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Microbicidal effectiveness of essential oil from *Zataria multiflora* and its main components

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ABSTRACT

Objective: To evaluate the antimicrobial activities of essential oil from *Zataria multiflora* and its main components against four Gram-positive bacteria, three Gram-negative bacteria and two fungi. **Methods:** The essential oil was prepared by water-distillation, and its composition was analyzed by GC-MS. Antibacterial and antifungal activities of the essential oil, thymol, carvacrol, p-cymene and γ-terpinene were assessed by the agar disk diffusion test. **Results:** Carvacrol (29.2%), thymol (25.4%), p-cymene (11.2 %), linalool (9.6 %) and γ-terpinene (8%) were detected as the main components of the oil. At concentration of 25-100 μg/mL essential oil, thymol and carvacrol significantly inhibited *Salmonella typhi*, *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Bacillus subtilis*, Aspergilus niger and *Candida albicans* with a similar manner. There were no any significant differences between essential oil, thymol and carvacrol. However, p-cymene and γ-terpinene could not show any such activity at used concentrations. *Pseudomonas aeroginosa* was resistance to all of tested compounds. **Conclusions:** The present study provides an additional data for supporting the use of thymol and carvacrol bearing essential oils as a safe and effective antimicrobial agent in traditional reminders for the treatment of infective disease.

1. Introduction

A large portion of the world's population currently uses traditional medicines and herbal therapy, which mainly 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. In recent decades, herbal therapy widely employ for treatment of several of human diseases. For example, essential oils or natural products derived from numerous traditional herbs have been examined for their antioxidant, antibacterial, antiparasitic and antifungal, antiviral and cytotoxic activities and for food preservation and for food safety[1-5].

Zataria multiflora Boiss, known as Avishan Shirazi in Persian, grows only in warm parts of Iran, Afghanistan and Pakistan. This aromatic plant belongs to the *Lamiaceae* family and is used in flavoring and preserving food and drinks for its antiseptic, analgesic and carminative properties in Iranian traditional medicine. The radical scavenging, antibacterial, antifungal, immunomodulatory and radio-protective activities of this plant has been reported in previous studies[6-11]. 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 still very little information about the biological activity of essential oil and its main constitute of this plant. Thus, in the present study, the effect of *Z. multiflora* essential oil and its main components on the inhibition of selected bacteria and fungi was investigated. Differences between the biological activity of the essential oil and the main components for the first time were assessed. In addition compounds which responsible to antimicrobial activities of the essential oil was identified.

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2. Materials and methods

2.1. Plant materials and essential oil preparation

The aerial parts (leaves) of *Z. multiflora* were obtained from wild plants in mountains of Arsenjan (Fars, Iran). The plant was taxonomically identified by a senior plant taxonomist, Prof. Ahmad Reza Khosravi at the Department of Biology, Shiraz University, Shiraz, Fars Province, Iran. A voucher specimen (24985) was deposited at the Herbarium of the Department of Biology, Shiraz University. The leaves of the plant (5 years-old) were separated from the stem and dried for 72 h in the shade. The air-dried leaves (100 g) were hydro-distilled for 3 h using an all-glass Clevenger-type apparatus according to the method outlined by the British Pharmacopeia[12]. The essential oil thus obtained was dried over anhydrous sodium sulphate (Sigma-Aldrich) stored in sealed vial at -20 ℃ before gas chromatography - mass spectrometry (GC-MS) analysis and further experiments.

2.2. Essential oil analysis and identification

GC analysis was carried out using a Agilent-technology chromatograph with HP-5 column (30m×0.32 mm *i.d.*×0.25 μm). Oven temperature was performed as follows: 60 ℃ to 210 ℃ at 3 ℃/min; 210 ℃ to 240 ℃ at 20 ℃/min and hold for 8.5 min, injector temperature 280 ℃; detector temperature, 290 ℃; carrier gas, N_2 (1 mL/min); split ratio of 1:50. GC-MS analysis was carried out using a Agilent 7890 operating at 70 eV ionization energy, equipped with a HP-5 MS capillary column (phenyl methyl siloxane, 30m×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 (RRI, HP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley GC/MS Library, Adams Library, Mass Finder 2.1 Library data published mass spectra data[13,14].

