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Essential oils in the treatment of respiratory tract diseases highlighting their role in bacterial infections and their anti-inflammatory action: a review†

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Abstract: The appearance of multidrug resistant bacteria and growing antibiotic resistance is leading to a continuous need for discovering new drugs and alternative treatments against infections. The investigation of the antibacterial effect of essential oils (EOs), which are commonly used nowadays in cosmetics, health care, traditional medicine and food industry, could be one of the promising solutions for this worldwide problem. EOs have a complex mode of action due to their multiple composition. Respiratory tract diseases (RTDs) associated with bacterial infection and inflammation affect a large number of people from every age group worldwide. Because of volatility, EOs can easily reach the upper and lower parts of the respiratory tract via inhalation. Moreover, due to their antimicrobial and anti-inflammatory potency, they offer an effective treatment in respiratory tract infections (RTIs). The purpose of this review is to describe the most frequently developing infections of the upper and lower respiratory tract and to show methods used for the determination of the antibacterial activity of EOs by gaseous contact. The mode of action of EOs on bacterial cells and their anti-inflammatory action are also discussed. Results coming from recently performed *in vivo* animal studies as well as human trials are also reported. Patents deal with the role of EOs and their volatile constituents in the treatment of RTIs are also introduced. On the whole, this review aimed at showing EOs as potential antimicrobials and as anti-inflammatory agents to alleviate symptoms and signs of RTDs including RTIs. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: essential oil; vapour phase; antimicrobial activity; respiratory tract infections; *in vivo* study; human trial; patent

Introduction

Essential oils (EOs) are the mixture of volatile compounds, mainly mono- and sesquiterpenoids, phenylpropanoids, etc., containing hundreds of individual chemical constituents, which may have biological activity. They are secreted in special cells, in secretion ducts or cavities or in glandular hairs from which they are extracted by distillation (water-steam or hydro-distillation), pressing, enfleurage, solvents or by supercritical fluid extraction.[1] In general, gas chromatography–mass spectrometry (CG-MS) is used to determine the chemical composition of EOs. It is well known that EO composition is influenced by many factors, e.g. environmental conditions, soil type, plant part, chemotype of plant species, isolation process, etc.,[2] which determine its biological activity.

Because of the spread of multidrug resistant bacteria and the growing antibiotic resistance to them many research groups have focused their research programmes on investigating the antimicrobial activities of plants and their extracts in the hope of discovering new antibiotics. Therefore, the number of publications about the *in vitro* antimicrobial activity of EOs has been dramatically increasing, in most cases without any innovation. It has been previously demonstrated that the oxygenated terpenoids in EOs, e.g. alcohols, aldehydes, esters, ketones, peroxides, and phenols, are responsible for strong antimicrobial activity and influence bacterial growth.[3] *In vitro* studies about antimicrobial activity of EOs describe a wide range of assays, e.g. disc diffusion, agar diffusion, broth dilution, etc., with different parameters (bacterial or fungal strains, agar recipes, incubation time, solvents, etc.) thus their results are difficult to compare with each other. In most cases their reliability is questionable. Because of volatile and non-water soluble properties of EOs, the common screening methods (e.g. disc diffusion or agar absorption) are not appropriate for their antimicrobial testing. Hood *et al.*[4] compared the results of different methods used for determination of antimicrobial activity of EOs. The authors concluded that the disc diffusion method does not provide true results because only the more water soluble components diffuse into the agar medium. The broth dilution method is mostly acceptable but the Tween concentration used for enhancing the solubility of EOs must be taken into consideration, because it may increase bacterial growth or alter cell permeability. Tween may show antagonistic or synergistic effect with EO resulting in lower or higher antimicrobial activity. Therefore, it is highly important that the parameters of our methods will be optimized before investigations are carried out to produce reproducible antimicrobial results of EOs.

According to the data of World Health Organization (WHO), lower respiratory tract infections (LRTI) are responsible for 5%

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(3.1 million people) of deaths worldwide regarding both sexes. This number was 6% in the female group and 5% in the male group.[5] In 2012, pneumonia was responsible for 13% of causes of death among post-neonatal (1–59 month) children.[6] Based on WHO data, LRTIs and chronic obstructive pulmonary disease (COPD) have remained the top major killers during the past decade.[7] Although WHO has a well-organized global vaccine action plan against most bacteria or viruses causing RTIs, many people suffer from influenza, pneumonia or tuberculosis and without proper treatment these diseases can kill many people worldwide. EOs may possess a preventive role in the treatment of RTIs. The application of EOs via inhalation seems to be the most effective way to cure patients, because of their volatile nature they can reach the site intended to be treated.[8]

In this review we give a short overview of the infections of the upper and lower respiratory tracts focusing on the most frequently developing diseases in patients. Among *in vitro* methods the vapour phase test (VPT) can be acceptable to detect the antimicrobial activity of volatile compounds in the airways. Therefore, VPT will be introduced in this review. The mode of antibacterial and anti-inflammatory actions of EOs will also be highlighted. Furthermore, our article focuses on some of the recently published *in vivo* studies as well as human clinical trials. Recent patents concerning potential uses of EOs or their volatile constituents in the treatment of RTDs are also described.

Infections of the upper and lower respiratory tracts

The respiratory system can be divided into upper and lower tracts. The upper respiratory tract (URT) includes the epiglottis and surrounding tissues, larynx, nasal cavity, and the pharynx (throat). The pharynx has a tube-like structure and is divided into three parts: nasopharynx; oropharynx and laryngopharynx. The oro- and nasopharynx are lined with stratified squamous epithelial cells, which contain microbial flora. It is important to highlight that upper respiratory tract infections (URTIs) may spread and become more serious because the mucous membrane of the upper tract is continuous with the mucosal lining of the sinuses, eustachian tube, middle ear, and lower respiratory tract.[9]

Upper respiratory tract infections (URTIs)

The URTIs include laryngitis, laryngotracheobronchitis, epiglottitis, pharyngitis, peritonsillar abscesses and rhinitis. Although most of the URTIs are caused by viruses, other pathogens (e.g. bacteria) can also be involved in these diseases. The most relevant bacteria and viruses that can cause URTIs are summarized in Table 1 and Table 2.

Acute laryngitis is almost invariably caused by viruses and is usually associated with influenza or common cold. The characteristic symptoms include hoarseness and deepening voice.

