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Ovim putem želela bih da se zahvalim mojim dragim roditeljima i bratu cimeru. Neizmerno hvala na bezgraničnoj podršci i uverenju da smo tim, da nema nerešivih problema, samo usputnih prepreka koje kada se savladaju samo nas ojačaju.
Takođe želim da se zahvalim dragoj Kaći, takođe članu porodice. Veliko hvala za svaki minut pažnje, za savete i druženja.
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Još jednom hvala svima od srca!

Abstract

Infections in the respiratory tract are concerning the public health sector worldwide. According to studies carried out in different countries of Europe colds incite many patients to consult their doctors and pharmacists seeking for Over-The-Counter (OTC) remedies. In fact, since long plants and their extracts have been the prime targets of research. Many scientists and also some pharmaceutical industries carry out a lot of clinical trials in order to provide more information for their usage in disease prevention and pathogen control, along with reduction of undesirable side effects. In this review, the biological activities, such as anti-inflammatory, antimicrobial and antitussive activities, as well as, the mechanism of action of different essential oils, on respiratory diseases, are presented. The aim of this review is to show that essential oils represent an important potential source of novel drugs, since their active constituents possess many pharmacological properties.

Abstract

Atemwegsinfektionen bewegen das Gesundheitswesen weltweit. Laut Studien, die in verschiedenen Ländern Europas geführt wurden, sind Over-The-Counter (OTC) Produkte sehr beliebt bei Schnupfen und anderen Erkrankungen des Respirationstraktes. Pflanzen und ihre Extrakte stellen schon seit vielen Jahren für viele Forscher, sowie auch für die Pharma-Industrie, ein neues Interessen-Gebiet dar. Zahlreiche klinische Studien werden durchgeführt, um mehr Informationen über ihre Verwendung in der Krankheitsprävention und Pathogenkontrolle, wie auch die Verringerung der unerwünschten Nebenwirkungen zu sammeln. In dieser Übersicht werden die biologischen Aktivitäten wie entzündungshemmende, antimikrobielle und antitussive Aktivitäten, sowie der Wirkmechanismus verschiedener ätherischer Öle bei Atemwegserkrankungen vorgestellt. Das Ziel ist zu zeigen, dass ätherische Öle eine wichtige potentielle Quelle für die Entwicklung neuer Arzneimittel darstellen, da ihre Wirkstoffe viele pharmakologische Eigenschaften besitzen.

Contents

Introduction

Worldwide, there are more than half a million of plants, but only about 5 percent of them, have a scientifically proven biological activity. It has been well-known since ancient times, that some plants and their extracts, are an important source of natural products used in the treatment of various diseases. That being the case many essential oils are targets to evaluate for use on respiratory infections. Respiratory pathologies are a public health concern worldwide and effective natural products have been the prime targets of research especially in the last decades, with intensified studies of natural therapies. Plants could be an important source of novel drugs as natural alternatives [1-4].

Essential oils are derived from the secondary metabolism of the plant. They are hydrophobic, concentrated liquids, characterized by a strong odor. They are complex mixtures of different volatile compounds, composed mainly of terpenoids, including monoterpenes and sesquiterpenes. A variety of other low molecular compounds may also occur, such as aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones, phenylpropane and also simple alkanes and alkenes. Essential oils appear to have the greatest concentration in special cells or groups of cells or in glandular hairs in various parts of the plant [5]. Therefore leaves, fruits, roots, peels, barks can be used for the extraction.

Respiratory tract diseases are pathological conditions that affect the air passage organs including the nose, the bronchi and the lungs. Cough is scientifically proven to be one of the most common symptoms of many acute and chronic diseases. Acute cough symptom is, in the majority of cases, the result of infection, specifically known as the Upper Respiratory Tract Infection (URTI). This is the most common disease among people of all ages. Common cold and allergens can also cause acute cough [1, 2]. Respiratory diseases range from mild and self-limiting, such as the common cold, to life-threatening like bacterial pneumonia, acute asthma and lung cancer. Respiratory tract diseases can be classified by the etiology in two groups.

On the first group, there are the non-infective diseases such as the famous chronic diseases bronchitis and asthma bronchial and on the second, there are the respiratory tract infections caused by bacteria, viruses and fungi. Infections can affect any part of the respiratory system. They are traditionally divided into upper and lower respiratory tract infections. About 30-60% of the medical consultation and 30% of the hospitalization costs, occur due to respiratory tract infections. The most common upper respiratory tract infection is the common cold. However, infections of adjacent organs such as sinusitis, tonsillitis, otitis media, pharyngitis and laryngitis are also considered upper respiratory tract infections. Respectively, the most common lower respiratory tract infection is pneumonia, which is an infection of the lungs, usually caused by bacteria, particularly *Streptococcus pneumoniae* in Western countries. When this infection affects kids and elderly people as well, especially in developing countries the developed complications can even lead to death. Viruses and fungi can cause pneumonia as well [6]. Eccles' research findings [7,8] show, that the cool sensation and relief of the nasal congestion and dyspnoea experienced by menthol and other plant extracts, as well as essential oils, can be explained by a physiological mechanism situated in the nose. After the inhalation through the airway afferent nerves, menthol stimulates the cold receptors in the nasal mucosa membrane allowing this signal to be forwarded to the larynx via the trigeminal nerve. Isenberg and Schäffer et al. [9] reported that this mechanism can be explained by the depolarization of cold receptors, caused by the inhibition of calcium receptors in the cell.

From the 3000 essential oils known, 300 of them, approximately, are important to the pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries (Bakkali et al., 2008 [5] Gilles et al., 2010 [10]). However, the rational and safe use of many of them has to be scientifically justified (Kumar et al., 2007 [11]). For example, herbal cough treatments with proven clinical efficacy, that include ivy, primrose, thyme-based preparations, are recommended as expectorants in current European guidelines [12]. Essential oils extracted from plants, may have antibacterial properties with synergistic interactions to each other. They usually consist of a large number of components and it is likely that their action module

involves many targets inside the bacterial cells. A number of essential oil's components have been identified as antibacterial, such as carvacrol, citral, eugenol, geraniol and thymol. In addition, essential oils possess antifungal, antiviral, and antiparasitic attributes. Nowadays, researchers indicate an increased interest in finding novel drugs of plant origin and thus essential oils have risen as a big potential alternative in the prevention and the treatment of respiratory tract diseases [3].

Eucalyptus

Originating from Australia, *Eucalyptus*, from the family of Myrtacae, grows nowadays in both tropical and subtropical climates in all over the world. Of the different species known, *Eucalyptus globulus* is one of the most thoroughly researched [13]. Research done on other species from the *Eucalyptus* family has demonstrated that the main constituent of the essential oil with 70% is 1,8-cineole (also known as eucalyptol) which has been reported to possess many healing properties, such as stimulation of respiration, cough relieving , mucolytic and relaxation of the respiratory muscles [14,15]. Many traditional healers from all over the world use the leaves (dry or fresh) of different species to treat illnesses, such as asthma, cough, cold, sore throat, bronchitis and pneumonia [16, 17, 18].

Eucalyptus grandis essential oil

Essential oils are obtained by hydrodistillation, from the fresh and the dry leaves of *E. grandis*. This essential oil has been tested against respiratory tract infections caused by *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Moraxella catarrhalis* [19] and others [20].

Antimicrobial activity

Respiratory tract diseases, such as pneumonia, bronchitis, asthma and pulmonary diseases, affect the air passages (nose, bronchi, lungs) [21, 22]. *Klebsiella* is a genus of nonmotile Gram-negative bacterium, found in the human nose, mouth and gastrointestinal tract as normal flora. *K. pneumoniae* is the most important member of the *Klebsiella* genus of the enterobacteriaceae. However, it can also evolve into a human pathogen and lead to a wide range of diseases. For instance, if it is aspirated, it can cause destructive changes to human lungs resulting to bloody sputum. The symptoms are chills, fever, coughing and chest pain [21, 23, 24]. *M. catarrhalis* is a fastidious and also nonmotile Gram-negative bacterium known to cause respiratory infections in the upper respiratory tract leading to otitis combined with sinusitis, shortness of breath, chronic bronchitis and cough. [21, 22]. *S. aureus* is a Gram-positive coccal bacterium that is often found in the nose, respiratory tract and on the skin. It is usually a commensal bacterium that colonizes asymptomatically about 30% of the human population, but it can sometimes cause diseases, especially in the lower respiratory tract, like *K. pneumoniae*. *S. aureus* in particular causes bacteremia and infective endocarditis. Moreover, when mucosal barriers have been opened it can also cause various skin infections. [21, 25].

In the research led by Soyingbe et al. [19], the chemical composition of the essential oils was captured by gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS). The essential oil obtained by hydrodistillation from the fresh leaves of *E. grandis* revealed 31 compounds and the most abundant of them were α-pinene (which is a bicyclic monoterpene), p-cymene (which is an alkylbenzene-monoterpene), 1,8-cineole (which is a cyclic ether-monoterpene) and α-terpineol (which is an alcohol-monoterpene). Whereas the main components of the essential oil obtained by hydrodistillation from the dry leaves of *E. grandis*, are 1,8-cineole, limonene (which is a monocyclic monoterpene), α-pinene and spathulenol (a tricyclic sesquiterpene alcohol). The antimicrobial assay of Soyingbe et al. [19] was carried out using two techniques, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The MIC is the lowest concentration of the sample, at which no visible microbial growth is

observed. The MBC is the lowest concentration of the sample, at which inoculated bacterial strains are completely killed. Bacteria treated with ampicillin and neomycin were used as positive controls. The results obtained from the MIC and MBC revealed that the essential oils of both (fresh and dry) leaves of *E. grandis* possess properties against respiratory tract bacteria. However, the fresh leaf's oil appeared to possess a more significant antibacterial activity than the dry leaf oil, especially against *K. pneumoniae*, which is the most sensitive microorganism with the lowest MIC and MBC. The reason to these differences could be the drying process of the plant, which is mostly done because it reduces microbial growth and makes the storage of the material easier. Drying a plant material, cannot only accelerate the distillation, but can lead also to the breaking of the cells where the essential oil is stored. Reactions such as dehydration, oxidation, and glycosylation can occur during the drying process. Obviously, all of them affect the composition as well as the concentration of the components of an essential oil. For example, while the amount of α-pinene and p-cymene decreased in the dry leaf, the amount of 1,8-cineole and limonene increased. [19,26]. To conclude, the essential oil of *E. grandis* demonstrated a similar antimicrobial ability following the standards and showed a broad spectrum activity as it reacted against, both Gram-negative and Gram-positive bacteria. Furthermore, the oil inhibited the growth of MDR bacteria. The reason for such good antimicrobial activity reports could be the presence of compounds like 1,8-cineole, α- and β-pinene and limonene which have been reported [27] to have antimicrobial properties.

The mechanism of that antimicrobial activity of essential oils was barely studied. Soyingbe et al. [20] tested the effect of essential oils on the DNA and the membrane of bacteria. From the results acquired from the DNA's clearage studies, it is evident that the essential oil from *E. grandis* did not damage the microbial DNA, meaning it did not have any impact on it. The essential oil however, showed an impact on the microbial membrane since it damaged the membrane's integrity, causing the release of lactate dehydrogenase (LDH) which is a cytosolic enzyme. This result can also be correlated to previous studies [28, 29] which have also shown that essential oils damage the microbial cell membrane. The prime target of essential oils is the negative charge of bacterial cell surfaces which damages their cytoplasmic

membrane resulting into higher permeability of the cell membrane, as well as cell lysis and loss of intracellular constituents. It is interesting to note that the LDH release test [20] was carried out on *Escherichia coli* (*E-coli*), which is a Gram-negative bacterium with most of its strains being harmless and part of the normal gut flora. The pathological strains can cause serious food poisoning. Also *E-Coli* is the most studied prokaryotic model organism and plays an important role in the biotechnology. *Bacillus pumilus* does not play a big role for humans. *Enterobacter cloacae* is a Gram-negative bacterium, member of the physiological gut flora of humans and is not usually an important pathogen. *B. subtilis* is a Gram-positive bacterium and part of the normal human gut flora and an important model organism. Soyingbe et al. [19] repeated exactly the same LDH release assay, two years later, on three very important respiratory tract infectious bacteria. The very low levels of LDH released from the cytosol, suggest that the damage of the cell membrane has only a little effect in the bacteria's death. In fact, living cells such as bacteria possess a mechanism that helps them expel toxic substances. This mechanism (efflux pump) has the function of a pump, is found in the inner membrane of the bacteria and releases toxic substances, including antibiotics [30].