2.3. Antibacterial and antifungal activities assay

All microorganisms were obtained from the Persian type culture collection (PTCC), Tehran, Iran. Essential oil, thymol (Fluka, purity> 98%), carvacrol (Fluka, purity> 98%), *p*-cymene (Fluka, purity> 97%) and γ-terpinene (Fluka, purity> 97%) were individually tested against four gram negative bacteria (*Pseudomonas aeruginosa* PTCC 1074 (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 in Luria and Bertani (LB) medium at 37 ℃ for 16-24 h. Antimicrobial tests were carried out by the disk diffusion method in which a microbial suspension (50 μL of 1×108 colony-forming units (CFU/mL) was spread on a nutrient agar plate (Farazbin, Iran)[15]. The disks (Whatman No. 1, 6 mm diameter, Padtan Teb, Iran) were impregnated with 20 μl of different concentrations of essential oil, thymol, carvacrol, *p*-cymene and γ-terpinene (0, 25, 50, 100 μg/mL) and placed on the inoculated agar. The inoculated plates were incubated at 37 ℃ for 24 h. DMSO was used as the negative control. Positive control disks included (all at 10 mg/plate and purchased from Padtan Teb) Gentamicin, Ampicillin and Ketoconazole 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 inhibition of bacterial or fungal growth.

2.4. 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 (ANOVA) test at $P<0.01$ using statistical package for the social sciences (SPSS, Abaus Concepts, Berkeley, CA, version 16) and Prism 5 (Graph Phad, San Diego, USA) software.

3. Results

3.1. Chemical composition

The essential oil was prepared by water-distillation, and its chemical composition was analyzed by GC-MS. GC-MS analysis of the essential oil indicated the main components are; carvacrol (29.2%), thymol (25.4%), *p*-cymene (11.2 %), linalool (9.6 %) and γ-terpinene (8%). The yield of essential oil from leaf material was 2.2% (w/w) (Table1).

3.2. Antibacterial Activity

The antibacterial activity was assessed by measuring the zone of bacterial growth inhibition. Our results indicated that, essential oil at concentrations of 25-100 μg/mL significantly ($P<0.01$) inhibited the growth *S. typhi*, *K. pneumonia*, *E. coli*, *S. aureus*, *S. epidermi* and *B. subtilis* significantly (Table 2). Carvacrol at concentrations of 25-100 μg/mL significantly ($P<0.01$) inhibited the growth *S. typhi*, *K. pneumonia*, *E. coli*, *S. aureus*, *S. epidermi* and *B. subtilis* significantly (Table 3). Thymol at concentrations of 50-100 μg/mL significantly ($P<0.01$) inhibited the growth *S. typhi*, *K. pneumonia*, *E. coli*, *S. aureus*, *S. epidermi* and *B. subtilis* significantly (Table 4). However, *P. aeroginosa* is resistance ($P>0.01$) to essential oil, carvacrol and thymol. Accordingly, essential oil and thymol at concentration of 25-100 μg/mL and carvacrol at concentration of 50-100 μg/mL significantly

Table 1
Antibacterial and antifungal activity of essential oil against selected bacterial strains.

Microorganism	Zone of inhibition in diameter (mm) 0 μg/mL	25 μg/mL	50 μg/mL	100 μg/mL	GM/AM/KN
P. aeroginosa	5.9±0.8	6.2±0.5	6.5±0.7	6.65±1.0	14.6±1.0
S. typhi	5.9±0.8	21.0±3.6	30.0±2.7	36.4±1.3	14.6±1.0
K. pneumonia	5.9±0.8	22.3±2.2	30.6±1.7	39.3±2.4	14.6±1.0
E . coli	5.9±0.8	22.0±3.3	30.7±4.1	42.0 ± 3.0	14.6±1.0
S. aureus	5.9±0.8	30.9±4.1	38.4±4.0	43.6±2.8	14.6±1.0
S. epidermis	5.9±0.8	25.85±2.5	32.5±2.9	43.4±2.2	14.6±1.0
B. subtilis	5.9±0.8	36.7±3.9	42.1±3.2	48.5±4.0	14.6±1.0
A. niger	5.9±0.8	13.6±0.9	22.8±1.8	33.2±1.7	14.6±1.0
C. albicans	5.9±0.8	14.2±0.8	23.8±0.5	30.0 ± 1.0	14.6±1.0

The antibacterial and antifungal activity was assessed by measuring the zone of microbial growth inhibition. Data are expressed as mean ± SD of inhibition zone diameter (mm) for different concentration of essential oil against selected microbial strains. Gm = Gentamicin, AM = Ampicilin, KN = Ketoconazole. Essential oil at all used concentration significantly (P<0.01) inhibits all microbial strain except *P. aeroginosa*.

