

In vitro* antimicrobial activity, phytochemical analysis and total phenolic content of essential oil from *Mentha spicata* and *Mentha piperita

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Abstract

To find the efficacy of *Mentha spicata* and *Mentha piperita* essential oils against selected clinical isolates. The oil from both common herbs has been evaluated for phytochemical constituents, TLC bioautography assay and total phenolic content. The antimicrobial potential of mint species essential oils was evaluated by agar well diffusion method against selected clinical isolates. Preliminary phytochemical analysis and total phenolic content was analyzed. The antibacterial effect was investigated using the TLC-bioautographic method. Antimicrobial activity of mint species essential oils was assessed on 11 bacterial and 4 fungal clinical isolates. Both the essential oils showed maximum activity against *S. aureus* 1, producing the maximum zone of inhibition 21 ± 0.09 mm in *Mentha spicata* and 19.2 ± 0.07 mm in *Mentha piperita*. Preliminary phytochemical analysis demonstrated the presence of most of the phytochemicals including flavonoids, saponins, cardiac glycosides, reducing sugars and steroids in both the essential oils tested. Thin layer chromatography and bioautography assay in *Mentha spicata* essential oil demonstrated well defined growth inhibition zones against *Acinetobacter* spp. in correspondence with alkaloids observed at *Rf* value ranging from 0.76 to 0.90. Total phenolic content shows that *Mentha piperita* had the highest contents of total phenolic (12.63 ± 0.878 μ g GAE) followed by *Mentha spicata* (9.41 ± 0.594 μ g GAE). Based on the present study, the essential oils from mint species possess antimicrobial activity against several clinical isolates tested and thus can be a good source of natural antimicrobial agent.

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Keywords

Antimicrobial activity

Essential oils

Mentha spicata

Mentha piperita

TLC bioautography assay

Total phenolics

Introduction

Herbs are an ancient source of medicine, flavouring, beverages, dyeing, fragrances and cosmetics uses that have attracted biotechnology, cosmetics, pharmaceutical and food industries. *Mentha* is a genus of widely distributed aromatic perennial herbs that grows in the temperate regions of Eurasia, Australia and South Africa. The mint species possesses both medicinal and commercial importance. The leaves, stems and flowers of *Mentha* species are used in various foods to offer aroma and flavour and is also used in herbal teas. It has also been used as a folk remedy for treatment of fevers, headaches, digestive disorders, bronchitis, ulcerative colitis, liver complaints etc. Spearmint (*Mentha spicata*) and Peppermint (*Mentha piperita*) are among the important members of the *Lamiaceae* family.

Spearmint is an aromatic herbal plant used widely in cosmetic, confectionary, chewing gum, food, toothpaste, pharmaceutical industries and for essential oil productions. It is an important herb used fresh and dried for folk medicine such as stimulant and carminative. The essential oil is extracted from

freshly harvested mint leaves or from dried leaves via distillation process. The essential oil obtained has been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties (Singh and Aggarwal, 2013). The essential oil contains significant amounts of limonene, dihydrocarvone, and 1, 8- cineol (Hussain *et al.*, 2010). The distinctive smell of spearmint oil is because of its most abundant compound carvone.

Peppermint is a perennial 50–90 cm high, strongly scented and a prototypical member of the mint family. The *Mentha piperita* plant is aromatic, stimulant and used for allaying nausea, headache and vomiting. The leaves of peppermint are commonly used in herbal tea and for culinary purpose to add flavour and aroma. The oil obtained is widely used essential oils in food products, dental preparations, mouthwashes, cosmetics, pharmaceuticals, soaps and alcoholic liquors. It is also found to possess astringent, antiseptic, antipyretic, antispasmodic, antimicrobial rubefacient, stimulant, emmenagogue and anti-aging properties (Ali *et al.*, 2002). The distinctive smell and flavor of this *Mentha* species is because of its high menthol content. Essential oils are dominated by

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monoterpenes mainly menthol, menthone and their derivatives (Jeyakumar *et al.*, 2011).

Resistance of pathogens to antimicrobial compounds has lethal effects as the development of drug resistance outpaces the development of new drugs. Infectious diseases, a leading cause of untimely death worldwide, have become a global concern. The clinical effectiveness of many existing antibiotics is being threatened by rapid emergence of multidrug resistant pathogens (Penner and Medson, 2005). Down the ages there has been an increasing interest in the use of plant extracts and essential oils as alternative remedies for the treatment of various infectious diseases. Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties (Burt, 2004; Kordaly *et al.*, 2005). Essential oil obtained from plants is safe, cost effective and has no side effects as compared to costly synthetic drugs having adverse effect. Keeping in view the above issues, the present study was aimed to determine the *in vitro* antimicrobial activity, phytochemical analysis, TLC bioautography assay and total phenolic content of essential oils from *Mentha spicata* and *Mentha piperita* common herbs against Gram-positive, Gram-negative and fungal clinical isolates.

