CHEMICAL COMPOSITION, RADICAL SCAVENGING, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF *ZATARIA MULTIFLORA* BIOSS ESSENTIAL OIL AND AQUEOUS EXTRACT

AMIN MAHAMMADI PURFARD and GHOLAMREZA KAVOOSI[1]

Institute Of Biotechnology, Faculty of Agriculture, University Of Shiraz, Shiraz 71441-65186, Iran

[1]Corresponding author.
TEL: 098(711)2272805;
FAX: 098(711)2272805;
EMAIL: ghkavoosi@shirazu.ac.ir

Received for Publication February 28, 2012
Accepted for Publication May 22, 2012

doi:10.1111/j.1745-4565.2012.00384.x

ABSTRACT

The composition, reactive oxygen species, reactive nitrogen species, hydrogen peroxide and thiobarbituric acid reactive substances scavenging, antibacterial and antifungal activities of aqueous extract and essential oil from *Zataria multiflora* was examined. Carvacrol (29.489%), thymol (25.701%), *p*-cymene (11.247%), linalool (9.363%) and γ-terpinene (8.054%) were detected as the main components of the oil. The inhibitory concentrations for total radical scavenging activities were between 1.92–3.15 and 2.46–4.58 μg/mL of aqueous extract and essential oil, respectively. The minimum inhibitory concentration for *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermi*, *Bacillus subtilis*, *Aspergillus niger* and *Candida albicans* were 2.65 ± 0.7, 2.72 ± 0.8, 2.85 ± 0.5, 3.02 ± 0.8, 3.53 ± 1, 3.65 ± 0.9, 2.2 ± 0.5 and 2.8 ± 0.8 μg/mL of essential oil. However, aqueous extract cannot inhibit all of the bacteria and fungi tested. Thus, *Z. multiflora* essential oil has antioxidant and antimicrobial activity and can be used in the therapy of oxidative damage and in the therapy of infective diseases and can be used as a tea or additive in foods and traditional remedies for the treatment of infectious diseases.

PRACTICAL APPLICATIONS

Oxidative stress-related and infective diseases are one of the most common health problems in the worldwide population. A large portion of the world's population currently uses traditional medicines and herbal therapy, which involves the use of the aqueous extract of a plant in the form of an herbal tea. Essential oil derived from herbal medicines that have been known for their biological activities for many decades and should not be toxic to man and could replace chemical antioxidant, antibacterial and antifungal agents. This study clearly demonstrates the potential of essential oil from *Zataria multiflora* as antioxidant against reactive oxygen species, reactive nitrogen species, hydrogen peroxide (H_2O_2) and thiobarbituric acid reactive substances and the potential of essential oil from *Z. multiflora* as antimicrobial against *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermi*, *Bacillus subtilis*, *Aspergillus niger* and *Candida albicans*. The chemical composition of essential oil was identified. Thus, identification of such compounds also helps to discover of new antioxidant, antibacterial and antifungal agents.

INTRODUCTION

A large portion of the world's population currently uses traditional medicines and herbal therapy, which involves the use of the aqueous extract of a plant in the form of an herbal tea. Although herbal medicines are regarded to have therapeutic potential against several diseases, neither their active components nor their mechanisms of action are fully understood.

 © 2012 Wiley Periodicals, Inc.

In the recent decade, herbal therapy is widely employed for treatment of several human and animal diseases, e.g., essential oils or natural product derived from traditional herbs have been experienced for antioxidant (Sze *et al.* 2010), antibacterial (Solorzano-Santos and Miranda-Novales 2011), antiparasitic (Anthony *et al.* 2005) and antifungal, antiviral and cytotoxic (Reichling *et al.* 2009) activities, and for food preservation and food safety (Tajkarimi *et al.* 2010).

Zataria multiflora Boiss, with the Persian name Avishan Shirazi, grows only in warm parts of Iran, Afghanistan and Pakistan. This aromatic plant belongs to the *Lamiaceae* family. In traditional medicine in the Middle East, *Zataria* is used in flavoring and preserving food and drinks for its antiseptic, analgesic and carminative properties (Amirghofran *et al.* 2011; Zomorodian *et al.* 2011). Even though the composition, antiviral, antifungal and antibacterial activities of the essential oil of many other medicinal and aromatic species have been previously studied, there is very little information about the biological activity of this plant. Thus, in the present study, the composition and reactive oxygen species (ROS), reactive nitrogen species (RNS), hydrogen peroxide (H_2O_2) and thiobarbituric acid reactive substances (TBARS) scavenging activities of *Z. multiflora* essential oil and aqueous extract were determined. In addition, the effect of *Z. multiflora* essential oil and aqueous extract on the inhibition of *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermi*, *Bacillus subtilis*, *Aspergillus niger* and *Candida albicans* were investigated.