Table 2.
Antibacterial and antifungal activity of carvacrol against selected bacterial strains.

Microorganism	Zone of inhibition in diameter (mm) 0 μg/mL	25 μg/mL	50 μg/mL	100 μg/mL	GM/AM/KN
P. aeroginosa	5.9 ± 0.8	6.0 ±0.5	6.5 ± 0.5	7.0 ± 1.0	14.6±1.0
S. typhi	5.9 ± 0.8	16.8±0.5	22.5±0.5	26.5 ± 0.8	14.6±1.0
K. pneumonia	5.9 ± 0.8	17.9±0.8	22.5 ±1.3	27.1 ± 3.0	14.6±1.0
E. coli	5.9 ± 0.8	13.3±1.2	20.6±0.9	40.0 ± 1.7	14.6±1.0
S. aureus	5.9 ± 0.8	13. 0±2.7	22.0 ± 2.4	26.5 ± 2.8	14.6±1.0
S. epidermis	5.9 ± 0.8	13.5±1.3	22.5 ± 2.1	29.0 ± 2.4	14.6±1.0
B. subtilis	5.9 ± 0.8	18.8±1.7	27.4±1.4	42.7 ± 1.7	14.6±1.0
A. niger	5.9 ± 0.8	165.8±1.6	30.8 ±1.8	37.0 ± 2.0	14.6±0.6
C. albicans	5.9 ± 0.8	19.0±2.5	32.4 ± 2.4	40.6 ± 1.3	14.6±0.6

The antibacterial and antifungal activity was assessed by measuring the zone of microbial growth inhibition. Data are expressed as means ±SD of inhibition zone diameter (mm) for different concentration of carvacrol against selected microbial strains. Gm = Gentamicin, AM = Ampicilin, KN = Ketoconazole. Carvacrol at all used concentration significantly (P<0.01) inhibits all microbial strain except *P. aeroginosa*.

inhibited bacterial growth with a similar manner. However, p-cymene and γ-terpinene cannot show any such activity at used concentrations.

3.3. Antifungal activity

Antifungal activity was evaluated by measuring the zone of fungi growth inhibition. Our results indicated that, essential oil at concentrations of 25-100 μg/mL significantly (P<0.01) inhibited the growth *A. niger* and *C. albicans* significantly (Table 2). Carvacrol at concentrations of 50-100 μg/mL significantly (P<0.01) inhibited the growth *A. niger* and *C. albicans* significantly (Table 3). Thymol at concentrations of 25-100 μg/mL significantly (P<0.01) inhibited the growth *A. niger* and *C. albicans* significantly (Table 4). Accordingly, essential oil and thymol at concentration of 25-100 μg/mL and carvacrol at concentration of 25-100 μg/mL significantly inhibited fungi growth with a similar manner. However, *p*-cymene and γ-terpinene cannot show any such activity at used concentrations.

Table 3
Antibacterial and antifungal activity of thymol against selected bacterial strains.

Microorganism	Zone of inhibition in diameter (mm) 0 μg/mL	25 μg/mL	50 μg/mL	100 μg/mL	GM/AM/KN
P. aeroginosa	5.9 ± 0.8	6.0 ± 0.5	6.5 ± 0.5	7.0 ± 1.0	14.6±1.0
S. typhi	5.9 ± 0.8	16.8±0.5	22.5±0.5	26.5±0.8	14.6±1.0
K. pneumonia	5.9 ± 0.8	17.9±0.8	22.5 ±1.3	27.1±3.0	14.6±1.0
E . coli	5.9 ± 0.8	13.3±1.2	20.6±0.9	40.0±1.7	14.6±1.0
S. aureus	5.9 ± 0.8	13.8 ± 2.7	22 ± 2.4	26.5±2.8	14.6±1.0
S. epidermis	5.9 ± 0.8	13.5 ± 1.3	22.5±2.1	29.0±2.4	14.6±1.0
B. subtilis	5.9 ± 0.8	18.8±1.7	27.4±1.4	42.7±1.7	14.6±1.0
A. niger	5.9 ± 0.8	165.8±1.6	30.8 ±1.8	37.0±2.7	14.6±1.0
C. albicans	5.9 ± 0.8	19.0 ± 2.5	32.4 ±2.4	40.6±1.3	14.6±1.0

The antibacterial and antifungal activity was assessed by measuring the zone of microbial growth inhibition. Data are expressed as means ± SD of inhibition zone diameter (mm) for different concentration of carvacrol against selected microbial strains. Gm = Gentamicin, AM = Ampicilin, KN = Ketoconazole. Carvacrol at all used concentration significantly (P<0.01) inhibits all microbial strain except *P. aeroginosa*.

