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ORIGINAL ARTICLE

Antibacterial activity of *Zataria multiflora* Boiss essence oil against ESBL producing isolates of *Escherichia coli*

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ABSTRACT
Escherichia coli is the most common pathogen of bacterial infections worldwide that most frequently associated with ESBL production. E.coli with ESBL may cause urinary tract infections that can sometimes progress to more serious infections like blood poisoning. The increase in ESBL producing E. coli among community-onset urinary tact infections is an important public health concern as these organisms are resistant to multiple antimicrobial agents. Plant essential oils rich in carvacrol and thymol have gained importance for their antimicrobial activity. We determined the composition of Zataria multiflora essential oil of the Jandagh area in Iran and measured its activity against ESBL producing urinary isolates of Escherichia coli. The essential oil of the aerial parts of Zataria multiflora from Jandagh area in Iran was prepared by hydrodistillation and its chemical constituents were analyzed by a combination of capillary GC and GC-MS. Twenty five components were identified of which, carvacrol (50.6 %), thymol (13.4 %) and pcymene (8.3 %) were the main constituents. The antibacterial activity of the oil was determined against 10 clinical isolates of Escherichia coli and 6 Gram positive and Gram negative bacterial standards by disc diffusion. Minimum inhibitory and bactericidal concentrations (MIC and MBC) were determined by broth microdilution assay. Considerable inhibitory activity was shown against all test bacteria shown by disc diffusion (except P. aeruginosa) and MIC and MBC values were within the range of 0.015 - 2.01 mg/ml for the susceptible organisms.
Key words: *Antibacterial activity, Escherichia coli, essential oil, Zataria multiflora, Iran.*

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INTRODUCTION

Extended-spectrum beta lactamases (ESBL) mediate resistance to broad-spectrum cephalosporins and are important causes of multidrug resistance in Gram-negative bacteria [1, 2]. Among these, Escherichia coli and *Klebsiella pneumoniae* are most frequently associated with ESBL production [3]. *E. coli* is an opportunistic pathogen that causes a significant proportion of community and hospital acquired infections especially urinary tract infections (UTI) [4].
Studies have shown that ESBL producing *E. coli* have rapidly spread worldwide and pose a serious threat for health care associated infections [5].These problem show the necessity of seeking alternative antibacterial agents such as plant natural products.

Plant products have been recognized as antimicrobial agents for some time [6]. Among these, few plants have been reported for their biological activities against multidrug resistant bacteria including the ESBL producing *E.coli* and *K. pneumoniae* [7-9]. *Zataria multiflora* Boiss (Avishan-e-Shirazi in Persian and Sa'atar or Zaatar in the Old Iranian medical books) is a thyme-like plant and a member of Labiatae family that grows wild in central and southern parts of Iran [10]. It is used in traditional folk remedies for its antiseptic, analgesic (pain-relieving) and carminative (anti-flatulence and intestine-soothing) properties [10, 11]. The antibacterial activity of *Z .multiflora* has been shown against a number of Gram-positive and Gram-negative bacteria [12-16]. We determined the chemical composition of the essential oil of *Zataria multiflora* from the Jandagh area and its antibacterial activity against clinical isolates of Escherichia coli as well as standard ATCC cultures. To the best of our knowledge, there are a few published reports on antimicrobial effects of the *Zataria multiflora* oil, especially against the clinically important antibiotic resistant microorganisms.

MATERIAL AND METHODS

Plant material

The aerial parts of *Zataria multiflora* were collected at full flowering stage (April/May 2008) from Jandagh in Isfahan Province at an altitude of 1230m. A voucher specimen was deposited at the Herbarium of Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran.

Essential oil preparation

The essential oil of the air-dried sample (100g) was isolated by hydro distillation for 3 h, using a Clevenger-type apparatus according to the method recommended in British Pharmacopoeia (1988). The distillated oil was dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4°C until analysis.