Efflux pump inhibition

Nowadays even though a wide range of antibiotics exists, the resistance of pathogens keeps growing and a big necessity arises for developing new antibacterials, either by improving the drug design or by developing efflux pump inhibitors [31]. Efflux pumps are proteinaceous active transporters that can be found in the cytoplasmatic membrane of all kinds of cells. Efflux pumps are highly efficient in drug extrusion and broad substrate specificities. It is very important to underline their role in the developing of multidrug resistance in bacterial pathogens. Multidrug resistance is the bacteria’s capability, to survive lethal doses of structurally diverse drugs which are usually capable of eradicating non-resistant strains. Multidrug resistance is defined by the World Health Organization as a major threat for the public health. Drug extrusion by the multidrug efflux pumps, in addition to target alteration, drug inactivation, decreased permeability and increased efflux have a big impact on the mechanism of multidrug resistance. Efflux pumps

can expel a variety of antibiotics and develop additional resistance mechanisms, by lowering the intracellular antibiotic concentration and promoting the mutation accumulation. An over-expression of multidrug efflux pumps has been found to be associated with drug resistance. Efflux pump inhibitors can act against multidrug resistance pumps by directly binding on the pump and blocking it [32].

Soyingbe et al. [19] tested the abilities of the essential oils for their multidrug resistance inhibition of Rhodamine 6G's (R6G) accumulation, using the method of Maesaki et al. [33]. The accumulation of the R6G was expressed as percentage in the cells after exposure to glucose, essential oils and the standard inhibitor (berberine). The results revealed that essential oils were able to increase the accumulation of R6G inside bacterial cells, which shows that essential oils might be used as efflux pump inhibitors. But R6G seems to be bacteria-strain specific. In fact, on the one hand essential oils from the fresh leaf have shown more effective results for *K. pneumoniae* and *M. catharralis*, because the R6G absorption was better for them than for *S. aureus*. On the other hand essential oils from the dry leaf had a better impact on *S. aureus*. The first interesting thing to notice is that the accumulation of R6G in the fresh leaf's oil was even higher than that of the standards and secondly that the essential oils from dry leaves were more effective for *S. aureus*. So, the R6G concentration can be increased by plant extracts [19]. It is also important to mention the significant difference between these bacteria: *S. aureus* is a Gram-positive bacterium, with only one layer of cell wall which makes them more receptive to antibiotics, than Gram-negative bacteria which have a double membrane. This could explain the higher percentage of accumulation for *S. aureus* [34].

Antioxidant activity

Soyingbe et al. [20] reported, that various pathogens affecting the respiratory tract may cause oxidative stress which finally triggers asthmatic attack, able to destroy the bacterial membrane and block the efflux pump mechanism.

Free radicals are either an atom or a group of atoms, such as superoxide radicals ($O_2^{\bullet}$) and hydroxyl radicals ($OH^{\bullet}$) that possess an unpaired electron and are therefore very reactive and unstable. There are also non-radicals, for example: the hydroxyl anion (OH^-), since the unpaired electron is resolved by the addition of an electron and singlet oxygen (O_2) too. These four radicals are commonly called Reactive Oxygen Species (ROS), because they are generated during the breathing (aerobic) process. So, free radicals can be produced during normal physiological function as a result of metabolic activities, mitochondrial respiration, liver oxidase and xanthine oxidase for example. But they may also turn into a pathological condition when they are produced as a result of smoking, stress conditions, air pollutants and drugs. The exposure to all of these conditions increases the amount of the ROS [35]. Given the facts, antioxidants become important in the oxidative stress treatment, because they interact with free radicals and neutralize them. In fact, they can decrease the ROS concentration by reactions with the radicals, by chelating and scavenging oxygen, so the chain reaction is neutralized before damaging of tissues and organs. A misbalance between radical-generating, radical-scavenging and oxidative stress development may lead to pathophysiological conditions. In the human body, molecules such as, DNA, RNA, proteins and cell membrane are more susceptible to be attacked of free radicals than others. This situation can automatically lead to cell damage and damage on extracellular constituents, as nucleic acids, proteins and carbohydrates. This can utterly lead to neurodegenerative diseases, autoimmune diseases, arthritis, and cardiovascular diseases and even rapid aging [36, 37, 38].

During the past years, interest for natural antioxidants has grown among the scientists and the antioxidant capability of essential oils has been thoroughly examined. Researches carried out with the essential oil of *Nepeta deflersiana* (Lamiaceae), displayed the essential oil's ability to reduce DPPH (1,1-Diphenyl-2-picrylhydrazyl) but only moderately. The insignificant antioxidant activity observed was associated with the low content of phenolic volatile compounds, such as thymol and carvacrol [39]. Kadri et al. [40] who focused their work on the antioxidant activity of the essential oil of *Artemisia alba* stated that the antioxidant properties of this essential oil may be applied in both, the pharmaceutical industry for the

prevention of a variety of diseases and in food as potential natural antioxidant additive. These essential oils could also be models for new free radical-scavenging drugs.

The essential oil of *E. grandis* was screened for its antioxidant activity by measuring the scavenging action of DPPH and NO (nitric oxide radicals) radicals and the Fe^{2+} chelating. The results were expressed in inhibitory concentration providing 50% inhibition (IC_{50}). The high scavenging action of DPPH and NO radicals indicated that the essential oil of *E. grandis* is a powerful natural antioxidant [20].

Eucalyptus odorata **essential oil**

Streptococcus pyogenes, *S. pneumoniae*, *S. agalactiae*, *S. aureus*, *Pseudomonas aeruginosa*, *K. pneumonia* and *Haemophilis influenzae* are the most important respiratory tract pathogens and they show the biggest resistance towards antibiotics. Elaissi et al. [41] evaluated the antimicrobial activity of 8 different eucalyptus species against the 7 bacteria cited above. *E. odorata* is the one with the highest antimicrobial action against *S. aureus*, *H. influenza*, *S. agalactiae*, *S. pyogenes* and *S. pneumoniae*. It also possesses great antifungal properties, however *E. odorata* showed also a higher cytotoxic effect.

In the chemical composition twenty five main compounds were reported which were used for the principal components analysis (PCA) and hierarchical cluster analysis (HCA) as well. 3 groups (A, B and C) were composed and each group constituted a chemotype. *E. odorata* was classified in Group A and was characterized by the highest mean percentage of crypton and the lowest of 1,8-cineole. Group B (*E. maidenii*, *E. lehmannii*, *E. sideroxylon* and *E. cinerea*) was characterized as the essential oils with the highest concentration of limonene and α-terpineol. Finally, essential oils of group C (*E. astringens*, *E. leucoxylon* and *E. bicostata*) were attributed of having the highest mean percentage of epiglobulol [41]. It has been reported that *E. cinerea*, *E. sideroxylon*, *E. bicostata*, *E. maidenii*, *E.*

leucoxylon, *E. lehmannii* and *E. astringens* contain 1,8-cineole as the major compound [41-46]. It was also reported that variations in the chemical composition within species are depending on factors, such as climatic and environmental conditions. For example, *E. cinerea* that is growing in Morocco has a higher percentage of 1,8-cineole than the one growing in Tunisia [43].

Antibacterial activity

In the antibacterial activity test, 5 groups and subgroups of bacterial strains were formed according to the sensitivity levels of each essential oil. The antibacterial tests against pathogens showed that, *E. odorata* possessed the greatest counteraction capacity against *S. aureus*, followed by *S. agalactiae*, *H. influenza*, *S. pyogenes* and *S. pneumoniae*. *E. maidenii* has a quite good antibacterial activity against *S. aureus* as well. This high sensitivity of *E. maidenii* and *E. odorata* can be explained by the high concentration of p-cymene in their essential oils [41]. On the one hand previous studies have reported that *S. aureus* is very sensitive to essential oils with a high percentage of p-cymene [5], on the other hand, another study related this high sensitivity of *S. aureus* with the presence of only one layer in Gram-positive bacteria which makes them more susceptible to antibacterial attack [47]. The results of the antibacterial activity of *E. odorata* were equally comparable to the inhibition of antibiotics like gentamicin, erythromycin, vancomycin and benzylpenicillin. Only compared to fosfomycin the essential oil has shown lower activity.

To conclude, the activity of essential oils varied from strain to strain. Generally, the high activity was not only related to the presence of high percentage of 1,8-cineole, but also to other minor compounds. [41]

Cytotoxicity

The cytotoxicity assay showed significant high levels of cytotoxicity for the essential oil of *E. odorata*. A real connection between chemical composition and cytotoxicity could not be made, but the lowest cytotoxicity was attributed to essential oils that contained high percentage of 1,8-cineole and lower percentages

of limonene and α-pinene, for example the essential oil of *E. maidenii*. However, these essential oils can be considered safe because their cytotoxicity is still lower compared to previous studies [41]. The reason for the high levels of cytotoxicity of *E. odorata* could be the lower percentage of 1,8-cineole and its richness in cryptone (a ketone), then p-cymene (a monoterpene hydrocarbone), and phelandral and cuminal which are aldehydes [48].

Eucalyptus globulus **essential oil**

The *Eucalytus* genus consists of about 900 species and subspecies which can provide a big source of essential oils. These essential oils can be found in the leaves of 300 species of this genus and 20 of them are exploited by pharmaceutical and cosmetic industries because of their richness in 1,8-cineole [41].

Antibacterial and synergistic activity

Pereira et al. [49] focused their research on the antibacterial activity of one of the most studied species, namely *E. globulus*. They also tested the synergistic effects of essential oils and extracts combined with an antibiotic against one famous respiratory tract bacterium (*P. aeruginosa*) and its isolates.

P. aeruginosa, is a Gram-negative bacterium affecting plants, animals and humans. This bacterium is very problematic because of its resistance against many drugs and all sorts of treatments. Therefore, it can be called a prototypical MDR. *P. aeruginosa* causes serious illnesses, especially nosocomial infections. As a matter of fact, it is considered opportunistic and mostly attacks patients with co-morbidities, especially their respiratory tract [50, 51].

In this study two different essential oils were used with respectively 79 % (oil A) and 83% (oil B) of 1,8-cineole. These two oils were acquired in food stores. Even though both were obtained by hydrodistillation, oil A derived from industrial processing, whereas oil B from a smaller quantity production. The results have shown that Oil B exerted better results of inhibition against the different isolates.

However, it was reported that the antibiotics had lower MIC, therefore they were more effective against the isolates than the essential oils. As a conclusion, it is interesting to note that only 3 isolates have shown antagonism when the synergism was tested and the combination of essential oils and antibiotics has demonstrated additive effects for more than 50% of the cases [49].

Immunomodulatory and Anti-inflammatory effects

Inflammation is the physiological response of body tissues to noxious stimuli, such as cell damaging and pathogens. This mechanism can be defined as protective. In fact, the organism is protecting himself against harmful stimuli. The aim of inflammation is at first to eliminate the prime cause of the cell damaging, as well as to repair the damaged tissues and to restore the homeostasis at the infected areas. Calor, dolor, rubor and tumor (heat, pain, redness and swelling), are all typical signs of an inflammation. Inflammation is considered to be a mechanism of innate immunity that is why immune cells, blood vessels and mediators are part of the response. Two types of inflammation exist, either acute or chronic.

The acute type is a short-term process that usually appears within some minutes or hours and withdraws after the repairing process is completed, because of the presence of a negative feedback. It is characterized by the moving of plasma and leukocytes from the blood to the damaged tissue as a response of the organism to a harmful stimulus. At the start of an inflammation, inflammatory mediators are massively released from the macrophages and the mast cells. These mediators infiltrate into compartments, where they are usually not present in such high amounts resulting into a boost of the inflammatory response.

An extended, long-term inflammation also called chronic, is characterized by synchronic destruction and healing of the tissue. In that case, the body cannot find the appropriate solution to the noxious stimuli and the body fails in the regulating of its own mechanism. Chronic inflammation implies a progression of the disease. Asthma is one example of this type of inflammation. The main goal of anti-inflammatory therapy is to reduce the number of inflammatory mediators [4].