Table 4.
Physico-chemical properties of carvacrol, thymol, p-cymene, γ-terpinene and linalool from *Z. multiflora* essential oil.

Properties	Carvacrol	Thymol	p-Cymene	Linalool	γ-Terpinene
Percentage in oil	29.2	25.4	11.2	9.6	8.0
Physical occurrence	liquid	crystalline	liquid	liquid	liquid
Furmula	C10H14O	C10H14O	C10H14	C10H18O	C10H16
Molar mass (D)	150.22	150.22	134.24	154.25	136.24
Density (g/mL)	0.977	0.960	0.857	0.860	0.853
Melting point (℃)	< -20	51	< -20	< -20	< -20
Boiling point (℃)	233	232	177	198	183
Water solubility (g/L)	830	846	6	754	1
Octanol solubility	soluble	soluble	soluble	soluble	soluble
P(o/w)	3.6	3.3	4.1	3.8	4.5
Phenol content	Yes	Yes	No	No	No
H-band capacity	Yes	Yes	No	Yes	No

4. Discussion

In Iran *Z. multiflora* is used in traditional medicine for its antiseptic, analgesic and carminative properties. Despite its remarkable array of medical applications, to our knowledge, little research has been carried out on the biological activity

of the essential oil and its main components of this plant. The composition of essential oils depends on the species, climate, and altitude, time of recollection and stage of growth. Accordingly, the composition of *Z. multiflora* from different parts of Iran with different climate is very variable. The main components of the various essential oils were thymol (5.5-56%) and carvacrol (4-78%) with a high percentage and few other compounds[6-11]. However, in our study the main components were carvacrol, thymol, *p*-cymene, linalool and γ-terpinene. Thus, the plant analyzed in the research is a new chemotype of *Z. multiflora* that analyzed previously.

The antibacterial assay indicated that, among these compounds only essential oil, thymol and carvacrol have showed strong antibacterial activity with a similar manner. At concentration of 25-100 μg/mL essential oil, thymol and carvacrol significantly inhibited bacterial growth with a similar manner. There is no any significant difference between essential oil, thymol and carvacrol. Both *p*-cymene and γ-terpinene cannot show any such activity at used concentration. These antibacterial activities which recognized in the essential oils from several medicinal plants established that the most effective individual antimicrobial agent was phenolic monoterpenes carvacrol and thymol [16-21]. In addition, carvacrol and thymol could inhibit the growth of bacteria through permeabilization and depolarization the cytoplasmic membrane [22,23].

Antifungal activity indicated that, among these compounds only essential oil, thymol and carvacrol have showed strong antifungal activity with a similar manner. At concentration of 25-100 μg/mL essential oil, thymol and carvacrol significantly inhibited fungi growth with a similar manner. There is no any significant difference between essential oil, thymol and carvacrol. However, *p*-cymene and γ-terpinene cannot show any such activity at used concentration. The antifungal activities of the essential oils from several medicinal herbs were evaluated against *A. flavus* and *A. niger*, *C. albicans*. The essential oil samples generally displayed potent fungicidal activity and antifungal potencies varied and appeared to be intensified by increasing carvacrol and thymol contents[24,25]. In addition, carvacrol and thymol could inhibit the growth of fungi through permeabilization and depolarization the cytoplasmic membrane which, ultimately leading to cytoplasmic membrane disruption, induction of dose-dependent $Ca2_+$ bursts and cell death[26-29].

