



Biological activity of various extracts and phenolic content of *Micromeria persica* and *M. hedgei*

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Abstract

Background and objectives: Lamiaceae members have long been used in Iranian Traditional Medicine (ITM) for their various medicinal properties. The main objective of this study was to evaluate the antioxidant capacity and antimicrobial activity as well as the total phenolic content (TPC) of the various extracts and fractions of two Iranian endemic *Micromeria* (*M. persica* and *M. hedgei*). **Methods:** Plant materials were extracted with methanol by maceration for 24 h. Then, the methanol extract (ME) was further fractionated to obtain the chloroform (M-C) and water (M-W) fractions. The antimicrobial activity was investigated against seven Gram-positive and -negative bacteria and three fungi. Antioxidant activity was evaluated by DPPH method and the data were compared with their total phenolic contents. **Results:** The nonpolar sub fractions (M-C) of both plants were active against pathogens especially *Staphylococcus epidermidis* and *Bacillus subtilis* with equal MIC values of 3.75 and 7.5 mg/mL, respectively. Antioxidant activity evaluation showed that the polar fractions of both *Micromeria* species were stronger than nonpolar fractions, while the more considerable effect was observed for the water soluble fraction of the extract for *M. hedgei* with IC₅₀ value of 59.1 µg/mL in comparison to *M. persica* (IC₅₀ = 76.3 µg/mL). The highest gallic acid equivalent (GAE) total phenolic contents was found to be 263.5 ± 1.5 and 256.3 ± 3.1 mg/g dry weight for M-W extracts of *M. hedgei* and *M. persica*, respectively. **Conclusion:** The results indicated that the two species might be suggested as new potential sources of natural antioxidant and antimicrobial agents.

Keywords: antimicrobial, antioxidant, Lamiaceae, *Micromeria*, phenolic content

Introduction

The genus *Micromeria* (Lamiaceae) is represented in Iran by five species, among which *M. persica* and *M. hedgei* are endemic [1,2]. In Iranian and Turkish folk medicine, *Micromeria* species are used as herbal teas due to their pleasant aroma and medicinal properties and as a substitute for mint. The aerial flowering parts of the plants are locally used for treatment of cold. Several *Micromeria* species have been reported

as antiseptic, abortifacient, antirheumatic, CNS stimulant, and tonic [3]. They are also used for treatment of heart disorders, indigestion and headaches and as topical anaesthetic for toothache and wounds, inflamed eyes, skin infections and chest pains [4,5]. Some *Micromeria* species have also shown antioxidant and antimicrobial properties [6-8]. Antioxidant compound can prevent the oxidative stress which

is involved in many acute and chronic diseases including cancer, cardiovascular troubles and neurodegenerative diseases [9]. However, interest in naturally occurring antioxidants has increased while the synthetic antioxidant like BHA and BHT are suspicious for liver damage and carcinogenesis [10]. Also according to microbial resistance towards conventional preservatives, it is always essential to find new sources of antimicrobials [11]. The main objective of this study was to evaluate the antioxidant capacity and antimicrobial activity as well as the total phenolic content (TPC) of the various extracts and fractions of two Iranian endemic *Micromeria* (*M. persica* and *M. hedgei*) for the first time.

Experimental

Plant material

The aerial parts of *Micromeria hedgei* and *M. persica* were collected during flowering stage in April and May 2015 at altitude of ca. 900 and 2200 m, respectively. *M. hedgei* was collected from Bokhoon, Hajiabad, Bandar Abbas (Hormozgan Province, Iran) and *M. persica* was collected from mountains around Abadeh (Fars province, Iran). Voucher specimens (MPH-291 and MPH-386, respectively) were deposited in the Herbarium of Medicinal Plants and Drugs Research Institute of Shahid Beheshti University, Tehran, Iran.

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical and sodium carbonate were purchased from Fluka (Neu-Ulm, Germany). Folin-Ciocalteu reagent, 2,6-di-tert-butyl-4-methylphenol, butylated hydroxytoluene, (BHT) and other reagents and solvents were obtained from Merck (Darmstadt, Germany).

Preparation of the extracts

Air-dried and ground plant materials (20g) of both *Micromeria* species were extracted with methanol by maceration method for 24 h. Methanol was removed under reduced pressure and the concentrated extract was partitioned with

water and chloroform (1:1). The final fractions, the methanol extract (ME), water soluble methanol extract (M-W) and chloroform soluble extract (M-C), were examined in further evaluations.

