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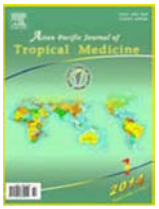

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Antibacterial activity of ethyl acetate and aqueous extracts of *Mentha longifolia* L. and hydroalcoholic extract of *Zataria multiflora* Boiss. plants against important human pathogens

Saeide Saeidi[1], Kazem Hassanpour[2], Mehdi Ghamgosha[3], Mohammad Heiat[4], Ramezan Ali Taheri[5], Ali Mirhosseini[6], Gholamreza Farnoosh[4*]

[1]*Department of Agronomy and Plant Breeding, Agriculture Research Center, Zabol University, Zabol, Iran*

[2]*Medical School, Sabzevar University of Medical Sciences, Sabzevar, Iran*

[3]*Neurosciences Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran*

[4]*Applied Biotechnology Research Centre, Baqiyatallah University of Medical Sciences, Tehran, Iran*

[5]*Nanobiotechnology Research Centre, Baqiyatallah University of Medical Sciences, Tehran, Iran*

[6]*Applied Microbiology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran*

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ABSTRACT

Objective: To determine the potential antibacterial activity of ethyl acetate and aqueous extracts from *Mentha longifolia* L. (*M. longifolia*) and hydroalcoholic extract of *Zataria multiflora* Boiss. (*Z. multiflora*) against important human pathogens.

Methods: *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Klebsiella pneumoniae* (*K. pneumonia*), *Enterobacter cloacae*, *Salmonella typhi*, *Proteus mirabilis*, *Serratia marcescens*, *Bacillus cereus*, *Staphylococcus saprophyticus* and *Staphylococcus aureus* were kinds of pathogenic bacteria to determine the antibacterial effect of aqueous and ethyl acetate extracts of *M. longifolia* and hydroalcoholic extract of *Z. multiflora* using broth microdiluation method.

Results: The lowest minimum inhibitory concentration and minimum bactericidal concentration values for *K. pneumonia* and *Pseudomonas aeruginosa* (1.25 and 2.5 mg/mL) were observed by the hydroalcoholic extract of *Z. multiflora* and the lowest minimum inhibitory concentration and minimum bactericidal concentration values for *K. pneumonia* and *Serratia marcescens* (2.5 and 5 mg/mL) were observed by the aqueous extracts of *M. longifolia*.

Conclusions: In conclusion, it seems that *Z. multiflora* and *M. longifolia* extracts could inhibit the growth of all of the mentioned bacteria.

1. Introduction

Plants' diversity has a considerable importance as a source of pharmaceutically active substances[1-4]. The Labiates family comprised 220 genera of aromatic plants which are widely used for various purposes. *Mentha longifolia* L. (*M. longifolia*), commonly known as a wild mint named Puneh, is a fast-growing and perennial herb that has creeps along an underground rootstock, which can grow to 1-2 m tall. The leaves are grown in pairs opposite each other along the square-shaped stem[5]. Various biological activities have been reported for some species of *Mentha*, as antibacterial[6-8], antifungal[3], and insecticidal properties[9]. It is mainly used for ailments such as coughs, colds, stomach cramps, asthma, flatulence, indigestion and headaches. Externally, it has been used to treat wounds and swollen glands[10].

*Corresponding author: Gholamreza Farnoosh, Applied Biotechnology Research Centre, Baqiyatallah University of Medical Sciences, Tehran, Iran.
Tel/Fax: 021-88617711
E-mail: rzfarnoosh@yahoo.com

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Zataria multiflora Boiss. (*Z. multiflora*), locally known as "Saatar", belongs to Labiatae possessing fragrant odor like lemon and thyme. The plant consists of small ovate or nearly round, leathery leaves mixed with numerous fine flowers[11-13]. It is extensively used in folk medicines of Pakistan and in Iranian traditional medicine. The most effective compounds of *Z. multiflora* are thymol and carvacrol[14,15]. It is mainly used as diuretic, anti-parasite, anti-flatulence and appetizer[16]. The present study was carried out to determine the potential antibacterial activity of ethyl acetate and aqueous extracts of *M. longifolia* and hydroalcoholic extract of *Z. multiflora* against important human pathogens. To achieve that, we attempted to assay the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of these extracts against important human pathogens.

