Chapter

The Antibacterial Activity of *Mentha*

Monique Mancuso

Abstract

The topic of this chapter is the antibacterial activity of *Mentha* against several pathogenic bacteria. Some aromatic plants are recently being studied for their antibacterial properties, such as citrus essential oils, *Armoracia rusticana*, etc., showing inhibition against bacteria, fungi and yeasts. This chapter highlights the antibacterial characteristics of *Mentha piperita* (peppermint) and other *Mentha* sp. that are used daily as folk remedies and in food industry too. *Mentha* acts as counterirritant and analgesic with the ability to reduce pain and improve blood flow to the affected area. For these reasons, mint essential oils are well studied due to their antibacterial activities against both Gram-negative and Gram-positive ones and can be useful as a substitute to some antibiotics and combat the antimicrobial bacterial resistance.

Keywords: *Mentha* sp., antibacterial activity, plants, leaf extract, essential oils

1. Introduction

The essential oils (EOs) are a group of several natural chemicals that are characterised by their volatility and aroma [1].

The essential oils are produced by different plant parts (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots) as secondary metabolites [2]. The EOs are about 3000, 300 of which are used for pharmaceutical, agronomic, food, sanitary, cosmetic and perfusion purposes [2]. They are a complex of natural mixtures of lipophilic substances and consist of two fractions: volatile (from 85 to 99%) and non-volatile, the second one being a heavier fraction than the first one (from 1 to 15%) [3]. Hydrocarbon compounds and oxygenated compounds prevail in the volatile fraction of EOs. The oxygenated fraction gives the characteristic flavour to the essences, while terpenes and sesquiterpenes perform a support function. The separation process of terpenes, as well as improving the stability of the essence, allows to concentrate the oxygenate fraction that it brings a superior contribution to perfume and aroma. The non-volatile fraction consists of many classes of substances such as high molecular weight hydrocarbons, fatty acids, steroids, carotenoids, waxes, coumarins, psoralenes and flavonoids [2]. Several EOs extracted from plants contain compounds that are responsible for their antimicrobial effects [4–6]. The mechanisms by which different EOs are capable of damaging bacteria depend on their composition. Generally, antimicrobial activity is derived not only from a single mechanism of action but also from a cascade of reactions that involve the entire bacterial cell because EOs have several chemical structures in their composition and, consequently, several functional groups. In general, Gram-positive bacteria are more susceptible to the effects of EOs than Gram-negative

bacteria, due to significant structural differences in the cell wall of these two groups of bacteria [7, 8]. The structure of Gram-positive bacteria facilitates the penetration of hydrophobic molecules into the cell and acts on the bacterial wall, cytoplasmic membrane or cytoplasm [1].

The diseases caused by bacterial pathogens are a great concern all over the world [9]. Since the beginning of the 1980s, it is observed that the number of antimicrobial agents decreased considerably, while the resistance of the microorganisms to them has been growing in a fast way due to the development of new resistance mechanisms [10].

For these reasons, nowadays, there has been a growing interest in the determination of the biological and antimicrobial properties of herb extracts derived from several medicinal plants [11]. Among the species of plants from which essential oils are obtained, there is mint (*Mentha* sp.), in fact, which is used all over the world as flavouring agent in cosmetics, in pharmaceutical products as well as food including candy and gum and for liqueur [12]. The genus *Mentha*, family Labiatae, consists of about 25 species. Native from the temperate areas of the world is common in Eurasia, North America, southern Africa, and Australia, mints are widely distributed throughout. Mint essential oil is produced by their leaves [13–20]. Mint essential oils (MEOs) are used as scents in perfumery. Some species are commonly used in herbal medicine. The antibacterial effects of mint species, in particular peppermint oil from *Mentha piperita*, spearmint oil from *Mentha spicata* var. *crispa* and corn mint oil from *Mentha arvensis,* have great antibacterial activity against *Staphylococcus aureus, Streptococcus pyogenes* and *B. subtilis* [1, 9, 21]. *Mentha pulegium* showed activity against *S. aureus* and *Enterococcus faecalis* [11].

Mentha spicata and other *Mentha* species showed activity against Gram-negative bacteria; the former is active against biofilm cultures of *Vibrio* spp. [22]; *Mentha longifolia* is active against *Salmonella typhimurium* [23]; and *Mentha pulegium* inhibits the growth of *Pseudomonas sp., E. coli and Pseudomonas aeruginosa* [11, 24, 25].