Acute laryngotracheobronchitis or croup is closely related to laryngitis, and is a relatively common disease among young children (under 3 years of age). This illness is associated with fever, inspiratory stridor, hoarseness, and a harsh, barking, non-productive cough. If the infection (e.g. with parainfluenza viruses) affects not only the larynx but also the trachea or bronchi, the croup becomes a more serious disease.[9]

An infection of the epiglottis and other soft tissues above the vocal cords is called **epiglottitis**. The symptoms include fever, difficulty in swallowing, and respiratory obstruction with inspiratory stridor. In contrast to laryngitis, epiglottitis is usually caused by bacterial infection, mainly by *Haemophilus influenzae* type b.[9]

Table 1. Bacteria that can cause upper respiratory tract infections[9]

Organism	Disease
*Mycoplasma pneumoniae**	Laryngotracheobronchitis (croup)
Haemophillus influenzae type b	Epiglottitis
Streptococcus pneumoniae	
*Staphylococcus aureus***	
Streptococcus pyogenes Group C and G beta-hemolytic streptococci	Pharyngitis/tonsilitis
Arcanobacterium (Corynebacterium) haemolyticum	
Neisseria gonorrhoeae	Pharyngitis
Corynebacterium ulcerans	
Yersinia enterocolitica	
M. pneumoniae	Pneumonia/bronchitis/ pharyngitis
Fusobacterium necrophorum	Peritonsillar abscesses
S. pyogenes	
S. aureus	

* in few cases
** occasionally

The main symptoms of **pharyngitis** and **tonsillitis** can be characterized by erythematous and swollen tissues. Viruses and bacteria are also responsible for these infections.

Peritonsillar abscesses are most common in children (older than 5 years) and in young adults. The most important organisms in this infection are non-spore-forming anaerobes, e.g. *Fusobacterium necrophorum*. The treatment of peritonsillar abscesses is highly important, because it can spread to adjacent tissues, as well as erode into the carotid artery to cause an acute haemorrhage.[9]

Rhinitis (common cold) affects both children and adults and is mainly caused by viruses. It is characterized by fever, increased mucous secretion, sneezing, and watery eyes.[9]

Lower respiratory tract infections (LRTIs)

Trachea, bronchi and bronchioles belong to the lower part of the respiratory system. The most important diseases (acute or chronic bronchitis, bronchiolitis and pneumonia) and pathogens of the lower part of respiratory tract were summarized in Table 2 and Table 3.[9]

Acute bronchitis is an acute inflammation of the tracheobronchial tree accompanied by cough, fever and often clear sputum production. As the illness persists the sputum may become purulent. Acute bronchitis is mainly caused by viruses but in 40% of cases bacterial infection is responsible for the symptoms.[10]

In **chronic bronchitis** excessive mucus production leads to coughing up sputum on most days during at least 3 consecutive

Table 2. Viruses that can cause upper respiratory tract infections (URTIs) and lower respiratory tract infections (LRTIs)

Virus	Disease
URTIs	
Influenza virus, parainfluenza virus	Laryngitis
Rhinoviruses Adenoviruses Coronaviruses Human metapneumovirus	
Parainfluenza viruses Respiratory syncytial virus Adenoviruses Rhinoviruses* Enteroviruses*	Laryngotracheobronchitis (croup)
Human immunodeficiency virus-1	Pharyngitis
Rhinoviruses Coronaviruses Adenoviruses Parainfluenza and influenza viruses Respiratory syncytial virus (RSV)	Rhinitis (common cold)
LRTIs	
Influenza virus, Adenovirus, Coronavirus	Acute bronchitis
RSV, Parainfluenza viruses Rhinoviruses, Adenoviruses, Influenza viruses, Enteroviruses	Bronchiolitis
RSV, Human metapneumovirus, Parainfluenza-, Influenza-, Adenovirus	Community-acquired pneumonia (in children)
Adenovirus, Cytomegalovirus, Parainfluenza, Varicella, Rubeola, RSV	Community-acquired pneumonia (in adults)

* in few cases

Table 3. Bacteria that can cause lower respiratory tract infections[9,14]

Bacteria	Disease
Bordatella pertussis *B. parapertussis* *Mycoplasma pneumoniae*	Acute bronchitis
nonencapsulated *Haemophilus influenzae* *Streptococcus pneumoniae* *Moraxella catarrhalis*	Chronic bronchitis
Chlamydia trachomatis *Pneumocystis jiroveci* *M. pneumoniae*, *Chlamydia pneumoniaea* *S. pneumoniae* *H. influenzae* type b	Community-acquired pneumonia
S. aureus, *Legionella* spp., *Acinetobacter*, *M. catarrhalis*, *C. pneumoniae*, Meningococci	
Pseudomonas aeruginosa, *Enterobacter* spp., *Klebsiella* spp., other Enterobacteriaceae, MRSA, *Acinetobacter* spp., *S. pneumoniae*, anaerobes, *Legionella* spp., *H. influenzae*	Hospital-, ventilation- and healthcare-associated pneumonia
Mycobacterium tuberculosis	Pneumonia (patients with compromised immune system)

MRSA: methicillin-resistant *Staphylococcus aureus*

months for more than 2 successive years.[11] Patients with chronic bronchitis can suffer from acute flare-ups of infection. This illness is mainly caused by pathogenic bacteria, e.g. non-encapsulated *H. influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis*. Cigarette smoking and inhalation of fumes or dust are also relevant contributing factors.

Bronchiolitis is the inflammation of the smaller diameter bronchiolar epithelial surfaces and mainly develop during the first 2 years of life. The characteristic symptoms include wheezing, hyperinflation, cough, runny nose, rapid breathing, and respiratory distress. Respiratory syncytial virus (RSV) accounts for 40% to 80% of bronchiolitis cases showing marked seasonality (during winter and early spring).[9]

Pneumonia is the inflammation of the LRT involving the lungs and supporting tissues. It is a dangerous infection, because it can easily lead to the patients' death. There are two major categories of pneumonias: community-acquired pneumonia and pneumonia associated with hospital, ventilation or health care. The pathogenesis of pneumonias is complicated, and organisms can cause infection in many possible ways (e.g. by upper airway colonization or aspiration of organisms). Pneumonias are mainly caused by viruses (e.g. adenovirus, cytomegalovirus, parainfluenza, varicella, rubeola, or RSV), but after primary infection, viruses can inhibit host defence mechanisms leading to a secondary bacterial infection. In general, fever, chills, chest pain, and cough suggest pneumonia, but 10% to 30% of patients complain of headache, nausea, vomiting, abdominal pain, diarrhoea, and myalgias.[9] It should be highlighted that the aetiology of acute pneumonias is strongly dependent on age. More than 80% of pneumonias in infants or children are caused by viruses, whereas less than 10% of pneumonias in adults are viral.[9]

Unfortunately, pneumonia is the leading cause of death among hospitalized patients.[12,13] Some of these pneumonias occur due to contaminated equipment used for inhalation therapy. Moreover, patients with compromised immune systems, have a much higher risk of tuberculosis.