Essential oil analysis

GC-FID analysis of the oil was conducted using a Thermoquest-Finnigan instrument equipped with a DB-5 fused silica column (60 m x 0.25mm i.d., film thickness 0.25 μm). Nitrogen was used as carrier gas at the constant flow rate of 1.1ml/min. The spilt ratio was 1/50. The oven temperature was raised from 60°C to250°C at a rate of 5°C/min. The injector and detector (FID) temperatures were kept at 250°C and 280°C, respectively. GC-MS analysis was carried out on Thermoquest-Finnigan Trace GC-MS instrument equipped with the same column and temperature programming as mentioned for GC. The transfer line temperature was 250°C. Helium was used as the carrier gas at a flow rate of 1.1ml/min with a split ratio equal to 1/50. The constituents of the essential oil were identified by calculation of their retention indices under temperature-programmed conditions for n-alkanes (C6-C24) and the oil on a DB-5 column under the same conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley 7.0) or with those of authentic compounds and confirmed by comparison of their retention indices with those reported in the literature [17,18]. Semi-quantification data was obtained by FID area percentages without the use of correction factors.

Bacterial strains

Sixteen bacterial strains were employed including 10 clinical isolates *E.coli* and 6 reference bacterial strains (*Bacillus subtilis* ATCC 465, *Enterococcus faecalis* ATCC 29737, *Staphylococcus aureus* ATCC 25923, Escherichia coli ATCC 25922, *Klebsiella pneumoniae* ATCC10031 and *Pseudomonas aeruginosa* ATCC85327).

Antibacterial activity of *Zataria multiflora* measured by disc diffusion and broth microdilution

The antibacterial activity of the essential oil and its main components were determined by the discdiffusion method [19]. Briefly, 0.1 ml of a suspension of each test microorganism (10^8 cells/ml) wasspread on Mueller-Hinton agar plates and discs containing 4.4, 6.5 or 8.7 mg essential oil/disc were placed on the microbial lawns. For preparation of the discs, dried oil was reconstituted in Dimethlyl Sulfoxide (DMSO, Merck) (w/vol) before placing the appropriate amounts onto 6 mm sterile blank discs. For MIC and MBC determinations, the distilled dry oil was reconstituted in DMSO to make the starting stock before preparing the dilutions in Muller Hinton Broth (MHB, Merck) within the range of 64-125mg/ml.

Antibiotic discs (Padtan Teb, Tehran) including gentamycin (10μg), tetracycline (30μg) and imipeneme (10 μg) were also employed. The tests were carried out in triplicate and plates were incubated at 37°C for 24 h. The diameters of inhibition zones were measured following the incubation period and reported in mm. For quantitative determination of susceptibility, minimum inhibitory concentrations (MIC) were measured according to the CLSI protocol by the microdilution broth assay using serial two-fold dilutions of the essential oil in Mueller-Hintonbroth (MHB) containing 0.5 % Tween 80 [20]. Minimum bactericidal

concentrations (MBC) were determined by spreading 100 ml of the contents of all MIC wells that showed no bacterial growth over nutrient agar plates and incubated at 37°C for 24 h. The highest dilution showing at least 99 % inhibition (less than 5 colonies) was recorded as MBC.

RESULTS AND DISCUSSION

Hydrodistillition of *Zataria multiflora* aerial parts gave an oil yield of 2.5% (w/w) representing 97.98% of the total oil based on the plant dry weight. Table I shows that the *Zataria multiflora* essential oil contained 25 components. As shown, carvacrol was the major component constituting 50.57% followed by thymol (13.10%) and p-cymene (8.27 %). Other studies on chemical composition of *Z. multiflora* have reported different amounts of thymol and carvacrol. This range from 15.3 - 82.7 % for carvacrol and 0.1 - 38.8 % for thymol depending on the ecological regions the plants were collected from, since the environmental conditions affect plant chemical contents (21). Interestingly, it was also shown that the oil from Z. multiflora fresh plant has a higher thymol content as the major component while carvacrol is the major constituent of the dried plant (16).

Disc diffusion results showed that *Zataria multiflora* essential oil was highly active against all clinical isolates as well as the ATCC standards in comparison with the antibiotic discs (Table II). The inhibition zones obtained were 19 - 32 mm forth lowest oil concentration (4.4 mg /disc) followed by 25-39 mm (6.5 mg/disc) and 25-46 mm (8.7 mg/disc) against the ATCC standards except for *P. aeruginosa*. The same results were observed against the clinical isolates of *E. coli* and inhibition zones of 18-26 mm were obtained for the lowest oil concentration. The MIC and MBC values (Table II) were within the range of 0.031-2.01 mg/ml for all test organisms except for *P. aeruginosa* (32 mg/ml). The antibacterial activity of Z. multiflora has been reported against a range of microorganisms including the Gram-negative enteric bacteria [14,15]. This is the fact that MIC and MBC values were similar, shows the bactericidal activity of the oil. It may also explain that the target of the active oil components is the cell membrane. In fact it has been shown that among the phenolic compounds, carvacrol has the highest antimicrobial activity due to its hydrophobic nature and the presence of a free hydroxyl group which is essential for antimicrobial activity on cell membranes [21].