In vitro

In the past years many scientists investigated the anti-inflammatory properties of essential oils in the prevention and treatment of diseases. *E. globulus* oil, when compared to lavender and tea tree oil *in vitro*, has shown that it can increase the phagocytic activity whereas the two others cannot. In fact, it was reported that dose-dependently its essential oil stimulated the phagocytosis by macrophages without producing any pro-inflammatory effects. It is believed that the *E. globulus* essential oil's phagocytic ability is related to the microtubule network, because it ceased, when the essential oil's-stimulated cells were treated with a microtubule-destabilizer. In order to find out the reason of inflammation, the cytokine profile was examined. Three different groups were tested: makrophages with essential oils only, with lipopolysaccharide (LPS) only and with essential oils followed by LPS. In contrast to LPS, essential oils did not change the cytokine profile. Although LPS elevated some interleukins and the tumor necrosis factor-alpha (TNF-α), their inflammatory effect was reduced with the essential oil pretreatment. During an inflammation essential oils inhibit or decrease interleukin 4 (IL-4), interleukin 6 (IL-6), TNF-α and NF-κB, but they do not have any influence on IL-2, IL-10 and interferon-γ [52].

In vivo-Animal studies

The innate cell-mediated immune response was also observed *in vivo* in the peripheral blood of rats after the essential oil's application. After 15 days of oral treatment with essential oil, a significant increase of monocytes was noticed. A significant increase of CD44 and CD25 monocyte surface markers occurred too. But there was no effect noticed on the granulocytes and lymphocytes. Even though the treatment with essential oils was terminated, their effect continued for 5 more days and was defined as monocyte activation and extravasation. Immune-suppressive tests were also carried out on rats. A 5-fluorouracil (5-FU)/essential oil combination was used. Essential oils inhibited the 5-FU-induced myelotoxicity and at the same time they were increasing the phagocytic action. This renders the essential oil as a cell-mediated immuno-regulatory agent in immune-suppressive pathologies or

infectious diseases [52].

One study carried out in rats also demonstrated the efficacy of the *E. globulus* essential oil in LPS induced chronic bronchitis. At a dose of 300 mg/kg resulted a significant reduction of the bronchitis symptom harshness, less infiltration of inflammatory cells and decreased airway mucins [53].

Antibacterial action

In a study, the antimicrobial effect of *E. globulus* essential oil was tested, against various bacterias from human specimen samples (120 isolates of *S. pyogenes*, 20 isolates of *S. pneumoniae*, 40 isolates of *S. agalactiae*, 20 isolates of *S. aureus*, 40 isolates of *H. influenza*, 30 isolates of *Haemophilus parainfluenza*, 10 isolates of *K. pneumoniae* and 10 isolates of *Stenotrophomonas maltophilia*). The most sensitive of them were *H. influenza*, *H. parainfluenza* and *S. maltophilia*. *S. pneumoniae* and *S. agalactiae* showed little sensitivity versus the oil. Very little antibacterial effect was delivered against *S. pyogenes* and *S. aureus*. The oil had no effect against *K. pneumoniae*. In the same study, 21 plants (and different dilutions) against 6 bacterial species (*E-coli*, *K. pneumoniae*, *P. aeruginosa*, *Pseudomonas vulgaris*, *B. subtilis* and *S. aureus*) were put into trial. 19 oils acted antibacterially against at least one of the species. To conclude, *E. globulus* oil showed the lowest impact against these bacteria [54].

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (MTB). It generally affects the lungs and other body parts. There are two forms, the latent tuberculosis and the active form TB. Most of the infections are of the latent type without symptoms. Around 10% of the latent infections turn to an active form that can be lethal especially if it stays untreated. The main syptoms of the active form are: chronic cough with blood containing sputum, fever, sleep hyperhydrosis and weight loss. The disease is spreadable via the air, but only people that have the active form can infect others by coughing, spiting and speaking. The treatment of tuberculosis is very difficult because of the high MDR-TB rate, which often includes many antibiotics for a long period of time [55].

As an example, a 28-year old woman, who was diagnosed with TB by her sputum culture results and her chest x-Ray, refused to undergo the classical therapy and chose to inhale *E. globulus* oil. Three weeks, three times a day she inhaled with 3 mL essential oil to 500 mL boiling water. In her first results, just 10 days later, her malaise and cough reduced significantly, whereas her appetite and weight went back to normal levels. Her body temperature was normalized and the sputum cultures became negative. Unfortunately, the x-ray did not show any improvements and the erythrocyte sedimentation remained very high. Anyway, the patient did finally continue with the conventional treatment after 3 weeks of essential oil treatment. *In vitro* effects of the essential oil of *E. globulus* were unfortunately not reported. In the author's opinion it is anyway not possible to expect the chest x-ray to change after 10 days [56].

Antioxidant activity

An antioxidant is a molecule that inhibits the oxidation of other molecules. By oxidation free radicals can be produced and this can lead to chain reactions that damage cells.

Eucalyptus oils have shown a biological capability as antioxidants. However *E. globulus* seems to have a poor antioxidant action compared to the other species [57]. Similarly when it was compared with 10 other essential oils for their free radical scavenging activity, *E. globulus* essential oil achieved relatively low results. In the author's opinion, this poor scavenging ability of this oil could be due to its high concentration of monoterpenes [58].

Nasal ciliary beat frequency (CBF)

The mucociliary clearance is a self-clearing mechanism of the bronchi. The passage of airway in the respiratory tract that goes from the bronchi down to the alveoli has a surface called epithelium. This surface is covered with cilia which are hair-shaped structures. The cilia are surrounded by mucus which is considered to be the initial defense of the airway. Mucus is trapping small inhaled particles, also microbes. With the ciliary movement and rhythmic beating the mucus is propelled

in direction towards the pharynx. Finally, in the throat it is either swallowed and destroyed by products of the stomach or expelled via coughing and sneezing. Important for good mucociliary clearance are the structure of the cilia, the number, their activity and coordinated movement [59].

In a CBF study, the oils of sesame, peanut, soy, thyme, lavender, eucalyptus (species not specified) and menthol were tested. All essential oils were diluted to miglyol 840 which is a neutral, low viscosity carrier oil. Their activity was tested on the inferior nasal turbinate. To complete the test, ciliated epithelial brushings were placed on slides and they were exposed to test dilutions at different times, with 20 minutes being the maximum. It was reported that all oils, except thyme oil and miglyol 840, increased the CBF. The best results were noticed for eucalyptus essential oil. The dilution of 0.2% oil increased the CBF by reaching a peak 20% at 10 minutes and remained at the same levels for another 20 minutes. The dilution of 2% oil increased the CBF towards 11.8% at the first 5 minutes, but as time was passing, it continually decreased after 10 minutes and more [60].

***Eucalyptus tereticornis* essential oil**

Analgesic effect

Analgesia is the relief from pain and the group of drugs used for that purpose are called either analgesic or painkiller. These drugs act in various ways on the peripheral and central nervous systems.

A study tested the analgesic effect of the essential oils of three different species *Eucalyptus citriodora*, *E. tereticornis* and *E. globulus*. Compared to morphin, these three essential oils showed dose-dependent and time-dependent peripheral and central analgesic properties in rodents. The greatest anti-inflammatory effect was shown with *E. tereticornis* in a model of rat paw edema. The anti-inflammatory activity of essential oils was also compared to dexamethasone and the results have shown 75% (for the essential oils) of inhibition

of neutrophil migration in the rat peritoneal cavity, whereas dexamethasone has shown 97% of inhibition. A vascular permeability test which was also carried out, revealed pain reduction but the results were very much varying from species to species and they were varying also depending of the permeability agent [61].

Antioxidant activity

In a study lead on the free radical scavenging abilities of *E. tereticornis* oil, very good results were reported. Oils from fresh or decaying leaves and also from separate constituents of the oil were tested against superoxide anion and hydroxyl radical. All of them displayed very high antioxidant activities. The results were comparable to or even surpassed the standard antioxidants ascorbic acid and t-butylhydroxytoluene, respectively. It is interesting to note that all the major constituents did not beat the result of the whole oil which clearly shows the synergistic effect of the combination [62].

***Eucalyptus radiata* essential oil**

Antibacterial activity

A study *in vitro* tested the antibacterial effect of vaporized *E. radiata* and other essential oils (cinnamon bark, lemongrass, perilla, thyme, peppermint, tea tree, coriander, lavender, rosemary and citron) against six strains of bacteria (*H. influenza*, *S. pyogenes*, penicillin-susceptible *S. pneumoniae*, *S. aureus* and *E-coli*). All the cited oils showed to be effective, but the eucalyptus essential oil only to a small extent. When it comes to the susceptibility of the bacterias *E-coli* was the least and *H. influenza* the most susceptible. Between them were *S. pneumoniae* and *pyogenes* and then *S. aureus*, respectively (from most to least) [63].

Essential oils from other *eucalyptus* species

Antibacterial action

A study examined *E. sideroxylon* and *E. torquata*) for their antimicrobial activity against nine bacterial strains. The essential oils were extracted from different plant parts (leaf, stem and flower). The results have shown that the Gram-positive bacteria are more sensitive to the essential oils activity. *S. aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis* and *B. subtilis* have shown the highest sensitivity. Whereas, out of five Gram-negative bacteria only 2 exerted a moderate sensitivity (*K. pneumonia* and *Proteus mirabilis*) and the 3 other *E-coli*, *P. aeruginosa* and *Salmonella typhi* revealed very little to none sensitivity [64].

Antioxidant activity

The antioxidant abilities of E. *polyanthemos*, *E. globulus* and *E. perriniana* were examined. It was reported that *E. polyanthemos* essential oil possesses the greatest activity. Its antioxidant effect is even comparable to α-tocopherol, since it inhibited during minimum of 30 days the oxidation of hexanal to hexanoic acid. The standard α-tocopherol at 50 μg/mL inhibited the oxidation of hexanal by 98%. In comparison, 500 μg/mL of *E. polyanthemos*, *E. globulus* and *E. perriniana* essential oils inhibited the oxidation by 99%, 55% and 16%, respectively [57].

Eucalyptol

This cyclic ether and monoterpenoid also known as 1,8-cineole is a natural organic compound that was found and identified for the first time in *E. globulus* oil. In some of the eucalyptus species eucalyptol can even be found in a concentration up to 90%. It can also be found in camphor, rosemary, tea tree and other aromatic plants and it is obtained by fractional distillation of eucalyptus oil. Several studies have shown that this isolated compound has similar properties as the plant from which it is

obtained (Eucalyptus).

Immunomodulatory/Anti-inflammatory effects *in vitro*

Juergens et al. [65] reported that 1,8-cineole exerts inhibitory effects on the stimulated cytokine production in human lymphocytes and monocytes. The authors defined this monoterpene as a strong inhibitor of IL-1β and TNF-α and it also possesses small effects on chemotactic cytokines. In fact, at 1.5 μg/mL, 1,8-cineole inhibited the cytokine production in lymphocytes by 92% of TNF-α, by 84% of IL-1β, by 70% of IL-4, by 65% of IL-5. The cytokine production was also inhibited in monocytes with even greater results: by 99% of TNF- α, by 84% of IL-1β, by 76% IL-6 and by 65% of IL-8. A dose-dependency was shown when the concentration was at 10-fold lower. The cytokine inhibition declined. At 0.15 μg/mL the production of TNF- α was inhibited by 16% and the production of IL-1β by 36% (in lymphocytes), whereas in monocytes the inhibition was reported to be at 77% and 61%, respectively. So, at the lower concentration 1,8-cineole showed a greater effect on the inhibition of the production in monocytes, but at the higher concentration the effects were comparable to lymphocytes and monocytes. In the author's opinion, since 1,8-cineole controls the airway mucus hypersecretion, it might exerted an impact on asthma-, sinusitis- and chronic obstructive pulmonary disease- (COPD) exacerbation.

LPS that stimulated human monocyte cells showed an increased synthesis of early growth response factor-1 (Erg-1) in the nucleus and the whole cell. This factor's function is: transcriptional regulator as it plays a role in the regulation of cell proliferation and apoptosis. With 1,8-cineole pretreated monocytes have shown dose-dependently (1-100 mg/L) a lowered expression of Erg-1. No change in the expression of nuclear factor kappa B (NF-κB) was noticed [66]. In another similar study, α-pinene (a less abundant compound in eucalyptus essential oil) has shown an inhibiting NF-κB activity with its nuclear translocation being reduced [67]. It is interesting to notice that the NF-κB activity was not reduced by the essential oil of *E. globulus* (as a whole) [68].