The following table (Table 4) represents the most relevant bacteria which lead to pneumonia:

Mycobacterium tuberculosis is often an aetiological agent in chronic lower respiratory tract infections.[15] In some cases fungi, e.g. *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Cryptococcus neoformans*, also cause acute pneumonia. The incidence of pneumonia is increasing among older patients and in patients with chronic obstructive lung disease or diabetes. The impairment of some factors, e.g. decreased mucociliary function, decreased

Table 4. Bacteria that can cause pneumonia[9,14]

Bacteria	Disease
Chlamydia trachomatis	Community-acquired pneumonia (in neonates)
Pneumocystis jiroveci	Community-acquired pneumonia (in infants)
Mycoplasma pneumoniae, *Chlamydia pneumoniaea*	Community-acquired pneumonia (in school-age children)
S. pneumoniae,*H. influenzae* type b,*Staphylococcus aureus*, *Legionella* spp., *Acinetobacter* spp., *M. catarrhalis*, *C. pneumoniae*, Meningococci	Community-acquired pneumonia (in adults)
Pseudomonas aeruginosa, *Enterobacter* spp., *Klebsiella* spp., other Enterobacteriaceae, methicillin-resistant *S. aureus*, *Acinetobacter* spp., *S. pneumoniae*, anaerobes, *Legionella* spp., *H. influenzae*	Hospital-, ventilation- and healthcare-associated pneumonia
Mycobacterium tuberculosis	Pneumonia in patients with compromised immune system

cough reflex, may contribute to a greater incidence of pneumonia in the elderly.[9]

Cystic fibrosis (CF) is a serious genetic disease that leads to persistent bacterial infection in the lungs resulting in airway cell wall damage and chronic obstructive lung disease.[16] It affects both children and adults. A very mucous *Pseudomonas*, produced by a large amount of extracellular capsular polysaccharide, can be isolated from the sputum of infected patients. Moreover, other pathogens e.g. *S. aureus*, are also involved in the pathogenesis of CF.[16]

Essential oils in the treatment of respiratory tract infections

In the European Pharmacopoea,[17] more than 25 essential oils are official. Among them, e.g. the essential oil of anise, bitter fennel fruit, eucalyptus, peppermint, tea tree and thyme are frequently used for the treatment of respiratory tract diseases. According to the Community herbal monographs of the Committee on Herbal Medicinal Products (HMPC), these oils can be applied generally based upon long-standing use. In this part, these essential oils will be shortly described.

Anise oil

Anise oil (*Anisi aetheroleum*) is obtained by steam distillation from the dry ripe fruits of *Pimpinella anisum* L., and star anise oil from *Illicium verum* Hook. Anise oils are clear, colourless or pale yellow liquids. They can be jellified at 14-16°C because of their *trans*-anethole content.[17] The fruits contain 2-6% of essential oil. The major components of anise oil are *trans*-anethole (80-95%) and anisaldehyde, and *trans*-anethole and methyl-cavicol in the star anise oil.[18]

Anise oil can be used for the treatment of respiratory complaints, mainly as an expectorant in cough associated with cold.[19] A single dose of anise oil is 50–200 μL, three times daily, but it should not be taken for more than two weeks. Its use in children and adolescents under 18 years of age is contraindicated because of the lack of data and the presence of estragole. Persons with known sensitivity to anethole should avoid anise and its oil.[19,20] Preparations containing the EO or alcoholic extracts should not be used during pregnancy and lactation because mild oestrogenic activity and the antifertility effects of anethole have been demonstrated in rats.[21] Allergic reactions affecting the skin or the respiratory system may occur, but their frequency is not known.[20]

Bitter fennel fruit oil

Bitter fennel fruit oil (*Foeniculi amari fructus aetheroleum*) is obtained by steam distillation from the ripe fruits of *Foeniculum vulgare* Miller, ssp. *vulgare* var. *vulgare*. The EO is a clear, colourless or pale yellow liquid with a characteristic odour. The main constituents of the oil are fenchone (12.0-25.0%) and *trans*-anethole (55.0-75.0%).[17]

The traditional herbal medicinal products of bitter fennel fruit oil are used as an expectorant in cough associated with cold. In the case of adults and the elderly 200 μL of EO, as a single dose per day or in multiple divided doses, can be taken for not more than two weeks. Its use in children and adolescents under 18 years of age is contraindicated because of the lack of data and the presence of estragole. Hypersensitivity to the active substance (e.g. *trans*-anethole) may develop. Because of the oestrogenic activity of *trans*-anethole, excessive doses of fennel oil may affect hormone therapy, oral contraceptive pill and hormone replacement therapy. Allergic reactions to fennel oil affecting the respiratory system may occur, but their frequency is not known.[22]

Eucalyptus oil

Eucalyptus oil (*Eucalypti aetheroleum*) is obtained by steam distillation and rectification from the fresh leaves or the fresh terminal branchlets of various species of *Eucalyptus* rich in 1,8-cineole. The most frequently used species are *Eucalyptus globulus* Labill., *E. polybractea* R.T. Baker and *E. smithii* R.T. Baker. This oil is a colourless or pale yellow liquid with an aromatic and camphoraceous odour and a pungent and camphoraceous taste.[17] The plants have a 0.5-3.5% essential oil content with the main component of 1,8-cineole (not less than 70%), while minor components include α-pinene (2-8%) and camphor (less than 0.1%). To achieve these parameters and to minimize less desirable substances such as aldehydes, the oil obtained from initial steam distillation is rectified by alkaline treatment and fractional distillation.[19]

The primary use of eucalyptus oil includes the treatment of cough, cold, bronchitis, and symptomatic relief of colds and catarrh of the upper respiratory tract. For inhalation 12 drops per 150 ml of boiling water, or a 1.5% V/V solution prepared from 1 tablespoon (15 ml) per litre of warm water can be applied, and the treatment may be repeated up to three times daily. Eucalyptus oil is used in ointments containing 1.3% V/m oil, for adults and children over 12 years, as a thick layer, up to three times daily.[19] Eucalyptus oil and its preparations should not be applied to the

face, especially the nose, of babies and little children. Since human data are not available, eucalyptus should not be used during pregnancy and lactation without medical advice.[19]

Peppermint oil

Peppermint oil (*Menthae piperitae aetheroleum*) is obtained by steam distillation from the fresh aerial parts of the flowering plant of *Mentha* × *piperita* L. The EO is a colourless, pale yellow or pale greenish-yellow liquid. It has a characteristic odour and taste followed by a sensation of cold.[17] The EO yield of peppermint is 1.2-3% and contains menthol (30-55%), menthon (14-32%), isomenthone (1.5-10%), menthyl acetate (2.8-10%), menthofuran (1-9%), 1,8-cineole (3.5-14%), limonene (1-5%), not more than 3% of pulegone and not more than 1% of carvone, with a higher ratio of cineole compared to that of limonene.[19]

The therapeutic use of peppermint oil includes the symptomatic treatment of digestive disorders (e.g. flatulence, irritable bowel syndrome), and the symptomatic treatment of coughs and colds. 3–4 drops of oil added to hot water can be applied by inhalation.[19] Peppermint oil is contraindicated in children under 2 years of age, because menthol can induce reflex apnoea and laryngospasm.[23] Direct application of peppermint oil preparations to the nasal area or chest of infants and small children must be avoided because of the risk of laryngeal and bronchial spasms. Inhalation of menthol can cause apnoea and laryngoconstriction in susceptible individuals. Menthol can cause jaundice in newborn infants (due to glucose-6-phosphate dehydrogenase deficiency).[19] Peppermint oil should not be used during pregnancy without medical advice because of the lack of evidence.

