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Chemical Composition and Antibacterial and Antioxidant Properties of Essential Oils of *Zataria multiflora*, *Artemisia deracunculus* and *Mentha piperita*

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ABSTRACT

Background and objectives: Utilization of essential oils instead of chemical preservatives has received significant attention in recent years. The present study aims to evaluate chemical composition and antibacterial and antioxidant properties of essential oils of *Zataria multiflora*, *Artemisia deracunculus* and *Mentha piperita*.

Methods: Chemical profile of the essential oils was analyzed by gas chromatography/mass spectrometry. The microwell dilution and agar disk diffusion methods were used to evaluate the antibacterial properties of the essential oils. Total phenolic content, β-carotene-linoleic acid bleaching test and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays were carried out to determine the antioxidant properties.

Results: Menthol (39.18%) and mentone (21.64%) were the main components of the essential oil of *M. piperita*, while estragol (34.75%) and limonene (15.72%) were the major components of the essential oil of *A. dracunculus*. The main components of the essential oil of *Z. multiflora* were carvacrol (36.81%) and thymol (33.04%). The essential oils of *M. piperita* and *Z. multiflora* showed greater antimicrobial effects. Moreover, *Z. multiflora* showed the greatest antioxidant activity among the essential oils. The total phenolic content of *Z. multiflora* was 228.14±0.45 mg gallic acid equivalent/g.

Conclusion: Given their favorable antioxidant and antimicrobial properties, the essential oils of *Z. multiflora*, *A. deracunculus* and *M. piperita* can be used as natural food preservatives.

Keywords: *Zataria multiflora*, *Artemisia deracunculus*, *Mentha piperita*, antibacterial effect, antioxidant effect.

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INTRODUCTION

Foodborne or food spoilage microorganisms can cause illness in consumers and significantly reduce the shelf life of foods. *Staphylococcus aureus* can rapidly grow in foods, and is known as the most common foodborne microorganism (1). *Listeria monocytogenes* is another important foodborne bacterium, which can survive in extreme conditions, such as low temperature, high salinity and acidity (2). *Bacillus cereus* is commonly found in the environment and has the ability to form spores and grow under cold conditions. Moreover, inadequate cooking of foods contaminated with these microorganisms can lead to food poisoning (3). Consumption of *Escherichia coli*-contaminated food can cause asymptomatic gastrointestinal disease or more serious complications, which might lead to death if not treated (4). Despite the global advances in food safety, salmonellosis is still one of the most important public health issues. *Salmonella*, the main causative agent of this disease, especially serovar *typhimurium*, is resistant to multiple drugs, which has made this infection difficult to treat (5).

Due to the harmful effects of synthetic additives on human health, special interest has been given to characterization of plant essential oils, which are important components of foods and beverages (6). One of the main benefits of using essential oils in food products is their favorable antibacterial activity against a wide range of microorganisms (6, 7). The inhibitory effects of essential oils against the growth of many gram-negative or gram-positive microorganisms and fungal species have been reported in several studies (7-9). Essential oils rich in phenolic compounds and other phytochemicals exhibit significant antioxidant and free radicals scavenging activities, and therefore can be used to extent shelf life of foods (10). Peppermint (*Mentha piperita*) is an important medicinal plant that grows in many countries. Certain parts of this plant, such as leaves, are used as herbal tea or spice for their known flavor and fragrance, while its extract/essential oil is used in cosmetic, pharmaceutical, and food industries (11). Peppermint is a hybrid of water mint (*M. aquatica* L.) and spearmint (*M. spicata* L.). Certain aroma compounds found in *Artemisia dracunculus* (Tarragon), such as monoterpenes and sesquiterpenes also have medicinal effects (10). *Zataria multiflora*, from the family *Lamiaceae*, is another important medicinal plant that grows wild in Iran, Pakistan, and Afghanistan (12). *Z. multiflora* has pain-relieving, immune-stimulating, antinociceptive, antibacterial, antifungal, antiviral, and antioxidant properties (12-14).

The aim of the present study was to determine the chemical composition and antioxidant and antibacterial properties of essential oils from *M. piperita*, *A. dracunculus*, and *Z. multiflora*.

