

Plant Essential Oil: An Alternative to Emerging Multidrug Resistant Pathogens

Abstract

The emergence of multidrug resistant bacteria and rising antibiotic resistance is leading to an incessant need for discovering novel drugs and alternative treatments against infections. The outcome of investigations on the antibacterial effect of Essential oils (EOs) which are commonly used in recent times could be one of the promising solutions to this worldwide problem. EOs as well as their volatile constituents has been discovered to be a potential for preventing and treating infectious diseases because of their biological activities. The clinical efficacy of most of the marketed antimicrobials is found to be threatened by the quick emergence of multidrug resistant pathogens which increases the need to find alternatives. In recent times, many EOs have been discovered to have significant cytotoxic, antiparasitic and antimicrobial activity against a wide range of pathogens. As a result of this, EOs and their components have been utilized as a source of new antimicrobials in combating infectious diseases.

Keywords: Antimicrobial activity; Infectious disease; Drug resistance

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Abbreviation: EO: Essential Oils; ESBL: Extended Spectrum β -Lactamases; MRSA: Methicillin-Resistant *Staphylococcus Aureus*; OMEO: *Ocimum Micranthum* EO; PIEO: Pistacia Integerrima EO; MPO: Myeloperoxidase; AOM: Acute Otitis Media; OPEO: Orange Peel EO; MDA: Malondialdehyde; RSV: Respiratory Syncytial Virus; UTIs: Urinary Tract Infections

Introduction

Multidrug resistance is a challenge across different types of microorganisms, bacterial and viral pathogens. This is because they account for a significant outcome of clinical infections observed. The tendency of microorganisms to acquire and disseminate resistance genes among themselves via horizontal gene transfer makes resistance patterns to become widely distributed. Bacterial pathogens exhibit an array of resistance phenotypes, the most important of which is the production of resistance enzymes. These include extended spectrum beta-lactamases, carbapenemases and AmpC which have been widely reported to confer resistance to different classes of antibiotics.

Drug resistance is observed in a range of pathogenic and non-pathogenic bacteria; however, methicillin-resistant *Staphylococcus aureus* (MRSA) is most commonly reported [1]. Multidrug resistance is also on the increase in Gram-negative bacteria (e.g., *Acinetobacter spp.* and *Klebsiella spp.*) associated with the production of extended spectrum β -lactamases (ESBLs). Clinicians are having very few treatment choices left for combating infectious diseases, with no new drugs on the horizon. This problem is not just restricted to the hospital or healthcare environment. In recent times, many drug-resistant strains are seen in the community and also among animals (both domestic and farmed). This has resulted in a number of changes in practice in the treatment of infectious diseases. Owing to these problems, there has been a renewed interest in research into alternative

antimicrobials and more targeted treatment strategies, including therapies employed before the advent of antibiotics, with a return to traditional remedies and medicines such as EOs.

Medicinal and sweet-smelling plants are crucial pillars of healthcare and traditional medicinal systems of the world [2]. EOs is plant derived oil with great medicinal benefits as they contain the essence of herbs and flowers in concentrated form. They are used in medicine to satisfy the priority of health care needs of the population. Until now, more than 3000 EOs have been described; of which about one tenth are of relevance to the pharmaceutical, nutritional or cosmetic industries. EOs has been found to boast many useful bioactivities particularly the antimicrobial activities which have formed the basis for the development of new alternative remedies/therapeutics for the control and prevention of human diseases [3]. Applications of EOs are often considered quite safe and effective with no side effects. These oils have been historically used for the treatment of infectious diseases and inflammation in traditional medicine across the world [4]. They are administered orally, topically, or via aromatherapy, depending on historical use and chemical composition.

