses, while aciclovir is effective after attachment inside the infected cells. [85] Then, some essential oils from the family Myrtaceae, like cajeput (*Melaleuca leucadendra*), clove (*Syzygium aromaticum*), kanuka (*Kunzea ericoides*) and manuka *(Leptospermum scoparium)* were analyzed by Schnitzler et al. One of the focus of this study was to evaluate the oils cytotoxicity in a standard neutral red assay (NRU). This is is a cell survival assay based on the ability of viable cells to incorporate and bind neutral red (NR). Maximum noncytotoxic concentrations for *Melaleuca leucadendra* oil and *Syzygium aromaticum* oil were determined at 0.006%, *Kunzea ericoides* oil and *Leptospermum scoparium* oil were more cytotoxic with a maximum noncytotoxic 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. [86] Also the antiviral activity of lemon balm oil, the essential oil of *Melissa officinalis*, Lamiaceae, against enveloped Herpes viruses was investigated. The major constituents of *Melissa officinalis*, analyzed by GC-MS analysis, 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 IC(50) 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 noncytotoxic concentrations, lead 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 analyze 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. [87] In another paper the focus was the essential oil of star anise (*Illicium verum*, 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 lead 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 lead 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 similiar high selectivity index of 140. In conclusion, star anise essential oil, phenylpropanoids and sesquiterpenes are promising candidates as aniviral agents against HSV-1.[88] 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 (*Melaleuca 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 lead 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 tested drugs lead to an interaction in a dose-dependent manner with HSV particles. They inactivated viral infection. Furthermore, the study showed that among the analyzed 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 ten-fold higher selectivity index and a lower toxicity than its isolated single monoterpenes. [89]

Garazzo et al. investigated the *in vitro* antiviral activity of *Melaleuca alternifolia* essential oil (teatree 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, like terpinen-4-ol, terpinolene, α-terpineol, possess an inhibitory effect on influenza A/PR/8 virus subtype H1N1 replication at non-cytotoxic concentartions. Furthermore, TTO showed an ID(50) value of 0.0006% (v/v), which was much lower than its CD(50) 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. [90]

The inhibitory effect of essential oils of *Lippia alba* (Verbenaceae), *Lippia origanoides* (Verbenaceae), *Oreganum vulgare* (Lamiaceae) and *Artemisia vulgaris* (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 CC(50) values less than 100 μg/ml and the MIC values (Minimal Inhibitory Concentration) of 3.7 and 11.1 μg/ml. The CC(50)/MIC ratio was of 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 lead 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, there was no reduction of virus yield observed. In conclusion, the tested oils showed antiviral activity against YFV through direct virus inactivation. [91]

# 8. 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.

2007 Sharififar et al. investigated the antioxidant and free radical scavenging activities of the essential oils from flowers and fruits of *Otostegia persica* (Lamiaceae). By GC/MS analysis about 30 compoments were identified in each oil. The major constituents of the essential oil flowers (EOFL) were α-pinene (17.2%), 1-octen-3-ol (13.4%) and cubenol (7.3%). The major constituents of the essential oil of the fruits (EOFR) were diisooctyl phthalate (45%) and 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: the 2,2-diphenyl-1-1-picrylhydrazyl (DPPH) free radical-scavenging test and the ammonium thiocyanate. The results showed that EOFL possess greater antioxidant and radical scavenging activity in both tests. In the DPPH free radical-scavenging, EOFL showed antioxidant activity with an IC(50) value of 19.8 +/- 1.8 µg/ml. In the second test system, EOFL exhibited an inhibition rate of oxidation of linoleic acid of 93.5 +/- 2.8. The high amount of oxygenated monoterpenes in EOFL is maybe the reason for its higher antioxidant activity.[92]

Chaieb et al. investigated the antioxidant properties of the essential oil of clove (*Eugenia caryophyllata*, Myrtaceae). The major components, analyzed 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 antiradical dose required to cause a 50% inhibition (IC50) was recorded. The results exhibited that the oil showed a very strong radical scavenging activity with an IC(50) value of 0.2 µg/ml. A comparison was made with the synthetic antioxidant tert-butylated hydroxytoluene which exhibited an IC(50) value of 11.5 µg/ml. Furthermore, the oil showed promising antifungal effects.[93]