Material and Methods

Acquisition of essential oils

Commercial brands of *Mentha spicata* and *Mentha piperita* essential oil was purchased from Delhi, India. As per manufacturer's information, it was prepared by steam distillation. The oil was further distilled by rotary evaporator. The essential oil was dissolved in methanol (0.3 ml oil/2 ml methanol). The oil was transferred into sterile vials and stored at 4°C till further analysis.

Microbial cultures and Growth conditions

The microbial cultures included multi-drug resistant isolates of *Escherichia coli*, *Salmonella typhi*, *Salmonella paratyphi*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter* spp. The cultures of bacteria were maintained on nutrient agar slants at 4°C throughout the study. Four fungal isolates studied includes *Aspergillus niger*, *Aspergillus* spp., *Candida albicans* and *Rhizopus nigricans*. The cultures were maintained on potato dextrose agar at 4°C. These microbial isolates served as test pathogens for studying antimicrobial activity.

Antimicrobial activity assay

The agar well diffusion method was employed with slight modifications to determine the antibacterial activities of *Mentha* species essential oils in methanol (Dahiya and Manglik, 2013). All tests were performed in triplicate and the antimicrobial activity was expressed as the mean of inhibition.

Phytochemical analysis

The oils dissolved in methanol (0.3 ml oil/2 ml methanol) were evaluated for the presence of different phytochemicals. The essential oil was tested for the presence of various phytoconstituents including alkaloids, anthraquinones, cardiac glycosides, flavonoids, reducing sugars, saponins, steroids, tannins and phlobatanins by using wet reactions (Ling *et al.*, 2011; Ghamba *et al.*, 2012).

TLC bioautography assay

Mentha spicata essential oil exhibiting significant antimicrobial potential against *S.aureus* 1 and *Acinetobacter* spp. as determined by agar well diffusion method was analyzed using TLC bioautography assay (Dahiya and Purkayastha, 2012).

Analysis of total phenolic content

A total phenolic content (TPC) was determined according to Folin-Ciocalteu's method (Singleton *et al.*, 1999). The essential oils were diluted to a suitable concentration for analysis. Half millilitres of essential oil, 1 mL of 1N Folin-Ciocalteu's reagent and 1 mL of 20% Na₂CO₃ (w/v) were mixed. The mixture was incubated for 2 h with continuous shaking at ambient temperature. The optical density was measured at 765 nm. Different concentrations of gallic acid (10-90 µg/mL) were determined to be a calibration curve. The results were shown as µg gallic acid equivalents (GAE)/5mg essential oil.

Statistical analysis

The antimicrobial activity of oils was indicated by clear zones of growth inhibition. The resultant clear zones around the agar wells were measured in mm. All the experiments were conducted in triplicate and the data are presented as mean values ± standard deviation.

Results

The evaluation of antibacterial activity of *Mentha spicata* and *Mentha piperita* essential were found to effectively inhibit the clinical isolates tested, however, with differing sensitivity. The antibacterial activity of

Table 1. Phytochemical analysis of *Mentha spicata* and *Mentha piperita* essential oil

Phytoconstituents	<i>Mentha spicata</i> oil	<i>Mentha piperita</i> oil
Alkaloids	+	+
Reducing sugar	+	+
Saponins	+	+
Steroids	+	+
Anthraquinone	-	-
Cardiac glycoside	+	+
Flavonoids	+	+
Phlobatanins	-	-
Tannins	-	-

+: Present, -: Not present

both the essential oils was assessed using the agar well diffusion method by measuring the diameter of zones of inhibition as represented in Table 1. In both the oils tested, Gram positive organisms (*Staphylococcus aureus*) were found to be more susceptible than the Gram negative organisms (*E. coli*, *P. aeruginosa*, *Salmonella* spp.). Among the Gram positive bacteria, both the essential oils showed maximum activity against *S. aureus* 1, producing the maximum zone of inhibition 21 ± 0.09 mm in *Mentha spicata* and 19.2 ± 0.07 mm in *Mentha piperita*. Among Gram negative organisms tested, *Acinetobacter* spp. was more sensitive with 18 ± 0.11 mm zone of inhibition in *Mentha spicata* essential oil as compared to *E. coli* (14 ± 0.05 mm) and *Klebsiella* spp. (12.7 ± 0.07 mm). No inhibition was observed in case of *S. typhi*, *S. paratyphi* and *P. aeruginosa*. With respect to the fungal clinical isolates tested, *C. albicans* showed a higher inhibition zone of 11.7 ± 0.12 mm as compared to *Rhizopus nigricans* possessing inhibition zone of 8.3 ± 0.05 mm in *Mentha piperita* essential oil. Whereas, excellent antifungal activity against *Aspergillus niger* with inhibition zone 15.7 ± 0.09 mm was exhibited by *Mentha spicata* essential oil. The oil also possesses antifungal activity against *Aspergillus* spp. (13 ± 0.13 mm) and *Candida albicans* (11.8 ± 0.10 mm).