2. Materials and methods

2.1. Plant material

The leaves of *M. longifolia* and *Z. multiflora* were collected from Zabol and Kerman (south-eastern of Iran, the natural sources of these plants). After drying, they were stored in the dark place at room temperature. Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory[17].

2.2. Extraction

Plants were properly dried and pulverized into a coarse powder as reported by Hanafy *et al*[17]. Each of 20 g grinded powders of *M. longifolia* was soaked in 60 mL ethyl acetate and water separately for 1 d (by shaking) and 20 g of *Z. multiflora* was soaked in 60 mL ethanol (95%, v/v) plus water separately for 1 d. After dissolving process, materials were filtered (Whatman No. 1 filter paper) and dried using rotary evaporator. Finally 0.97 g of dried extracts were obtained and then stored at 4 °C in air tight screw-cap tube[18].

2.3. Bacterial cultures

Bacterial strains were obtained from standard laboratory of Veterinary Department in Islamic Azad University, Kerman Branch, Iran. Antibacterial activity of the plant extracts were investigated using Gram-negative bacteria strains [*Pseudomonas aeruginosa* (ATCC 9027) (*P. aeruginosa*), *Shigella dysenteriae* (ATCC 1013) (*S. dysenteriae*), *Klebsiella pneumonia* (ATCC 13183) (*K. pneumonia*), *Salmonella typhi* (ATCC 1006) (*S. typhi*), *Proteus mirabilis* (ATCC 49565) (*P. mirabilis*), *Serratia marcescens* (ATCC 21074) (*S. marcescens*), *Enterobacter cloacae* (ATCC 13047) (*E. cloacae*)] and strains of Gram-positive bacteria [*Bacillus cereus* (ATCC 4010) (*B. cereus*), *Staphylococcus saprophyticus* (ATCC 15305) (*S. saprophyticus*) and *Staphylococcus aureus* (ATCC 6538p) (*S. aureus*)]. The cultures of bacteria was sub-cultured on nutrient agar (Oxoid) and stored at 4 °C until required for study.

2.4. Antibacterial activity assay by determining MIC and MBC

The broth microdilution method was used to determine MIC and MBC. All tests were performed in Mueller-Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/v). Briefly, serial dilutions of the extract were prepared in a 96-well microtiter plate ranging from 0.3 to 10.0 mg/mL. To each well, 10 μL of indicator solution (prepared by dissolving 10 mg extract in 2 mL of dimethyl sulfoxide) and 10 μL of Mueller-Hinton broth were added. Finally, 10 μL of bacterial suspension (10^6 CFU/mL) was added to each well to achieve a concentration of 10^4 CFU/mL. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then incubated at 37 °C for 18-24 h. The color change was then assessed visually. The lowest concentration at which the color change occurred was considered as the MIC value. The three values of the tested extract were provided for the MIC and MBC assay[19].

The MIC is defined as the lowest concentration of the extract at which microorganism does not exhibit the visible growth. The microorganism growth was indicated by turbidity. The MBC was defined as the lowest concentration of the extract at which the incubated microorganism was completely killed[10,20,21].

3. Result

All plants assayed in this study are commonly used as medicinal plants in different areas of Iran and other parts of the world as well. The antibacterial effect and potency of the extracts was quantified by the presence

of inhibition. *Z. multiflora* extracts showed antibacterial activity against human pathogen bacteria with varying strength. The levels of MIC and MBC ranging from 2.5 to 5 mg/mL and from 2.5 to 10 mg/mL, respectively are shown in Table 1. The lowest value of MIC and MBC were observed by hydroalcoholic extract of *Z. multiflora* against *K. pneumonia* and *P. aeruginosa* (1.25 and 2.5 mg/mL) (Table 1). Ethyl acetate and aqueous extracts of *M. longifolia* showed inhibitory activity against Gram-negative and Gram-positive bacteria with varying strength and these effects were dose-dependent shown in Table 2.