Da Silva et al. investigated the antioxidant activity of essential oil and methanol extract of *Aniba canelilla* (Lauraceae). 1-Nitro-2-phenylethane was identified as the main volatile component with 70.2-92.1%. The oils exhibited a DPPH scavenging activity (EC(50)) of 198.17 +/- 1.95 µg/ml. This is low in comparison with the EC(50) value of

wood methanol extracts (4.41 +/- 0.12 µg/ml), which was equivalent to that of Trolox, used as an antioxidant standard. The high antioxidant activity of this species is maybe based upon the high amount of total phenolics (710.53 +/- 23.16 mg of GAE/g). [94] Furthermore, the chemical composition and the antioxidant capacity of the essential oil of *Lippia schomburgkiana* (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* lead to an inhibition of the DPPH radical, showing an EC(50) value of 16.1 +/- 0.7 µg/ml. This EC(50) value is only three times lower than that of trolox with 4.7 +/- 0.4 µg/ml. This leads to the conclusion that *Lippia schomburgkiana* possesses a high antioxidant activity. Moreover, the brine shrimp bioassay was used to measure the LD(50) 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 +/- 0.4 µg/ml). [95]

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*, Rutaceae), pink grapefruit (*Citrus paradise*, Rutaceae), coriander (*Coriandrum sativum,* Apiaceae), and clove (*Caryophyllus aromaticum*, 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. [96] Another Rutaceae was investigated by Kambouche et al. in order to evaluate the chemical composition and the antioxidant potential of *Ruta montana* essential oil (Rutaceae), which is growing in the Oran region in the west of Algeria. The main constituents were analyzed by GC/MS. About twenty 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 *Ruta montana* essential oil possesses antiradical 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. [97]

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 DPPH radical with an IC(50) 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 IC(50) value of 0.75 µg/ml. [98]

Lopes-Lutz et al. focused their research on the Asteracen plants and studied the chemical composition, antimicrobial 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 testet 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 β-carotene/linoleate model. The determination of the DPPH radical scavenging activities exhibited only weak activities for these oils. [99]

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* (Lamiaceae) leaves, which are rich in essential oil and antioxidative phenolic substances. In an experimental field in Austria twelve accessions were grown. The focus of this study was to analyze 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 decoloration, 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 works by measuring the amount needed of the substance being tested to inhibit the oxidation of the reagent. The Folin-Ciocalteu reagent is a mixture of phosphomolybdate and phosphotungstate. The 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 FRAP assay. Similiar 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. [100]

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* (Lamiaceae) essential oil, growing in different habitats. The major components, analyzed 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 postdistilled dry samples. The prevalent compounds of *Salvia officinalis* methanolic extracts were Rosmarinic acid, carnosol, and carnosic acid. The results of this study showed that *Salvia 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. [101]

By Laouer et al. its essential oil from the aerial part was analyzed as to its compostion and antioxidant activity. Thirty-seven compounds were identified by GC-MS. The major constituent was germacrene D with 45.7%. The antioxidant activity was determined using three *in vitro* assays: scavenging effect on DPPH, the ABTS test and the phosphomolybdenum method. The ABTS test (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) is used to observe the reaction kinetics of specific enzymes. ABTS turnes into a green end-product which can easily be identified at 420nm with a spectrophotometer. The phosphomolybdenum method is based upon the oxidation to a phosphomolybdenum blue complex by the addition of nitrite. The results of this study showed that the oil presented an antioxidant activity. Furthermore, another focus of the study was the antimicrobial activity, but the essential oil showed no effects on the tested microorganisms. [102] Schmidt et al. investigated the chemical composition and the antioxidant effects of the essential oil from *Mentha x piperita* (Lamiaceae). The main constituents, analyzed 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 *Mentha x piperita* possessed antiradical activity with respect to DPPH and hydroxyl radicals. Moreover, the oil exhibited a stronger antioxidant impact

on the hydroxyl radical. The IC(50) value were 860 µg/ml for DPPH and 0.26 µg/ml for hydroxyl radicals. Furthermore, the essential oil of *Mentha x piperita* exhibited antioxidant activity in a linoleic acid emulsion system in terms of inhibiting conjugated dienes formation by 52.4% and linoleic acid secondary oxidized products generation by 76.9% (at 0.1% concentration). [103] Also the chemical composition and the antioxidant activity of the essential oil from cornmint (*Mentha canadensis*, Lamiaceae) was investigated by the same author group. The major constituents, again analyzed by GC/FID and GC/MS, were menthol (41.2%) and menthone (20.4%). The antiradical activity of cornmint oil with respect to the DPPH and hydroxyl radicals was established. The results of this study showed an IC(50) 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 exhibited as well antioxidant activity in a linoleic acid emulsion system. At concentration of 0.1%, the oil lead to an inhibition of the formation of conjugated dienes by 57.1% and the generation of secondary oxidized products of linoleic acid by 76.1%. [104] Yang et al. investigated the antioxidant activities of six popular and commercially available essential oils. The testet oils were from lavender (*Lavandula angustifolia*, Lamiaceae), peppermint (*Mentha piperita*, Lamiaceae), rosemary (*Rosmarius officinalis,* Lamiaceae), lemon (*Citrus limon*, Rutaceae), grapefruit (*Citrus paradise,* Rutaceae), and frankincense (*Boswellia carteri*, Burseraceae). The major components of the testet essential oils, analyzed 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 RC(50) 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. [105] Finally, the antioxidant activty of the essential oils from five spice plants, which are widely used in a Mediterranean diet, were studied by Viuda-Martos. The tested oils were oregano (*Origanum vulgare*, Lamiaceae), thyme (*Thymus vulgaris*, Lamiaceae) rosemary (*Rosmarinus officinalis,* Lamiaceae), sage (*Salvia officinalis,* Lamiaceae) and clove (*Syzygium aromaticum,* 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 (the ferric reducing antioxidant power) value (1.47 TEAC – trolox equivalent antioxidant capacity). Moreover, the highest percentage inhibition of TBARS (thibarbituric acid reaczive 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. [106]