Tea tree oil

Tea tree EO (*Melaleucae aetheroleum*) is obtained by steam distillation from the foliage and terminal branchlets of *Melaleuca alternifolia* (Maiden and Betch) Cheel, *M. linariifolia* Smith, *M. dissitiflora* F. Mueller and/or other species of *Melaleuca*. It is a clear, colourless to pale yellow liquid with a characteristic odour.[17] Plants contain approx. 2% EO with the major components of monoterpenes, such as terpinen-4-ol (minimum 30%), γ-terpinene (10-28%) and 1,8-cineole (less than 15%).[18]

Tea tree oil can be used for the treatment of respiratory infections (e.g. cold, influenza, bronchitis). For external application liquid or semi-solid preparations containing 5-10% m/m of tea tree oil can be used. Rarely contact dermatitis develops. During pregnancy tea tree oil should not be applied, because there is no data in connection with its safe use.[19]

Thyme oil

Thyme oil (*Thymi aetheroleum*) is obtained by steam distillation from the fresh flowering aerial parts of *Thymus vulgaris* L., *T. zygis* Loefl. ex L. or a mixture of both species. It is a clear, yellow or very dark reddish-brown liquid with a characteristic, aromatic, spicy odour, reminiscent of thymol.[17] The dried herbal substance contains up to 2.5% EO. The thyme oil contains phenols, mainly thymol and/or carvacrol, and terpenoids.[19]

The therapeutic application of thyme oil includes the respiratory disorders (bronchial catarrh, supportive treatment of pertussis). For inhalation 4–5 drops of thyme oil can be used. Children under 5 years of age and patients with epilepsy or diseases of the thyroid gland and pregnant women should not use thyme oil.[19]

Vapour phase test (VPT) for demonstrating the antimicrobial activity of essential oils by gaseous contact

The antimicrobial activity of EOs *in vitro* has been extensively investigated and demonstrated against a number of microorganisms, generally using disc diffusion or agar dilution methods as a direct-contact assay.[24–28] These methods used different parameters (e.g. strains of microorganism, agar recipes, incubation time, etc.) usually without any innovations, therefore the results from the assays are difficult to compare with each other and sometimes their reliability is questionable. It should not be forgotten that EOs are non-water soluble substances, thus the common screening methods (disc diffusion, agar absorption) may be not appropriate for their antimicrobial testing.

EOs have traditionally been applied for respiratory tract infections via inhalation.[29] Among *in vitro* methods, the vapour phase test (VPT) demonstrates the vapour activity of EOs in the most appropriate way, and these results may be useful to understand the antimicrobial activity of them in the respiratory tract. In VPT, generally, a paper disc containing EO is placed on the inside surface of the upper lid of a Petri-dish. The lower lid contains agar, and a suspension of test microorganism containing approximately 10^6 cfu/mL is spread over this surface. The plate is immediately inverted on top of the lid and sealed with parafilm to prevent leakage of the vapour. After incubation an inhibition of bacterial growth on the agar plate can be detected, which is the measure of EO activity. This method provides only relative values, because only a few authors define the minimum inhibitory concentration (MIC) in atmosphere (MIC_{air}) by applying airtight boxes.[30]

In 1959, the vapour activity of EOs was published first by Maruzzella *et al.*[31] and Kienholz.[32] Today the inverted Petri-dish technique is used by several researchers.[33,34] In some cases an airtight box of 1 L air capacity was applied to increase the vapour concentration of EO.[35] A review published by Al Yousef in 2014[36] gives an overview of test methods (inverted Petri-dish, disc volatilization, vapour agar contact, airtight box, divided Petri-dish) used for determination of the antimicrobial activity of EOs or their components by gaseous contact method. Moreover, factors (e.g. volatility, evaporation speed and stability of EO, exposure time, chemical composition of EO, incubation temperature, growth phase and location of microorganism tested, and concentration of EO in VP) affecting the efficacy of vapour activity of EOs were also discussed. Based on this review, EOs can be used as air disinfectants in healthcare environments to control RTIs due to their antibacterial activity observed at optimized VP conditions.

Unfortunately, there are only a few number of articles in which respiratory tract pathogens were tested. Inouye *et al.*[35] investigated 14 EOs and their main constituents in the gaseous state against *H. influenzae*, *S. pneumoniae*, *S. pyogenes* and *S. aureus*. Cinnamon bark, lemongrass and thyme oils (wild-, red- and geraniol-type) showed the lowest minimal inhibitory dose (MID, mg/L in air), followed by EOs containing terpene alcohols. EOs rich in ketone, ether and hydrocarbon had high MIDs. The authors also concluded that antibacterial activity of EOs was most effective at high vapour concentration and short exposure time. *H. influenzae* was the most sensitive strain, followed by *S. pneumoniae*, *S. pyogenes*, and *S. aureus*. Among the EO main constituents, cinnamaldehyde

and thymol showed the highest activity (6.25 mg/L in air), followed by citral, perillaldehyde, octanal and nonanal. Menthol, terpinen-4-ol and linalool showed moderate activity, while esters (geranyl and linalyl acetate) exhibited very weak activity against *S. aureus* (800 mg/L in air).

In another study the vapours of geranium, lemongrass and their mixture (BioScent™) were tested against methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *Enterococci* by direct contact and vapour diffusion.[34] A special machine, ST Pro™, was used to disperse BioScent™ into the environment. In a sealed box environment, the growth of MRSA was reduced by 38% after 20 h exposure to BioScent™ vapour. In an office environment, this EO mixture produced 89% reduction of airborne bacteria in 15 h. These results suggested that geranium/lemongrass oils could be used for air disinfection.[34]

The airborne anti-tuberculosis activity of *Eucalyptus citriodora* EO was investigated by Alvarenga *et al*.[37] In this study, biochemometrics, 3D analytical approaches involving high-resolution CCC fractionation, GC-MS, bioactivity measurements, and chemometric analysis were used. The anti-tuberculosis activity was measured by an inverted Petri-dish technique. In the *E. citriodora* EO, 32 active compounds were identified, e.g. citronellol, linalool, isopulegol, α-terpineol, spatulenol and β-eudesmol. Artificial mixtures (AMxs) method was used to demonstrate the interaction between the EO components. The AMxs containing citronellol, citronellal and eucalyptol showed anti-tuberculosis activity, while citronellal, the major constituent of *E. citriodora* oil, had weak activity on its own. The authors highlighted that potentiation, additive, and/or synergistic effects among major and minor phytoconstituents cannot be disregarded.[37]

It is very rare when antiviral activity of an EO's aerosol or vapour is investigated. In one study the antiviral effect of tea tree (TTO) and eucalyptus oil (EUO) aerosols in range concentrations was tested against Influenza A virus. Both aerosols had strong antiviral activity within 5–15 min of exposure.[38]

