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Antimicrobial activity and essential oil composition of *Zataria multiflora Boiss* in two regions of Iran

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Abstract

This study was led with the purpose of evaluating the antimicrobial activity and identifies the essential oil composition of *Zataria multiflora Boiss* obtained from Jahrom and Kerman Provinces of Iran. Essential oils were obtained by hydro-distillation using Clevenger type apparatus during approximately 3 hours and analyzed using gas chromatography/mass spectrometry (GC/ MS). Forty-seven components were identified in *Z. multiflora* oil (collected from Jahrom) that represented 99.96% of the oil. The main components of the oil were, Thymol (38.89%), Carvacrol methyl ether (31.63%), γ-Terpinene (7.08%) and δ-3-Carene (6.67%). Forty-nine components were identified in *Z. multiflora* oil (collected from Kerman) that represented 99.91% of the oil. The main components of the oil were, Carvacrol (47.19%), α-Terpinyl acetate (16.40%), δ-3-Carene (8.46%), 1,8-cineole (5.50%) and γ-Terpinene (4.58%). Antimicrobial activity of the essential oils were evaluated the disc diffusion methods. The essential oil showed strong antimicrobial activity against *Candida albicans and E. coli* two medically important pathogens.

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Introduction

With the growing interest in the use of essential oils in both the food and the pharmaceutical industries, a systematic examination of plant extracts for these properties has become increasingly important. The use of natural antimicrobial compounds is important not only in the preservation of food but also in the control of human and plant diseases of microbial origin (Baratta *et al.*, 1998).

Essential oils of herbs and their components, which are products from the secondary metabolism of plants, have many applications in ethno-medicine food, flavoring and preservation as well as in the fragrance and pharmaceuticals industries (Edris, 2007).

Zataria multiflora is a plant belonging to the labiatae family that geographically grows only in Iran, Pakistan and Afghanistan. This plant with the vernacular name of Avishan Shirazi (in Iran) has several traditional uses such as antiseptic, anesthetic and antispasmodic (Rastegar *et al.*, 2012).

The aim of this study was to determine the chemical composition and antimicrobial activity of essential oils of *Z. multiflora* Boiss. collected from Jahrom and kerman Provinces of Iran.

Materials and methods

Plant material

Aerial parts of *Zataria multiflora* Boiss were collected in February 2013 in pre flowering stage in Jahrom and Kerman from Iran. The harvested plants were dried at room temperature (25°C) for 2 weeks. Then, air- dried plants ground and powdered with mixer for essential oil extraction.

Essential oil extraction

Essential oil was obtained from dried aerial parts (100 g) from *Z. multiflora* Boiss that were subjected to hydro-distillation using a Clevenger-type apparatus (w/w) for 3 h. Then the oils drying by anhydrous sodium sulfate. The isolated oils were stored in tightly closed vials at 4°c until analysis.

Essential oil analysis

Essential oil was analyzed by Hewlett – Packard GC/MS (model 7890 series II) operating at 70e V ionization energy, equipped with a HP–5 capillary column phenyl methyl siloxane (30m' 0.25mm, 0.25 µm film thickness) with helium as the carrier gas and a split ratio of 1:20. The retention indices for all the components were determined according to the Van Den Dool method (Van den dool and Kratz, 1963) 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 and Mass finder 3 libraries or with the published mass spectra (Adams, 2001).

Micro –organisms

Standard strain of *Candida albicans* (ATCC No.10231) and Gram-negative bacteria *E. coli* (ATCC 25922), were obtained from Persian Type Culture Collection (PTCC) in Iranian Research Organization for Science and Technology.

Determination of antifungal/antibacterial activity by the disc diffusion method

In vitro antimicrobial activity of the essential oil of *Z. multiflora* (collected from Jahrom and kerman) were evaluated by disc diffusion method, with determination of inhibition zones (IZ), according to the National Committee for Clinical Laboratory Standards using 100 µL each suspension of the tested microorganisms containing 2.0×10^6 CFU/ml for bacteria and 2.0×10^5 CFU/ml (0.5 McFarland) spore for fungi strain. The bacteria inoculate was prepared by suspending overnight colonies from sabouraud dextrose agar (SDA) media, and the *C. albicans* was prepared by suspending colonies from 48 hold potato dextrose agar (PDA) cultures respectively. Fungal or bacterial suspension were seeded into Petri dishes (9 cm) containing 20 ml sterile sabouraud dextrose agar (SDA) or potato dextrose agar (PDA) using a sterile cotton swab. The sterile paper discs (6 mm in

diameter) were individually impregnated with 20 µl of the oil and then placed on the agar plates which had previously been inoculated with the tested microorganisms. The plates were inoculated with bacteria incubated at 37°C for 24 h and at 30°Cfor 48 h for the *C. albicans* strain. After incubation, the mean inhibition zone diameter for each concentration was measured in millimeters. All the studies were performed in triplicate. Blank discs containing 20 µl DMSO were used as negative controls (Alizadeh, 2013).