However, not only essential oils from Lamiacean plants possess an distinct antioxidant activity. Also other plants were studied in order to detect "new" natural antioxidants. E.g., Martins et al. investigated the chemical composition and the antioxidant activity of the volatile oil from the fruit peel of *Garcinia brasiliensis* (Clusiaceae). A total of 38 components were identified. The major constituents, analyzed 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 only a poor antioxidant activity. [107] Then, the essential oil composition and antioxidant activity of *Pterocarya fraxinifolia* (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 *Pterocarya fraxinifolia* bark and leaves was investigated by six *in vitro* assay systems. The DPPH free radical-scavenging assay showed an IC(50) value of 3.89 +/- 0.09 for leaves and 41.57 +/- 1.30 µg/ml for bark. Furthermore, the leaf extract showed a promising reducing power at 2.5 and 80 µg/ml which was comparable with Vitamine C ($p > 0.05$). The extracts showed as well $Fe^{2+}$ chelating ability as weak nitric oxide-scavenging activity. The extracts lead 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. [108]

Gholivand et al. investigated the chemical composition and the *in vitro* antioxidant activity of the essential oil and methanol extracts of *Psammogeton canescens* (Umbelliferae). The chemical composition, analyzed 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 reducing power assay. The highest radical-scavenging activity in the DPPH system was executed by the polar subfraction of methanol extract (49.5 +/- 1.21µg/ml). Moreover, in the second case the inhibition capacity of the polar subfraction of 92.40% +/- 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 *Psammogeton canescens* possess significant antioxidant activities. [109] Another Myrtaceae as above was the focus of an investigation by Singh et al.. He studied the chemical composition and the antioxidant activity of the essential oil from fresh and decaying leaves of *Eucalyptus tereticornis* (Myrtaceae). The main components of the fresh leaf oil, analyzed 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 superoxide anion. The results of this study showed that both essential oils possess a strong radical scavenging activity against the DPPH radical with IC(50) values of 110 and 139.8 µg/ml for fresh and decaying leaf oil. Moreover, the essential oils at concentrations of 400 µg/ml showed as well 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 *Eucalyptus tereticornis* are a rich source of monoterpenoids exhibiting antioxidant activity. [110] A very prominent and often used plant was the focus of interest of Sarikurkcu et al.. He 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* (Verbenaceae) fruits from Turkey. The chemical composition was analyzed 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 +/- 1.22 µg GAEs/mg extract. Furthermore, 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. [111]

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## 10. FIGURES:

[a] Figure 1: Inhibitory effect of piperitenone oxide (PO) on the nociceptive reaction to intraperitoneal acetic acid injection in mice (newly drawn according to Sousa et al., 2009).

[b] Figure 2: Inhibitory effect 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., 2009)

[c] Figure 3: The inhibition of cell growth achieved by alpha-humulene and isocaryophyllene. (newly drawn according to Legault et al., 2007)

[d] Figure 4: The composition of the essential oil of Tanacetum gracile. (newly drawn according to Verma et al., 2008)

## 11. TABLES

[A] Table 1 : The potential of Fructus Evodia, Radix Saposhnikoviae, Rhizoma Atractylodes lancea Radix Aucklandiae, Radix Curcuma wenyujin, Rhizoma and Radix Notopterygii, Lignum Aquilariae Resinatum and Herba Schizonepetae to multiple the enhancive permeation.

[B] Table 2: The activation energy for LHCl in water, in vehicle per se and in presence of 5% w/v basil oil.

[C] Table 3: LC(50) values and LT(50) values of *Cinnamomum camphora*, *Allium cepa*, *Mentha piperita*, *Matricaria chamomilla*, *Rosmarinus officinalis* and D-phenothrin

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