Mode of antibacterial and anti-inflammatory actions of EOs

Currently, the knowledge about the mode of action of EOs against pathogens has been increasing, but in many cases *S. aureus* was used as test microorganism. In one study, the cellular effects of *Inula graveolens* and *Santolina corsica* EOs on *S. aureus* ATCC 6538P strain were investigated.[39] The effects of time and treatment dose on cell viability were determined by time-kill and bacteriolysis assays. Transmission electron microscopy was used to observe the marked structural changes caused by the EO treatments. The authors suggested that the cytoplasmic membrane and the cell wall are involved in the toxic action of *I. graveolens* and *S. corsica* EOs. Muthaiyan *et al*.[40] investigated the antimicrobial effect and mode of action of terpeneless cold pressed Valencia orange (CPVO) EO on MRSA. Results showed that 0.1% of CPVO oil induced cell wall stress stimulus was consistent with the inhibition of cell wall synthesis and triggered cell lysis observed with transmission electron microscopy. Since EOs can disrupt the cell membrane structure, they indirectly affect the toxin secretion of different bacteria.[39]

To date, studies have demonstrated that the bacterial cell targets of EOs include the cell wall and membrane, thereby disturbing ATP production and pH homeostasis. Moreover, EOs can influence the cellular transcriptome, proteome, and the quorum-sensing system.[41] It is well-known that Gram-negative bacteria are more resistant to EOs than Gram-positive bacteria because of the difference in their cell wall structure. In 2013 Nazzaro *et al*.[42] published a review about the mode of action of EOs on pathogenic bacteria. They concluded that EOs and their components have single target or multiple targets during their antimicrobial activity.

As we mentioned previously, there are only a few number of publications in which the mechanism of an EO on bacteria caused by RTIs was published. In the study performed by Bouhdid *et al*.[43] the activity of cinnamon EO (CEO) containing 73.3% of *E*-cinnamaldehyde was investigated against *P. aeruginosa* and *S. aureus*. The cell membrane permeability of *P. aeruginosa* was more disturbed after CEO treatment than in the case of *S. aureus*. However, flow cytometric analysis revealed that in the presence of CEO *S. aureus* entered in a viable but nonculturable state. In this state the virulence potential of bacteria is preserved and the cycle of infection will restart when bacterial cells recover their full metabolic capacity. This fact must be taken into consideration when EO activity is evaluated. Previously, our workgroup had demonstrated the effect of CEO and clove EO (CLEO) on outer membrane protein (OMP) composition of *P. aeruginosa*.[44] The oils were administered to the culture at concentrations of 0.5 x MIC and 2 x MIC and incubated for 60 min. Some proteins disappeared after the treatment of CEO and CLEO. Decreases in the amount of some proteins may be explained by the protein synthesis inhibiting effect of these oils. We received similar results to the study published first by Burt *et al*.[45] The mode of antimicrobial action of EOs is highly related to their chemical composition.[41,42] For example, in a study aromadendrene as the main compound of *Eucalyptus globulus* oil (EGO) showed higher MIC value against *P. aeruginosa* than the 1,8-cineole-rich EGO.[46] Aromadendrene having cyclopropane ring can cause alkylation of proteins, and results in their altered conformation.[47] Furthermore, the chemical change (e.g. oxidation) of EO components may contribute to their antibacterial action.[35] Oxidation of citral, *d*-limonene and α-pinene in air has been already experienced and their 'products' contributed to higher antibacterial action.[48,49]

EO constituents can also interact with transient receptor potential (TRP) ion channels located in the airways.[18] This TRP superfamily of cation channels is divided into six subfamilies based on sequence homology, including TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPA (ankyrin), TRPP (polycystin) and TRPML (mucolipin).[50] These ion channels are thought to play a key role in respiratory diseases such as asthma, chronic obstructive pulmonary (COPD) and cough. They can be activated by a diverse range of chemicals, e.g. capsaicin and citral (TRPV1 agonists), menthol and 1,8-cineol (TRPM8 agonists), allyl isothiocyanate, carvacrol and cinnamaldehyde (TRPA1 agonists) and physical stimuli (e.g. temperature, membrane potential changes and osmotic stress).[18] The investigation of the selective blockers (antagonists) of these channels, which is a rapidly growing field, may provide attractive novel strategies to treat characteristic features of respiratory diseases. Previously, it has been demonstrated in experimental animals and patients with airway diseases that a marked hypersensitivity to cough was induced by TRPV1 agonists.[51] Therefore, TRPV1 receptor antagonists have been proposed as therapeutic candidates.[52] (−)-Menthol is a TRPV1 receptor antagonist.[53] Inhaled (−)-menthol (30 μg/L) decreased evoked cough in guinea pigs by 56%.[54] An inhaled mixture of 75% (−)-menthol and 25% 1,8-cineole significantly reduced cough evoked by citric acid in healthy individuals.[55] In 2013, a review on the

chemical and biological properties (e.g. cooling effects and toxicity) of menthol was published.[56]

In a review published in 2011, Banner *et al.*[50] summarized the evidence that modulation of selected TRP channels may have beneficial effects in targeting key features of several respiratory diseases including inflammation of the airways, hyper-reactivity of the airways, mucus secretion and cough. They concluded that the introduction of selective TRP channel blockers in the clinical practice may open an exciting new chapter in the evaluation of TRP channel modulators as therapeutic agents, but despite the rapid and significant advances in the understanding of TRP channels in recent years important gaps in the pharmacological and physiological knowledge remain.[50] Because of the great number of constituents, EOs seem to have several potential cellular targets.[41]

In vivo studies

It is well known that the results coming from the *in vivo* animal models cannot be directly extrapolated to humans, but may provide valuable data about the mechanism of constituents tested. Before the full clinical application, further and more comprehensive *in vivo* studies are still required. In this part the results of the more recently performed animal studies dealing with the EOs action during respiratory tract diseases are summarized.

CF is a severe respiratory tract disease and its morbidity and mortality are related to lung alterations characterized by a vicious circle of obstruction, infection and chronic inflammation of the airways.[57] Bronchial infection induces an intense inflammatory process characterized by a massive invasion of neutrophils and leucocytes including eosinophils, lymphocytes and monocytes.[57] CF patients are often infected by *S. aureus*, *H. influenzae* and *P. aeruginosa*.[16] Recently, in the treatment of CF, studies focus on the application of hypertonic salt solutions or osmotic agents such as mannitol,[58] systemic corticosteroids,[59] ibuprofen,[60] azithromycin[61] and S-nitrosoglutathione (GSNO) reductase inhibitors.[62] EOs may play a role in the treatment of CF due to their antimicrobial and anti-inflammatory effects.