Toxicity Activities of Essential Oils to Pathogens

Antifungal activity

Antifungal properties of EOs have been an interesting new therapeutic alternative to the synthetic drugs. These products have significant importance by the fact that, similar to bacteria, fungal drug-resistant strains are increasing rapidly [5]. Dias Ferreira et al. [6] in his study on EOs revealed that *Curcuma longa L.* EO was toxic for *Aspergillus flavus*, and inhibited aflatoxin production. Aflatoxins are toxins produced by mold that can damage the liver and may lead to liver cancer in humans. It is a naturally occurring mycotoxin produced by two types of mold: *Aspergillus flavus* and

Aspergillus parasiticus. However, the impending effects of EOs against aflatoxin production are of abundant interest [7]. The Ephedra major Host EO was reported to minimized growth and aflatoxin production by *Aspergillus parasiticus* [8]. Likewise, the EO extracted from *Chenopodium ambrosioides* L. was revealed to be toxic against a broad species of fungi and also found to repress the aflatoxin B1 production by the aflatoxigenic strain of *Aspergillus flavus* [9].

Hammer et al. [10] had shown the complex mechanisms which represent the basis of antifungal activities of EOs. In their study, they emphasize the modifications of the cell membrane by enhancing its permeability, alteration and disorganization of the membrane proteins, inhibiting of the cell breathing, modifying of the transportation processes of ions, intra and extra-cellular in human cells. The antifungal effects were proven against: *Candida Albicans*, (causative agent of vagina infection) *C. glabrata*, *Saccharomyces cervisiae* and *Aspergillus fumigates* [10].

Antibacterial activity

EOs has also been reported to be of great importance against multidrug resistant bacteria. Yap et al. [11] in their study demonstrated that cinnamon bark as well as lavender EO induced unalterable membrane damage to multidrug resistant *E. coli* strain and also inhibit quorum sensing evidenced by reduced production of bioluminescence. The most polar fraction obtained from *Cistus ladaniferus* Gouan ex Steud EO, which was mainly constituted by mono- and sesquiterpene alcohols, were reported to induce cell wall distortion with an outer cytoplasmic membrane detachment in a multidrug resistant strain of *Enterobacter aerogenes* [12]. Likewise, Knezevic et al. [13] reported that *Eucalyptus camaldulensis* Dehnh. and *Myrtus communis* L. EOs were tested against multidrug resistant *Acinetobacter baumannii* wound isolates and were found to demonstrate antibacterial effects when administered alone, but also reported synergistic effects when combined with antibiotics.

Antiparasitic activity

Plant EOs has also been used as substitutes against endo and ectoparasites. In this way, protozoan parasites *Plasmodium falciparum* and *Leishmania donovani* had been reported to be resilient to orthodox drugs which have led to the high rate of mortality and morbidity [14]. However, *Lavandula angustifolia* Mill. and *Lavandula x intermedia* EOs have also been reported to have antiparasitic activity when assayed against the human protozoal pathogens *Giardia duodenalis* and *Trichomonas vaginalis* and also against the fish pathogen *Hexamita inflata*.

Cytotoxicity activity

Numerous researchers on EOs have shown a strong interest in the cytotoxic ability of EOs. Abundant efforts have been executed in order to investigate the potential therapeutic effects of EOs against several diseases especially those characterized by excessive cell growth and proliferation such as cancer or bacterial infections [15]. The major mechanisms that mediate the cytotoxic effects of EOs comprise the induction of cell death by activation of apoptosis and/or necrosis processes, cell cycle arrest, and loss of function of essential organelles. Several of these effects are attributed to the lipophilic nature and low molecular weight

of the key components that comprise EOs which allow them to cross cell membranes, alter membrane composition and increase membrane fluidity, leading to leakage of ions and cytoplasmic molecules.

The cytotoxic effects of EOs are largely made through disrupting the structure of membranes, leading to bacterial cell permeability. As a result of membrane permeability, all other cellular functions including membrane potential, efflux pump activity or respiratory activity are also compromised [16]. Moreover, it has been evidenced, using flow cytometry experiments, that the mode of action mediated by bacterial cell permeabilization is similar in both Gram-positive and negative bacteria [17]. The ability to maintain the membrane potential and pH gradient is necessary for cell survival, and a decrease in these parameters is indicative of significant damage to the cell membrane [18].