Another *in vitro* study performed on LPS-stimulated human monocytes showed that

1,8-cineole (10 µg/mL) has a significant inhibiting effect on TNF-α (by 99%), IL-1β (by 74%), leukotriene (LT) B4 (by 47%) and thromboxane B2 (by 91%). This was noticed after 20 hours and is also dose-dependent. At the same concentration of 1,8-cineole a reduction of TNF-α by 98% was shown for IL-1β stimulated macrophages [69].

Asthma (human clinical trials)

Asthma is a very common chronic disease of the airways. About 235 millions of persons in the world suffer from this long-term inflammatory disease. It is characterized by symptoms, such as reversible airflow obstruction, bronchospasm, wheezing, coughing, chest tightness and shortness of breath. All these vary in severity and frequency individually depending on the person. The causes are not completely clear. It is assumed that the condition is triggered by a combination of genetic predisposition and environmental factors, for example allergens. But also specific medication (beta blockers) can be a trigger. Other triggers are emotional factors and also physical activity. The diagnosis is based on various symptoms, the reaction to therapy and the spirometry. The stages are classified according to parameters as frequency of symptoms, results of forced expiratory volume in one second (FEV1) and peak expiratory flow rate. Pathophysiological asthma results from a chronic inflammation of the air tract, of which the bronchi are especially affected. The surrounding smooth muscles tend to contract easier. During the attack the lining of the bronchial tubes swell leading to narrowing of the airway and to reduced air flow. Increased eosinophils and thickening of the lamina reticularis are typical. With the years the smooth muscle can get bigger and the number of mucous glands can raise. Components of the immune-system such as, lymphocytes, macrophages, neutrophils, cytokines, histamine, leukotrienes and chemokines are also involved. Although asthma cannot be cured with appropriate management and strategies of prevention, the disease can be controlled, so both quality and quantity of life can be increased. Asthma has a relatively low fatality rate compared to other chronic diseases [70].

LTB4 and prostaglandin E2 (PGE2) are both produced in the pathway of arachidonic acid metabolism. In a study with a total of 22 patients the productions of LTB4 and PGE2 were measured. All patients were pretreated for three days with 1,8-cineole (200 mg three times daily). From them, 10 patients suffered from bronchial asthma leaving the remaining 12 as healthy controls. After the pretreatment, measurements were performed in stimulated monocytes from the bronchial asthma patients and from the healthy controls. Two parameters (FEV_1 and airway resistance RAW) were measured, one day before the treatment, during and after discontinuing it. Both groups of patients have shown significant results when it comes to inhibition of LTB4 and PGE2. After three days of 1,8-cineole treatment the FEV_1 increased by 23.7% and RAW decreased by 26.1%. Another lung check after four days has shown significantly improved FEV_1 and RAW [71]. The same author performed a double-blind, placebo controlled trial in order to demonstrate the anti-inflammatory effect of oral 1,8-cineole. Asthma patients were taking for 12 weeks three times per day 200 mg of 1,8-cineole. Before the treatment patients were using a dose of 5-24 mg of prednisolone (average of 11 mg) per day. The required oral glucocorticoid dosage was decreased in the control group by a mean of 0.91 mg and 3.75 mg for the cineole group. It is interesting to note that the rescue medication (Salbutamol-Albuterol) was increased almost double in the control group, when the prednisolone dosage was lowered by 2.5 mg. whereas, in the cineole group there was no such increase in rescue medicine even when the dosage of prednisolone was decreased by 5 mg. At that reduction rate four patients from the cineole group and 11 from the placebo group quitted. The cineole group preserved lung function capacity (peak expiratory flow rate, FEV1 and RAW) four times longer than the control group even at a lower prednisolone dosage [72].

Rhinosinusitis (human clinical trial)

This inflammation of the sinuses is also known as sinus infection. Commonly the symptoms are thick nasal mucus, a plugged nose and pain in the facial area. Other signs may be fever, headache, no sense of smell, cough and sore throat. A sinusitis can be caused by infections, allergies or air pollutants, mostly due to a viral infection. Annually, about 10% to 30% persons from the USA and Europe

are affected. It affects more often women than men. If it lasts less than 12 weeks then it is an acute rhinosinusitis. If the symptoms persist for more than 12 weeks then it is defined as chronic. Chronic rhinosinusitis affects around 12% of people [73].

A study has shown the positive effects of 1,8-cineole in the therapy of rhinosinusitis. A total of 150 persons took part in a double-blind, placebo-controlled trial. All the persons that were randomized for the trial suffered from subjective symptoms of sinusitis, such as headaches (with or whiteout bending), pressure point in the zone of the trigeminal nerve, plugged nose and nose secretions (rated by quantity and viscosity). For seven days the patients were treated with oral 1,8-cineole (200 mg three times daily) and also 100 μg of xylometazoline (a decongestant) three times per day in order to relieve nasal congestion. The treatment group, that contained 75 persons, has shown over 80% of improvement after these seven days. While the placebo group revealed less than 50% of improvement. In order to rate the improvement a symptoms-sum-score was set. At the end of the study an ultrasonography was executed. The results have demonstrated that sinus shadowing remained in 37 individuals of the placebo group, whereas in the cineole group only in four patients [74].

COPD (human clinical trial)

Chronic obstructive pulmonary disease (COPD) is an obstructive disease with a long-term poor airflow. Typical symptoms are: shortness of breath and cough with sputum production. It is a progressive disease meaning that every time these symptoms get worse, so, even walking up the stairs will eventually be difficult in time. The main reason for this disease is tobacco smoking along with air pollution and genetics (the last two play a smaller role). The diagnosis is based on a lung function test and the airflow rates. A breathing test called "spirometry" can measure how much and how quickly a person can forcibly exhale air. Opposite to asthma the airflow reduction in COPD does not improve with the use of a bronchodilator. It affects equally both male and female and it typically occurs after the age of 40. It causes also many deaths. In 2015 it was reported that around 3 million persons died

because of COPD [75].

A double-blind, placebo-controlled, six month trial, revealed the efficacy of 1,8-cineole in COPD patients. The study counted 242 COPD patients who were receiving 200 mg of 1,8-cineole three times a day. Their previous medication was not changed during the study. A significant decrease was noticed in the exacerbation frequency. In the placebo group it occurred 0.9 times in 6 months, whereas in the cineole group it occurred 0.4. Moreover, the severity was rated by a subjective scoring and also the duration. In the cineole group an average duration of 4.0 days was reported versus 5.7 days for the control group. There was no visible difference between the control and the cineole group in the lung function tests. Also, no significant differences were shown in typical COPD symptoms, such as 'trouble breathing' and dyspnoea. Both parameters showed improved scores in both groups [76].

Analgesic effect

In order to test the activity of 1,8-cineole in rats or mice, the animals were injected with pro-inflammatory substances. 1,8-cineole exerted an inhibitory effect on pain sensation. It was also shown that 1,8-cineole is not effective by the μ-opioid receptors in body. The opioid antagonist naloxone did not reverse the analgesic activity of 1,8-cineole [15].

A pain evaluation that used rats and mice revealed that 1,8-cineole has a comparable analgesic activity to morphine. Just as morphine, also eucalyptol exerts analgesic effects on both, central and peripheral nervous system. Moreover, a synergistic effect was observed between these two. Naloxone did not antagonize the activity of cineole. The use of eucalyptus essential oil lowers the needed dose of morphine with the strength of the analgesic effect remaining the same. β-Pinene exhibited an anti-nociceptive supraspinal effect in rats, but opposite to cineole an opioid-antagonist effect was noticed. This antagonistic activity towards morphine is comparable to naloxone [77].

A study used rat superior cervical ganglion to evaluate the nerve excitability. 1,8-cineole (at concentrations 0.1, 1.0, 3.0 and 6.0 mM) was injected

intracellularly and the excitability was recorded. An inhibition was shown at 1.0, 3.0 and 6.0 mM. At 6.0 mM a significant lowering of excitability was shown resulting in a total action potential block in all the tested neurons. In the author's opinion the mechanism can be explained by the depolarization of the neuronal cytoplasmic membrane. This is the indirect reason of the mechanism of action [78].

Antispasmodic effect

Spasmolytic agents suppress muscle spasms. A study compared the antispasmodic effect of the essential oil of *E. tereticornis* and 1,8-cineole. Their effect was evaluated chemically and electrically with induced contraction of guinea pig tracheal smooth muscle. An inhibition of potassium-induced smooth muscle contraction was reported for both. *E. tereticornis* essential oil has shown an inhibition at 200-1000 μg/mL, with 50% concentration inhibition at 248 μg/mL. While cineole has shown an inhibition at 600-1000 μg/mL with 50% contraction inhibition at 446 μg/mL. When the contractions were induced by acetylcholine, *E. tereticornis* essential oil (200-400 μg/mL) increased the contractions but caused relaxation at 800-1000 μg/mL, whereas cineole significantly strengthened the acetylcholine-induced contractions at all concentrations (10-1000 μg/mL) [79].

A further study has shown that, when 1,8-cineole was applied in guinea pig tracheal smooth muscle a significant reduction in contraction was noticed. Moreover, cineole in combination with ovalbumin also significantly relaxed smooth muscle. The animals were previously sensitized with ovalbumin. Since it did not affect muscarinic-induced contractions, the effect is related with the sympathetic part of the nervous system [80].

Another study reported that 1,8-cineole vapor had few to no effect on citric-acid-induced cough in guinea pig. But the authors noted that even though they failed to increase the concentration to verify, there might be a dose-dependent antitussive effect on the respiratory tract. Different essential oils might have better results depending on their constituents and their synergistic effects [81].

Tea tree oil

Eucalyptus essential oil and tea tree oil have very similar terpenoid molecules as constituents. The main difference is the amount of 1,8-cineole and terpinen-4-ol. 1,8-cineole is dominating in eucalyptus essential oil with about 45%, while there is only little or no trepinen-4-ol. Whereas in tea tree oil terpinen-4-ol is dominating with 30% or more and 1,8-cineole about 15% or less [82].

Antibacterial action

A study evaluated several essential oils against *S. aureus*. The essential oils were injected in the nutrient broth and the result was an inhibition of cell growth. Tea tree oil has shown the greatest activity. The growth inhibition was 13 mm for tea tree oil, 12.5 mm for chamomile essential oil and 12 mm for *E. globulus* essential oil. A 100% inhibition was attained with 100 µL of eucalyptus essential oil. Chamomile inhibited cell growth above 50 µL, while tea tree oil caused an inhibition already at only 10 µL. It was also shown that three times the amount of *E. globulus* essential oil as tea tree oil was necessary to reach a 100% inhibition. This was demonstrated with the alginate-bead method. Moreover, it was reported that tea tree oil is binding on cells with an affinity double bigger than the other oils. This strong cell binding could be the reason for a greater antibacterial activity [83].

In 2006 a review reported the medicinal properties of tea tree oil. Antimicrobial activity was found on 27 bacteria strains and 24 fungal strains, antiviral, antiprotozoal and anti-inflammatory activities were also noticed [86]. In some cases tea tree oil antimicrobial activity might be stronger than eucalyptus oil antimicrobial activity, but there are very little studies on oral use of tea tree oil. It is believed that its activity is mainly due to terpinen-4-ol and α-terpineol. These two components are minor in eucalyptus essential oil, but major in tea tree oil [82].
Although a 2006 review [84] reported a therapeutic resistance with common antibiotics, it did not occur with tea tree oil. Two further studies reported the opposite, a three days use of tea tree oil at sub-lethal antibacterial doses does affect the activity of antibiotics against MRSA. The efficacy is reduced [85, 86].

Tea tree oil inhalation revealed very good results in the therapy of tuberculosis. Two women (age: 41 and 33) were unwell for 12 months and 3 weeks, respectively. Both patients had high ESR levels, positive cultures of antibiotic sensitive *M. tuberculosis* and in the chest x-ray was showing bilateral consolidation. The younger patient also had effusion on the right pleura. Before starting the conventional tuberculosis medication, tea tree oil was inhaled for 10 days (41-year-old) and for 5 days (33-year-old). It was reported that the sputum cultures were no longer positive for *M. tuberculosis* on the fourth and on the fifth day, respectively. The physical symptoms declined also. A chest radiography has shown a clearance of the right pleura effusion in the 33-year-old woman. After that both patients underwent conventional tuberculosis therapy [87].