MATERIALS AND METHODS

Plant Materials and Aqueous Extract and Essential Oil Preparation

The aerial parts of *Z. multiflora* were obtained from wild plants in mountains of Arsenjan (Fars Province, Iran). The taxonomic identification of the plant was confirmed by a senior plant taxonomist, Prof. A. R. Khosravi at the Department of Biology, Shiraz University, Shiraz, Fars Province, Iran. A voucher specimen (24,985) has been deposited at the Herbarium of the Department of Biology, Shiraz University. The leaves of plant materials were separated from the stem and dried in the shade for 72 h.

For the aqueous extract, the air-dried leaves (100 g) were successively extracted with 300 mL of pure water for 72 h at room temperature. The aqueous extracts were filtered using Whatman filter paper and then concentrated *in vacuum* using a rotary evaporator. The resulting powdered material was stored at –20C until use.

For the essential oil, the air-dried leaves (100 g) were hydro-distilled for 3 h using an all-glass Clevenger-type apparatus (Alphalab, Tehran, Iran) according to the method outlined by the British Pharmacopeia (British Pharmacopeia 1998). The essential oil thus obtained was dried over anhydrous sodium sulphate (Sigma-Aldrich, St. Louis, MO) and stored at 4C before gas chromatography–mass spectrometry (GC–MS) analysis and further experiments.

Identification of the Oil Components

GC analysis was carried out using an Agilent-technology chromatograph with HP-5 column (30 m × 0.32 mm i.d. × 0.25 μm). Oven temperature was performed as follows: 60–210C at 3C/min; 210C to 240C at 20C/min and hold for 8.5 min, injector temperature 280C; detector temperature, 290C; carrier gas, N_2 (1 mL/min); split ratio of 1:50. GC–MS analysis was carried out using an Agilent 7,890 operating at 70 eV ionization energy, equipped with a HP-5 MS capillary column (phenyl methyl siloxane, 30 m × 0.25 mm i.d × 25 μm) with He as the carrier gas and split ratio 1:50. Retention indices were determined using retention times of n-alkanes that were injected after the essential oil under the same chromatographic conditions. The retention indices for all components were determined according to the method using n-alkanes as standard. The compounds were identified by comparison of retention indices (HP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley GC–MS Library, Adams Library, MassFinder 2.1 Library data published mass spectra data (Joulain *et al.* 2001; Adams 2007; Mc Lafferty 2009).

ROS Scavenging Assay

ROS scavenging activity of the essential oil was determined as previously described (Re *et al.* 1999). Briefly, 10 μL of the aqueous extract (0, 2, 4, 6, 8, 10 μg/mL in water) or essential oil (0, 2, 4, 6, 8, 10 μg/mL in dimethyl sulfoxide [DMSO]) was added to 1.0 mL of diluted 2,2′-azino-di (3-ethylbenzthiazoline sulphate) (ABTS) radical solution (7 mM ABTS and 2.54 mM potassium persulfate). After mixing, the absorbance was read at 734 nm using spectrophotometer (Pharmacia, Uppsala, Sweden). The percentage of ROS scavenging was obtained by the equation: $(A734_{blank} - A734_{sample}) / A734_{blank} \times 100$. The concentration providing 50% inhibition (IC_{50}) was calculated from a graph plotting inhibition percentage against different essential oil concentrations.

RNS Scavenging Assay

NO scavenging activity of the essential oil was determined as previously described (Marcocci *et al.* 1994). Briefly, 10 μL of the aqueous extract (0, 2, 4, 6, 8, 10 μg/mL in water) or essential oil (0, 2, 4, 6, 8, 10 μg/mL in DMSO) was incubated with 0.5 mL of sodium nitrite (10 μg/mL in 100 mM sodium

citrate pH 5) at 37C for 2 h. After incubation, 0.5 mL of Griess reagent was added and the absorbance was read at 540 nm using a spectrophotometer (Pharmacia). The percentage of RNS scavenging was obtained by the equation: $[(A540_{blank} - A540_{sample}) / A540_{blank}] \times 100$. IC_{50} was calculated from the graph plotting inhibition percentage against different essential oil concentrations.