Generally, the main components of Zataria oil are phenolic monoterpenes (carvacrol and thymol). They are biosynthesized from-terpinene through *p*-cymene. Therefore, these two compounds are always present in oils containing carvacrol and thymol. All of these compounds are monoterpenic nature containing methyl and isopropyl function groups in para position to each other. The main difference between γ-terpinene or *p*-cymene and carvacrol or thymol is the substitution of hydroxyl group on the phenol ring in the thymol and carvacrol and the only difference between carvacrol and thymol is the position of hydroxyl group[30]. However, the antimicrobial activity of these compounds is very different. The extent of antibacterial activity induced by monoterpenes can be related to intrinsic hydrophobicity. The hydrophobicity index can be determined experimentally by its partition coefficient in octanol/water (Po/w). Carvacrol, thymol, *p*-cymene and γ-terpinene have a log Po/w of 3.3, 3.64, 4.1 and 4.7, respectively[30,31]. However, thymol and carvacrol could successfully diffuse through the cell membrane and disrupt cells. Thus in addition to carbon skeleton the hydroxyl group is required for biological activity[31,32]. Collectively, it was concluded that essential oil bearing thymol and carvacrol might exhibit its antimicrobial activity through cytoplasmic damages and membrane depolarization. The main driving force for ATP synthesis is transmembrane electric potential and proton-motive force across cytoplasmic and mitochondrial membranes. Phenolic monoterpenes pass through cytoplasmic membranes, disrupts the structure of lipid bilayer and change membrane permeability[22-25]. Permeabilization of membrane enhances the leakage of proton from cell, disrupt membrane electric potential, and reduce proton motive force and finally ATP synthesis. In addition reduction of membrane potential enhances the leakage of ions, ATP, amino acids and proteins from the cell. The leakage of ions out of the cell is a clear indication of membrane damage and cell death. In addition essential oil bearing thymol and carvacrol might specifically upregulate metabolic and energy pathways, stress response, autophagy, and drug efflux which mediate cell death[26-29].

Accordingly, these results indicated that essential oils bearing thymol and carvacrol have antimicrobial activities and can inhibit pathogenic bacteria and fungi. However, further study is also necessary to confirm antimicrobial activities of the phenolic monoterpenes in vivo conditions. Thus, the present study is provided an additional data for supporting the use of phenolic monoterpenes and essential oil bearing thymol and carvacrol as safe and effective source of natural antimicrobial agents in additive foods and traditional remedies for the treatment of infectious disease.

Declaration of interest

The authors report no declarations of interest.

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References

[1] Sze SC, Tong Y, Ng TB, Cheng CL, Cheung HP, Herba epimedii.

Anti-oxidative properties and its medical implications. *Molecules* 2010; **15**: 7861-7870.

[2] Solorzano-Santos F, Miranda-Novales MG. Essential oils from aromatic herbs as antimicrobial agents. *Curr Opin Biotechnol* 2011; **23**:1-6.

[3] Anthony JP, Fyfe L, Smith H. Plant active components. A resource for antiparasitic agents? *Trends in Parasitol* 2005; **21**: 462-468.

[4] Reichling J, Schnitzler P, Suschke U, Saller R. Essential oils of aromatic plants with antibacterial, antifungal, antiviral and cytotoxic properties. Forch Komplementmed 2010; **16**: 79-90.

[5] Tajkarimi MM, Ibrahima SA, Cliver DO. Antimicrobial herb and spice compounds in food. *Food Control* 2010; **21**: 1199-1218.

[6] Karimian P, Kavoosi G, Saharkhiz MJ. Antioxidant, nitric oxide scavenging and malondialdehyde scavenging activities of essential oils from different chemotypes of *Z. multiflora*. *Nat Prod Res* 2011; in press.

[7] Mansour A, Enayat K, Neda MS, Behzad A. Antibacterial effect and physicochemical properties of essential oil of *Z. multiflora* Boiss. *Asian Pac J Trop Med* 2010; **3**: 439-442.

[8] Zomorodian K, Saharkhiz MJ, Rahimi MJ, Bandegi A, Shekarkhar G, Bandegani A, et al. Chemical composition and antimicrobial activities of the essential oils from three ecotypes of *Z. multiflora*. *Pharm Mag* 2011; **7**: 53-59.

[9] Saei-Dehkordi SS, Tajik H, Moradi M, Khalighi-Sigaroodi F. Chemical composition of essential oils in *Z. multiflora* Boiss from different parts of Iran and their radical scavenging and antimicrobial activity. *Food Chem Toxicol* 2010; **48**: 1562-1567.

[10] Amirghofran Z, Hashemzadeh R, Javidnia K, Golmoghaddam H, Esmaeilbeig A. *In vitro* immunomodulatory effects of extracts from three plants of the Labiatae family and isolation of the active compound. *J Immunotoxicol* 2011; **8**: 265-73.

[11] Hosseinimehr SJ, Mahmoudzadeh A, Ahmadi A, Ashrafi SA, Shafaghati N, Hedayati N. The radioprotective effect of *Z. multiflora* against genotoxicity induced by I^3 irradiation in human blood lymphocytes. *Cancer Biother Radiopharm* 2011; **26**: 325-329.