We believe that this is the first report on the biological activity of Z. multiflora against ESBL producing clinical isolates of E. coli. The increasing rate of ESBL production in E. coli clinical isolates is alarming and the fact that most of these organisms are multidrug resistant causes serious concern in eradication of these infections. Considering that few antibiotics could be used for the treatment of ESBL producing E. coli, seeking alternative therapeutic agents such as natural plant products are extremely important. Carvacrol and thymol are the most likely candidates for this biological activity.

Table 1. Chemical composition of *Zataria multiflora* essential oil

Compound	RI*	%	Compound	RI*	%
A-Thujene	927.27	0.08	Carvacryl acetate	1348.9	3.83
A-Pinene	936.74	2.00	trans-caryophyllene	1431.0	3.5
Camphene	950.76	0.09	eudema-3,7-dien	1448.0	0.1
3-Octanone	965.91	0.18	aromadendrene	1451.2	2.03
B-Pinene	978.03	0.16	α-humulene	1463.5	0.2
Mycerene	982.95	0.68	cyclosativene	1471.0	0.12
P-Cymene	1017.5	8.27	ledene	1502.9	1.07
B-Terpineol	1026.0	0.9	spathulenol	1577.3	1.08
Γ-Terpinen	1053.1	2.84	Caryophylene oxide	1584.7	1.45
Linalool	1085.7	1.27	Monoterpene hydrocarbons		11.46
P-Menth-1-En-4-Ol	1168.2	1.04	Oxygenatedmonoterpenes		76.97
P-Menth-1-En-8-Ol	1180.3	1.12	sesquiterpene hydrocarbons		7.02
Carvacrol Methyl Ether	1228.0	1.62	Oxygenated sesquiterpenes		2.53
Thymol	1268.7	13.10	sesquiterpene hydrocarbons		7.02
Carvacrol	1284.7	50.57	oxygenated sesquiterpenes		2.53
Thymyl Acetate	1329.7	0.68	other		
			Total		**97.98**

* Retention Index

Table 2. Antibacterial activity of *Zataria multiflora* essential oil against clinical isolates

Microorganism	Inhibition Zone (mm)[a] Oil (mg/disc)						MIC[b] (mg/ml)	MBC[c] (mg/ml)
	4.36	6.54	8.72	Gm	Te	Im		
*B. subtilis**	30	38	44	23	30	nt	0.015	0.015
*E. faecalis**	19	23	34	6	11	nt	2.01	2.01
*S. aureus**	31.6	39	46	17	17	nt	0.015	0.03
*E. coli**	22	32	40	20	20	nt	0.25	0.25
*K. pneumoniae**	24.6	25	25	20	24	nt	0.50	0.50
*P. aeruginosa**	6	6	13	20	20	nt	32	32
E. coli UI 6	18	nt[d]	nt	13.4	nt	17.4	0.50	0.50
E. coli UI 12	20.7	nt	nt	11.8	nt	17.1	0.25	0.25
E. coli UI 16	22	nt	nt	16.5	nt	24.1	0.25	0.25
E. coli UI 19	23.7	nt	nt	15.9	nt	18.4	0.125	0.125
E. coli UI 21	25	nt	nt	16.1	nt	19.8	0.31	0.31
E. coli UI 28	24	nt	nt	15.8	nt	18.9	0.125	0.125
E. coli UI 37	23.6	nt	nt	14.3	nt	18.2	0.125	0.125
E. coli UI 41	25.6	nt	nt	16.4	nt	22.1	0.062	0.062
E. coli UI 52	21.9	nt	nt	14.1	nt	19	0.25	0.25
E. coli UI 60	24.8	nt	nt	13.8	nt	15.8	0.031	0.031

[a]No inhibition zones (6 mm); resistant, 10-14; intermediately resistant and >15; sensitive.
[b]Minimum inhibitory concentration
[c] Minimum bactericidal concentration
[d]Not tested
* ATCC standard cultures
►Values are means of 3 repeats

