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Full Length Research Paper

Antibacterial activity of *Satureja bakhtiarica bung* essential oil against some human pathogenic bacteria

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Bacterial infections are a major problem in humans; as such, replacing the chemical drugs used against them with plant drugs is an important research. Here, the chemical compositions and antibacterial activity of the essential oils obtained from *Satureja bakhtiarica bung* against ten human pathogenic bacteria were evaluated. Chemical compositions of essential oil were analyzed by gas chromatography-mass spectrometry (GC-MS) method. Antibacterial activity of essential oil was evaluated by well diffusion method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by the macrodilution method. The GC-MS spectrums showed 13 compounds, in which the highest chemical composition was related to phenol (37.36%), thymol (22.65%) and *p*-cymen (19.29%) compounds. The essential oil of *Satureja bakhtiarica bung* showed good activity against all tested bacteria, except for *Pseudomonas. aeruginosa*, which is possibly due to the high levels of phenol in their compositions. The MIC and MBC values of the bacterial strains sensitive to the essential oil were in the ranges of 0.8 to 25 and 50 to 100 mg/ml, respectively. However, the essential oil of *Satureja bakhtiarica bung* is a suitable plant drug against human pathogenic bacteria.

Key words: *Satureja bakhtiarica,* antibacterial, essential oil.

INTRODUCTION

There is a popular and scientific interest to screen essential oils of plants used medicinally all over the world (Salehi et al., 2005). The use of medicinal plants as anti-bacterial and anti-inflammatory drugs in folk medicine is a practice common in Iran, although in most cases, the active principles of the plants are unknown (Hajhashemi et al., 2002). In recent years, multiple drug resistance in human pathogenic microorganisms has been developed due to the indiscriminate use of commercial anti-microbial drugs commonly used in the treatment of infectious diseases. This situation forced scientists to search for new antimicrobial substances from various sources like medicinal plants (Clark, 1996; Cordell, 2000). *Satureja* is a genus of the well-known medicinal plant of *Lamiaceae* family and it comprises numerous species growing wild in the Mediterranean area (Bezi et al., 2005). Many members of the genus *Satureja* have aromatic and medicinal characteristics (Sahin et al., 2003) and have been used also to treat various ailments such as cramps, muscle pains, nausea, indigestion, diarrhea and infectious diseases (Leung, 1996; Hajhashemi et al., 2000; Hajhashemi et al., 2002). More so, it has shown antispasmodic, anti-diarrhoeal, anti-oxidant, sedative as well as antimicrobial properties (Zargari, 1990). *Satureja bakhtiarica* is an endemic plant and one of the most important of the twelve species of Iranian *Satureja* species which is widely distributed in the southern region of Iran. There are some published investigations regarding the antimicrobial activity of

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Abbreviations: **GC-MS**, Gas chromatography-mass spectrometry; **MBC**, minimum bactericidal concentration; **MIC**, minimum inhibitory concentration; **RI**, retention indices; **RT**, retention times; **MHA**, Muller Hinton Agar; **NIST**, National Institute of Standards and Technology; **NBS**, National Bureau of Standards; **DMSO**, dimethyl sulfoxide; **SD**, standard deviation.

various *Satureja* species. Unfortunately, so far no reports are known which included the antimicrobial effect of the essential oil of *Satureja bakhtiarica* on human pathogenic bacteria. Therefore, the aim of this work is to evaluate the chemical composition of *Satureja bakhtiarica* by using gas chromatography-mass spectrometry (GC-MS) analysis and to determine the antibacterial activity by the well diffusion method.

MATERIALS AND METHODS

Plant material

Apparently, healthy leaves of *Satureja bakhtiarica bung* were collected and identified during the flowering period, from the mountain region of the Fars province in Iran.

Isolation of essential oil

Air-dried plant material (100 g) was hydro distilled for 3 h using a Clevenger type apparatus. The essential oils were collected over water, separated and dried over anhydrous sodium sulphate, after which they were stored in sealed vials at 4 to 6°C prior to chemical analysis and antimicrobial screening.