Antioxidant activity (DPPH assay)

Scavenging activity of ME, M-W and M-C fractions of both *M. hedgei* and *M. persica* was assessed using the method described by Bozin *et al.* [12]. Samples in various concentrations (10 - 1000 µg/mL) were mixed with 1 mL of 90 µM DPPH solution and adjusted to the final volume of 4 mL with 95% methanol. The reagent and samples were shaken in a dark place for 1 h at room temperature. The absorbance was read against a blank at 517 nm. The Inhibition percentage of free radical, DPPH radicals was calculated as follows:

$$In\% = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

Extract concentration providing 50% inhibition (IC₅₀) was calculated from the graph of inhibition percentage vs samples concentration. Synthetic antioxidant reagent, butylated hydroxytoluene (BHT), was used as the positive control and all tests were carried out in triplicate.

Total phenolic content (TPC)

Total phenolic content of *M. hedgei* and *M. persica* methanol extracts and fractions were determined by modified literature methods involving Folin-Ciocalteu reagent and gallic acid (ranging from 0-1000 mg/L) as the standard [13]. Aliquots (20 µL) of each fraction (0.01 g/mL) were transferred to test tubes and diluted with 2 mL of distilled water and mixed with 100 µL of Folin-Ciocalteu reagent. After 3 min, 300 µL of sodium carbonate 7% w/v was added. Test tubes were shaken for 2 h at room temperature. Absorbance was measured at 765 nm. The same procedure was repeated for all standard gallic acid solutions. Tests were carried out in triplicate.

The results were expressed as mg gallic acid equivalents per g dry weight of extracts (mg GAE/ g of extract).

Microorganisms

Four Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 465, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228), three Gram negative bacteria (*Klebsiella pneumoniae* ATCC 10031, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 85327) and three fungi (*Aspergillus niger* ATCC 16404, *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 9763) were used in the experiments.

Antimicrobial activity assessment

In vitro antimicrobial activity of the extracts was evaluated by disc diffusion method using Mueller-Hinton agar for bacteria and Sabourou Dextrose agar for fungi with determination of inhibition zones (IZ). Each concentrated extract was dissolved in pure DMSO (10 mg/mL) and 10 μ L of each solution was delivered on a disc and then disc papers (disc diameter 6 mm) were placed in the inoculated plates. DMSO (10 μ L) was used as the negative control. Minimum inhibitory concentration (MIC), defined as the lowest concentration of the fractions that resulted in a complete inhibition of visible growth in the broth, was measured by microdilution broth susceptibility assay recommended by NCCLS [14]. The incubation conditions were 24 h at 37 °C for bacteria and 48 h at 24 °C for fungi. Tetracycline and gentamicin for bacteria and nystatin for fungi were used as positive standards in order to control the sensitivity of the microorganisms.

Results and discussion

DPPH radical scavenging activity and total phenolic contents of various extracts of *M. hedgei* and *M. persica* and BHT (as the positive control) are presented in table 1. The methanol extracts and polar subfractions of both

Micromeria species were stronger than the nonpolar subfractions. In addition, the strongest effects were observed for the ME and M-W of *M. hedgei* with IC₅₀ value of 80.1 and 59.1 μ g/mL in comparison to *M. persica* with IC₅₀ of 120.3 and 76.3 μ g/mL, respectively; while BHT IC₅₀ was 20.2 μ g/mL. The nonpolar (M-C) subfraction of the species could bleach DPPH' in high concentrations. The amount of total phenolic was highest in the methanol extracts and polar subfraction (M-W) rather than in nonpolar subfractions (M-C). The highest value was recorded for M-W of *M. hedgei* (263.5 mg GAE/g extract) and the lowest for the non-polar subfraction (M-C) of *M. hedgei* (73.2 mg GAE/g extract). Comparing the two species, the polar fractions of *M. hedgei* possessed more phenolic compounds while the nonpolar fractions showed to have less phenolic compounds. By considering the results in table 1, the phenolic content was high in polar extracts. It seems that presence of polar phenolic was fundamental in the evaluation of free radical scavenging. Besides, the highest activity, observed for the methanol extracts and polar subfraction of the methanol extracts reflected the radical scavenging characteristics of these phenolics. The key role of phenolic compounds as scavengers of free radicals has been emphasised in several reports [15,16]. Assessing the antioxidant activity of the methanol extract of *Micromeria cilicica* by the DPPH assay from Turkey has shown an IC₅₀ value of 74.7 μ g/mL [6].