Preliminary phytochemical analysis revealed the presence of flavonoids, saponins, cardiac glycosides, reducing sugars and steroids in both the essential oils tested (Table 2). None of the tested essential oil had shown the presence of tannins, phlobatannins and anthraquinone. Bioautographic assay is usually used to screen for antimicrobial activity by separating components onto the surface of chromatographic plates and overlaying the TLC plate with molten bacterial agar. TLC bioautography was performed

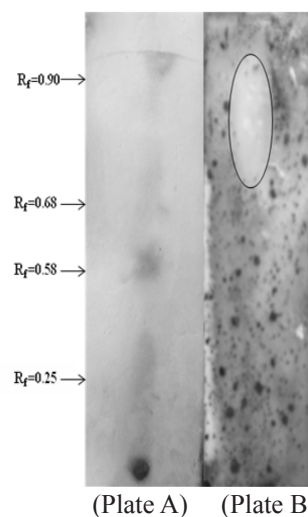


Figure 1. Chromatogram and Bioautogram of essential oil of *Mentha spicata* against *Acinetobacter* spp. Arrow (Plate A) indicates spots visualized when sprayed with vanillin/sulfuric acid reagent. Zones of inhibition (Plate B) are encircled and observed as clear spots against pink background

for *Mentha spicata* essential oil against *S.aureus* 1 and *Acinetobacter* spp. Inhibition zones against the growth of *S. aureus* 1 and *Acinetobacter* spp. was observed on the TLC plates of essential oil as clear spots on pink background when sprayed with aqueous solution of 2, 3, 5 Triphenyl tetrazolium chloride. One large inhibition zone with R_f value ranging from 0.76 to 0.90 which was also positive when the TLC plate was sprayed with dragendorff's reagent. This result suggests that the antimicrobial activity present in *Mentha spicata* essential oil against *Acinetobacter* spp. may be due to the presence of alkaloids. It is possible that the observed inhibition was likely due to one or more active compounds which overlap possibly due to the solvent system used for screening. In addition to the components with antimicrobial activity several compounds on the reference chromatogram were visible in UV light at 235 nm (data not shown) and others that were visible by using vanillin/sulphuric acid reagent, many of these compounds did not coincide with the antimicrobial components. No inhibition zone was observed corresponding to the spots with the R_f value of 0.25, 0.58 and 0.68 respectively on reference chromatogram (plate A). Likewise, antibacterial activity was not found in the assayed oil for the spots with R_f values below 0.68 (Figure 1).

The phenolic compounds prove the importance of antioxidant behaviour and contribute significantly to the total antioxidant activity of aromatic herbal plant samples. The total phenolic contents of the two essential oils were expressed as equivalents of gallic acid (GAE/5 mg of essential oil). *Mentha piperita*

Table 2. Antimicrobial activity of *Mentha spicata* and *Mentha piperita* essential oil determined by agar well diffusion method

Test Microorganisms	Zone of Inhibition (in mm)	
	<i>Mentha spicata</i> oil	<i>Mentha piperita</i> oil
Bacterial isolates		
<i>Acinetobacter</i> spp.	18±0.11	15.9±0.13
<i>E.coli</i> 1	14±0.05	-
<i>Klebsiella</i> spp.1	12±0.12	17.3±0.07
<i>Klebsiella</i> spp. 2	12.7±0.07	-
<i>Klebsiella</i> spp. 3	-	-
<i>Salmonella typhi</i>	-	-
<i>Salmonella paratyphi</i>	-	-
<i>S. aureus</i> 1	21±0.09	19.2±0.07
<i>S. aureus</i> 2	11.4±0.11	12±0.12
<i>S. aureus</i> 3	15±0.085	14.9±0.08
<i>P. aeruginosa</i>	-	-
Fungal isolates		
<i>Aspergillus</i> spp.	13±0.13	-
<i>Aspergillus niger</i>	15.7±0.09	-
<i>Candida albicans</i>	11.8±0.10	11.7±0.12
<i>Rhizopus nigricans</i>	-	8.3±0.05

Zone of inhibition is expressed as mean ±standard deviation, -: no inhibition

had the highest contents of total phenolic followed by *Mentha spicata*. For each 5 mg essential oil, *Mentha piperita* had 12.63± 0.878 µg GAE and *Mentha spicata* had 9.41 ± 0.594 µg GAE of total phenolic.