[12] British pharmacopeia. HMSO; *London*: 1988. P. 137-138.

[13] Adams RP. *Identification of essential oil components by gas chromatography/mass spectrometery*. Illinois,USA: Allured Publishing Corporation; 2007.

[14] Joulain D, Kong WA, Hochmuth DH. *Terpenoids and related constituents of essential oils. Library of Mass Finder*, 2.1. Humburg: 2001.

[15] Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1996; **45**: 493-496.

[16] Nostro A, Marino A, Blanco AR, Cellini L, Di Giulio M, Pizzimenti F, et al. *In vitro* activity of carvacrol against staphylococcal preformed biofilm by liquid and vapour contact. *J Med Microbiol* 2009; **58**: 791-797.

[17] Nostro A, Roccaro AS, Bisignano G, Marino A, Cannatelli MA, Pizzimenti FC, et al. Effects of oregano, carvacrol and thymol on *Staphylococcus aureus* and Staphylococcus epidermidis biofilms. *J Med Microbiol* 2007; **56**: 519-523.

[18] Garcia-Garcia R, Lopez-Malo A, Palou E. Bactericidal action of binary and ternary mixtures of carvacrol, thymol, and eugenol against Listeria innocua. *J Food Sci* 2011; 76: M95-100.

[19] Rua J, Fernandez-Alvarez L, De Castro C, Del Valle P, De Arriaga D, Garcia-Armesto MR. Antibacterial activity against foodborne S. aureus and antioxidant capacity of various pure phenolic compounds. *Foodborne Pathog Dis* 2011; **8**: 149-157.

[20] Sokovic M, Glamoclija J, Marin PD, Brkic D, van Griensven LJ. Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. *Molecules* 2010; **15**: 7532-7546.

[21] Sozmen F, Uysal B, Oksal BS, Kose EO, Deniz IG. Chemical composition and antibacterial activity of O. saccatum P.H. Davis essential oil obtained by solvent-free microwave extraction: comparison with hydrodistillation. *J AOAC Int* 2011; **94**: 243-250.

[22] Xu J, Zhou F, Ji BP, Pei RS, Xu N. The antibacterial mechanism of carvacrol and thymol against *Escherichia coli*. *Lett Appl Microbiol* 2008; **47**: 174-179.

[23] Paparella A, Taccogna L, Aguzzi I, Chaves-Lopez C, Serio A, Marsilio F, et al. Flow cytometric assessment of the antimicrobial activity of essential oils against L. monocytogenes. *Food Control* 2008; **19**: 1174-1182.

[24] Bisht D, Pal A, Chanotiya CS, Mishra D, Pandey KN. Terpenoid composition and antifungal activity of three commercially important essential oils against *A. flavus* and *A. niger*. *Nat Prod Res* 2011; **25**:1993-1998.

[25] Vardar-Unlu G, Yagmuroglu A, Unlu M. Evaluation of in vitro activity of carvacrol against *C. albicans* strains. *Nat Prod Res* 2010; **24**: 189-193.

[26] Vale-Silva LA, Silva MJ, Oliveira D, Goncalves MJ, Cavaleiro C, Salgueiro L, et al. 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* 2012; **61**:252-260.

[27] Zuzarte M, Vale-Silva L, Gonçalves MJ, Cavaleiro C, Vaz S, Canhoto J, et al. Antifungal activity of phenolic-rich Lavandula multifida L. essential oil. *Eur J Clin Microbiol Infect Dis* 2011; in press.

[28] Ahmad A, Khan A, Akhtar F, Yousuf S, Xess I, Khan LA, et al. Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against Candida. *Eur J Clin Microbiol Infect Dis* 2011; **30**: 41-50.

[29] Rao A, Zhang Y, Muend S, Rao R. Mechanism of antifungal activity of terpenoid phenols resembles calcium stress and inhibition of the TOR pathway. *Antimicrobial Agents Chemother* 2010; **54**: 5062-5069.

[30] Baser KHC, Demirci F. *Chemistry of essential oils*. In: Berger RG Ed, Flavours and Fragrances: Chemistry, Bioprospecting and Sustainability. Heidelberg: Springer; 2007: P.43-86.

[31] Dorman HJD, Deans SG. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J Appl Microbiol* 2000; **88**: 308-316.

[32] Griffin SG, wyllie SG, Markham JL, Leach D. The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. *Flavour Fragr* 1999; **14**: 322-332.