In 2000 a study examined the effect of tea tree oil on the viability of the wall-less bacterium *Mycoplasma pneumoniae*. Wall-less bacterias commonly known as L-form bacteria, are strains of bacteria that lack cell walls. *Mycoplasma*, that is a parasitic species of bacteria, also lacks a cell wall. But it is not considered to be a real L-form, since it is not derived from bacteria that normally have cell walls. *M. pneumoniae* is a small bacterium and it is a human pathogen causing pneumonia. This form of pneumonia is an atypical bacterial pneumonia. The bacterium is characterized by the absence of a peptidoglycan cell wall. This causes the development of resistance to many antibacterial agents. *M. pneumoniae* is able to imitate the surface composition of host cells and this results to a persistance of the bacteria even after the treatment [88]. The minimum inhibitory concentration was determined at 0.006% (v/v) of tea tree oil for the wild type and 0.003% (v/v) for the mutants of *M. pneumoniae* that lost the host cell adhering ability. The authors advised tea tree oil for mouth washing and also inhalation with tea tree oil in the case of *M. pneumoniae* infection [89].

Anti-inflammatory effect

Inhaled tea tree oil exerts a strong anti-inflammatory effect. The mechanism was explained on stimulated immune system of mice. It was shown that

the hypothalamic-pituitary-adrenal axis settles the effect [90].

Camphor

The fragrant camphor tree (*Cinnamomum camphora*) from the Lauraceae family is native to Asian countries such as Japan, China and Taiwan. It has also been naturalized in other parts of the World. Its products, such as the camphor oil, have a long history of traditional use especially in the East. For instance, Chinese used camphor as a circulatory stimulant and analeptic. Camphor was also used in the 14th century during the Black death as fumigant [91]. Camphor belongs to one of the most well-known and commercially relevant aroma chemicals. Therefore, it has an annual market value of approximately 100 million US$ [92]. It is known that camphor possesses various biological properties such as antimicrobial, antiviral, anti-nociceptive and also antitussive activities. The essential oil is traditionally obtained by distillation of the wood of the camphor tree. Its major component is the active (1*R*)-(+)-camphor. Natural camphor can be found in many essential oils of aromatic plant species such as *Salvia fruticosa* and *Rosmarinus officinalis*. Camphor is a white and waxy solid that possesses a strong aromatic odor. It is a terpenoid ($C_{10}H_{16}O$) and two enantiomer forms are existing: (1*S*)-(-) - and (1*R*)-(+)-camphor. Although they have the same aromatic odor it is still unknown which impact the stereochemistry bears on the biological activity [93]. Synthetic camphor is most of the time synthesized from α-pinene which is obtained from the turpentine oil.

Antitussive activity

Cough is nowadays a very common symptom but many of the current therapies are ineffective. Inhalation with aromatic vapors has been used traditionally for ages, especially in the treatment of upper respiratory tract diseases, because of their known antitussive effect. A study tested the effects of camphor vapor on two parameters: nasal resistance to airflow and nasal sensation of airflow. It was demonstrated that camphor had no impact on nasal resistance to airflow, but a

sensation of improved airflow and a cold sensation were reported. These results of Burrow et al. have shown that the antitussive effect of camphor is due to a stimulation of the cold receptors in the nose [94]. Another study reported the action of camphor on the cough reflex in conscious guinea pigs. Three different concentrations of camphor vapor (50, 133 and 500 mg/L) were tested. At 500 mg/L, camphor reduced the cough frequency significantly (33%). At the same time an increase of latent, asymptomatic cough was noticed [95]. Further studies have shown that camphor activated cold receptors, the minty-cool ion channel (TRPM8). But the mechanism how the activation of TRPM8 inhibits cough is still unclear [96, 97]. Kumar et al. [98] tested the antitussive activity of camphor and camphor lactam in guinea pig in citric-acid induced cough. At the start camphor was used to synthesize camphor lactam. For that it was treated with hydroxylamine-O-sulfonic acid and glacial acetic acid with a Beckmann-like rearrangement in structure. It is interesting that this little change in the structure significantly increased cough latency while it was also reducing cough frequency. Moreover, it was noticed that at the same concentrations (125, 250 and 500 µg/L) camphor lactam provided higher cough inhibitory levels than camphor [98].

However it is important to note that camphor is also a very toxic substance which very often causes poisoning when it is ingested. Reports about the lethal dose exist and it ranks between 50-500 mg per kg bodyweight. Cautious use is very important, especially for kids.

***Carum copticum* essential oil**

C. copticum is commonly known as "Ajwain" and belong to the Apiaceae family. The plant originates from Egypt but grows in different regions in Europe, Asia, especially India and Iran. In the traditional medicine this plant has a wide range of use, in fact Persians have been using it for thousands of years. *C. copticum* possesses an aromatic odor due to thymol. It is widely used as a spice in the curry powder

because of its spicy taste. The therapeutic uses of *C. copticum* include treating of common cold and acute pharingitis, due to the bronchodilatory, antitussive and antidysponea effects of the plant [99]. Since *C. copticum* grows in different areas of the world, the chemical composition of the essential oil varies. Essential oils of *C. copticum* from different areas contain different compounds. Commonly an examination by GC and GC-MS analysis gives the total amount of essential oil and the components. Different studies and cultivations have shown that in general the main components of the essential oil of *C. copticum* are: thymol, carvacrol, p-cymene, γ-terpinene and sometimes also o-cymene, terpinolene and nerolidol [100].

Respiratory Effects

The effect on the respiratory system is one of the therapeutic effects of *C. copticum*. In traditional medicine it is used in asthma and dyspnoea symptoms. *C. copticum* has shown significant relaxant effect on tracheal smooth muscles, but it was reported that the effect was not due to thymol or competitive antagonistic effect on cholinergic receptors. α-Pinene, a constituent of the plant's oil showed anticholinergic activity [101]. Another study has reported about the relaxant effect of different fractions in guinea pig's tracheal smooth muscle. For the preparation of four fractions the essential oil was freezed overnight at 0℃. The white crystals were collected by filtration, air dried, and exposed to NMR analysis. 1 mL of filtrate was chromatographed on a silica gel (70-230 mesh). It was eluted with solvent mixtures of petroleum ether (40-60℃) and chloroform with various concentrations (4:1). 25 mL fractions were collected and fractions were mixed if their TLC (Thin Layer Chromatography) profile was analog. Sulfuric acid (50% v/v) was used for the visualization of the spots. The results revealed that the relaxant effect of the fraction 2 (suggested to be carvacrol) was comparable to the theophylline effect. And fraction 2 has also shown better effects than other fractions. For instance, fraction 3 exerted also a relaxant effect but with less intensity. The results have also ascertained that this relaxant effect of fraction 2 and 3 was not the result of inhibition on muscarinic- or stimulation of β-adrenergic receptors [102]. Another study has also shown that carvacrol, one of the main constituent of *C. copticum,* exerted a considerable relaxant effect on tracheal smooth muscle of guinea pigs. This relaxant

effect was even greater than the effect of theophylline [103].

An evaluation of the bronchodilatory effect of *C. copticum* seeds extract was made on guinea pig trachea in presence of high K^+ (50 mM) and carbachol. The results revealed a dose-dependent relaxation with *C. copticum* doses from 0.1 to 1 mg/mL. It was also demonstrated that a possible mechanism is a blocking effect on the calcium channel [104].

The relaxant effects on tracheal smooth muscle were also demonstrated for other plants containing carvacrol, *C. carvi* among others [106]. Therefore, carvacrol, the main constituent of *C. carvi* may exert a relaxant effect on the tracheal smooth muscle.

One study examined the possible mechanisms that cause this relaxant effect of carvacrol on tracheal smooth muscle. For this its effect on histamine receptors was tested in trachea smooth muscle in guinea pigs. EC_{50} histamine (effective concentration of histamine causing 50% of maximum response) was measured in presence of carvacrol and chlorpheniramine. The results have shown that carvacrol is a competitive antagonist towards H1 histamine receptors. In addition, a stimulation of β-adrenergic receptors and also a blocking effect of muscarinic receptors were reported [106]. In fact, this stimulatory effect of carvacrol on β2-adrenoceptors was proved in a further study. Measurements of EC_{50} and isoprenaline (which is a $\beta_{1/2}$-Agonist, used in the past in the treatment of asthma) concentration response curve were taken into evaluation. These two were performed in presence of carvacrol, propranolol and saline, on tracheal smooth muscle of guinea pigs. The first group of guinea pig was incubated with chlopheniramine, in order to block H1 histamine receptors, whereas the other group was not. A reduction of EC_{50} levels was observed in presence of carvacrol. In presence of propranolol, EC_{50} levels were higher, compared to the ones of saline. Moreover, the results have shown a parallel leftward shift of isoprenaline concentration response curve [107]. These results indicated that carvacrol possesses a stimulatory effect on β_2-adrenoceptors.

Inhibition of muscarinic receptors is another possible mechanism for the relaxant effect of carvacrol on the tracheal smooth muscle. This was proven by performing a metacholine-response curve and a measurement of EC_{50} in presence

of different concentrations of carvacrol compared with saline. The results have shown both a rightward shift in the metacholine-response curve and increased EC_{50} levels. This suggested that carvacrol may have competitive antagonistic effects on muscarinic receptors [108]. According to these results the relaxant sensation felt is due to the mechanism of inhibitory effects on muscarinic and histamine receptors and stimulatory effects on β_2-adrenoceptors or a combination of the three mechanisms.

Even though carvacrol shows potent relaxant effects on tracheal smooth muscle it does not show an antitussive effect. A review tested the antitussive effect of aerosols of two different concentrations of carvacrol, codeine, saline and aqueous and macerated extracts. Animals were exposed to these aerosols of different solutions and 10 minutes later the number of citric-acid induced cough was enumerated. The results revealed comparable results for aqueous and macerated extracts and codein. But carvacrol, one of the constituent of *C. copticum* that possesses bronchodilatory effects, did not exert an antitussive effect. This implies that cough and bronchoconstriction have a different afferent neural route [109].

Several studies also examined the effects of *C. copticum* and carvacrol in inflammatory respiratory diseases such as asthma. One study made a comparison between bronchodilatory effects of boiled extract from *C. copticum* (oral intake) and theophylline in asthmatic patients. These two different drugs were given and 15 minutes later various pulmonary function tests were addressed. The test measurements continued for 180 minutes after the drug administration. *C. copticum* has proven to exert a bronchodilatory effect in the asthmatic airways. At the same concentrations this bronchodilatory effect was even comparable to the effect of theophylline. This study documented that *C. copticum* could be very interesting as a bronchodilator in the therapy of obstructive airway disease [110].

Other studies examined the anti-inflammatory and the immunomodulatory effects of carvacrol. In fact an inhibition of TNF-α, IL-1β and TGF-β was demonstrated in one trial. In that study the effect of carvacrol was tested on a cell culture of macrophages induced in porcine alveolar inflammation [111]. Carvacrol

also inhibited secretion of TNF-α and IL-1β in porcine alveolar macrophage [112]. Anti-inflammatory effects of carvacrol were also revealed with inhibitory effects on COX-1 and COX-2 and 5-lipoxygenase. Exudates volume and leukocyte migration in plural cavity were measured *in vivo* and *in vitro*. Carrageenan was injected in the cavity, causing exudation and migration. The results have shown a preventive effect of carvacrol on exudates volume and leukocytes migration [113].

Another main constituent of *C. copticum* is thymol which possesses antispasmodic properties and bears an impact on ciliary motion. One review demonstrated the effect of thymol on tracheal and ileum smooth muscles and ciliary motion in the respiratory tract of rats. The results revealed a dose-dependent spasmolytic effect of thymol and the mucus transfer increased due to stimulatory effects on ciliary clearance and ciliary motion [114]. Thyme extract has demonstrated antispasmodic effects as well. It was suggested that phenolic oil compounds such as thymol could be the reason for this effect in thyme extract [115].

Lemongrass and Peppermint essential oils

Antifungal activity

Al Yousef [116] reported the antifungal activity of volatile compounds from Lemongrass and Peppermint oils. These oils were tested against some species of *Aspergillus* that cause respiratory pathologies. Nowadays, the use of essential oils in the treatment of fungal infections has risen. Volatiles from essential oils have gained importance due to various resistances that the strains acquire against certain drugs [116].

Lemongrass (*Cymbopogon citratus*), belongs to the grass family and is native to Asia, Africa and Australia. Their smell as well as their flavor resembles to lemon and they are commonly cultivated as culinary (Asia) and medicinal (India) herbs. Biological activities such as antibacterial and antifungal are attributed to

lemongrass [117-119], as well as analgesic and anti-inflammatory activities [120].