H_2O_2 Scavenging Assay

H_2O_2 scavenging activity of the essential oil was determined as previously described (Buyukbalci and Ei 2008). Briefly, 10 µL of the aqueous extract (0, 2, 4, 6, 8, 10 µg/mL in water) or essential oil (0, 2, 4, 6, 8, 10 µg/mL in DMSO) was incubated with 1.0 mL of H_2O_2 (50 mM in 100 mM phosphate buffer pH 7.4) at 37C for 60 min. After incubation, the absorbance was read at 230 nm against a blank solution containing phosphate buffer without H_2O_2 using a spectrophotometer (Pharmacia). The percentage of H_2O_2 scavenging was obtained by the equation: $(A230_{blank} - A230_{test}) / A230_{blank} \times 100$. IC_{50} was calculated from the graph plotting percentage inhibition against different concentrations of tested compound.

TBARS Scavenging Assay

TBARS scavenging activity of the essential oil was determined as previously described (Hodges *et al.* 1999). Briefly, 10 µL of the aqueous extract (0, 2, 4, 6, 8, 10 µg/mL in water) or essential oil (0, 2, 4, 6, 8, 10 µg/mL in DMSO) were incubated with malondialdehyde (50 µM in acetic acid pH 4) at 37C for 12 h. After incubation, one volume of thiobarbithoric acid (200 µM in acetic acid; pH 4) was added. The mixture was incubated at 95C for 1 h. After cooling at room temperature, the absorbance was read at 532 nm using a spectrophotometer (Pharmacia). The percentage of TBARS scavenging was obtained by the equation: $(A532_{blank} - A532_{sample}) / A532_{blank} \times 100$. IC_{50} was calculated from a graph that plotted inhibition percentage against different concentrations of the tested compound.

Antibacterial and Antifungal Assay Using Disk Diffusion Method

All microorganisms were obtained from the Persian Type Culture Collection (PTCC), Tehran, Iran. The essential oil and aqueous extract were individually tested against four gram-negative bacteria (*Pseudomonas aeruginosa* PTCC 1074 [American Type Culture Collection {ATCC} 9027], *Salmonella typhi* PTCC 1609 [Iran isolate], *Klebsiella pneumoniae* PTCC 1053 [ATCC 10031] and *Escherichia coli* PTCC 1330 [ATCC 8739]), three gram-positive bacteria (*Staphylococcus aureus* PTCC 1112 [ATCC 6538], *Staphylococcus epidermi* PTCC 1114 [ATCC 12228] and *Bacillus subtilis* PTCC 1023 [ATCC 6633]), and two fungi (*Aspergillus niger* PTCC 5010 [ATCC 9142] and *Candida albicans* PTCC 5027 [ATCC 10231]). Microorganisms were cultured at 37C for 16–24 h and the densities were adjusted to 0.5 McFarland standards at 530 nm (10^8 cfu/mL). Antimicrobial tests were carried out by the disk diffusion method (Bauer *et al.* 1996). The microbial suspension (100 µL of 10^8 cfu/mL) was spread on nutrient agar plates (Farazbin Kimia Co., Teharan, Iran). Disks (Whatman no. 1, 6 mm diameter, Padtan Teb, Iran) were impregnated with 20 µL of different concentrations of essential oil and aqueous extract (0, 25, 50 and 100 µg/mL) and placed on the inoculated agar. The inoculated plates were incubated at 37C for 24 h. DMSO was used as the negative control. Positive control disks included gentamicin, ampicillin and ketoconazole (all Padtan Teb, Iran, 10 mg/disk) for gram-negative bacteria, gram-positive bacteria and fungi, respectively. All tests were performed in triplicate. Antibacterial activity was evaluated by measuring the zone of bacterial growth inhibition.

Antibacterial and Antifungal Assay Using Minimum Inhibitory Concentration Determination

Minimum inhibitory concentration (MIC) was determined against serial dilutions of the essential oil and aqueous extract (0–100 µg/mL) using microdilution method recommended by Clinical and Laboratory Standards Institute (Wayne 2006a,b). Bacteria and fungi strains were suspended in Luria–Bertani media and the densities were adjusted to 0.5 McFarland standards at 570 nm (10^8 cfu/mL). Bacteria and fungi suspensions (100 µL) and the essential oil and aqueous extract (100 µL) were added to microtiter plates and incubated at 37C for 24 h. Medium without bacteria and fungi was used as sterility control. Medium with bacteria but without essential oil and aqueous extract was used as growth control. The growth in each well was compared with that of the growth in the control well. MIC were visually determined and defined as the lowest concentration of the compounds produced >95% growth reduction compared with the growth in the control well.