Table 1. Antioxidant activity and total phenolic contents of various extracts of *Micromeria hedgei* and *M. persica*

Samples		DPPH assay	Total phenolic content
		IC ₅₀ (μ g/ml)	GAE mg/g dry extract
<i>M. persica</i>	ME	120.3 \pm 2.5	175.1 \pm 2.5
	M-C	440.6 \pm 2.8	79.4 \pm 1.3
	M-W	76.3 \pm 0.4	256.3 \pm 3.1
<i>M. hedgei</i>	ME	80.1 \pm 0.6	253.1 \pm 1.2
	M-C	768.5 \pm 1.5	73.2 \pm 1.1
	M-W	59.1 \pm 0.9	263.5 \pm 1.5
BHT		20.2 \pm 0.7	-

ME, Methanol extract; M-C, Chloroform fraction of methanol extract; M-W, Water soluble fraction of methanol extract. Results are given as mean \pm standard deviation of three different experiments

Table 2. Antimicrobial activities of chloroform fractions (M-C) of methanol extract of *Micromeria hedgei* and *M. persica*

Microorganism	<i>M. persica</i>		<i>M. hedgei</i>		Gentamicin (10 µg/disc)		Tetracycline (30µg/disc)		Nystatin (30µg/disc)	
	IZ (mm)	MIC (mg/mL)	IZ (mm)	MIC (mg/mL)	IZ (mm)	MIC (mg/mL)	IZ (mm)	MIC (mg/mL)	IZ (mm)	MIC (mg/mL)
<i>Bacillus subtilis</i>	18	7.5	20	7.5	-	-	21	3.2	-	-
<i>Staphylococcus aureus</i>	16	7.5	19	7.5	-	-	20	3.2	-	-
<i>Staphylococcus epidermidis</i>	20	3.7	22	3.7	-	-	34	1.6	-	-
<i>Enterococcus faecalis</i>	14	15	16	7.5	-	-	9	6.4	-	-
<i>Pseudomonas aeruginosa</i>	na	-	na	-	12	6.4	-	-	-	-
<i>Escherichia coli</i>	15	7.5	17	7.5	23	3.2	-	-	-	-
<i>Klebsiella pneumonia</i>	11	15	13	15	20	3.2	-	-	-	-
<i>Aspergillus niger</i>	na	-	na	-	-	-	-	-	16	6.4
<i>Saccharomyces cerevisiae</i>	na	-	na	-	-	-	-	-	18	1.6
<i>Candida albicans</i>	na	-	na	-	-	-	-	-	18	3.2

IZ, Inhibition zone including diameter of disc (6 mm); MIC, Minimum inhibitory concentration as mg/mL; na, not active; -, not tested

In another study, the methanol extract of *Micromeria fruticosa* ssp. *serpyllifolia* growing in Turkey has exhibited significant antioxidant activity in DPPH assay, providing 50% inhibition at 70.9 µg/mL concentration. The gallic acid equivalent total phenolic content of the the methanol extract of this species was found to be 55.2 µg/mg dry weight extract [3]. The water soluble fraction (M-W) of the methanol extract of *M. persica* has shown almost the same type of antioxidant activity compared to the results published for *M. cilicica* [6]. In case of *M. hedgei*, the M-W fraction of the methanol extract showed higher antioxidant activity than *M. persica* as well as the two other species studied from Turkey [3,6].

Table 2 shows the antimicrobial activity of the nonpolar fraction of both *Micromeria* species. The ME and M-W of *M. hedgei* and *M. persica* were inactive against microorganisms; therefore, just the antimicrobial activity of M-C of the

species were measured. The chloroform fraction of *M. hedgei* was more active than that of *M. persica*. For example, the M-C of *M. hedgei* with 22 mm IZ and 3.75 mg/mL MIC value exhibited stronger activity against *Staphylococcus epidermidis*. Both *Micromeria* species failed to show any activity against fungi.

This was the first study about the antioxidant and antimicrobial activity of two Iranian endemic *Micromeria* species. They showed a good to moderate antioxidant and antimicrobial activity. These results encourage complementary and more studies on the chemical composition of the plant extracts with the aim of separation and structure elucidation of their active components and evaluation of biological activity of each compound separately.

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Declaration of interest

The author declares that there is no conflict of interest. The author alone is responsible for the content of the paper.

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