Essential Oils in the Treatment of Respiratory Tract Infections

In the European Pharmacopeia, [19] more than 25 EOs are official. Some of which are the EOs of anise, bitter fennel fruit, tea tree and thyme. These are the frequently used EOs for the treatment of respiratory tract diseases.

Anise oil

The key components of anise EO are trans-anethole (80-95%), anisaldehyde, trans-anethole and methyl-cavicol in the star anise oil [20]. Anise oil had been reported to be active in the treatment of respiratory infection; mainly as an expectorant in cough associated with cold [21]. A single dose of anise oil of about 50-200 μ L for three times daily, but not taken for more than two weeks was prescribed for its effectiveness. Though, allergic reactions affecting the skin or the respiratory system had been reported, their frequency is not known [22].

Bitter fennel fruit oil

The Bitter fennel fruit 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%) [19]. 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 μ L of Bitter fennel fruit EO, as a single dose per day or in multiple divided doses, was prescribed to be taken for not more than two weeks. However, hypersensitivity to the active substance (e.g. trans-anethole) had been reported to develop after usage. Also, due of the oestrogenic activity of trans-anethole, excessive doses of fennel oil may also affect hormone therapy, oral contraceptive pill and hormone replacement therapy [23].

Tea tree oil

Tea tree EO (Melaleuca aetheroleum) is gotten 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 [19]. The plant contain approximately 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%). Tea tree oil had been used for the treatment of respiratory infections such as cold, influenza and bronchitis [20]. For external application, liquid or semi-solid preparations containing 5-10% m/m of tea tree oil was prescribed. Rarely contact dermatitis had also been reported to develop after usage [19].

Thyme oil

Thyme oil (*Thymi aetheroleum*) is gotten by steam distillation from fresh flowering aerial parts of *Thymus vulgaris*, *Thymus zygis* 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 [19]. The dried herbal substance contains up to 2.5% EO. The thyme oil contains phenols, mainly thymol and/or carvacrol, and terpenoids [21]. The therapeutic application of thyme oil includes the respiratory disorders such as bronchial catarrh and the supportive treatment of pertussis. For inhalation, 4-5 drops of thyme oil was prescribed [21].

In vivo Studies on EOs' Action on Respiratory Tract Diseases

Animal trials

Cystic fibrosis is a severe respiratory tract infection and its morbidity and death rate are related to lung alterations branded by a brutal circle of obstruction, infection and chronic inflammation of the airways [24]. Bronchial infection induces an intense inflammatory process characterized by a massive invasion of neutrophils and leucocytes including eosinophils, lymphocytes and monocytes [24]. Cystic Fibrosis patients are often infected by *S. aureus*, *H. influenzae* and *P. aeruginosa* [25]. In another study, the anti-inflammatory property of *Ocimum micranthum* EO (OMEEO) was assessed [26]. In rat trachea, OMEEO relaxed contraction induced by carbachol. Inhaled OMEEO applied as aerosol prevented tracheal hyper responsiveness to carbachol in ovalbumin-sensitized animals. The authors concluded that OMEEO 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 components of OMEEO [27].

Zhou et al. [28] studied the effect of thymol constituents on allergic airway inflammation in an OVA-induced mouse asthma model. The mice 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 reduced the airway hyper responsiveness and blocked the activation of NF- κ B pathway. The authors, therefore, concluded that thymol may be involved in the treatment of allergic asthma [28]. In another research, the EO isolated from *Pistacia integerrima* EO (PIEO) was tested in an LPS-induced inflammation model [29]. This medicinal plant is traditionally used in India for the combating asthma and chronic bronchitis. PIEO (7.5, 15 and 30 mg/kg) was administered for four days to animals before LPS administration. The doses were selected based on LD50 value and preliminary efficacy studies were done. The result revealed that EO treatment lowers 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 revealed the protective effect of PIEO treatment. According to these findings, PIEO have a role in the treatment of bronchial asthma because of its complex mode of action [29].