Statistical Analysis

All data are expressed as the means plus standard deviations of at least three independent experiments. The significant differences between treatments were analyzed by one-way analysis of variance test at $P < 0.01$ using the Statistical Package for the Social Sciences (SPSS) version 16 (Abaus Concepts, Berkeley, CA) and Prism 5 (Graph Phad, San Diego, CA) software.

TABLE 1. CHEMICAL COMPOSITION OF ESSENTIAL OIL FROM *Z. MULTIFLORA*

Components	RI	Rel. %
α-Thujene	924	0.499
α-Pinene	931	1.234
Camphene	945	0.269
β-Pinene	974	0.575
3-Octanone	983	0.267
Myrcene	988	0.729
A-Terpinene	1,015	0.562
p-Cymene	1,025	11.247
Limonene	1,027	0.448
1,8-Cineol	1,029	0.447
γ-Terpinene	1,059	8.054
cis-Sabinene hydrate	1,064	0.451
trans-Linalool oxide	1,070	0.56
cis-Linalool oxide	1,086	0.602
Linalool	1,104	9.363
Borneol	1,163	0.846
Terpinene-4-ol	1,175	0.486
A-Terpineol	1,195	0.326
Thymol methyl ether	1,232	0.533
Carvacrol methyl ether	1,242	2.4
Linalyl acetate	1,264	0.188
Thymol	1,297	25.701
Carvacrol	1,310	29.489
Thymol acetate	1,353	0.219
Carvacrol acetate	1,371	0.735
(*E*)-caryophyllene	1,417	1.331
Aromadendrene	1,436	0.258
Viridiflorene	1,491	0.223
Spathulenol	1,574	0.585
Caryophyllene oxide	1,580	1.369

RI, retention index.

RESULTS

Plant Materials

The essential oil was prepared by water-distillation, and its chemical composition was analyzed by GC–MS. As shown in Table 1, GC–MS analysis of the essential oil indicated the main components were carvacrol (29.489%), thymol (25.701%), *p*-cymene (11.247%), linalool (9.363%) and γ-terpinene (8.054%). The yield of essential oil from leaf material was 2.2% (w/w). The yield of the aqueous extract from leaf material was 0.6% (w/w). At this time the chemical composition of the aqueous extract was not assessed.

Radical Scavenging

The IC_{50} for ROS, RNS, H_2O_2 and TBARS scavenging activities were estimated to be 2.46 ± 0.7, 2.48 ± 0.8, 3.84 ± 1.3 and 4.58 ± 1.4 μg/mL of essential oil, respectively (Table 2). The IC_{50} for ROS, RNS, H_2O_2 and TBARS scavenging activities were estimated to be 1.92 ± 0.6, 2.17 ± 0.8, 2.86 ± 0.5 and 3.15 ± 1.2 μg/mL of aqueous extract, respectively (Table 2). At concentration > 10 μg/mL both essential oil and aqueous extract significantly ($P < 0.01$) scavenge ROS, RNS, H_2O_2 and TBARS by 100%. Thus, the radical scavenging capacity of the aqueous extract was similar to the radical scavenging of essential oil and there is no significant difference between radical scavenging of essential oil and aqueous extract ($P > 0.01$).

Antibacterial Activity

Antibacterial activity was assessed by measuring the zone of bacterial growth inhibition and MIC determination. The results of disk diffusion assay were summarized in Table 3. The results indicated that essential oil at all tested concentrations significantly ($P < 0.01$) inhibited the growth *S. typhi*, *E. coli*, *K. pneumonia*, *S. aureus*, *S. epidermi* and *B. subtilis*. However, *P. aeroginosa* was resistant to the essential oil ($P > 0.01$). MIC for *S. typhi*, *E. coli*, *K. pneumonia*, *S. aureus*, *S. epidermi* and *B. subtilis* were 2.65 ± 0.7, 2.72 ± 0.8, 2.85 ± 0.5, 3.02 ± 0.8, 3.53 ± 1 and 3.65 ± 0.9 μg/mL of essential oil, respectively. At concentration > 5 μg/mL, essential oil significantly ($P < 0.01$) reduced the growth of all bacteria by 100%. However, the aqueous extract did not show any such activity at any concentration used.