MATERIALS AND METHODS

Areal parts of *M. piperita*, *A. dracunculus* and *Z. multiflora* were purchased from a local spice store in Gorgan, Iran. Identification of the plants was carried out in University of Agriculture and Natural Resources of Gorgan, Iran. A Clevenger-type apparatus was used for extraction of the essential oils. The extractions were dried over anhydrous sodium sulfate and then stored at 4 ^{o}C (15). Components of the essential oils were identified according to a method described by Moradi et al. (16). Gas chromatography/mass spectrometry (GC/MS) analysis of the essential oils was carried out using a Hewlett Packard 5890 system equipped with an HP-5MS capillary column (30 × 0.25 mm ID × 0.25 mm film thickness). Flow rate of helium was 1 ml/min. Initial temperature of the column was 50 ^{o}C, which was progressively increased to 120 ^{o}C at a 2 ^{o}C/min rate for 3 min, and finally increased to 300 ^{o}C for 5 min. The MS procedure was operated with ionization energy of 70 eV. Compounds were identified by comparing their retention indices with those of standard samples and mass spectral data available in the Wiley library (Wiley-VCH 2001 data software, Weinheim, Germany).

Three gram-positive bacteria including *S. aureus* (PTCC 1015), *L. monocytogenes* (PTCC 1298), and *B. cereus* (PTCC 1665) and two gram-negative bacteria including *E. coli* (PTCC 1533) and *S. thyphimurium* (PTCC 1730) were obtained from the microbial collection of the Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

One microliter of bacterial culture (18 hours) at a concentration of 10^6 CFU/ml equivalent to 0.5 MacFarland standard was spread on Mueller-Hinton agar (Merck Darmstadt, Germany). Sterile paper disks (diameter: 6mm, purchased from Padtan Teb, Iran) were soaked

in 10 µl of essential oil and placed on the agar media. The plates were incubated at 37 ^{o}C for 24 hours, and diameter of inhibition zone was measured in mm (16).

The microwell dilution method was used to determine the minimum inhibitory concentration (MIC) of the essential oils against the bacteria. Bacterial suspensions were taken from 18h cultures in broth (10^6 CFU/ml equivalent to 0.5 MacFarland standard). The essential oils were dissolved in 10% dimethyl sulfoxide. Subsequently, the solutions were diluted to the highest concentration (100000 µg/ml) as a stock solution, and then two-fold serial dilutions were prepared in a concentration range of 100000 to 1562.5 µg/ml in nutrient broth. Aliquots of 160 µl Brain Heart Infusion (BHI) broth (Merck Darmstadt, Germany) and 20 µl inoculums were dispensed into 96-well micro plate. Then, 20 µl of the essential oil were added to each well. Negative control (180 µl of uninoculated BHI broth+20 µl of essential oil) and positive control (180 µl of BHI broth+20 µl of inoculums) were also considered in the last wells. Final volume of each well was 200 µl, and the final concentration of the essential oils was between 10000 and 156.2 µg/ml. In addition, final bacterial suspension in each well was approximately 1.5×10^5 CFU/ml. The lowest concentration at which no visible bacterial growth was observed was determined as the MIC (15).

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was carried out based on a method described previously with slight modifications (17). Briefly, 50 µl of different concentrations of the essential oils and a reference antioxidant (BHT) were added to 2 ml of DPPH methanolic solution (24 µg/ml). The mixture was shaken and kept in dark and at room temperature for 60 min. Then, absorbance was read at 515 nm using a spectrophotometer (LKB Novaspec II; Pharmacia, Sweden). The same procedure was performed for a blank, which contained no antioxidant. The DPPH scavenging capacity of the essential oils was determined based on the following equation:

$$I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

The essential oil concentration causing 50% inhibition (IC50) was recorded according to the inhibition percentage curve for each sample.