Statistical analysis

All data were done in three replicate and analyzed by analysis of variance (ANOVA) and mean values were compared with Duncan's multiple range test using SPSS software (version 9.2 for windows).

Result and discussion

Essential oil components

The chemical composition of the essential oil of *Z. multiflora* Boiss (collected from Jahrom and Kerman) and the retention indices are presented in (Table 1). Forty-seven components were identified in *Z. multiflora* oil (collected from Jahrom) that represented 99.96% of the oil. The main components of the oil were, Thymol (38.89%), Carvacrol methyl ether (31.63%), γ-Terpinene (7.08%) and δ-3-Carene (6.67%). Forty-nine components were identified in *Z. multiflora* oil (collected from Kerman) that represented 99.91% of the oil. The main components of the oil were, Carvacrol (47.19%), α-Terpinyl acetate (16.40%), δ-3-Carene (8.46%), 1,8-cineole (5.50%) and γ-Terpinene (4.58%). The composition of the essential oil of *Z. multiflora* has been examined previously by other researchers. Shafiee and Javidnia, (1997) found that the major components of *Z. multiflora* Boiss. obtained from Iran were phenolic compounds, carvacrol (61.29 %) and thymol (25.18%). Alipour-eskandani, (2011) found that the major components of *Z. multiflora* obtained from Shiraz Province of Iran was Carvacrol (71.1%). Mahmoodi *et al.*, (2012) reported, phenolic monoterpene carvacrol (71.1%) , γ- terpinene (7.34%) and α-pinene (4.26%) were the main components of *Z. multiflora* essential oil. Khosravi *et al.*, (2009) reported carvacrol (61.99%) and thymol (25.18%) were the main components of *Z. multiflora* essential oil.

Variation in the compositions of essential oils from *Z. multiflora* may be due to the geographical region, variety, age of the plant and the method of drying and extraction of the oil (Alizadeh *et al.,* 2013 and Dehghanzadeh *et al.*, 2012).

Antimicrobial activity

Antimicrobial activities of *Z. multiflora* oil (collected from Jahrom and Kerman) were analyzed by disc diffusion method against *Candida albicans* and *E. coli,* are reported in (Table 2). There was considerable variability in size of zone of inhibition depending the different volumes of oil was used. Greater zones of inhibition were observed when 20µL of essential oils were used. The inhibition zones for *Z. multiflora* collected from Jahrom ranged from 25-58.67 mm for *Candida albicans*(ATCC No.10231) and10.33-45.33 mm for the *E. coli*(ATCC 25922) and the inhibition zones for *Z. multiflora* collected from Kerman ranged from 22-77.67 mm for *C. albicans* (ATCC No.10231) and 13.33-40.67mmfor the *E. coli*(ATCC 25922). The antimicrobial activity of *Z. multiflora* essential oil collected from Kerman was higher than essential oil collected from Jahrom. Also antifungal activity of the essential oils obtained from two regions have greater than antibacterial activities (Table 2). The antimicrobial activity of *Z. multiflora* essential oil exhibited that this oil has great antimicrobial activities against two tested microorganism.

The antimicrobial activity of essential oils (EOs) and their components has long been recognized, and interest has increased in recent years. Some Researchers reported there is relationship between the chemical structures of the most abundant compounds in the tested essential oils and the antimicrobial activity. In this study antimicrobial activity of *Z. multiflora* can be attributed to the presence of Thymol, Carvacrol and 1,8- cineol.

Studies that have been performed on the antimicrobial activity of carvacrol and thymol have shown that they have a broad spectrum of antimicrobial activity against almost every Gram-positive and Gram negative bacteria tested Friedman *et al.*, (2002). Besides this antibacterial activity, carvacrol and thymol have been described as antifungal, antitoxigenic, insecticidal and anti-parasitic (Lindberg *et al.*, 2000). Also, the antimicrobial properties of this plant can be due to the 1,8-cineole, because this compound can be penetrated into the cell membrane and destroyed it and has high antibacterial activity (Ben-Afra *et al.*, 2002).

Table 1. Essential oil components of *Zataria multiflora* Boiss. (collected from Jahrom and Kerman) analyzed by (GC/MS).