Antifungal Activity

Antifungal activity was assessed by measuring the zone of fungal growth inhibition and MIC determination. The results of disk diffusion assay were summarized in Table 3. The results indicated that essential oil at all tested concentrations significantly ($P < 0.01$) inhibited the growth of *A. niger* and *C. albicans*. MIC for *A. niger* and *C. albicans* were 2.2 ± 0.5 and 2.8 ± 0.8 μg/mL of essential oil, respectively. At concentration > 5 μg/mL, essential oil significantly ($P < 0.01$) reduced the growth of both fungi by 100%. However, the aqueous extract did not show any such activity at any concentration used.

TABLE 2. RADICAL SCAVENGING ACTIVITY OF *Z. MULTIFLORA* AQUEOUS EXTRACT AND ESSENTIAL OIL

Compounds (μg/mL)	IC_{50} for ROS	IC_{50} for RNS	IC50 for H_2O_2	IC_{50} for MDA
Aqueous extract	1.92 ± 0.6	2.17 ± 0.8	2.86 ± 0.5	3.15 ± 1.2
Essential oil	2.46 ± 0.7	2.48 ± 0.8	3.84 ± 1.3	4.58 ± 1.4

IC, inhibitory concentration; IC_{50}, effective concentration of the test compound which scavenge the radical by 50%; MDA, malondialdehyde.

TABLE 3. ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL AGAINST SELECTED BACTERIAL STRAINS

	Essential oil (µg/mL)				GM/AM/KN	MIC
Microbia	0	25	50	100	10 µg/mL	µg/mL
Pseudomonas aeruginosa	5.9 ± 0.8	6.2 ± 0.5	6.5 ± 0.7	6.65 ± 1	14.6 ± 0.96	–
Salmonella typhi	5.9 ± 0.8	21 ± 3.6	30 ± 2.7	36.35 ± 1.3	14.6 ± 0.96	2.65 ± 0.7
Escherichia coli	5.9 ± 0.8	22 ± 3.3	30.7 ± 4.1	42 ± 3	14.6 ± 0.96	2.72 ± 0.8
Klebsiella pneumoniae	5.9 ± 0.8	22.3 ± 2.2	30.62 ± 1.7	39.25 ± 2.4	14.6 ± 0.96	2.85 ± 0.5
Staphylococcus aureus	5.9 ± 0.8	30.85 ± 4.1	38.42 ± 4	43.57 ± 2.8	14.6 ± 0.96	3.02 ± 0.8
S. epidermis	5.9 ± 0.8	25.85 ± 2.5	32.5 ± 2.9	43.35 ± 2.2	14.6 ± 0.96	3.53 ± 1
Bacillus subtilis	5.9 ± 0.8	36.65 ± 3.9	42.12 ± 3.2	48.5 ± 4	14.6 ± 0.96	3.65 ± 0.9
Aspergillus niger	5.9 ± 0.8	13.6 ± 0.9	22.8 ± 1.8	33.2 ± 1.7	10.6 ± 0.6	2.2 ± 0.5
Candida albicans	5.9 ± 0.8	14.2 ± 0.84	23.8 ± 0.5	30 ± 1	10.6 ± 0.6	2.8 ± 0.8

The antibacterial activity was assessed by measuring the zone of microbial growth inhibition. Data are expressed as means ± standard deviation of inhibition zone diameter (mm) for different concentration of essential oil against selected microbial strains.
MIC was defined as the lowest concentration of the compounds produced >95% growth reduction compared with the growth in the control well.
Gm, gentamicin; AM, ampicilin; MIC, minimum inhibitory concentration.

DISCUSSION

Z. multiflora is a thyme-like plant that grows broadly in the central and southern parts of Iran. Despite its remarkable array of medical applications, to our knowledge, little research has been carried out on the biological activity of the essential oil constituents of this plant. GC–MS analysis of the essential oil indicated the main components to be carvacrol, thymol, *p*-cymene, linalool and γ-terpinene. The composition of the essential oil from *Z. multiflora*, which was previously analyzed by other research groups, was shown to depend on the species, climate, altitude, time of collection and growth stage. In those studies, the main components of the essential oil were only thymol (5–56%) and carvacrol (5–78%), both at a high percentage and with few other compounds (Karimian *et al.* 2011; Zomorodian *et al.* 2011). However, in our study, the main components were carvacrol, thymol, *p*-cymene, linalool and γ-terpinene. Thus, the plant analyzed in this research was a new chemotype of *Z. multiflora*.