Peppermint (*Mentha* x *piperita*) is a hybrid mint. It is a cross between watermint and spearmint. The plant is cultivated worldwide. Peppermint is the oldest and most popular flavor of mint-flavored products. Its main constituent menthol is known for activating the TRPM8 cold receptors in the skin and mucosa. This mechanism is the source of the cooling sensation of peppermint oil [7-9]. Peppermint oil and peppermint leaf have been used as antispasmodic in gastrointestinal tract problems and in the treatment of irritable bowel syndrome. Other medicinal properties such as carminative, cholagogue, antibacterial and secretolytic are attributed to the plant. Peppermint has not only shown great results in the treatment of gastrointestinal tract diseases, but also in the treatment of catarrh of the respiratory tract and inflammation of the oral mucosa [121].

An inhalation of spores of the fungus *Aspergillus fumigatus* can cause aspergillosis. Once the spores have reached the lungs they form a knotted mass of fungus fibers and blood clumps. The expansion of fungus increases continuously and results to a destruction of lung tissue, but they do not always expand to other body parts [118]. The majority of clinical antibiotics were used to cure this infection. But due to their toxicity, drug-drug interaction, low fungicidal efficacy, cost and arising of resistance strains (caused by frequent use), there is a big necessity of novel anti-fungal substances, especially ones with higher efficiency and lower toxicity, compared to the ones currently on the market [122].

Aspergilus spp. (*A. flavus*, *A. niger* and *A. fumigatus*) were isolated from the deep sputum pulmonary of the most severe tuberculosis patients. Lemongrass and peppermint leaves were distilled for 2.5 hours. Later the oils were separated and dried over anhydrous sodium sulfate. Then they were analyzed with the help of (GC/MS). Their effects on mycelia growth, spore germination and fungal morphology were tested. Analysis has demonstrated that citral is the main constituent in lemongrass oil (70.17%) and menthone plus menthol in peppermint oil (total 52.96%). Citral, or lemonal, with the molecular formula $C_{10}H_{16}0$, is a mixture of two isomeric acyclic monoterpene aldehydes.The *E*-isomer is geranial or citral A, while the *Z*-isomer is neral or citral B [123]. Besides citral, myrcene was

also found in lower concentration in lemongrass oil. In peppermint oil, 1,8-cineole, isomenthone and methyleacetate were found in lower concentrations [116].

The essential oils and their constituents were then assessed for antifungal activity. The antifungal activity against 7 days old cultures of *Aspergilus* ssp. was tested at doses of 5, 10, 15 and 20 μL/0,4L air space by the inverted Petri dish method. After an incubation of 5 days at 30℃, a linear growth of the mycelium was measured and expressed as average values (mm). Results have shown various antifungal effects for each essential oil. For example lemongrass oil revealed high antifungal activity at 5 μL/0.4L air space against *A. niger* and *A. fumigatus*. Lemongrass oil volatiles (at 15 μL/0.4L air space) inhibited *A. flavus* only moderately. Concerning peppermint oil, a very weak suppressing activity towards *A. niger* and *A. flavus* was reported (15 and 20 μL/0,4L air space, respectively). And *A. fumigatus* was continuously growing at a concentration 20 μL/0.4L air space [116].

In order to test the activity on fungal spore germination, 300 μL of spore suspension (from 7 day old cultures) were spread on glass slides and incubated with essential oils at 30℃ for 24 hours. After the incubation, every glass slide was fixed with lacto-phenol-cotton blue statin. The spore germination was observed under the light microscope. Since spores of *A. niger* and *A. fumigatus* were completely inhibited in presence of lemongrass oil at 10 μL/0.4L air space and since spores of *A. flavus* lost their activity when exposed to lemongrass oil at 15 μL/0.4L air space, this oil proved to be very powerful against spore germination. Whereas peppermint oil volatiles proved to be weak against spore germination of spores of *A. flavus* and *A. fumigatus* at 20 μL/0.4L air space. The same concentration resulted in complete inhibition of *A. niger* spore germination [116].

These results suggest that the degree of antifungal activity is varying according to the function tested of the essential oil. The maximum antimycotic activity was exhibited by lemongrass followed by peppermint. These two plants inhibited both, the fungal mycelium and spore germination. Previous studies reported that volatile aromatic plants exhibit stronger antimicrobial activity in comparison to nonaromatic ones [124]. In this study lemongrass and peppermint

prove that idea [116].

MIC and MLC of volatiles of lemongrass, peppermint oils and their main constituents were demonstrated using a two-fold series. The plates were inoculated and then incubated for 5 days at 30℃. The lowest concentration which inhibited fungal growth was recorded as MIC. The fungistatic and fungicidal (lethal) activity of the essential oils was determined by transferring the fungal disc from the treated plates, where no growth was observed, to new plates without essential oils. These plates were then incubated for 10 days at 30℃. Fungi with absence of mycelial growth were considered to have shown a fungistatic answer to the essential oils. Finally MLC represented by the lowest essential oil concentration which allows for no fungal re-growth. The lowest MIC and MLC values for lemongrass were reported against *A. niger* and *fumigatus*. Peppermint oil has shown intermediate inhibitory effect against *A. niger*. As to the constituents of the essential oils, *A. niger* proved to be more susceptible towards citral and citral+myrcene mixture than *A. flavus* and *A. fumigatus*. Myrcene alone did not show any effect against *Aspergillus* spp. The mixture menthone+menthol (both components of peppermint oil) exhibited the highest MIC values for all the tested *Aspergillus* strains. The same results were noticed for menthone alone, even if *A. niger* was more sensitive to menthol, when compared to *A. flavus* and *A. fumigatus* [116].

Al Youssef [116] has reported that in general, crude oils exhibit greater fungal inhibiting activity than the separated compounds. In fact, citral, the major constituent of lemongrass oil, has shown great antifungal activity. On the contrary, myrcene did not exert any activity. Moreover, a mixture of these two showed low MIC values (lower than citral alone), suggesting that the mixture's efficacy is due to synergistic effects. These results suggested that a single component of lemongrass, citral, could substitute the whole oil at the same concentration level. Although myrcene does not possess any antifungal activity it enhances the activity of citral, and the dose level can be reduced [116]. Several other studies demonstrated synergistic effects of compounds in essential oils [125,126]. Another study published in 2010, has reported similar results. The whole essential oil possesses higher activity than each separated compounds [127]. Silva et al. and Saddiq and

Suzan [128,129] have shown that lemongrass, as well as citral, exhibit effectively antimicrobial activity. A parallel was also made between lemongrass antimicrobial potential and citral concentration [130]. Peppermint oil was also shown to be fungistatic [116]. Further studies reported the same results for peppermint [131].

Fungal morphology changes were examined under the light microscope. After treatment with lemongrass oil volatiles some morphological transformations (decreased sporulation, less pigmentation, reduction of conidiophores) were noticed in *A. niger* [116]. This fungicidal effect of lemongrass was also reported in earlier studies [132,133].

In conclusion, the present study suggests that the two presented oils could be used for the treatment of respiratory pathologies caused by fungi. Nevertheless, further examinations are needed for their applicability and possible toxicity especially *in vivo* [116].

Silver Fir essential oil

Antibacterial and antioxidant

Abies alba, the European Silver Fir is native to the mountains of Europe. It is a large evergreen tree growing up to 40 or 50 meters high. Its essential oil is known for a distinctive and refreshing pine-forest fragrance. The interest for this essential oil keeps growing because it is known to help in respiratory pathologies. Interesting are especially its ability of soothing effect for muscle. A study investigated the chemical composition, antibacterial and antioxidant activities of a commercial silver fir essential oil. The major components of the silver fir were identified using GC-MS methods. In total 20 significant peaks were reported. Bornyl acetate was dominating (30.31%) followed by camphene, 3-carene, tricyclene, dl-limonene, α-pinene, caryophyllene, β-phellandrene and borneol. The results revealed very poor antibacterial activity, among the 6 tested strains (*S. aureus*, *S. mutans*, *Listeria*

monocytogenes, *Acinetobacter baumannii*, *E-coli* and *Vibrio parahaemolyticcus*) the essential oil exhibited only mild activity against *S. aureus*. Whereas the essential oil possesses strong antiradical activities against DPPH and ABTS radicals. It was reported that the essential oil was able to reduce both radicals dose-dependently and it was also noticed that less concentration was required for a 50% reduction (RC_{50}) against DPPH radicals than for ABTS radicals [134].

Lippia sidoides **essential oil**

L. sidoides is a bush from the family of Verbenaceae. The plant is native to Brazil, where it is popularly known as "alecrim pimenta". *L. sidoides* is used in traditional medicine topically on the skin and mucous membrane as an antiseptic. This effect is due to the thymol presence. Previous studies reported about the well-known antimicrobial activity of the plant. The essential oil's activity was tested against two bacterial strains: *Candida albicans* and *S. mutans* [135,136]. Two further studies have demonstrated anti-inflammatory, antioxidant and gastroprotective effects [137,139]. The plant's main constituent, thymol, has shown very good antimicrobial activity [139]. Therefore, the therapeutic effect of *L. sidoides* is related to the presence of thymol but also the direct contact between the essential oil with the microorganism.

In microbiological investigations, the most common bacteria that are isolated from the sputum samples are: *S. aureus* and *P. aeruginosa* [140,141]. And these two strains usually colonize or infect the upper respiratory tract [142]. But several articles reported about the frequent resistance to commonly used antibiotics for these two bacterial species [143,144]. With the raise of bacterial strains resistant to antibiotics there is a need for new antimicrobial agents or supplements that would affect the current antibiotics therapies. There are indications that essential oils can lower microbial resistance if implemented as a therapy. The heterogeneity and complexity of the components of the oil

may make it increasingly difficult for the microorganism to adapt to the different work mechanisms the individual substance may have. So, essential oils may be a potential source of novel drugs that fight against the development of microbial resistance [145].

The chemical composition of *L. sidoides* fresh leaves essential oil was obtained by hydrodistillation. In total 7 compounds were identified by GC-MS analysis. Thymol (84.9%), ethyl-methyl-carvacrol (5.33%) and p-cymene (3.01%) were reported as main components of the essential oil. The concentration of thymol can vary though.

Antibacterial activity

The antibacterial activity of essential oil from *L. sidoides* essential oil and thymol was examined using gaseous contact. The results have shown that *S. aureus* is more susceptible to the essential oil. Previous studies have already reported the antibacterial activity against *S. aureus* of *L. sidoides* essential oil and thymol by direct contact method [146,147]. Other studies demonstrated that the antibacterial activity is related to the presence of small terpenoids and of phenolic compounds, such as thymol, carvone, carvacrol, menthol and muurolene. These components possess also antifungal activity [147,148]. The results of this test correspond to previous reports indicating that Gram-negative bacteria are more resistant to essential oils than Gram-positive [149,150].

Moreover, the antibiotic activity of gentamycin against *S. aureus* has been enhaced in the presence of thymol and the essential oil of *L. sidoides*. Gentamycin is an antibiotic that belongs to the aminoglycoside group. These antibiotics exhibit antibacterial activity against Gram-negative strains and generally not against Gram-positive bacterias. The enhancement was demonstrated with essential oils, in fact a considerable increase of antibiotic activity of gentamycin at different concentrations was observed. An enhancement was also observed when neomycin and amikacin were combined with volatiles of the essential oil. Even though results have shown antibiotic activity as well as an

enhancement of antibiotic activity (of amikacin and neomycin) against *S. aureus* in presence of thymol. When the results of thymol were compared to the results of the essential oil, it was observed that thymol was less effective against *S. aureus*. This suggests that other compounds present in the essential oil are also important for the antimicrobial activity [151].

When it comes to the tests against *P. aeruginosa*, the results did not reveal any differences in the antibiotic activity of the volatile constituents of the essential oil and thymol. An enhancement of antibiotic activity of all antibiotics was noticed for both the essential oil and thymol [151].

Different mechanisms of interaction can be noticed between essential oils and antibiotics, and these can involve the bacterial membrane for example but also the composition of the essential oil is very important. In fact other compounds found in the essential oil such as carvacrol, p-cymene, β-caryophyllene and 1,8-cineole also show antimicrobial activity. These compounds may act synergistically and therefore enhance the antimicrobial activity of the essential oil [151].