No	Compounds	RI[a]	Percentage in oil of Jahrom	Percentage in oil of Kerman
1	α-Thujene	923	0.09	0.20
2	α-Pinene	930	0.99	1.83
3	Camphene	944	0.13	0.10
4	Sabinene	969	0.21	0.44
5	β-pinene	973	0.54	0.66
6	3-Octanone	981	0.61	0.89
7	Myrcene	987	0.21	0.27
8	3-Octanol	991	0.06	0.09
9	n-Decane	995	0.02	0.03
10	α-Phellandrene	1002	0.64	1.18
11	δ-3-Carene	1008	6.67	8.46
12	α-Terpinene	1014	0.28	0.37
13	*p*-Cymene	1022	0.10	0.15
14	Limonene	1025	0.46	0.60
15	β-Phellandrene	1026	0.07	0.04
16	1,8-Cineole	1028	2.84	5.50
17	(Z)-β-Ocimene	1032	0.17	0.15
18	Benzene acetaldehyde	1039	0.11	0.07
19	(E)-β-Ocimene	1043	0.09	0.14
20	γ-Terpinene	1055	7.08	4.58
21	cis-Sabinene hydrate	1063	0.22	0.23
22	trans-Linalool oxide	1068	0.30	0.03
23	Terpinolene	1084	0.20	0.03
24	Linalool	1100	0.05	0.03
25	Hotrienol	1102	0.02	0.06
26	Borneol	1162	0.29	0.23
27	Terpinene-4-ol	1174	0.52	0.04
28	α-Terpineol	1187	0.75	0.60
29	γ-Terpineol	1201	0.09	0.03
30	trans-Dihydrocarvone	1210	0.25	1.22
31	Carvacrol methyl ether	1240	31.63	1.07
32	Thymol	1290	38.89	0.34
33	Carvacrol	1300	0.06	47.19
34	α-Terpinyl acetate	1349	0.09	16.40
35	Thymol acetate	1354	0.08	0.02
36	Eugenol	1357	0.09	0.03
37	Carvacrol acetate	1372	2.42	0.12
38	α-Gurjunene	1406	0.33	0.09
39	(E)-Caryophyllene	1418	0.14	2.62
40	Unknown	1424	0.13	0.04
41	γ-Elemene	1431	0.04	0.04
42	Aromadendrene	1436	0.26	0.67
43	α-Humulene	1450	0.03	0.20
44	allo-Aromadendrene	1457	0.05	0.12
45	γ-Gurjunene	1468	0.06	0.09
46	Viridiflorene	1492	0.52	0.07
47	δ-Cadinene	1519	1.08	0.51
48	Spathulenol	1574	–	1.10
49	Caryophyllene oxide	1579	–	0.94
Total			99.96	99.91

[a]RI, retention indices in elution order from HP – 5 column.

Table 2. Antimicrobial activity of the essential oil of *Zataria multiflora* (collected from Jahrom and Kerman).

		Inhibition Zone (mm)							
Collected *Z. multiflora*	Microorganisms	Essential oil (µl/disk)							
		20	10	5	2.5	1.25	0.63	0.32	0.16
Jahrom	*E. coli*	45.33 c	39.0 c	34.67 b	28.0 b	25.33 b	15.33 b	13.0b	10.33 b
Jahrom	*C. albicans*	58.67 b	55.0 ab	52.67 a	35.0 a	32.67 a	31.33 a	25.0 a	11.0 b
Kerman	*E. coli*	40.67 d	22.67 d	20.33 c	19.33c	18.0 c	16.3 b	13.0 b	12.0 b
Kerman	*C. albicans*	77.67 a	59.33 a	51.0 a	34.67 a	31.0 a	28.0 a	25.0 a	22.0 a

Each value in the table was obtained by calculating the average of three experiments.

Diameter of inhibition zone including disc diameter of 6mm.

In each column, means with the same letters are not significantly different at 5% level of Duncan· s new multiple range test.

The antimicrobial activity of the essential oil of *Z. multiflora* has been examined previously by other researchers. For example, it has been reported that was strongly inhibited the growth of the *Bacillus cereus* by the essential oil of Z. *multifora* (Alipour-eskandani, 2011) and other researchers reported that the essential oil from of *Z. multiflora* has a great potential for application as a natural antimicrobial agent to preserve food (Mahmoodi *et al.*, 2002).

Conclusion

In this study it was found that *Z. multiflora* (collected from Jahrom and Kerman) essential oil had a good antimicrobial activity against *Candida albicans* and *E. coli*. In conclusion, present results suggested that the *Z. multiflora* essential oil might be a source of antibacterial and antifungal activity against food borne and human pathogens.

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