Gas chromatography mass spectrometry analysis

GC-MS analysis of the oil was conducted using a Hewlett Packard 6890 instrument operating on EI mode and equipped with a 5MS-HP fused silica column (30 m×0.25 mm × 0.25 µm film thickness capillary column). Helium (99.99%) was used as the carrier gas at the constant flow of 1 ml/min. The oven temperature was held at 60°C for 1 min, then programmed to 210°C at a rate of 6°C/min, and then held for 10 min. The injector and detector (FID) temperatures were kept at 250 and 280°C, respectively. The components of the oil were identified by comparison of their mass spectra with those obtained from authentic samples and/or the National Institute of Standards and Technology/ National Bureau of Standards (NIST/NBS) and Wiley mass spectral database. They were also confirmed by comparison of their retention indices (RI) (Van den Dool and Kratz, 1963) and retention times (RT), either with those of authentic compounds or with published data (Adams, 2001).

Antibacterial activity determination

The microorganisms used in this study were *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonasa aeruginosa* ATCC 27853, *Kellebsiella pneumoniae* ATCC27881, *Salmonella typhi* ATCC 10556, *Serratia marcesens* ATCC 35668, *Shigella dysenteriae* ATCC 15303, *Shigella flexneri* ATCC 15305, *Enterobacter aerogenes* ATCC 25933 and *Proteus vulgaris* ATCC 25942, provided from the Institute pasture of Iran.

The antimicrobial activity of essential oils was tested by the agar well diffusion method on Muller Hinton Agar (MHA). Using a cork borer, five wells (6 mm in diameter) were made in the agar medium (one well was in the center and four were at the corner), and inoculums containing 1.5×10^6 CFU/ml of the test bacteria were spread onto the surface of the medium with a sterile swab. In the case of essential oil, 10, 20, 30, 40 and 50 µl of the essence was pipetted into the wells, whilst 50 µl of dimethyl sulfoxide (DMSO) was served as a control. Gentamycine disk was used as a positive control. The agar plates were incubated for 24 h at 37°C and the diameter of the zone of inhibition surrounding the wells was measured. Assays were performed in triplicate and the data were shown as mean ± standard deviation (SD).

Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC of the essential oil against the test microorganisms was determined by the broth macrodilution method. An eight-fold serial dilution of the essence in DMSO, including 100, 50, 25, 12.5, 6.25, 3.12, 1.6, 0.8 and 0.4 mg/ml was prepared in sterile test tubes. These dilutions were added to 1 ml Muller Hinton Agar medium containing 1.5×10^6 CFU/ml bacteria. Two test tubes served as positive and negative control, respectively. The test tubes were incubated at 35°C for 24 h. The concentration at which complete inhibition of the growth was observed was recorded as MIC. The MIC for each of the test bacteria was determined in triplicate assays and the data were shown as mean ± SD. To determine MBC, broth was taken from each well and inoculated in MHA for 24 h at 37°C.

RESULTS

The results obtained in the qualitative and quantitative analyses of essential oil are shown in Table 1. From the essential oil of *Satureja bakhtiarica*, 13 constituents were identified, of which phenol (37.36%), thymol (22.65%) and *p*-cymene (19.29%) were the major components.

The results regarding the antibacterial activity of the essential oil from *Satureja bakhtiarica* are indicated in Table 2. The essential oil of *Satureja bakhtiarica* showed good activity against all test bacteria, except for *Ps. aeruginosa*, as compared to gentamycine antibiotic (as positive control). *E. coli*, *K. pneumonia* and *S. aureus* were the most susceptible. The control treatment (DMSO) did not show an inhibitory effect on any of the bacteria. However, the antibacterial activity of essential oil in all concentrations (from 10 to 50 µl) was found for the tested bacteria.

The MIC value of the essential oil of *Satureja bakhtiarica* for all test bacteria is shown in Table 3. As shown in Table 3, the MIC value of the bacterial strains sensitive to the essential oil was in the range of 0.8 to 25 mg/ml. The lowest MIC was found for *Staphylococcus aureus, Shigella dysenteriae* and *Proteus vulgaris* (0.4 mg/ml), whereas the maximum MIC was found for *Ps. Aeruginosa* (25 mg/ml) and *K. pneumoniae* (12.5 mg/ml). The MBC value of the essential oil of *Satureja bakhtiarica* for all test bacteria is shown in Table 4. As shown in Table 4, the MIC value of the bacterial strains sensitive to the essential oil was in the range of 6.25 to 25 mg/ml. The lowest MBC was found for *Shigella dysenteriae* and *Shigella flexneri* (6.25 mg/ml), whereas the maximum MIC was found for *Ps. aeruginosa* (50 mg/ml).