Table 1

Antibacterial effects of hydroalcoholic extract of *Z. multiflora* against human pathogenic bacteria. mg/mL.

Standard bacteria	MIC values	MBC values
S. aureus	2.50	5.0
B. cereus	2.50	5.0
E. cloacae	2.50	5.0
S. saprophyticus	5.00	10.0
K. pneumonia	1.25	2.5
S. typhi	2.50	5.0
S. dysenteriae	2.50	5.0
P. mirabilis	2.50	5.0
P. aeruginosa	1.25	2.5
S. marcescens	2.50	5.0

Table 2

Antibacterial effects of ethyl acetate and aqueous extracts of *M. longifolia* extracts against human pathogenic bacteria. mg/mL.

Standard bacteria	Ethyl acetate extract		Aqueous extract	
	MIC values	MBC values	MIC values	MBC values
S. aureus	1.25	2.50	2.5	5
B. cereus	2.50	5.00	5.0	10
E. cloacae	0.62	1.25	2.5	5
S. saprophyticus	5.00	5.00	10.0	10
K. pneumonia	1.25	2.50	2.5	5
S. typhi	1.25	2.50	5.0	5
S. dysenteriae	2.50	5.00	5.0	10
P. mirabilis	1.25	2.50	5.0	10
P. aeruginosa	1.25	2.50	5.0	10
S. marcescens	2.50	5.00	2.5	5

4. Discussion

Plants products have been used for relieving pain in human's diseases. Several books on herbal medicine have been published by Iranian scientists in a few centuries like Avicenna and Razi. The aim of this paper was to substantiate the antibacterial activity of ethyl acetate and aqueous extracts of *M. longifolia* and hydroalcoholic extract of *Z. multiflora* against some important human pathogens. Our study results show that the lowest values of MIC and MBC belong to the hydroalcoholic extract of *Z. multiflora* on *K. pneumonia* and *P. aeruginosa* (1.25 and 2.5 mg/mL). According to study of Rahman *et al.*, *P. aeruginosa* ATCC 27853 had the most sensitive to ethanol and methanol extracts of *Z. multiflora*, respectively[19]. The antibacterial and antifungal activity of *Z. multiflora* have also been reported. According to study of Ghasem *et al.*, MIC of *Z. multiflora* on *Salmonella enteritidis* and *Escherichia coli* (*E. coli*) was enumerated at 6 250 ppm, whereas the MIC of antimicrobial agent for *Listeria monocytogenes* and *S. aureus* were found at 3 125 ppm[22]. In 1999, Marino *et al.* studied the effect of *Zataria* flower as well as leaf extracts on six species of Gram-positive pathogenic bacteria together with nine species of Gram-negative bacteria[23]. The results showed that *Zataria* flower extracts in comparing with the leaf extracts was more effective on bacterial species. It is important for us to observe the highest sensitivity of *E. coli* to *Zataria* flower extracts, which is the main causative agent for gastroenteritis in Iran[24,25]. In the research of Hafedh *et al.*[25], the lowest values of MIC and MBC were observed by the aqueous extracts of *M. longifolia* against *K. pneumonia* and *S. marcescens* (2.5 and 5 mg/mL). At this study, they found that the MIC values of the *M. longifolia* oil have a broad antibacterial activity especially for *S. aureus* and *Micrococcus luteus*. The noted values are 0.78 and 0.19 mg/L, respectively[23]. Nikšić *et al.* proved that the strongest antibacterial activity effect of *M. longifolia* oil was on Gram-negative strains: *E. coli*, *P. aeruginosa* and *Salmonella enterica*[26]. The study of Akroum *et al.* demonostrated that the ethanolic extract was able to inhibit the growth of *S. aureus*, *B. cereus* and *Bacillus subtilis*[27]. The current studies provide the evidences for the presence of active and effective constituents in the hydroalcoholic extract of *Z. multiflora* and ethyl acetate and aqueous extracts of *M. longifolia* as those can inhibit the growth of Gram-positive and negative bacteria. The results of present study recommend that *Z. multiflora* and *M. longifolia* should be explored for their potential use in treatments of bacterial infectious diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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