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Chemical composition and antimicrobial effect of the essential oil of *Mentha longifolia* L.

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ABSTRACT

The aim of this study was to determine the composition and antimicrobial effect of *Mentha longifolia* L. essential oil in "in-vitro" condition. For this purpose, the chemical composition of the essential oil which obtained by hydro-distillation was examined by GC/MS and the antimicrobial effect was studied on the growth of bacterial species including *Bacillus cereus, Pseudomonas aeruginosa and Proteus vulgaris* using micro-dilution method. The minimum inhibitory concentration (MIC) and minimum bactericidal or fungicidal concentration (MBC, MFC) were determined for the essential oil. Chemical composition analysis of the essential oil identified a total of 66 compounds. The main components of essential oil were Carvone (32%), 1,8-cineole (19%), piperitone (12%), limonene (9%) and limonene (6%) representing 78% of the total oil. Other separated components accounted for less than 22% of the oil. Results of antimicrobial analysis showed that *Bacillus cereus* (MIC=58 and MBC=232 μg/ml) was more resistance than two other bacterial species. The results of the present study indicated that *Mentha longifolia* L. essential oil had significant antimicrobial activity; therefore, it can be used as a natural preservation to increase the shelf life of food products.

INTRODUCTION

Food borne disease mediated by pathogenic microorganisms or microbial toxins is an important global public health problem because they take a huge toll on human health and mortality[1]. It has been estimated that as many as 30% of people in the industrialized countries suffer from food borne diseases each year caused by microbes[2]. Food additives have been used for centuries in the food processing practices for several purposes including the prevention of microbial growth and increase in the food shelf lives[3]. Due to the excessive use of food preservatives which some of them are doubtful to be carcinogenic and teratogenic and also increasing consumer demand to natural foods with a long shelf life and without chemical preservatives, food producers trend to replace chemical preservatives with natural forms such as oils and herbal extracts as antibacterial additives[4,5]. In the recent years, efforts have been devoted to find new antimicrobial materials from natural resources for food preservation[6]. Reports indicated that many extracts and essential oils of edible plants had properties to prevent against a wide range of fungal contamination of foods[4,6-11].

The mints, *Mentha* species belonging to the family Labiatae (Lamiaceae), are widely distributed in Eurasia, Australia, and South and North Africa. Various species of *Mentha* have been used as folk remedies for treatment of bronchitis, flatulence, anorexia, ulcerative colitis and liver complaints, due to their anti-inflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue, and anticatharral

activities[12].

Regarding to *Mentha longifolia* L., many studies have focused on chemical and functional characteristics of the plant, but the aim of present study was to evaluate the composition and potential antimicrobial activities of essential oil of another variety of *Mentha longifolia* L. (collected from Khorasan-Iran) on the growth of some bacteria which had not been studied.

MATERIALS AND METHODS

Plant material and extraction of essential oil

Aerial parts of the *Mentha longifolia* L plant were collected in 2013 from Khorasan 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 longifolia* L. 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[23].

Organisms and inoculation conditions

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

To prepare microbial suspension, 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[13,14]. Bacterial suspensions were standardized to concentrations of 1.5×10^8 CFU/ml[14].

Minimum inhibitory concentration (MIC) test

Mentha longifolia L. essential oil dissolved at 5% dimethyl sulfoxide (Aplichem, Germany) and Then, it diluted to the highest concentration (30000 µg/ml), and then serial twofold dilutions were made in a concentration range from 7.25 to 7500µg/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 longifolia* L. 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 s 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[13,14].

RESULTS AND DISCUSSION

Chemical composition of *Mentha longifolia* L. essential oil

Chemical composition analysis of the essential oil identified a total of 66 compounds. The main components of essential oil were Carvone (32%), 1,8-cineole (19%), piperitone (12%), limonene (9%) and limonene (6%) representing 78% of the total oil. Other separated components accounted for less than 22% of the oil.

It has been accepted that the anti-microbial activity of most essential oils is related to their phenolic monot-

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erpenes[15]. According to the findings, *Mentha longifolia* L. essential oil is a good source of oxygenated mono-terpenes which have significant anti- microbial properties.

Effect of essential oil of *Mentha longifolia* L. on microbial species

Antimicrobial activity of essential oil of *Mentha longifolia* L. was determined via the microwell dilution method against three bacteria. 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 TABLE 1. Results obtained from the microdilution method, followed by measurements of MIC and MBC indicated that essential oil of *Mentha longifolia* L. exhibited significant antibacterial activity against tested bacteria and the sensitivity was as follows: *P. vulgaris*>*P.aeroginosa*> *B.cereus*.oxygenated mono-terpenes 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[16].

TABLE 1 : Minimum inhibitory concentration (µg/ml) and minimum fungicidal or bactericidal concentration (µg/ml) of essential oil of *Mentha longifolia* L. essential oil

Microorganisms	MIC (µg/ml)	MBC or MFC (µg/ml)
Bacillus cereus	58	232
Pseudomonas aeruginosa	14.5	58
Proteus vulgaris	29	58

The values in the table are an average of 3 experiments

High proportion of mono-terpenes present in *Mentha longifolia* L. and its combination with phenolic monoterpens has shown to led to a synergistic activity resulting in destabilization of the microbial membrane[17,18].

The existence of other antimicrobial constituents such as 1,8-cineole[19] combined with other minor constitutes might be involved in improving overall antimicrobial activity of essential oils.

CONCLUSION

Mentha longifolia L. 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 longifolia* L.essential oil endemic to Khorasan province in Iran. In conclusion, the results of the present work showed that *Mentha longifolia* L. essential oil had an antimicrobial activity and can be used as an antimicrobial additive in food systems. However, further studies are needed to evaluate the organoleptic and pharmaceutical effects and practical effectiveness of this application.

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