REFERENCES

1. Bradford PA. (2001). Extended-spectrum ß-lactamases in the 21st century. Characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev. 14: 933-51.
2. Paterson DL, Bonomo RA. (2005). Extended-spectrum ß-lactamases: a clinical update. Clin Microbiol Rev. 18: 657-86.
3. Jacoby GA, Han P. (1996). Detection of extended-spectrum ß-lactamases in clinical isolates of *Klebsiella pneumoniae* and Escherichia coli. J Clin Microbiol. 34: 908-11.
4. Podschun R, Ullman U. (1998). Escherichia spp. As nosocomial pathogens: epidemiology, taxonomy, typing methods and pathogenicity factors. ClinMicrobiol Rev. 1998; 11: 589-603.
5. .Doi Y, Park YS, Rivera JI, et al. Community-associated extended-spectrum β-lactamase-producing Escherichia coli infection in the United States. Clin Infect Dis 2013; 56:641.
6. Cowan MM. (1999). Plant products as antimicrobial agents. ClinMicrobiol Rev. 12: 564-82.
7. Abdel-Massih R, Abdou E, Baydoun E, Daoud Z. (2010). Antibacterial activity of the extracts obtained from *Rosmarinus officinalis, Origanum majorana and Trigonella foenum-graecum* on highly drug resistant Gram-Negative Bacilli. J Botany. 20-23. Article ID 464087, 8 pages doi:10.1155/2010/464087.
8. Uma B, Prabhakar K, Rajendran S. (2009). Phytochemical analysis and antimicrobial activity of Clitoreaternatea Linn against extended spectrum beta lactamase producing enteric and urinary pathogens. Asian J PharmaceutClin Res. 2009; 2: 94-6.
9. N'guessan JD, Dinzedi MR, Guessennd N, et al. (2007). Antibacterial activity of the aqueous extract of Thonningiasanguinea against Extended-Spectrum-β-Lactamases (ESBL) producing Escherichia coli and Klebsiella pneumoniae strains. Trop J Pharmaceut Res. 6:779-83.
10. Amin G. (1991). Popular medicinal plants of Iran deputy minister of research publication, ministry of health, treatment and medical education, Tehran; 1:40.
11. Mozzaffarian V. (1996). A dictionary of Iranian plant names. 2nd ed. Farhang Moaser Publication Tehran, 360.
12. Moshafi MH, Mansouri SH, Sharififar F, Khoshnoodi M. (2007). Antibacterial and antioxidant effects of the essential oil and extract of *Zataria multiflora* Boiss. J Kerman Uni Med Sci. 14: 33-43.
13. Sadeghzadeh L, Sefidkon F, Owlia P. (2006).Chemical composition and antimicrobial activity of the essential oil of Zataria multiflora. Pajouhesh and Sazandegi. 71: 52-6.
14. Saleem M, Nazli R, Afza N, Sami A, Ali MS. (2004). Biological significance of essential oil of *Zataria multiflora* Boiss. Nat Prod Res. 18: 493-97.
15. Abbasgholizadeh N, Ettehad GH, Arab R, et al. (2008). Antibacterial effects of *Zataria multiflora* Boiss (Shiraz oregano essence) on Enterobacteriaceae species. Res J Biol Sci. 3: 345-47.
16. Ettehad GH, Arab R. (2007).Evaluation of antibacterial effects of Shiraz oregano essence (*Zataria multiflora* Boiss) on Salmonella typhi and comparing to antibiotics. Res J Biol Sci. 2: 674-76.
17. Adams, R.P. (2001). Identification of essential oils components by gas chromatography/quadruple mass spectroscopy. Allured Publishing Co., Carol Stream Ill, USA.

18. Shibamoto T. (1987), Retention indices in essential oil analysis. In: Capillary Gas Chromatography in Essential Oil Analysis (Sandra P. and Bicchi C., eds.). Huethig Verlag, New York.
19. National Committee for Clinical Laboratory Standards. (2000). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically—Fifth Edition: Approved Standard M7-A5. NCCLS, Wayne, PA.
20. Standards for antimicrobial susceptibility testing (2001). 9th International Supplement 100-S10. Wayne, PA, USA.
21. Clinical and Laboratory Standards Institute (CLSI) (2005). Performance standards for antimicrobial susceptibility testing; 15th Informational Supplement., M100- S15.