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Chemical composition and antimicrobial activity of the essential oil of *Mentha piperita* endemic in Khorasan-Iran

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ABSTRACT

The aim of this study was to determine the composition and antimicrobial effect of *Mentha piperita* essential oil in "in-vitro" condition. The chemical composition of the essential oil obtained by hydro-distillation was examined by GC/MS and the antimicrobial effect was studied on the growth of seven microbial species including *Bacillus cereus, Pseudomonas aeruginosa and Proteus vulgaris* using micro-dilution method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. Chemical composition analysis identified a total of 28 compounds in which the main components were Menthol (32%), Mentone (13.4), Menthyl acetate (12%), 1,8-Cineole (8.2%) and Neomenthol (4%) representing 69.6 % of the total oil. Other separated components accounted for less than 30.4% of the oil. Results of antimicrobial analysis showed that the MIC values for *Bacillus cereus, Pseudomonas aeruginosa and Proteus vulgaris* was respectively 50, 200 and 100 µg/ml and the MBC was determined at 200, 400 and 200 µg/ml respectively. The results of the present study indicated that *Mentha piperita* essential oil had significant antimicrobial activity. © 2015 Trade Science Inc. - INDIA

KEYWORDS

Mentha piperita;
Antimicrobial activity;
Essential oil composition.

INTRODUCTION

One of the more alarming recent trends in infectious diseases has been the increasing frequency of antimicrobial resistance among microbial pathogens causing nosocomial and community-acquired infections[1]. In the recent years, efforts have been devoted to find new antimicrobial materials from natural resources for food preservation[2]. Reports indicated that many extracts and essential oils of edible plants had properties to prevent against a wide range of fungal contamination of foods[3-5].

Essential oils have long been applied as flavoring agents in foods, they have shown a wide spectrum of antimicrobial activity on food borne pathogens and spoilage bacteria[6]. There are more than 1300 plants with defined antimicrobial compounds but characterization of preservative properties is available for only few Essential oils[7].

The well-known and widely used peppermint (*Mentha piperita* L.) (Lamiaceae) is a cultivated natural hybrid of *Mentha aquatica* L. (water mint) and *Mentha spicata* L. (spearmint). Although a native genus of the Mediterranean region, it is cultivated all over the world

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for its use in flavor, fragrance, medicinal, and pharmaceutical applications. Peppermint oil is one of the most widely produced and consumed essential oils[8].

Peppermint (Mentha piperita) is a herbaceous rhizomatous, fast spreading, perennial and winter hard plant. It grows 30–90 cm tall, with smooth stems, square in cross section[9].

Besides its uses in food, herbal tea preparations, and confectioneries, the medicinal uses of mint, which date back to ancient times, include carminative, antiinflammatory, antispasmodic, antiemetic, diaphoretic, analgesic, stimulant, emmenagogue, and anticatharrhal application. It is also used against nausea, bronchitis, flatulence, anorexia, ulcerative colitis, and liver complaints. Mint essential oils are generally used externally for antipruritic, astringent, rubefacient, antiseptic, and antimicrobial purposes, and for treating neuralgia, myalgia, headaches, and migraines[8].

The aim of present study was to evaluate the composition and potential antimicrobial activities of essential oil of *Mentha piperita* (collected from Khorasan-Iran) on the growth of some food born pathogens.

MATERIALS AND METHODS

Plant material and Extraction of essential oil

Aerial parts of the *Mentha piperita* plant were collected in 2013 from Khorasan-Razavi Province (the northeast of Iran). The plant confirmed by Medicinal Plants Institute, Ferdowsi University, Mashhad, Iran. The essential oil of aerial parts of the *Mentha piperita* was extracted with water steam distillation using a clevenger apparatus according to the method of British Pharmacopoeia. The distilled essential oils were dried with anhydrous sodium sulfate and stored in the sterilized vial at 4°C until use[5].

Analysis of the essential oil

The chemical composition of the essential oil was analyzed using GC–MS technique. The mass spectrometer was Agilent 6890 N GC/5973MSD-SCAN (Agilent Technologies, Palo Alto, CA, USA) in the electron impact (EI) ionization mode (70ev) and HP- 5MS (bonded and cross-linked 5% phenylmethylpolysiloxane,30 mm-0.25 mm, coating thickness 0.25 mm) capillary column (Restek, Bellefonte, PA). Injector and detector temperatures were set at 220°C. The oven temperature was held at 50°C for 30 min, then programmed to 240°C at rate of 3°C/min. Helium (99.99%) was the carrier gas at a flow rate of 1 ml/min. Diluted samples (1/100 in hexane, v/v) of 1.0 were injected manually. The identification of the components was based on the comparison of their retention times and mass spectra with the data given in the literature, National Institute of Standard and Technology (NIST), Wiley and our own created library[10].

Organisms and inoculation conditions

The test organisms used in this study included *Bacillus cereus* PTCC1023, *Pseudomonas aeruginosa PTCC1310, Proteus vulgaris PTCC1449* which were obtained from Persian Type Culture Collection (PTCC), Iran.