It is known that two pathways are involved in mediating the effects of inhaled EO constituents: the neurological pathway, which acts on the central nervous system via the olfactory nerve,[63] or the pharmacological pathway, which acts through the bloodstream.[64] It is thought that the effect of EOs due to the ratio of their constituents may differ from the original ratio when the EO is absorbed. Satou *et al.*[65] studied the distribution of EO components after inhalation of single or mixed constituents (α-pinene, *p*-cymene, 1,8-cineole and limonene) in mice. After inhalation for 90 min, the level of these constituents was measured in the brain and the liver. The results showed that the amount of α-pinene in the brain and liver was twofold greater after mixed-component inhalation than after single-component inhalation. In a comparison of the components of the mixed inhalation, the ratio of α-pinene increased to about three times that of 1,8-cineole. The *in vivo* investigation of the distribution of EO constituents may contribute to understanding their action.

Several studies focus on the effect of tea tree oil (TTO). In a study, the anti-inflammatory action of inhaled TTO in mice was investigated. Animals received Zymosan intraperitoneal (ip) to elicit peritoneal inflammation, and were submitted for TTO inhalation. After inhalation (15 min), peritoneal leukocytes (PTLs) were isolated and counted. Levels of reactive oxygen species (ROS) and cyclooxygenase (COX) activity in PTLs were measured. The results demonstrated that TTO inhalation exerts a strong anti-inflammatory influence on the immune system stimulated by Zymosan injection, while having no influence on PTL number, ROS level, and COX activity in mice without inflammation. The hypothalamic-pituitary-adrenal axis was shown to mediate the anti-inflammatory effect of TTO.[66]

In another study, the anti-inflammatory property of *Ocimum micranthum* EO (OMEO) was evaluated.[67] In rat trachea, OMEO relaxed contraction induced by KCl or carbachol. Inhaled OMEO applied as aerosol prevented tracheal hyperresponsiveness to KCl or carbachol in ovalbumin-sensitized animals. Authors concluded that OMEO exerts peripheral analgesia in nociception of inflammatory origin and has antispasmodic activity on rat airways. These effects are mainly due to (*E*)-methyl-cinnamate, the main constituents of OMEO.

In a Chinese study, the protective effect of linalool on inflammation was investigated using lipopolysaccharide (LPS)-stimulated RAW 264.7 cells and an LPS-induced *in vivo* lung injury model.[68] Linalool alleviated the production of LPS-induced tumor necrosis-α (TNF-α) and interleukin-6 (IL-6) both *in vitro* and *in vivo*. Linalool decreased histopathological changes of the lungs *in vivo*. In another murine model the anti-inflammatory action of lavender EO (LEO) on experimentally induced bronchial asthma was studied. Animals (BALB/c mice) were sensitized by an ip injection of ovalbumin (OVA) at days 0 and 14, and subsequently challenged with nebulized OVA on days 28–30 (Control-Asthma group). In the LEO-Asthma group, mice inhaled the EO on days 14–31, and the allergic inflammatory response was determined on days 32 and 33.[69] In the LEO-Asthma group the inhibition of airway resistance was observed. Furthermore, lower total cell numbers, eosinophils, IL-5 and IL-13 cytokine levels were measured in bronchoalveolar lavage (BAL) fluids and peribronchial and perivascular tissues in the group treated with LEO. Reduced IL-4 and IL-5 mRNA expression was determined in lung tissue, compared with the Control-Asthma group. In addition, LEO treatment decreased *Muc5b* mRNA expression in the lungs but it had no effect on the Muc5a mRNA expression. The significance of this study is that LEO inhalation inhibits allergic inflammation and mucous cell hyperplasia with suppression of T-helper-2-cell cytokines and the *Muc5b* mRNA expression.[69] Therefore, this EO may be applied for the treatment of bronchial asthma.

Zhou *et al.*[70] studied the effect of thymol constituents on allergic airway inflammation in an OVA-induced mouse asthma model. Animals were orally treated with thymol in a dose of 4, 8, and 16 mg/kg body weight 1 h before OVA challenge. Thymol reduced the level of IgE, IL-4, IL-5 and IL-13, as well as the number of inflammatory cells in the airways. Moreover, thymol decreased the airway hyperresponsiveness and blocked the activation of NF-κB pathway. Authors concluded that based on these results thymol may be involved in the treatment of allergic asthma.[70]

In another experiment, the EO isolated from *Pistacia integerrima* (PI) was tested in an LPS-induced inflammation model.[71] This medicinal plant is traditionally used in India, e.g. for the treatment of asthma and chronic bronchitis.[72] PIEO (7.5, 15 and 30 mg/kg) was administered ip. for four days to animals prior to LPS administration. The doses were selected based on LD_{50} value and preliminary efficacy studies. The EO treatment reduced the LPS-induced increase in total cell count, neutrophil count, total protein and albumin levels in BAL fluid and myeloperoxidase (MPO) level in lung homogenates. Histopathological changes also showed the protective effect of PIEO treatment. According to these findings, PIEO

may have a role in the treatment of bronchial asthma because of its complex mode of action (inhibitory effects on NO level, macrophages and MPO, etc.).[71]

In some cases new techniques are involved in the investigation of the action of EOs. Nicolato *et al.*[73] used pharmacological magnetic resonance imaging to examine the secretory response induced by EOs (scotch pine, rosemary, and peppermint) in airway surface fluid. Scotch pine EO inhalation significantly increased the surface fluid in the middle portion of the trachea. Rosemary EO showed weaker secretory response, but no secretory response was detected after peppermint oil inhalation. With this technique the direct effect of EOs on the airways can be performed.

In the Traditional Chinese Medicine (TCM) *Mosla dianthera* as an aromatic herb is used for the treatment of cough, colds, fever, bronchitis, nasal congestion and headache.[74] Wu *et al.*[75] studied the chemical composition of *M. dianthera* EO (MDEO) and evaluated its anti-influenza effects in mice infected by influenza virus A (IVA) . Animals were treated with MDEO for 5 consecutive days in doses of 90–360 mg/kg after post-infection. Levels of serum IL-4 and IFN-γ and antioxidant parameters, e.g. malondialdehyde (MDA), superoxide dismutase (SOD), total antioxidant capacity (TAOC) and glutathione peroxidase (GSH-Px) were determined in lung tissue. In the MDEO, elemicin and thymol were the main constituents. MDEO had significant effects on decreasing lung viral titers, inhibiting pneumonia, reducing levels of serum IFN-γ and IL-4, and enhancing antioxidant activity in the lung tissue of IVA infected mice. Authors concluded that MDEO could provide a safe and effective therapeutic candidate for treatment of influenza and its subsequent viral pneumonia.[75]

Acute otitis media (AOM) is one of the most common viral upper respiratory tract infections in children.[76] In a study, the effect of orange peel essential oil (OPEO) microcapsules on oxidative injury was evaluated in mice with acute otitis media disease.[77] In three groups animals were fed with the diet containing OPEO microcapsules (5, 7, and 9%) daily for 15 days. Pharmacological findings showed that OPEO treatment could decrease serum and cochlea malondialdehyde (MDA), IgA, IgG, IgM levels and increase antioxidant enzyme activities. Therefore it can be concluded that the microcapsules containing OPEO could decrease oxidative injury in AOM rats.