2. Chemical composition of *Mentha* species essential oil

The essential oils from different *Mentha* species have been isolated by hydrodistillation using Clevenger apparatus or pharmacopoeia distillation apparatus [26].

The composition of MEOs that gives the characteristic peppermint aroma and flavour is menthol and pulegone [27], whereas for spearmint, it was reported that the flavour is due to carvone [28].

Several investigations have been carried out on the chemical composition of different samples of *Mentha* species from different geographical regions revealing that chemical composition and percentage varied depending upon the species and the harvesting time at different stages, and the geography as well as the extraction methods [29]. Some factors like physiological and environmental conditions, genetics and evolution also determine the chemical variability of *Mentha* essential oils [30]. Additionally, most of the species chemically characterised were rich in pulegone, menthone, menthol, carvone, 1,8-cineole, limonene and b-caryophyllene. For example, the chemical composition of the essential oil of *M. piperita* has abundant quantities of menthone, menthol and menthyl acetate, which varies based on different countries: in Serbia, menthone was 12.7%, menthol 37.4% and menthyl acetate 17.4% [31]. In Pakistan, the major components of *M. piperita* reported are menthone and menthol [32]. In India, menthol was (30–55%), menthofuran and menthyl acetate (1.0–9.2%) [33]. In Iran, *M. piperita EO* contains menthol (36.24%) and menthone (32.42%) as main constituents [34]. In Turkey, the reported chemical constituents of peppermint oil are menthone (44.1%), menthol (29.5%),

menthyl-acetate (3.8%) and menthofuran (0.9%) [35]. However, in Korea, *M. piperita* leaves EO has different composition and include limonene (64.5 and 94.2%), 1,8-cineole (46.1%), p-menth-2-en-ol (34.5%), menthol (33.4%) and linalyl-acetate (28.2%) as main components [36]. These differences can influence the antibacterial capacity with respect to one pathogenic bacteria species; it is important to note that it is not a single compound but the combination of the chemical compounds that carries the specific antimicrobial activity [37, 38]. The hydrophobicity is one of the major distinctiveness of essential oils, which enables their assimilation into the cell membrane. The MEO oil rich in menthol and compounds similar to menthol shows that the hydroxyl group and the presence of a system of delocalized electrons are important for the antimicrobial activity. These similar compounds destabilise the cytoplasmic membrane and, also, act as a proton exchanger, thereby reducing the pH gradient across the cytoplasmic membrane. The resulting collapse of the proton motive force and depletion of the ATP pool eventually lead to cell death [39].

3. Methods for testing the antibacterial activity of essential oils

The methods used for testing antimicrobial activity of EOs are the disc diffusion method, the determination of minimum inhibitory concentration (MIC) and the vapour phase method. Another method is the use of TLC-bioautography.

3.1 Agar diffusion test

In the agar diffusion test, the EO to be tested is placed on the top of an agar surface. There are two techniques: in the first one, the essential oil is adsorbed onto a sterile paper disk; in the second one, the EO is put inside a hole into the agar surface. Then, the agar plates are incubated according to the physiological characteristics of the tested bacteria. The antimicrobial agent tested by spreading in the medium inhibits bacterial growth, thereby creating halos of inhibition around the bacterial colonies; the size of inhibition zone is regarded as a measure for the antimicrobial potency of an essential oil [40]. But some lipophilic compounds such as farnesol, although the compound results in a strong inhibition in the serial dilution test [41], cause only small inhibition zones, i.e. against *Bacillus subtilis* [42]. Thus, strong inhibitors having low water solubility gave a poor or even negative result in the agar diffusion test. For this reason, it is better to perform different tests. Similarly, it is important to interpret the size of inhibition zones, which depends on both the diffusion coefficient and antimicrobial activity of every compound present in an essential oil [43].

3.2 Dilution test

In the dilution test, the essential oil to be tested is incorporated in a semisolid agar medium or liquid broth in several defined amounts. The absence of growth in agar plates or test tubes is determined with the naked eye after incubation. The minimum inhibitory concentration (MIC) is the concentration of essential oil present in the ungrown agar plate or test tube with the highest amount of test material. When essential oils are tested, the main difficulty is caused by their low water solubility. The addition of solvents (e.g. dimethylsulfoxide and ethanol) or detergents (e.g. Tween 20) to the growth medium is unavoidable, which however influences the MIC [44–46]. Another problem is the volatilisation of essential oils during incubation. Furthermore, MIC-influencing test parameters are the size of inoculum, the pH of growth medium and the incubation time.