DISCUSSION

Plant extracts are extensively used for traditional

Table 1. Percentage composition of the essential oils isolated from *Satureja bakhtiarica.*

RI	*Satureja bakhtiarica*	Compounds	
988	0.76	β-myrcene	1
1017	0.93	α-terpinene	2
1131	1.92	borneol	3
1268	22.65	*thymol*	4
1298	37.36	*phenol*	5
1567	2.02	caryophyllene oxide	6
1280	0.24	Carvacrole	7
1030	0.94	limonene	8
1010	19.29	p-cymene	9
1060	5.01	γ-terpinene	10
1076	4.92	L-linalool	11
1189	0.66	terpineol	12
1418	2.19	β-Caryophyllene	13

Table 2. Antibacterial activity of *Satureja bakhtiarica* essential oil.

Bacteria	Zone in inhibition (mm)						
	10µl	20µl	30µl	40µl	50µl	G.M	Control
E. coli	24.55±0.25	29.66±0.35	33.25±0.5	36.15± 0.25	38.22±0 .25	21.23±0.3	0
K. pneumoniae	25.25±0.25	32.22±0.29	36.25±0.22	38.66±0.63	39.25±0 .19	20.25±0.25	0
S. typhi	14.15±0.65	15.64±0.25	17.75±0.35	19.22±0.45	25.66±0 .15	26.55±0.34	0
S. aureus	24.95±0.15	26.66±0.15	27.55±0.45	35.22±0.25	38.15± 0.34	24.15±0.25	0
S. marcesens	16.82±0.35	18.83±0.25	21.25±0.45	23.83±0.50	27.66±0 .65	21.75±0.40	0
S. dysenteriae	14.44±0.40	17.35±0.65	22.66±0.15	26.66±0.40	28.66±0 .55	25.15±0.25	0
S. flexneri	16.25±0.50	21.22±0.55	25.55±0.40	30.44±0.25	32.22±0 .30	22.65±0.30	0
E. aerogenes	15.44±0.15	17.85±0.30	19.53±0.25	22.44±0.35	24.33±0 .44	20.55±0.40	0
P. vulgaris	17.22±0.65	19.73±0.15	24.75±0.65	25.55±0.55	29.22±0 .50	22.25±0.30	0
P. aeruginosa	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1 5.35±0.65	0

Table 3. MIC of the essential oil of *Satureja bakhtiarica.*

Bacteria	MIC (mg/ml)							
	100	50	25	6.25	3.1	1.6	0.8	0.4
E. coli	-	-	-	-	-	+	+	+
K. pneumoniae	-	-	+	+	+	+	+	+
S. typhi	-	-	-	-	-	+	+	+
S. aureus	-	-	-	-	-	-	+	+
S. marcesens	-	-	-	-	-	-	+	+
S. dysenteriae	-	-	-	-	-	-	+	+
S. flexneri	-	-	-	-	-	-	+	+
E. aerogenes	-	-	-	-	-	+	+	+
P. vulgaris	-	-	-	-	-	-	+	+
P. aeruginosa	-	-	+	+	+	+	+	+

Table 4. MBC of the essential oil of *Satureja bakhtiarica.*

Bacteria	MBC (mg/ml)								
	100	50	25	12.5	6.25	3.1	1.6	0.8	0.4
E. coli	-	-	-	-	+	-	-	-	-
K. pneumoniae	-	-	+	+	+	+	+	+	+
S. typhi	-	-	-	-	-	+	+	+	+
S. aureus	-	-	-	-	-	+	+	+	+
S. marcesens	-	-	-	-	-	+	+	+	+
S. dysenteriae	-	-	-	-	-	-	-	+	+
S. flexneri	-	-	-	-	-	-	-	+	+
E. aerogenes	-	-	-	-	-	+	+	+	+
P. vulgaris	-	-	-	-	+	+	+	+	+
P. aeruginosa	-	+	+	+	+	+	+	+	+

medicine in Iran. Essential oils have been used as flavoring agents in food and beverages and, due to the presence of antimicrobial compounds, they have a potential as natural agents for food preservation (Helander et al., 1998). Moreover, researchers have been interested in biologically active compounds isolated from plant species for the elimination of pathogenic micro-organisms because of the resistance they have developed to antibiotics (Cordell, 2000).