Human trials

As we mentioned earlier, the number of articles focusing on *in vitro* antimicrobial activity of EOs has been massively increasing. In contrast, the number of human trials has not increased to the same extent as *in vitro* studies. Previously, the individual components of 1,8-cineole[78] or menthol[79] have been extensively used in human experiments. The following respiratory activities of menthol have already been proved: antitussive in low concentration,[79] increases the sensation of nasal airflow giving the impression of decongestion,[79] depresses ventilation and the respiratory drive in comparatively higher concentration[80,81] and reduces respiratory discomfort and sensation of dyspnoea.[82] Because of the multi-faceted action of 1,8-cineole, e.g. antimicrobial,[83] antitussive,[84] bronchodilator,[85] mucolytic,[86] anti-inflammatory,[87] ciliary transport promotion and lung function improvement,[88] it can be used to treat a diverse range of respiratory conditions. There is a comprehensive summary[8] of human trials demonstrating the beneficial effects of 1,8-cineole in various respiratory conditions in the Handbook of Essential Oils edited by Can Baser and Buchbauer.[1] According to results, 1,8-cineole or eucalyptus EO can be effectively applied in the treatment of asthma, acute or chronic bronchitis, COPD, common cold and sinusitis. However, it is necessary to mention that EOs with a high content of menthol or 1,8-cineole should not be applied to the faces of infants or children.[18]

Today, articles focus not only on the EO of eucalyptus (Myrtaceae) or peppermint (Lamiaceae) but on EOs from other plant families. However, there is only a few number of human trials. It should be highlighted that some articles investigating the therapeutic application of EOs in RTDs have no abstract and they were published in Russian or Chinese. Therefore, these articles give information only for a narrow group of researchers.

According to a case report, a three-year old female patient affected by respiratory syncytial virus (RSV) was treated with an EO mixture containing *Lavandula latifolia*, *Thymus mastichina*, *Balsam abies* and *Mentha* x *piperita*.[89] Three drops were applied to a fibrous filter inserted into the base of a fan diffuser. The mixture was nebulized into the room every six hours and passively inhaled by the patient. Oxygen requirement was decreased to 1.5 litres per minute within 12 hours.

In a post-marketing observational study, the tolerability of Pinimenthol® ointment in adolescents (> or = 12 years) and adults suffering from upper respiratory tract infections (cold, acute or chronic bronchitis, bronchial catarrh or hoarseness) was examined.[90] 3060 patients were involved in the study. Pinimentol® ointment contains eucalyptus EO, pine-needle EO and menthol. This product was used for inunction (29.6% of patients), inhalation (17.3%) or inunction and inhalation (53.1%), respectively. The mean application time was 8.0 ± 3.4 days. The tolerability was rated as excellent or good by 96.7% of physicians and 95.7% of patients. Only 22 patients (0.7%) reported adverse drug reactions, e.g. hypersensitivity skin reaction (n = 10), cough (n = 6), obstructive respiratory tract symptoms (n = 5) and hypersensitivity reactions of mucous membranes (n = 4). According to these results, authors suggested the application of Pinimentol® ointment in URTIs in both adolescents and adults.[90]

Panahi *et al.*[91] investigated the effectiveness of a herbal product (Lamigex) containing the EO of *Syzygium aromaticum*, *Lavandula angustifolia* and *Geranium robertianum* in the treatment of acute external otitis (AEO) and compared its effects to those of ciprofloxacin. Seventy randomly assigned patients received ciprofloxacin 0.3% or Lamigex. Each group was administered with three drops every 12 hours for a week, in every case after cleansing the ear canal. After treatment, patients were examined by specialists for AEO symptoms. Moreover, ear discharge cultures were also checked at the beginning as well as at the end of this trial. Pain severity was also recorded with a visual analogue scale at the beginning, the third, and the seventh day of the study. Both antibiotic and Lamigex treatments improved the patients' conditions and reduced pain severity. However, the rate of pain improvement was different between the two groups. The number of positive cultures was also reduced by ciprofloxacin and Lamigex treatment by the end of the trial.[91] It is necessary to highlight that undiluted EOs should not be dripped into the ears, but diluted EOs may be placed on a cotton wad for partial insertion.[18]

In a smaller study,[92] 24 randomly assigned adults suffering from common cold inhaled air with either steam or a mix of 9% eucalyptus EO, 35% camphor and 56% menthol w/w for 1 hour. The mean concentration of EO compounds in the inspired air was 56 μg/L. In the inhalation group, only 6 out of 22 spirometric parameters significantly improved when measured after 20 min, and 14 improved after 1 hour. These parameters included forced vital

capacity (FVC: the total amount of air that can be forcibly exhaled after full inspiration), forced expiratory volume in one second (FEV_1: the amount of air forcibly exhaled in one second), forced expiratory volume in three seconds (FEV_3: the amount of air forcibly exhaled in three seconds), maximum expiratory flow rate (MEFR: maximum forced flow rate during full expiration), and forced expiratory flow 25% ($FEF_{25\%}$: the mean forced expiratory flow during the first 25% of FVC).[92,18]

In a prospective, randomized open study including 84 children of 5 to 15 year of age, the effect of *Nigella sativa* EO (NSEO) in the treatment of wheezing associated with lower respiratory tract illnesses was investigated.[93] The control group (n = 41) was administered with bronchodilators and the test group (n = 43) received NSEO orally in dose of 0.1 mL/kg body weight/day divided into two portions (every 12 h) for 14 days. Patients were examined on day 0 (before treatment), and on 3rd, 7, 10 and 14th day of treatment by measuring of the pulmonary index (PI) and peak expiratory flow rate (PEFR) parameters. According to findings, NSEO significantly reduced the PI compared to the control group during 14 days. There was more improvement in the PEFR value in the test group than in the control group but this difference was not significant.[93] However, the authors proved the beneficial role of NSEO in the management of wheezing associated with lower respiratory tract illnesses, more studies involving more children are necessary to make a final conclusion regarding the safe application of this EO.

In another prospective, randomized double-blind controlled trial, the activity of a spray containing EO of *Eucalyptus citriodora*, *E. globulus*, *Mentha* x *piperita*, *Origanum syriacum*, and *Rosmarinus officinalis* was studied in patients with URTI.[94] 34 patients in the test group used this spray 5 times a day (4 spraying each time) for 3 days. Then the change of the most debilitating symptoms (sore throat, hoarseness or cough) was assessed in patients. 20 minutes after the use of the spray, participants in the test group reported a greater improvement in symptoms compared to participants in the control group. There was no difference in symptom severity between the two groups after 3 days of treatment. Based on these results, authors suggested the local, rather than systemic, effect of this spray on the upper respiratory tract.[94]

In a multi-centre, randomized, double-blind, placebo-controlled clinical trial, the efficacy and tolerability of GeloMyrtol® (= Myrtol®) forte was studied in acute bronchitis.[95] 413 patients were included and randomized, 202 participants received GeloMyrtol® 300 mg, 4 capsules per day for 2 weeks. Investigators evaluated the patients' symptoms at baseline and after 7, 10 and 14 days of treatment, and participants recorded the intake of medication, their wellbeing and symptoms in their diaries. GeloMyrtol® reduced the day-time and night-time coughing periods, therefore patients did not suffer from sleep disturbance. Moreover, participants tolerated the treatment well. GeloMyrtol® has been a traditionally and frequently used product in Germany in the treatment of RTDs for many years.