Nevertheless, the serial dilution test in liquid broth was recommended for natural substances [47] and is standardised for the testing of antibacterial and antifungal drugs in liquid broth and agar plates [48]. Its use enables a link to data of pharmaceutical drugs and an easier interpretation of test results. All concentrations are recalculated in μg/ml [1, 49].

3.3 Vapour phase test

The components of EOs and their relative volatilities determine the characteristics of their vapours, which in turn affect the antimicrobial potential [50, 51]. For this test, a standardised method for testing the antimicrobial activity of essential oils does not exist. Recently, several studies confirmed that vapour phases of EOs are more effective antimicrobials than their liquid phases [51–53] probably because the lipophilic molecules in the aqueous phase associate to form micelles and thus suppress the attachment of the EOs to the organism, whereas the vapour phase allows free attachment [54].

3.4 TLC bioautography

Direct bioautography combined with thin layer chromatographic (TLC) separation is a rapid and sensitive screening method for the detection of antimicrobial compounds. Test microorganism cultures are capable of growing directly on the TLC plate, so each step of the assay is performed on the sorbent. Similar to the common antimicrobial screening methods, TLC bioautography must be carried out under controlled conditions, since the experimental conditions (e.g. solvent, sample application, resolution of compounds, type of microorganism and incubation time) may influence the result [55]. The advantages of direct bioautography are that it is suitable for evaluating complex plant extracts and facilitates rapid, economic and easy evaluation. The use of bioautography to detect antimicrobial compounds effective against plant and human pathogenic bacteria has been reported in the literature [56, 57].

4. Uses of mint essential oils

The mint species has always been widely used; the leaves, flowers, and stems of *Mentha* spp. are used traditionally in herbal teas or in several folk remedies for treatment [58, 59]. Recently, mint essential oil, as well as other plant essential oils, can be used as food preservative, in fact, there is a growing interest in the development of edible and biodegradable films for food made from bio-polymers, conservation and preservation instead of the synthetic preservatives and chemical additives once, that can cause intoxication, cancer and other degenerative diseases [60]. In addition, biobased active packaging facilitates continuous migration of active components into the food remaining at high concentrations for a prolonged time period [61]. Mint essential oil contains phenolic compounds such as α-pinene, citronellol, and methyl eugenol, which have antimicrobial activity against a wide range of microorganisms and antioxidant activity; for these reasons, MEOs are widely used as food additives and in pharmaceutical industries because they are considered as potent film additives that help in preventing lipid oxidation and microbial spoilage of foods [62]. Another interesting idea was to add mint essential oil (MEO) into gelatin-based edible films with an effective inhibition of microbial growth on the film surface [63]. Moreover, MEOs are also used both in agriculture to fight bacterial and fungal diseases [64] and to give other examples and in

aquaculture as an additive in fish feed to increase immune defences, but also as sedative and anaesthetic for farmed fish [65].

5. Conclusion

MEOs have antibacterial effects against a wide range of pathogenic microorganisms in humans, fish, and vegetables also. MEOs' antibacterial activity is linked to their chemical composition rich in pulegone, menthone, menthol, carvone, 1, 8-cineole, limonene and b-caryophyllene and phenolic compounds also such as α-pinene, citronellol and methyl eugenol. For these reasons, MEOs are widely used as food additives and in pharmaceutical industries to prevent microbial spoilage of foods. The most used methods to test the antimicrobial activity of EOs are the disc diffusion method, the determination of minimum inhibitory concentration (MIC), and the vapour phase method, and to have the most truthful analysis possible on the antibacterial characteristics, it is better to use more than one method. The use of MEOs, and in general of EOs, is very important because being natural substances and therefore easily biodegradable, it could be a promising alternative to synthetic materials to fight the increasingly common bacterial infections.

Conflict of interest

The author declares no conflict of interest.

Author details

Monique Mancuso[1,2]

1 Institute for Marine Biological Resources and Biotechnology (IRBIM), National Research Council (CNR), Section of Messina, Messina, Italy

2 Anton Dohrn Zoological Station (CIS-Italy), Italy

*Address all correspondence to: monique.mancuso@cnr.it

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