The *Z. multiflora* essential oil analyzed in this research showed potent radical scavenging activity. The radical scavenging activity of compounds is mainly due to their oxidation-reduction potential, which can play an important role in neutralizing free radicals. This activity is related to phenolic hydroxyl groups (Katalinic *et al.* 2006). According to GC–MS analysis; *Z. multiflora* contains high levels of phenolic compounds (thymol and carvacrol). The antioxidant activity of *Z. multiflora* essential oil is thus likely thus related to thymol and carvacrol (Zheng and Wang 2001). Our results indicated that the radical scavenging capacity of the aqueous extract was similar to the essential oil. This ability to scavenge radicals might be related to water-soluble phenolic monoterpenes. Thymol and carvacrol intrinsically have a hyrdrophobic character and are thus soluble in hydrophobic solvents (Baser and Demirci 2007). However, thymol and carvacrol are relatively hydrophilic and can be solubilized in water to some extent. Thus the radical scavenging of the aqueous extract might be related to water-soluble thymol and carvacrol and other water-soluble phenolic monoterpenes (Baser 2008).

Our results indicated that essential oil significantly inhibited the growth *S. typhi*, *E. coli*, *K. pneumonia*, *S. aureus*, *S. epidermi* and *B. subtilis*. However, *P. aeroginosa* was resistant to the essential oil. In addition, the aqueous extract did not show any such activity at any concentration used. It was thus concluded that *Z. multiflora* essential oil can effectively inhibit pathogenic bacteria and fungi. Essential oils probably exhibit their antibacterial activity through damage to the cytoplasmic membrane. Essential oil passes through cytoplasmic membranes, disrupts the structure of the lipid bilayer and alters membrane permeability (Nostro *et al.* 2009; Sokovic *et al.* 2010; Garcia-Garcia *et al.* 2011). Permeabilization of the membrane enhances the leakage of protons from cells, disrupts membrane electric potential and reduces proton motive force and finally adenosine triphosphate (ATP) synthesis. In addition, reduced membrane potential enhances the leakage of ions, ATP, amino acids and proteins from cells. The leakage of ions out of a cell is a clear indication of membrane damage and cell death (Paparella *et al.* 2008; Xu *et al.* 2008; Rua *et al.* 2011). The extent of membrane damage induced by an essential oil is related to the intrinsic hydrophobicity of its main components. Thymol, carvacrol, *p*-cymene, γ-terpinene and linalool are intrinsically hydrophobic (Baser and Demirci 2007). However, thymol and carvacrol could successfully diffuse through the cell membrane and disrupt cells. Thus, the hydroxyl group is also required for antibacterial activity (Griffin *et al.* 1999; Dorman and Deans 2000). Differences in the activity of the essential oil on the different bacteria tested are probably related to the lipopolysaccharide layer on the bacterial cell wall. In bacteria with a thick

lipopolysacharide layer, essential oil components cannot diffuse through the cell membrane at low concentrations. However, at higher concentrations this polysaccharide layer can be disrupted by essential oils. The difference in activity between the essential oil and aqueous extract is probably due to differences in the hydrophobic nature of these isolates. The aqueous extract has a lower hydrophobicity and cannot diffuse through the cell membrane and thus cannot disrupt the membrane, hence its weaker or less effective antibacterial activity.

Essential oil from *Z. multiflora* could inhibit both fungi, but was more effective against *A. niger* and *C. albicans*. However, the aqueous extract could not inhibit the growth of both fungi at any concentration used. This difference in activity between the essential oil and aqueous extract is probably due to differences in the hydrophobic nature of the isolates. The aqueous extract has lower hydrophobicity and cannot diffuse through the cell membrane and thus cannot disrupt the membrane. Essential oil-bearing phenolic monoterpenes generally displayed potent fungicidal activity while antifungal potency varied and appeared to be intensified by increasing carvacrol and thymol content and flow cytometry confirmed the occurrence of damage to the plasma membrane and cell death (Vardar-Unlu *et al.* 2010; Vale-Silva *et al.* 2012). In addition, fungicidal activity of thymol and carvacrol apparently originates from the inhibition of ergosterol biosynthesis and the disruption of membrane integrity (Ahmad *et al.* 2011). Furthermore, fungicidal activity might be due to the induction of calcium stress and upregulation of genes involved in metabolic and energy pathways, stress response, autophagy and drug efflux (Rao *et al.* 2010).