***Origanum vulgare* essential oil**

Antibacterial

It is interesting to mention the essential oil of *O. vulgare*, since it contains also thymol and carvacrol as main components. Due to their effect against the plasmic membrane, it is believed that thymol and carvacrol could cause disruption in the physical structure of the cell. The effects against the plasmic membrane are mechanisms such as: modifying the permeability of the membrane, denaturing its essential enzymes, changing the pH and electric potential. The last two mechanisms modify the proton motive forces and cause the enhancement of the antibiotics intake [152,153]. Carvacrol and thymol can also modify the activity of the calcium

channels and stimulate the expulsion of other important ions [154].

The direct or gaseous combination of compounds and essential oils with the microorganisms might also trigger impairment of bacterial energy systems [155,156]. Gram-negative bacteria possess an additional membrane that consists of lipopolysacharide (LPS). This provides a hydrophilic surface the main function of which is a permeability barrier for several hydrophobic agents [157,158]. Thymol is a potential membrane permeabilizer and could interact and disrupt the anionic LPS, this action could sensitize the bacteria to antibiotics [153,159]. Therefore, thymol has shown that it may suppress the growth of bacterial pathogens of respiratory tract infections, such as cystic fibrosis, and they can be auxiliary in the treatment of these diseases.

Pistacia integerrima **essential oil**

P. integerrima is a plant from the family Anacardiaceae. The species is native to Asia and is a very important medicinal plant in India, where it is known as Karkatashringi. Its galls have been valued in India in the traditional medicine for the treatment of various respiratory tract diseases, such as asthma, chronic bronchitis, phtisis and others. The essential oil has been reported to exhibit antibacterial, analgesic and anti-inflammatory activities among other activities (antispasmodic, carminative, antihelmintic) [160-163]. From the studies and reports above mentioned, it can be seen that the essential oil may have an effect on inflammatory conditions of bronchial asthma. This would explain the traditional medicinal use in hyperactivity of gut and airways disorder. But the ethnopharmacological uses are yet to be validated scientifically for the rational and safe use of the plant as well as its essential oil.

Asthma is characterized by airway hyper responsiveness which are the result of the release of granular mediators (such as histamine), newly synthesized mediators (such as leukotrienes, prostaglandins and platelet activating factor), and

cytokines (such as interleukins and tumor necrosis factor). In inflammatory cells, for example mast cells, eosinophils, macrophages, T lymphocytes and structural cells, the so called phosphodiesterase IV (PDE IV) enzyme is predominating. The PDE IV affects cellular signaling by degrading cyclic nucleotides that are important messengers. It also plays an important role in homeostasis [164,165]. Nowadays the therapy consists of a dual treatment: reliever therapy that aims a quick symptom relief and also a controller therapy that attacks the inflammatory component of asthma [166]. Establishing the appropriate asthma therapy has proven to be very difficult, because of the complexity of the disease process that involves various mediators' activities [167].

The essential oil of *P. integerrima* was obtained by hydrodistillation from 500 g dried and powdered galls. It is characterized by a colorless liquid, terebinthine odor and astringent taste. The chemical composition of the essential oil was determined by GC-MS and revealed that 4-carvomenthenol, levo-bornyl-actate, L-terpinen-4-ol, tetrahydrocarvone, borneol and (-)-spathulenol are the main compounds in the essential oil of *P. integerrima* [164].

Antioxidant activity

ROS are produced in the airways of an asthmatic person, these reactive oxygen species activate eosinophils, neutrophils, monocytes, and macrophages to produce superoxides (O_3^-). ROS also amplify the inflammatory response by activating NF-κB [168]. Shirole et al. [164] investigated *in vitro* the antioxidant activity of the essential oil using DPPH-scavenging assay. The results have shown a concentration dependent antioxidant activity. Significant results were reported at 10-100 μg/mL. But it is believed that the antioxidant potential is due to high polyphenolic contents [164]. Previous studies have also reported about the antispasmodic, antiasthmatic and anti-inflammatory activity of polyphenolic compounds [169].

Antiallergic activity

The main actors in type I hypersensitivity and allergic reactions are mast cells and basophils which are activated through IgE by specific antigens, this is followed by the release of various pro-inflammatory mediators such as leukotrienes, histamine and cytokines [170]. These mediators induce a very quick vascular permeability, leading to plasma extravasations, tissue edema, bronchoconstriction, mucus overproduction and leukocyte recruitment [171] Shirole et al. [164], investigated the antiallergic activity of *P. integerrima* by using *in vitro* mast cell degranulation assays. The effect of the essential oil on the compound 48/80- induced histamine release mast cells was tested. Compound 48/80 is a ionophore calcium channel opener, which can activate mast cell secretion. This action is associated with an influx of Ca^{2+} into the cell [174]. The essential oil of *P. integgerima* (33.33 µg/mL) showed 80.92% inhibition of compound 48/80 and therefore blocked mast cell secretion. A pretreatment with the essential oil reduced significantly compound 48/80 induced mast cell degranulation [164].

Spasmolytic activity

The effect of *P. integgerima* essential oil on histamine, acetylcholine (ACh) and KCl induced contraction of isolated guinea pig ileum was also investigated by Shirole et al. [164]. Previous studies only reported about the antihistaminic effect of the aqueous extract of the plant [173]. Due to inhibition of histamine and ACh induced contractions in guinea pig ileum, the essential oil of *P. integgerima* shows a very promising spasmolytic activity [164]. Smooth muscle contractions induced by Ach are mediated by a release of intracellular Ca^{2+} from the sarcoplasmatic reticulum and by a Ca^{2+} entry along voltage dependent and independent mechanisms [174]. The L-typ (Ca_v-L) and $Ca_v1.2$ are the amply expressed voltage gated calcium channels in the guinea pig ileum. In order to evaluate if the Ca_v channel was involved in the response of the essential oil, the effect of this essential oil on the guinea pig ileum pretreated with S-(-) Bay 8644 was tested. S-(-)-Bay 8644 is an L-typ agonist

that does not act by depolarization but by binding directly on the channel's α-subunit [175]. Under these conditions, the results revealed that *P. integgerima* essential oil induced a concentration dependent relaxation (EC_{50}=100 µg/mL). This suggested that the voltage gated L-subtyp calcium channel is involved in the essential oil's spasmolytic activity.

Angiogenesis

In western countries, high rates of angiogenesis and neovascularization are very important in the pathogenesis of many chronic inflammatory disease including asthma. Anti-angiogenic is a rising approach for treatment and prevention of chronic diseases [176]. The increase of vascularity in the bronchial mucosa of asthmatic patients has been a well characterized aspect of asthmatic airways. The inhalation of glucocorticoids leads to decreasing of airway vascularity and attenuating of increased blood flow [177]. The essential oil of *P. integgerima* posseses inhibitory activity when it comes to erythropoietin induced angiogenesis in Legghorn eggs. Erythropoietin group has shown a significant increase in blood vessel formation. An angiogenic response occurs within 72-96 h after stimulation. Around the region of the angiogenesis inducer such as erythropoietin, increased vessel density can be noticed. The drug treated and heparin treated group have demonstrated significant reduction in the number of blood vessels formation. The angiostatic activity of the drug treated group and the heparin group was comparable [164].

Anti-inflammatory activity

P. integgerima essential oil's anti-inflammatory activity was evaluated *in vivo* in LPS induced acute lung inflammation (neutrophilia). By a receptor mediated process, LPS triggers the activation of phagocytes. This results in the release of cytokines, including TNF-α which has been found to play a big role in the initiation, maintenance and progression of airway inflammation in asthma. Also, it induces increased adherence of neutrophils to endothelial cell, which causes large infiltration in the pulmonary space [178-180]. Shirole et al. [164]

reported that intratracheal instillation of LPS increases epithelial and endothelial permeability, influx of protein and albumin, white blood cell (WBC) migration, myeloperoxidas (MPO) activity and nitrate/nitrite levels. The essential oil ameliorated the LPS induced WBCs migraton, MPO activity and excessive production of pro-inflammatory mediators suggesting that the essential oil from *P. integgerima* plays a protective role in bronchial asthma.
Lefort et al. [183] reported that intraperitoneal injection of bacterial LPS can develop acute lung injury. This development can be measured by albumin extravasation or neutrophils myeloperoxidase activity in the lung parenchyma. The *in vivo* tests by Shirole et al. revealed that the administration of LPS intra-tracheal in female rats induced an invasion with neutrophils and an increased myeloperoxidase activity of the airway lumen. Already infiltrating neutrophils were also activated after the LPS administartion as it has been proven by the increased levels of MPO activity. It was noticed that the essential oil attenuated LPS induced neutrophilia in rats. In fact, the essential oil of *P. integgerima* inhibited leukocyte infiltration as a measure of total cell count in bronchoalveolar lavage (BAL) fluid. In the BAL fluid of rats that were treated with the essential oil, a significant decrease was seen on albumin levels. The reduction of leukocyte infiltration was related with the significant decrease in the neutrophil count and the reduction of the MOP activity in the BAL fluid [164].

Endogenous NO is produced by the inducible NO synthase (iNOS) and is very well known for its possible role in inducing asthma and other airway inflammation diseases by promoting the chemotaxis of inflammatory cells in lungs. NO levels in the BAL fluid of treated animal were found to be significantly lower than LPS control. This demonstrated the inhibitory effect of the essential oil on NO in LPS induced lungs inflammation in rats. The results suggest that NO inhibitors suppress airway inflammation by inhibiting inflammatory cells and mucus secretion in the lungs [164].

The major constituents found in the essential oil of *P. integgerima*, such as p-cymene, borneole, tetrahydrocarvone, 4-carvomenthenol, α-terpinenol, α-terpinene and β-caryophyllene, levobornyl acetate, may

contribute to the anti-asthmatic activity. It has been reported that β-caryophyllene reduces LPS-induced NF-κB activation and neutrophil migration in rat paws [182]. In LPS-activated cells, as macrophages, it is known that NF-κB regulates the expression of iNOS, TNF-α and interleukin. Another study reported about the anti-inflammatory activity of p-cymene in acute lung injury in mice. In this study intraperitoneal administration of p-cymene in LPS-induced lung injury, inhibited pro-inflammatory cytokins (TNF-α, IL-6, IL-1β) and mitogen-activated protein kinases (MAPK) signaling pathway [183]. *In vivo* studies on mices have shown that bornyl salicylate reduced neutrophil migration, cytokine release induced by zymosan, fluid leakage induced by acetic acid and NO production in makrophages. [184].

Effect against airway hyperresponsivness

The essential oil of *P. integgerima* was tested against airway hyperresponsiveness in guinea pigs. It has been noticed that the essential oil offers a significant protection against ovalbumin induced bronchoconstriction. This ovalbumin induced bronchoconstriction is dependent on IgE/IgG and leads to mast cell degranulation and severe bronchoconstriction when ovalbumin is inhaled [164].

In conclusion, rats treated with LPS show severe infiltration of inflammatory cells in the lungs and edema. Hyperplasia bronchial due to proliferation of fibrous connective tissue can be noticed. The essential oil from *P. integgerima* dose dependently reduced all the above mentioned inflammatory changes. This supports the hypothesis of its protective properties in bronchial asthma.. Also the pretreatment with essential oil ameliorated the condition of asthma.This anti-asthmatic activity is probably due to the rich content of terpenoids [164].

Citrus hystrix essential oil

C. hystrix is a plant from the family of Rutaceae native to Southeast Asia. It is a tropical herb commonly known as makrut lime. The valued parts of the plant are the leaves and the fruit peel. Makrut lime is very often used as ingredient in Asian cuisines. Two different essential oils can be extracted from makrut lime, the leaf oil and the fruit peel oil (makrut oil). Makrut lime has been reported in the past to be effective against 20 types of *Salmonella* and 5 species of other enterobacteria [185].

The gas chromatography study revealed that the predominant component was citronellal (80.04%) in makrut leaf oil, whereas in makrut oil the main components were limonene (40.65%), terpinen-4-ol (13.71%) and α-terpineol (13.20%) [186].

Antibacterial activity

The antibacterial activities were tested by disc-diffusion and broth microdilution methods against 411 isolates of groups A, B, C, F, G streptococci, *S. pneumoniae, M. catarrhalis*, *H. influenzae*, *S. aureus* (methicillin-resistant and -sensitive *S. aureus*) and *A. baumannii*, obtained from patients with respiratory tract infections. The results have shown very good activity for both makrut leaf oil and makrut oil, agianst several respiratory bacteria. The MIC and MBC for both oils were lowest against *M. catarrhalis* and *H. influenzae*, followed by *S. pneumoniae*, *Streptococcus* spp., *A. baumannii*, MSSA and MRSA, respectively. The results are very interesting because it was found that group A streptococci, the most common bacteria causing sore throat, was very sensitive to makrut lime essential oils [186].