Acute otitis media (AOM) is also one of the most common viral upper respiratory tract infections in children [30]. In an experiment, the effect of orange peel EO (OPEO) microcapsules on oxidative injury was assessed in mice with acute otitis media disease [31]. The three grouped animals were fed with the diet containing OPEO microcapsules (5, 7 and 9%) daily for 15 days. Pharmacological discoveries disclosed 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

The broad summary of human trials demonstrating the beneficial effects of 1,8-cineole (menthol) in various respiratory conditions was compiled by Harris [32] in the Handbook of EOs reviewed by Can Baser et al. [33]. According to reports from the study, 1,8-cineole or eucalyptus EO can be effectively applied in the treatment of asthma, acute or chronic bronchitis, COPD, common cold and sinusitis. Also, in 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* [34]. In a smaller study, 24 randomly assigned adults suffering from common cold were subjected to inhale 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 μ g/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 [35].

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* were studied in patients with URTI [36], 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. Twenty 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 [36]. In a multi-centre, randomized, double-blind, placebo-controlled clinical trial, the efficacy and tolerability of GeloMyrtol® (=Myrtol®) forte was studied in acute bronchitis [37], 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 [37].

Antimicrobial Effects of EOs on Multidrug Resistant Bacteria Responsible for Urinary Infections

Urinary Tract Infections (UTIs) are amongst the most common bacterial infections responsible for morbidity and mortality in all human populations [38], especially in women [39]. UTIs are revealed to be the second most common type of infections in human body, which are one of the most severe health challenges affecting millions of people yearly [40]. In recent years, UTIs has posed a real problem, particularly in developed countries [41], also the pathogens liable are commonly multidrug resistant bacteria to antibiotics [42]. The resistance against antibiotics has been spotted in several genera of bacteria which includes *Escherichia*, *Enterobacter*, *Klebsiella*, *Proteus*, *Salmonella*, *Serratia* and *Pseudomonas* [43], which has necessitated the insistence for new antimicrobial agents which will be used against treatment failures observed in UTIs related to multidrug resistance. In recent times, it has been clear that EOs as shown a promising and wealthy source of safe and effective new antimicrobial agents [44]. Therefore, EOs appears as a solution against treatment failures seen in UTIs.

The study of Zenati et al. [40] revealed that antimicrobial activity of EOs against strains collected from urinary infections showed the most important activity as recorded by *C. cassia*, since the oil showed significant activity against all Gram positive and negative strains including *P. aeruginosa*. Therefore, the resolution of UTIs problem depends more importantly on new antimicrobial agents in case of treatment failures. EOs such as *C. cassia* proved to exhibit an interesting antimicrobial activity against all studied bacterial species in the work of Yang et al. [45]. It has been clear that cassia oils holds strong antimicrobial activity with interesting MICs values which varies between 0.018 and 0.7mg/mL against Gram positive and negative bacteria. Also, cassia oil was active against *P. aeruginosa*; a specie which has been resistant to antibiotics [46]. Cassia oil seems to be an ingesting substitute treatment in nosocomial UTIs.

Conclusion

Among natural plant products, EOs deserves actual attention because of their uses in many different traditional healing approaches all over the world. Since antiquity, EOs and their constituents have been used to treat a large number of human illnesses. Today, EOs are used in alternative and holistic medicine for similar purposes and administered orally, topically or via aromatherapy. EOs are very interesting natural products and they possess various biological properties. An EO 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 high phenol content, for instance thyme and clove, have antiseptic properties. Due of their wide-ranging and complex effects, (such as antibacterial, antiviral, anti-inflammatory, mucolytic, bronchodilator, etc.) they can be used as valuable

materials to combat different respiratory tract diseases. Some EOs is applied completely 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. In recent times, *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, little periods of treatments (e.g. 3 days) are not sufficient for the analysis of results as well. Another limitation is associated with the safety use of EOs. However, in some cases, scientists have not observed any severe side-effects, but larger-scale studies should be designed in order to conclude the safety application of EO formulas. Moreover, it is tough 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. More well-designed clinical trials are needed in order to ascertain the real efficacy and safety of these plant products.

Conflict of Interest

The authors have declared that no conflict of interest exists.

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