Accordingly, *Z. multiflora* essential oil has antioxidant activity and can be used in the therapy of oxidative damage that tends to accompany some inflammatory conditions. In addition, *Z. multiflora* essential oil has antimicrobial activity and can inhibit pathogenic bacteria and fungi. The present study provides additional data for supporting the use of *Z. multiflora* as a tea or additive in foods and traditional remedies for the treatment of infectious diseases.

ACKNOWLEDGMENTS

This work was supported by the financial support from Shiraz University (grant no. 88-GR-AGRST-108). The authors thank their colleagues at the Plants and Drug Research Institute, University of Shahid Beheshti, for assistance with GC-MS analysis.

REFERENCES

ADAMS, R.P. 2007. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometery*, 4th Ed., p. 456, Allured Publishing Corporation, Carol Stream, IL.

AHMAD, A., KHAN, A., AKHTAR, F., YOUSUF, S., XESS, I., KHAN, L.A. and MANZOOR, N. 2011. Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against *Candida*. Eur. J. Clin. Microbiol. Infect. Dis. *30*, 41–50.

AMIRGHOFRAN, Z., HASHEMZADEH, R., JAVIDNIA, K., GOLMOGHADDAM, H. and ESMAEILBEIG, A. 2011. *In vitro* immunomodulatory effects of extracts from three plants of the *Labiatae* family and isolation of the active compound. J. Immunotoxicol. *8*, 265–273.

ANTHONY, J.P., FYFE, L. and SMITH, H. 2005. Plant active components. A resource for antiparasitic agents? Trends Parasitol. *21*, 462–468.

BASER, K.H. 2008. Biological and pharmacological activities of carvacrol and carvacrol bearing essential oils. Curr. Pharm. Dis. *14*, 3106–3119.

BASER, K.H.C. and DEMIRCI, F. 2007. Chemistry of essential oils. In *Flavours and Fragrances: Chemistry, Bioprospecting and Sustainability* (R.G. Berger, ed.) pp. 43–86, Springer, Heidelberg.

BAUER, A.W., KIRBY, W.M.M., SHERRIS, J.C. and TURCK, M. 1996. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. *45*, 493–496.

BRITISH PHARMACOPEIA. 1998. *British Pharmacopeia*, Vol. 4 pp. 137–138, HMSO, London.

BUYUKBALCI, A. and EI, S.N. 2008. Determination of *in vitro* antidiabetic effects, antioxidant activities and phenol contents of some herbal teas. Plant Foods Hum. Nutr. *63*, 27–33.

DORMAN, H.J.D. and DEANS, S.G. 2000. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J. Appl. Microbiol. *88*, 308–316.

GARCIA-GARCIA, R., LOPEZ-MALO, A. and PALOU, E. 2011. Bactericidal action of binary and ternary mixtures of carvacrol, thymol, and eugenol against *Listeria innocua*. J. Food Sci. *76*, M95–M100.

GRIFFIN, S.G., WYLLIE, S.G., MARKHAM, J.L. and LEACH, D. 1999. The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. Flavour Fragr. J. *14*, 322–332.

HODGES, D.M., DELONG, J.M., FORNEY, C.F. and PRANGE, R.K. 1999. Improving the thiobarbituric acid reactive substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta *207*, 604–611.

JOULAIN, D., KONG, W.A. and HOCHMUTH, D.H. 2001. Terpenoids and related constituents of essential oils. Library of Mass Finder, 2.1, Humburg, Germany.

KARIMIAN, P., KAVOOSI, G. and SAHARKHIZ, M.J. 2011. Antioxidant, nitric oxide scavenging and malondialdehyde scavenging activities of essential oils from different chemotypes of *Z. multiflora*. Nat. Prod. Res. DOI: 10.1080/14786419.2011.631136.

KATALINIC, V., MILOS, M., KULISIC, T. and JUKIC, M. 2006. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem. *94*, 550–557.

MARCOCCI, L., MAGUIRE, J.J., DROYLEFAIX, M.T. and PACKER, L. 1994. The nitric oxide scavenging properties of *Ginkgo biloba* extract EGB 761. Biochem. Biophys. Res. Commum. *201*, 748–755.

MC LAFFERTY, F.W. 2009. *Wiley Registry of Mass Spectral Data*, 9th Ed., p. 662, John Wiley and Sons, Inc., Hoboken, NJ.