The β-carotene-linoleic acid bleaching test was carried out based on a method described by Miraliakbari and Shahidi with slight modifications (18). In order to prepare the stock solution of β-carotene–linoleic acid, approximately 0.5 mg β-carotene (type I synthetic, Sigma–Aldrich) was dissolved in chloroform (1 ml) in a flask. Then, 20 µl of linoleic acid (Sigma–Aldrich) and 200 mg of tween 40 (Sigma–Aldrich) were added to the flask. Chloroform was removed at 40 ^{o}C using a rotary evaporator (Heidolph laborta 4003, SchwaBach, Germany). After adding 100 ml of distilled water, the mixture was shaken vigorously. Aliquots of the mixture (2.5 ml) were pipetted to test tubes containing 350 µl of the essential oils (concentration: 2 mg/ml). The same procedure was done with BHT and a blank. Absorbance of each tube was measured at 470 nm immediately and after two hours. The tubes were placed in a water bath at 50 ^{o}C. Finally, β-carotene protective capacity of the essential oils was determined based on the equation below:

$$I\% = (A_{\beta\text{ - carotene 2nd hour}} / A_{\beta\text{-carotene initial}}) \times 100$$

Total phenolic content of the essential oils was determined using the Folin-Ciocalteu assay. Gallic acid was used as standard in this assay (19). Briefly, 0.5 ml of the essential oil (2 mg/ml) was mixed with 250 µl of Folin-Ciocalteu reagent (Merck, Darmstadt, Germany) and 2.25 ml of distilled water. The mixture was vortexed and kept for 5 min. A 2 ml aliquot of Na_2CO_3 solution (7.5%) was added, and the mixture was incubated for 120 min at room temperature. Absorbance of the mixture was measured at 760 nm and data were expressed as mg gallic acid equivalent (GAE)/g of essential oil, relative to the values obtained from a standard curve of known concentrations of gallic acid.

All experiments were performed in triplicate. Statistical analysis of data was performed using SPSS, Inc, Chicago, IL software (version 16.0). Tukey's test was used to compare the differences between the mean values at significance of 0.05.

RESULTS

Table 1 shows the chemical composition of the essential oils of *M. piperita*, *A. dracunculus*, and *Z. multiflora*.

Table 1- Chemical components of the essential oils of *Z. multiflora, A. deracunculus* and *M. piperita* using GC/MS analysis

Zataria multiflora				*Artemisia deracunculus*			*Mentha piperita*		
No	Component	Retention Time (Min)	%	Component	Retention Time (Min)	%	Component	Retention Time (Min)	%
1	α-Thujene	17.34	0.82	Alpha pinene	9.48	6.94	Alpha-pinene	5.45	0.71
2	α-Pinene	17.78	1.03	Camphene	10.27	0.14	Sabinene	6.38	2.81
3	Camphene	18.17	0.23	Sabinene	10.72	0.57	β pinene	6.52	0.21
4	β-pinene	19.21	0.72	Myrcene	10.93	0.74	Alpha-terpinene	7.43	2.12
5	Myrcene	19.65	0.49	Limonene	11.78	15.72	Limonene	7.63	1.88
6	α-Phellandrene	20.20	0.29	Cis ocimene	12.01	2.21	1,8-Cineole	7.76	5.12
7	Terpinene-4-ol	20.70	0.57	Beta ocimene	12.16	4.11	Gamma terpinene	8.29	0.88
8	O-Isopropyltoluene	21.21	3.26	Estragol	14.74	34.75	Terpinolene	9.04	0.43
9	Limonene	21.30	1.08	L carvone	15.65	2.11	Linalool	9.33	0.36
10	11 Terpinolene	23.18	1.54	Bornyl acetate	16.59	1.21	Mentone	10.79	21.64
11	Linalool	23.40	4.58	Eugenol	17.61	2.25	Menthol	11.18	39.18
12	δ-3-Carene	23.49	0.22	Methyl cinnamate	18.02	2.12	Piperitone	11.48	2.1
13	Gamma terpinene	26.93	3.18	Methyl eugenol	18.39	8.16	Pipertitinone oxide	12.74	0.25
14	Thymol methyl ether	27.95	3.51	Beta-caryophyllene	19.12	2.89	Menthyl acetate	13.52	7.55
15	Thymol	30.10	33.04	Humulene	19.80	0.54	Beta-bourbonene	15.39	1.12
16	Carvacrol	30.79	36.81	Germacrene-D	20.21	4.29	Caryophyllene	15.89	1.57
17	Thymol acetate	31.90	0.28	Bicyclogermacrene	20.41	3.35	Germacrene-d	16.69	4.28
18	Eugenol	32.11	0.72	Spathulenol	21.59	4.24	Viridiflorol	18.95	5.11
19	β-Caryophyllene	33.57	0.17	7-methoxycoumarin	22.89	2.19			
20	3-Methylresacetophenone	33.71	0.69	Neophytadiene	24.92	0.12	----------	----------	-------
21	α-Humulene	34.40	0.78	----------	----------	-------	----------	----------	-------
22	Spathulenol	37.45	2.11	----------	----------	-------	----------	----------	-------
23	Caryophyllene oxide	37.62	1.23	----------	----------	-------	----------	----------	-------
24	1-Cycloheptene	38.25	0.12	----------	----------	-------	----------	----------	-------
25	*p*-Cemene	44.70	0.64	----------	----------	-------	----------	----------	-------
26	α-Terpinene	44.84	0.17	----------	----------	-------	----------	----------	-------
	Total		98.28			98.65			97.32