The pure major lipid components were chosen to investigate the active components responsible for the antibacterial activity. Citronellal, the major component in makrut leaf oil, was found to be very effective against *Streptococcus* spp., *A. baumannii*, MSSA and MRSA, even more effective than

the whole makut leaf oil. The same counts for α-terpineol and terpinen-4-ol, they were found to be more effective against *Streptococcus* spp., *A. baumannii* and *H. influenzae* than makrut lime oil. The obtained results ascertain that α-terpineol is indeed the most antibacterially effective component of the given oil, although it makes only 13.2% of it. Limonene, as the predominant component, shows very little antibacterial activity [186].

This study also revealed that all multi-drug resistant bacteria, such as *A. baumannii* and MRSA were very sensitive to makrut leaf oil and makrut oil. More than 80% of *A. baumannii* used in the study were found to be resistant to the common drugs tested in laboratory (aminoglycosides, ampicillin, cephalosporins, carbapenems), they were only sensitive to colistin which is highly nephro- and neurotoxic. MRSA was resistant to most drugs available. This increasing incidence of multi-drug resistant bacteria emphasized the need for effective novel alternative drugs [186].

Nepeta cataria **essential oil**

N. cataria is a plant from the family of Lamiaceae, it can be found in the Mediterranean coast and Asia. It is commonly known as Catmint. The plant has a characteristic lemony mint flavour, therefore it is used in herbal teas and also in cooking. It has been reported that the plant can be used medicinally in gastrointestinal and respiratory hyperactive disorders such as colic, diarrhoea, cough, asthma and bronchitis. The chemical composition varies from region to region, variety, climatic condition and other factors. The main constituents identified so far are: β-caryophyllene, caryophyllene oxide, 1,8-cineole, citronellol, geraniol, elemol, nerol, nerolidol, spathulenol, β-elemene, geranyl acetate, citronellyl acetate and geranial [187]. The major components found in the essential oil obtained from *N. cataria* of Pakistan were 1,8-cineole (21.00%), α-humulene (14.44%), α-pinene (10.43%) and geranyl acetate (8.21%) [188].

Myorelaxant activity and antispasmodic effect

Phosphodiesterase (PDE) inhibitors and calcium channel blockers (CCB) have been used for several years in respiratory disorders, especially in the treatment of asthma and cough. There is a possible presence of both constituents in the essential oil of *N. cataria, which* is why the myorelaxant effect of the essential oil was tested on guinea pig tracheal preparations. The results have shown that the essential oil of *N. cataria*, papaverine and verapamil, caused an inhibition of the calcium channel and of high kalium-induced contractions, suggesting non-specific tracheal relaxation. Papaverine is an antispasmodic drug. It has been demonstrated that the mechanism relays on a dual activity, including a calcium channel blocking and an inhibition of PDE [189,190]. Verapamil is a standard calcium channel blocker. When the inhibition effect of the essential oil was compared to papaverine, the potency turned to be very similar for both spasmolytics. Whereas verapamil was significantly more potent against the high K^+, as expected from Ca^{2+} antagonist, indicating that the essential oil possesses papaverine-like relaxant components, the PDE inhibition. The PDE inhibitory effect was also confirmed when the pretreatment with the essential oil of *N. cataria* increased the isoprenaline-induced inhibition of calcium retention capacity, constructed on calcium channel induced contractions. This effect is similar to papaverine, while verapamil did not show such an effect. These results confirmed that the essential oil mediates the myorelaxant effect by means of dual inhibition of PDE and Ca^{2+} channels. Moreover, it is well-known that PDE inhibitors can cause stimulatory side effects on the heart, CCB on the other hand can cause relaxant effects. But the essential oil did not exert such side effects at doses required for the potential indication [188].

Agastache mexicana ssp. mexicana essential oil

A. mexicana ssp. *mexicana* is native to Mexico. This plant from the Lamiaceae family is populary known as 'toronjil morado'. Methyl chavicol, linalool and D-limonene have been reported as major components of its essential oil [189]. Various uses of the medicinal plant have been reported, such as anti-inflammatory [190], antioxidant [191], spasmolytic [192] and tracheal relaxant [193]. Even though the plant is widely used in traditional medicine as a remedy to various respiratory disorders, there is only one study about the relaxant effect of extracts in rat trachea [193].

Navarrete et al. [194] investigated the relaxant effect of essential oil of *A. mexicana* in guinea pig isolated trachea rings. The essential oil was characterized by the presence of estragole (80.28%), D-limonene (17.56%) and linalyl anthranilate (2.16%). The study clearly demonstrated that the essential oil caused relaxation of contractions induced by carbachol and histamine in a concentration dependent manner. It is believed that this effect is mainly due to the presence of estragole and D-limonene, the major components of the essential oil of this plant. In order to understand the mechanism of the relaxant effect of the essential oil on guinea pig tracheal muscle further investigation about the downstream signaling was evaluated. The results have shown that the adenylyl cyclase, the K_{ATP} channel and the activation of β-adrenergic receptors were not involved in the relaxant effect on smooth muscle of trachea. But the essential oil was proven to be able to block the contractions induced by carbachol, histamine and calcium, suggesting that its action mechanism involves the blockade of calcium influx.

Herbal preparations

In a randomized double-blind trial, the effect on URTI symptoms (Upper respiratory tract infections) of an herbal preparation containing aromatic essential oils of five plants (*E. citriodora, E. globulus, M. piperita, Origanum syriacum,* and *R. officinalis*) was compared with a placebo spray. The herbal preparation was applied 5 times per day for 3 days. Patients reported a significant decrease in URTI symptoms 20 minutes after administration. Patients with severe symptoms reported an even more significant difference between the herbal preparation spray and the placebo group. An improvement was noticed after 3 days but no statistical difference between the herbal and the placebo group was detected. This suggested that the preparation possesses a local rather than systemic effect on the upper respiratory tract. The fast improvement within 20 minutes after application of the herbal spray could be explained by an anti-inflammatory and analgesic effect [195]. In fact, previous studies confirmed that eucalyptus [61] and mint oil [196] possess these effects. The antitussive effect of menthol and camphor [81] and the bronchodilatory effect of carvacrol [100-103] may also explain the fast relief of symptoms. This study has several limitations, but all in all it shows the advantages of aromatic preparations and that plant extract could be novel potential sources of antimicrobial agents. Therapies including herbal preparations may reduce prescribing antibiotics in cases when there is no indication, such as viral infections [195].

Conclusion

Aromatic plants have a centuries long tradition of being exploited as resources in traditional ethnomedicine. Their various pharmaceutical properties such as analgesic, diuretic, expectorant, spasmolytic, antioxidant, sedative and their potential health benefits were used since ancient times by traditional healers. This is why nowadays considerable attention has been given to the discovery of novel drugs capable of preventing and treating respiratory pathologies, especially those from plant origin. The important number of natural products that have been introduced into the market in the last decade prove the positive health effects.

Essential oils and their constituents, as important plant-derived products are a promising source of novel, alternative and natural drug. Their use is increasing in the contemporary medicine due to their pharmacological properties and so is the interest of scientists, who are running various investigations in order to get further informations about the uses and applications of essential oils. The aim of this work was to review the biological activities, as well as the mechanisms of actions of various essential oils in respiratory pathologies. It is important to mention that the various activities attributed to the different essential oils and their compounds cannot all be explained pharmacologically. But many of them possess activities comparable to synthetic drugs. Studies have revealed an important interest for eucalyptus oil and its major component 1,8-cineole. Taking in account all the results, eucalyptus essential oil is reported useful in many circumstances, particularly in respiratory problems including asthma, bronchitis and COPD. Eucalyptus oil and 1,8-cineole possess various effects, such as antimicrobial, anti-inflammatory, immunomodulatory, antioxidant, analgesic and spasmolytic ones. However, these activities and the potency varied significantly depending on the species and constituent composition. In general, these activities were not always related to a high content of the major compound 1,8-cineole, but also to the presence of other minor compounds, suggesting a synergistic activity. If one compares the antimicrobial activity of eucalyptus oil with other essential oils it might not always possess the greatest activity but the combination of safety and broad spectrum antimicrobial activity (including multidrug resistant strains and tuberculosis) render it very

attractive. Moreover, it is very unusual for an antimicrobial agent to possess also other activities, listed above. Tea tree oil has a very similar constituent composition as eucalyptus oil. The main difference is the lower amount of 1,8-cineole and the higher amount of terpinen-4-ol. Its antimicrobial activity might be stronger than eucalyptus oil, mainly due to terpinen-4-ol and α-terpineol, but the oral usage of tea tree oil has not yet been investigated enough. Except antibacterial activity, tea tree oil has shown good results in the therapy of tuberculosis and also as anti-inflammatory agent. Camphor is a multipurpose molecule with various applications, it may be used to treat medical conditions in humans but can also be a natural poison to kill insects. Natural camphor can be found in many essential oils of aromatic plants such as *C. camphora*, *S. fruticosa* and *R. officinalis.* Scientifically, numerous biological activities have been attributed to camphor including an antitussive one. This effect might be due to a stimulation of the cold receptors in the nose but the mechanisms is still unclear. The isolated terpenoid thymol and its phenol isomer carvacrol are the main constituents in 3 essential oils in this paper, *C. compticum*, *L. sidoides* and *O.vulgare.* Carvacrol has been found to have a relaxant effect on the tracheal smooth muscle but no antitussive effect. This bronchodilatory effect was even comparable to the effect of theophylline. This is why *C. copticum* could be very interesting as a bronchodilator in the therapy of obstructive airway disease. *N. cataria* essential oil may also have a myorelaxant effect on trachea and it has been demonstrated that the mechanism relays on a dual activity, including calcium channel blocking and an inhibition of PDE. Calcium influx blockade may be the mechanism of action of the essential oil of *A. mexicana.* It is believed that this relaxant effect is mainly due to the plant's essential oil main components etstragol and D-Limonene. But carvacrol has also shown anti-inflammatory and immunomodulatory effects and to be precise it may have preventive effects on exudates volume and leukocytes migration. The essential oil of *P. integgerima* may also reduce various inflammatory changes and its major constituents such as p-cymene, borneole and β-caryophyllene may contribute to the antiasthmatic activity, this renders the plant a potential protective drug in bronchial asthma. When it comes to the antimicrobial activity of thymol and carvacrol, they could cause disruption in the physical structure of the cell. Due to this permeabilizing effect against the plasmic membrane, thymol and carvacrol may suppress the bacterial growth in

respiratory tract infections and they can be auxiliary in the treatment of these diseases. In fact, thymol has shown that it may lower microbial resistance and enhance the antibacterial activity of antibiotics if they are implemented as a therapy. But when the results of thymol and *L. sidoides* essential oil were compared, it was observed that thymol as an isolate was less effective. This suggested that other compounds such as carvacrol are also important for the antimicrobial activity. It is believed that due to the heterogeneity and complexity of the components of essential oils it is increasingly difficult for the microorganisms to adapt to various work mechanisms the individual substance may have. However, citronellal, the major constituent in makrut laf oil, as well as α-terpineol and terpinen-4-ol, the major constituents in makrut oil, have shown that alone they possess greater antibacterial activity than the whole essential oils. And limonene, that is actually a predominant component, shows very little antibacterial activity. It was also revealed that multi-drug resistant bacteria may be very sensitive against makrut leaf oil and makrut oil. Essential oils from lemongrass and peppermint could be used in the treatment of respiratory pathologies caused by fungi. A single component of lemongrass oil such as citral can even substitute the whole oil at the same dose level. Although, myrcene, another constituent of the lemongrass oil that does not possess antifungal activity can enhance the activity of citral and the dose level can be reduced.

Finally, many plants, their essential oils and isolates have proven to be very interesting and useful in respiratory pathologies. However, not all isolated constituents possess a considerable pharmacological activity and also when an activity is present it cannot always be attributed to one single component. This suggests that the various biological activities are a result of synergy. Essential oils are known for their heterogeneity and complexity, this often makes it very difficult for scientists to understand the mechanisms of action. All in all many aromatic preparations are available for purchase over-the-counter, and have been tested as safe and distributed under European pharmaceutical law. This proves that they could be a very attractive alternative.

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