It is worth mentioning that in several human studies only patients' symptoms are measured during the experiments without microbiological investigations, since bacterial cultures were not checked at the beginning as well as at the end of trials. As we showed in the 'Infections of the Upper and Lower Respiratory Tracts' part, many kinds of microorganisms are responsible for the RTDs. To achieve the appropriate treatment of RTDs (e.g. administration of antibiotic or anti-inflammatory medicine or inhalation with EO), it is definitely important to diagnose the type of microbiological agent causing RTDs including RTIs.

Patents

The antimicrobial potency of EOs against respiratory tract pathogens has been well known for ages. Antimicrobial drugs often have a high price and side effects while the EOs are relatively cheap and safe natural materials. The application technique of these substances is variable depending on the symptoms of the disease and the treated area. The commonly used method is inhalation which can be divided into active and passive techniques. Active inhalation means that patients use an inhalation device or patch from where they can directly inhale the volatile components. EOs can be used with passive inhalation as well, when the EOs are applied into the environment via heating, vaporization or forced air ventilation.[89,96] An old-fashioned and the cheapest way for relieving the symptoms of respiratory diseases is vapour inhalation over a bowl of hot water containing a small amount of eucalyptus oil (EUO). The inhalation could be effective with the application of a towel over the head because in this case we could directly inhale the concentrated aromatic components.[97]

During the last few years several patents appeared, which tried to develop portable inhalation devices and suitable delivery systems for EOs. A cheap and practicable small pocket inhaler using EUO was invented for the treatment of respiratory conditions. In this simple device 70 drops of EO were applied to a fabric material in an appropriate glass vial, from where the patients can inhale the vapour directly through their nostrils or mouth. The best results were observed if the inhalation lasted for 3–5 minutes and it was repeated several times daily.[97]

A similar inhaler, which can ease nasal congestion and quell cough consisting of a paper wrapper around a fibrous cylindrical plug with aromatic substances (EUO or menthol) along the centreline was developed. Towards the plug an airtight aluminium foil guarantees that the device is hermetically sealed and aromatic substances remain fresh. The simple use, lower price (compared to plastic inhalers) and disposable hygienic form are the advantages of this device. Patients can directly inhale the EO through their nose, which leads to the reduction of nasal congestion.[98] A personal aromatherapy device can release volatile substances from its flexible inner container covered with a rigid outer shell. The flexible inner part contains fibrous material (e.g. cotton) saturated with an aromatic substance (e.g. EO). A cap closes the inner part including a flip nozzle, which operates between defined positions and permits release of effective aromas when the patient inhales. In a closed position the nozzle confirms that the vapour of the EOs cannot escape when the inhaler is not used. The device can easily be operated with one hand and the flexible container can deliver any kind of aromatic substances in an exact amount.[99]

Aromatherapy patch application is another possible solution for treating or preventing respiratory infections. In this case the use of a special carrier is also important which keeps the aromatic substances in fresh and constant form, and limits their release before the application.[100] The inventors presume that the living respiratory tract pathogens will be inactivated when getting into contact with the inhaled vapour, hence the saturated patch is usually placed in the vicinity of the nasal pathway with the application of an appropriate mask also. In a patent the adhesive patch contains safe and effective amounts of EOs (e.g. wintergreen, cinnamon, ginger, peppermint, lemon, clove, clary sage, and chamomile) alone or in combination. The patch is a unique vehicle with adhesive and a viscoelastic nature which maintains the stability and convenient use of EOs. When the patch was applied by the patient, the EO could continuously make contact with the skin of

the patient and the volatile components released. In some cases inventors apply cyclodextrines to carry active substances and to control the release of the volatile components.[96]

In another patent, an adhesive path containing a unique foraminous carrier was developed. The carrier can adhesively bind to the body parts (e.g. face, neck, and chest), where, after the release of the volatile substances (menthol, thymol, camphor, oil of peppermint, eucalyptus and ginger), they can be easily inhaled through the user's nose or mouth. A further benefit of the foraminous structure is that it can reduce the incidence of allergic reactions.[101]

In another patent by Vail W. B. and Vail M. L., the possible use of some EOs in the treatment of severe acute respiratory syndrome was described.[102] The authors recommend the inhalation of EO of *Eucalyptus globulus*, *E. citriodora*, *E. radiatto*, and *Melaleuca alternifolia* to reduce the risk of infection in public places. The duration and frequency of inhalation were precisely determined.

Further interesting patents are summarized in an article written by Sienkiewicz *et al*.[103] Finally, the encapsulation of EOs or their constituents is applied in the pharmaceutical technology, which decrease the volatility, stabilize the compounds, improve the shelf-life of products and prolong the biological effect.[104] Several patents being in the book chapter of Karlsen J.[104] describe the encapsulation and other programmed release techniques using EOs. We absolutely agree with the author that these technical solutions may open new areas of application of EOs, even in the treatment of RTDs.

Concluding remarks

EOs are very interesting natural products and they possess various biological properties. An essential oil may contain hundreds of individual chemical components, mainly mono- and sesquiterpenoids, and phenylpropanoids. For therapeutic purposes, they are administered via inhalation (e.g. eucalyptus oil), orally (e.g. peppermint oil) and trans-dermally (e.g. rosemary oil). Oils with a high phenol content, for instance thyme and clove, have antiseptic properties. Because of their wide-ranging and complex effects, e.g. antibacterial, antiviral, anti-inflammatory, mucolytic, bronchodilator, etc., they can be used as valuable materials in the treatment of different respiratory tract diseases. Some EOs are applied exclusively based upon long-standing use, but some EOs can be used based upon well-established use.

There are several *in vitro* techniques with which the antimicrobial activity of EOs can be tested. Today *in vivo* animal models of respiratory tract diseases offer good possibilities for testing their diverse biological effects. However, it should be highlighted that the number of well-designed human trials is still very low. Furthermore, some studies have several limitations. Firstly, the small sample size may limit the interpretation of results. Secondly, short periods of treatments (e.g. 3 days) are not sufficient for the interpretation of results, as well. Another limitation is associated with the safety use of EOs. However, in some cases investigators did not observe any severe side-effects, but larger-scale studies should be designed in order to conclude the safety application of EO formulas. In addition, it is difficult to perform a double-blind trial including EO or its individual constituent. Without a doubt, further studies, principally human trials, are needed to assess the efficacy and tolerability of EOs in respiratory tract diseases. More trials would also be important, because data coming from human studies may provide ideas for developing patents and might open novel perspectives for the development of products as well.

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