Based on the results of GC-MS analysis, menthol (39.18%) and mentone (21.64%) were the main components of *M. piperita*, while estragol (34.75%) and limonene (15.72%) were the major components of *A. dracunculus*. In addition, the major components of *Z. multiflora* were carvacrol (36.81%) and thymol (33.04%).

Results of the disk diffusion method demonstrated that the essential oils of *M. piperita* and *Z. multiflora* had higher antibacterial effect against *S. aureus*, *L. monocytogenes*, and *B. cereus* than the essential oil of *A. dracunculus* ($P<0.05$).

However, none of the essential oils had significant antibacterial effect against *E. coli* and *S. typhimurium*. As seen in table 2, the essential oil of *Z. multiflora* had the highest inhibitory effect against *S. aureus*. Overall, the essential oils of *M. piperita* and *Z. multiflora* showed promising antibacterial activity in the disk diffusion assay. Moreover, the essential oils were most effective against *S. aureus* and *B. cereus* (Table 2). *L. monocytogenes*, *S. typhimurium* and *E. coli* were the most resistant pathogens. The essential oil of *Z. multiflora* showed the highest antibacterial activity against *S. aureus* and *B. cereus* (156.2 ppm). On the other hand, the highest MIC of *A. dracunculus* and *M. piperita* essential oils were recorded against *E. coli* and *S. typhimurium*, respectively.

The antioxidant activity of the essential of *Z. multiflora* was 31.12±1.17 $\mu g.ml^{-1}$ in the DPPH test and 66.46±0.55% in the β-carotene-linoleic acid bleaching assay (Table 3). Furthermore, the total phenolic content of this essential was 228.14±0.45 mg GAE/ g.

Table 2- Antibacterial effect of the essential oils based on the disk diffusion (DIZ) and MIC (ppm) methods

Essential Oil	*S. aureus*		*L. monocytogenes*		*B. cereus*		*E. coli*		*S. typhimurium*	
	DIZ (mm)	MIC (ppm)	DIZ (mm)	MIC (ppm)	DIZ (mm)	MIC (ppm)	DIZ (mm)	MIC (ppm)	DIZ (mm)	MIC (ppm)
M. piperita	16.49±0.22^{D}	312.5	14.34±0.56^{C}	625	15.18±0.28^{D}	312.5	9.28±0.26^{A}	2500	12.52±0.45^{B}	1250
A. dracunculus	11.13±0.11^{B}	625	10.26±0.43^{B}	1250	11.48±0.33^{C}	625	7.81±0.17^{A}	2500	9.16±0.31^{B}	2500
Z. multiflora	18.76±0.36^{D}	156.2	16.58±0.47^{C}	312.5	16.74±0.17^{D}	156.2	14.2±0.22^{A}	625	16±0.53^{B}	625

Table 3- Antioxidant activity of the essential oils of *M. piperita*, *A. dracunculus*, and *Z. multiflora*

Sample	DPPH (IC_{50}) (µg.ml^{-1})	β-carotene-linoleic acid bleaching assay (%)	Total phenolic content (mg of GAE/g essential oil)
M. piperita	42.25±1.35^{B}	58.12±0.45^{B}	179.58±0.72^{C}
A. dracunculus	78.41±1.17^{C}	47.37±0.45^{C}	152.81±1.14^{B}
Z. multiflora	31.12±1.17^{D}	66.46±0.55^{D}	228.14±0.45^{A}
BHT	27.57±1.34^{A}	92.25±1.15^{A}	*

Results are expressed as mean ± standard deviation
*** Not examined**
Data marked with different subscripts within the same column indicate statistically significant difference (P<0.05).