To prepare microbial suspension, the bacterial species were cultivated on nutrient agar (Merck, Germany) slant at 37°C for 24 h. Finally, suspensions were adjusted to 0.5 McFarland standard turbidity[11]. Bacterial suspensions were standardized to concentrations of 1.5×10^8 CFU/ml[4,12].

Minimum inhibitory concentration (MIC) test

Mentha piperita essential oil dissolved at 5% dimethyl sulfoxide (Aplichem, Germany) and Then, it diluted to the highest concentration (25600 µg/ml), and then serial twofold dilutions were made in a concentration range from 12.5 to 6400 µg/ml.

MIC values of essential oil against microbial strains were determined based on a microwell dilution method. Ninety five µl of Mullerhinton broth (Merck, Germany) was dispended in to each 96 wells. One hundred µl of stock solution of *Mentha piperita* essential oil was added in to the first wells. Then 100 µl from their serial dilutions was transferred in to other consecutive wells except the well number 11 as positive control. Then 5 µl of the microbial suspension was added to each well except well number 12 as negative control. Contents of each well were mixed on a plate shaker at 300 rpm for 20 seconds and then incubated at 37°c for 24 h. Microbial growth was determined by detecting the absorbance at 630 nm using the ELX808 Elisa reader (Biotek Instrument Inc, USA). The MIC of essential oil was taken as the lowest concentration that showed no growth[11].

TABLE 1 : The main components of *Mentha piperita* essential oil in other studies

Main components					Origins of plant	References
Mentone (13.4)	Menthol (32%)	1,8-cineole (8.2%)	Menthyl acetate (12%)	Neomenthol (4%)	Iran, Khorasan	Current research
menthone (27.9%)	menthol (27.5%)	1,8-cineole (5.3%)	menthofuran (5.5%)	pulegone (6.4%)	Turkey	8
menthone 25%	menthol 51%	menthyl acetate 6%	1,8 cineole 7%	---	Algeria	13
Menthone (30.63 %)	menthol (25.16 %)	menthofuran (6.47 %)	β-phellandrene (5.59 %)	isomenthone (4.74 %)	Iran	14

TABLE 2 : Minimum inhibitory concentration (µg/ml) and minimum fungicidal or bactericidal concentration (µg/ml) of essential oil of *Mentha piperita* essential oil

Microorganisms	MIC (µg/ml)	MBC (µg/ml)
Bacillus cereus	50	200
Pseudomonas aeruginosa	200	400
Proteus vulgaris	100	200

The values in the table are an average of 3 experiments

RESULTS AND DISCUSSION

Chemical composition of *Mentha piperita* essential oil

Chemical composition analysis identified a total of 28 compounds in which the main components were Menthol (32%), Mentone (13.4), Menthyl acetate (12%), 1,8-Cineole (8.2%) and Neomenthol (4%) representing 69.6 % of the total oil. Other separated components accounted for less than 30.4% of the oil. Different studies have been done in other regions on chemical composition of the essential oil of different species of *Mentha piperita*. TABLE 1 shows and compares the main components of *Mentha piperita* essential oil in current research and other studies.

Effect of essential oil of *Mentha piperita* on microbial species

Antimicrobial activity of essential oil of *Mentha piperita* was determined via the microwell dilution method at 10 concentrations against three bacteria species. The results of in vitro antimicrobial activity assay showed that the essential oil possessed broad antimicrobial activity against the microorganisms tested.

The antimicrobial effect of essential oil against the microorganisms is shown in TABLE2. Results obtained from the microdilution method, followed by measurements of MIC and MBC indicated that essential oil of *Mentha piperita* exhibited significant antibacterial activity against tested bacteria and the sensitivity was as follows: *B.cereus*> *P. vulgaris*>*P.aeroginosa*.

It has been accepted that the anti-microbial activity of most essential oils is related to their phenolic monoterpenes[12]. According to TABLE 1, *Mentha piperita* essential oil is a good source of oxygenated mono-terpenes, in particular menthone which have significant anti- microbial properties. Although due to the differences in the test methods, bacterial strains, plant source, genetic constitution and harvest season making a direct comparison of the findings from different studies is difficult, but we can have a view on the composition of the essential oil in different environmental positions and conditions. Iscan et al. (2008), Benchikha et al. (2008) and Moghaddam et al. (2013) confirmed our findings about the chemical composition of the essential oil[8,13,14].

Oxygenated mono-terpenes including menthone are lipophilic in nature and act on the cell membrane which cause substantial morphological damage, resulting in a change in permeability and the release of cellular contents[15].

CONCLUSION

Mentha piperita is a popular and medicinal plant native to Iran. During recent years, more attention has paid to this plant due to its significant antimicrobial activity in food industry. This study characterized chemical composition and antibacterial properties of *Mentha piperita* essential oil endemic to Khorasan province in Iran. In conclusion, the results of the present work showed that *Mentha piperita* essential oil had an antimicrobial activity and can be used as an antimi-

crobial additive. However, further studies are needed to evaluate the organoleptic and pharmaceutical effects and practical effectiveness of this application.

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