NOSTRO, A., MARINO, A., BLANCO, A.R., CELLINI, L., DI GIULIO, M., PIZZIMENTI, F., SUDANO-ROCCARO, A. and BISIGNANO, G. 2009. *In vitro* activity of carvacrol against staphylococcal preformed biofilm by liquid and vapour contact. J. Med. Microbiol. *58*, 791–797.

PAPARELLA, A., TACCOGNA, L., AGUZZI, I., CHAVES-LOPEZ, C., SERIO, A., MARSILIO, F. and SUZZI, G. 2008. Flow cytometric assessment of the antimicrobial activity of essential oils against *Listeria monocytogenes*. Food Control *19*, 1174–1182.

RAO, A., ZHANG, Y., MUEND, S. and RAO, R. 2010. Mechanism of antifungal activity of terpenoid phenols resembles calcium stress and inhibition of the TOR pathway. Antimicrob. Agents Chemother. *54*, 5062–5069.

RE, R., PELLEGRINI, N., PROTEGENETE, A., PANNALA, A., YANG, M. and RICE-EVANS, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. *26*, 1231–1237.

REICHLING, J., SCHNITZLER, P., SUSCHKE, U. and SALLER, R. 2009. Essential oils of aromatic plants with antibacterial, antifungal, antiviral and cytotoxic properties. Forsch. Komplementmed. *16*, 79–90.

RUA, J., FERNANDEZ-ALVAREZ, L., DE CASTRO, C., DEL VALLE, P., DE ARRIAGA, D. and GARCIA-ARMESTO, M.R. 2011. Antibacterial activity against foodborne *S. aureus* and antioxidant capacity of various pure phenolic compounds. Foodborne Pathog. Dis. *8*, 149–157.

SOKOVIC, M., GLAMOCLIJA, J., MARIN, P.D., BRKIC, D. and VAN GRIENSVEN, L.J. 2010. Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an *in vitro* model. Molecules *15*, 7532–7546.

SOLORZANO-SANTOS, F. and MIRANDA-NOVALES, M.G. 2011. Essential oils from aromatic herbs as antimicrobial agents. Curr. Opin. Biotechnol. *23*, 1–6.

SZE, S.C., TONG, Y., NG, T.B., CHENG, C.L. and CHEUNG, H.P. 2010. Herba epimedii: Anti-oxidative properties and its medical implications. Molecules *15*, 7861–7870.

TAJKARIMI, M.M., IBRAHIMA, S.A. and CLIVER, D.O. 2010. Antimicrobial herb and spice compounds in food. Food Control *21*, 1199–1218.

VALE-SILVA, L.A., SILVA, M.J., OLIVEIRA, D., GONCALVES, M.J., CAVALEIRO, C., SALGUEIRO, L. and PINTO, E. 2012. Correlation of the chemical composition of essential oils from *O. vulgare* subsp. virens with the *in vitro* activity against clinical yeast and filamentous fungi. J. Med. Microbiol. *61*, 252–260.

VARDAR-UNLU, G., YAGMUROGLU, A. and UNLU, M. 2010. Evaluation of *in vitro* activity of carvacrol against *C. albicans* strains. Nat. Prod. Res. *24*, 189–193.

WAYNE, P.A. 2006a. *Clinical and Laboratory Standards Institute,* CLSIM7-A7. 7th ed. Clinical and Laboratory Standards Institute (CLSI). Metrhods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard.

WAYNE, P.A. 2006b. *Clinical and Laboratory Standards Institute*, CLSIM7-A7. 7th ed. Clinical and Laboratory Standards Institute (CLSI). Reference methods for broth dilution antifungal susceptibility testing of yeasts; approved standard.

XU, J., ZHOU, F., JI, B.P., PEI, R.S. and XU, N. 2008. The antibacterial mechanism of carvacrol and thymol against *Escherichia coli*. Lett. Appl. Microbiol. *47*, 174–179.

ZHENG, W. and WANG, S.Y. 2001. Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem. *49*, 5165–5170.

ZOMORODIAN, K., SAHARKHIZ M.J., RAHIMI M.J., BANDEGI, A., SHEKARKHAR, G., BANDEGANI, A., PAKSHIR, K. and BAZARGANI, A. 2011. Chemical composition and antimicrobial activities of the essential oils from three ecotypes of *Z. multiflora*. Pharm. Mag. *7*, 53–59.