Discussion

The traditional use of plants as medicines, increasing antibiotic resistance of pathogens and undesirable side effects of antibiotics suggested the use of *Mentha* essential oils as antibiotics or alternatives for the treatment of various infectious diseases. Various plant extracts and essential oils have been used as topical antiseptics and reported to possess antimicrobial properties. There is a need to investigate scientifically, the plant oils and extracts, which have been used in traditional medicines as potential sources of novel antimicrobial compounds (Mitscher *et al.*, 1987). In the present investigation, two mint species possessing both medicinal and commercial importance are studied. The essential oils have been screened for the presence of antimicrobial activity against clinical isolates, phytochemicals, total phenolic content and bioactive compound responsible for antibacterial potential by TLC bioautographic analysis.

In vitro antimicrobial potential of the two essential oils from *Lamiaceae* family was assessed quantitatively by agar well diffusion method. *Mentha spicata* and *Mentha piperita* essential oils investigated in the present study exhibited varying degree of

inhibitory effect against the selected Gram positive and Gram negative clinical isolates. Previous reports also revealed the antibacterial activity of the mint essential oils against *S. aureus*, *E. coli* and *Klebsiella* spp. (Jeyakumar *et al.*, 2011; Sujana *et al.*, 2013; Singh and Agarwal, 2013). The inhibitory effect of spearmint oil against *Escherichia coli*, *Bacillus subtilis*, *Aspergillus niger* and *Candida albicans* has also been reported by Suleiman *et al.* (2011). The results are consistent with the reports of previous investigators. However, Sartoratto *et al.* (2004) reported that peppermint oil was found to be strongly effective against *Salmonella* species which was not observed in the present investigation. Our results are in fair correlation with the studies in which spearmint and peppermint oil both showed antibacterial activities against Gram -ve and Gram +ve bacteria (El-Kady *et al.*, 1993; Pattnaik *et al.*, 1997; Singh *et al.*, 2011). The oil also possesses antifungal activity against *Aspergillus* spp. and *Candida albicans*. The differences in the antimicrobial activities with the reported one may be due to different geographical environment, age of the plant, different method followed for isolation of oil, cultivar type, seasonality etc.

Phytochemical analysis of the mint essential oils showed that the oils contain most of the phytoconstituents including flavonoids, saponins, cardiac glycosides, reducing sugars and steroids. Our results were in agreement with Singh *et al.* (2011) and Naidu *et al.* (2012) who observed the presence

of reducing sugar, flavonoids and alkaloids. None of the tested essential oil had shown the presence of tannins, phlobatannins and anthraquinone.

The bioactive components were separated on TLC followed by TLC bioautography of *Mentha spicata* essential oil against potential inhibitors, *S. aureus* 1 and *Acinetobacter* spp. The potential antibacterial activity present in *Mentha spicata* oil may be due to the presence of alkaloids which was confirmed when the TLC plate was sprayed with dragendorff's reagent. It is possible that the observed inhibition was likely due to one or more active compounds which overlap possibly due to the solvent system used for screening. No inhibition zone was observed corresponding to the spots with the *R_f* value of 0.25, 0.58 and 0.68 respectively on reference chromatogram. This could be attributed to evaporation of the active components, insufficient amount of the active component and photo-oxidation (Masoba and Eloff, 2005).

The antioxidant potential of mints greatly depends on the presence of phenolics. The major phenolic constituents of mints especially include rosmarinic acid and flavonoids, including flavones, flavanones and their glycosidic forms (Janicsak *et al.*, 1999; Areias *et al.*, 2001). Amongst the essential oils tested, *Mentha piperita* had the highest contents of total phenolic followed by *Mentha spicata*. Similar results were reported by Gharib and Silva (2013) and Nickavar *et al.* (2008) Whereas, Derakhshani *et al.* (2012) reported among mint species, spearmint had the highest TPC, though there was no significant difference between spearmint and peppermint. Mint extracts had good flavonoid content and total phenolic content (Naidu *et al.*, 2012). Thus, the antibacterial and antifungal activity of Mint species essential oil is attributable to the presence of phenolics and alkaloids. Food and cosmetic industries prefer antimicrobial agents and natural antioxidants for their potential benefits compared to their chemical counterparts. Due to volatile nature essential oils are the drugs for prevention and treatment of diseases. Hence it can be concluded that the *Mentha* species. essential oils can be a good source of natural antimicrobial agent.

Conclusion

Results from the present study indicated that the Mint essential oil can be used as a potential source of natural antimicrobial compound and the presence of phenolic and alkaloids possessing strong antioxidant potential. Hence it is essential to explore further by the identification of biologically active compounds, characterization and purification of the crude extracts

of *Mentha* species present.

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