DISCUSSION

Medicinal plants have long been used in traditional Iranian medicine for treatment of infections (20, 21). In addition, these plants and their products can be used as food additives to improve the shelf life and quality of different food products (8, 22, 23). Finding new essential oils with antimicrobial and antioxidant activities as well as adequate organoleptic properties has been the focus of many studies. Many studies have been carried out to identify the main constituents of the essential oils of *M. piperita*, *A. dracunculus*, and *Z. multiflora*. Samber et al. reported menthol (34.82%) and carvone (19.54%) as the major components of peppermint (24). Guerra et al. found menthol (30.3%) and isomenthone (26.7%) as the main components of *M. piperita* (25). In a study by Lesjak et al., methyleugenol (72.3%) and sabinene (12.4%) were the major components of the essential oil of *A. dracunculus* (26), which is inconsistent with our findings. Sharififar et al. reported thymol (37.59%) and carvacrol (33.65%) as the major components of *Z. multiflora* (13), which is also inconsistent with our findings. It should be noted that growth conditions, climate, soil condition and geographical location can affect the quality and quantity of the essential oil components (27).

In the present study, the essential oils of *M. piperita* and *Z. multiflora* were most effective against *S. aureus*. On the other hand, the essential oil of *A. dracunculus* showed the highest antibacterial activity against *B. cereus*. *E. coli* with inhibition zone diameter of 14.2 ± 0.22 mm was one of the most resistant microorganisms. In a study by Fatemi et al., the inhibition zone diameter of *E. coli* was 19.0 ± 1.00 mm (28). In general, gram-positive bacteria were more sensitive to the essential oils than the gram-negative ones, which could be due to the presence of divalent cations and polysaccharides in their outer membrane structure. The antimicrobial effect of *Z. multiflora* might be due to the high carvacrol and thymol content of its essential oil.

Antimicrobial activities of the essential oils were determined quantitatively using the MIC test. Similar to the disc diffusion assay, the essential oils showed greater antibacterial activity against gram-positive bacteria, which is in line with findings of some previous studies (29). The lowest MIC was related to the essential oils of *Z. multiflora*, *M. piperita* and *A. dracunculus*, respectively. In a study by Fazeli et al., *Salmonella typhi* was more resistant to *Z. multiflora* than to *E. coli*, which is not in line with our findings (30).

The DPPH assay is one of the most frequently used methods of determining the antioxidant properties of samples (31). The IC_{50} value of *Z. multiflora*, *M. piperita* and *A. dracunculus* was 31.12±1.17 µg.ml^{-1}, 42.25±1.35 µg.ml^{-1} and 78.41±1.17 µg.ml^{-1}, respectively. In this assay, the IC_{50} value of BHT that was used as the positive control was 27.57±1.34 µg.ml^{-1}. Similar findings were reported by Saei-Dehkordi et al. for the IC_{50} value of *Z. multiflora* (32).

The antioxidant activity of the essential oils was also evaluated using the β-carotene bleaching test, and the results were consistent with findings of the DPPH assay. Minor differences between the tests could be due to the presence of lipophilic compounds in the β-carotene bleaching test.

In order to establish a connection between the biological activity and the chemical

composition of the essential oils, the total phenolic content of each essential oil was determined using the Folin-Ciocalteu assay. In a previous study by Kaur et al., the total phenolic content of *M. piperita* was 399.8 ± 3.2 mg GAE/g, which is significantly higher than that of the *Mentha* species investigated in the present study. In another study, the total phenolic content of *A. dracunculus* was 24.10 ± 0.33 mg GAE/g, which is significantly lower than the value found in the present study. These variations may be due to the difference in the geographical location of the plants. Based on the findings, it can be concluded that thymol and carvacrol have high antioxidant activity.

CONCLUSION

Given the antibacterial and antioxidant activities of the essential oils tested in the present study, they can be used as natural food additives to extend the shelf life of foods. However, further studies should be carried out to determine the active phytochemicals responsible for the antibacterial and antioxidant activities of these essential oils.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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