Recently, many studies have focused on the antibacterial activity of the essential oil or extracts of *Satureja* species. These studies have revealed that the genus has antimicrobial activity against human, food and plant pathogens (Ciani et al., 2000; Baser et al., 2004; Ozcan and Erkmen, 2001; Gulluce et al., 2003; Adiguzel et al., 2006), due to the presence of phenolic components such as thymol and carvacrol. On the other hand, a large number of studies have reported that the essential oils of *Satureja* species are among the most potent essential oils regarding their antimicrobial properties (Sahin et al., 2003; Skocibusic et al., 2006; Vagionas et al., 2007).

The essential oils of many species of the genus *Satureja* are known to possess antibacterial and fungicidal properties, such as *S*. *brownie*, *S*. *montana*, *S*. *hortensis* and *S*. *thymbra* (Ozcan and Boyraz, 2000; Ozcan and Erkmen, 2001; Ciani et al., 2000; Sahin et al., 2003; Vagionas et al., 2007). To our knowledge, no data have been published on the biological properties of *Satureja bakhtiarica* essential oil, and there is a lack of information on its antimicrobial activity. Here, the *in vitro* antimicrobial activity of *Satureja bakhtiarica* essential oil against the microorganisms employed and its activity potentials were qualitatively and quantitatively assessed by the presence or absence of inhibition zones, zone diameters, MIC and MBC values. The results of the present study indicated that the essential oil of *Satureja bakhtiarica* have good antimicrobial effect on human pathogenic bacteria, which could be attributed to the high content of the compounds' essence with known antimicrobial activity, such as phenol (37.36%), thymol (22.65%) and *p*-cymene (19.29%). The essential oil extract did not display any antibacterial activity against *Ps. aeruginosa*. The basis of the varying degree of sensitivity of the test organisms of bacteria may be due to the intrinsic tolerance of microorganisms, and the nature and combinations of phytocompounds present in the essential oil (Ozturk and Ercisli, 2007).

In earlier investigations, other *Satureja* species such as *Satureja hortensis*, *Satureja icarica*, *Satureja pilosa* and *Satureja boissieri* were studied with respect to essential oil composition, and have been shown to be rich in components such as carvacrol, γ-terpinene, thymol and *p*-cymene (Gulluce et al., 2003; Adiguzel et al., 2006; Azaz et al., 2002), which was comparable to that of our study.

Essential oils rich in phenolic compounds such as cavracrol are widely reported to possess high levels of antimicrobial activity (Aligiannis et al., 2001; Baydar et al., 2004), which has been confirmed and extended in the present studies. This is the first study to provide data which show that the extracts of *Satureja bakhtiarica* plants evaluated against a wide range of microorganisms possess potential antibacterial.

Conclusion

Based on these results, it is possible to conclude that the usage of the essential oil of *Satureja bakhtiarica* as a natural antibacterial has a strong and broad spectrum of antibacterial activity against many human pathogenic bacteria, because the oil possesses strong antibacterial activity.

The antibacterial property of the essences of *Satureja bakhtiarica* is mostly attributable to the phenolic compound thymol and *p*-cymene. Further study is needed in order to obtain information regarding the practical effectiveness of *Satureja bakhtiarica* essential oil in order to prevent the growth of human pathogenic bacteria as antimicrobial agents in new drugs for the therapy of

infectious diseases in humans.

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REFERENCES

Adams R P (2001). Identification of essential oil components by gas chromatography/mass spectroscopy. Carol Stream, IL, USA: Allured Publishing Corp.

Adiguzel A, Ozer H, Kilic H and Cetin B (2010). Screening of Antimicrobial Activity of Essential Oil and Methanol Extract of *Satureja hortensis* on Foodborne Bacteria and Fungi. Czech J. Food Sci., 25: 81–89.

Aligiannis N, Kalpoutzakis E, Mitaku S, & Chinou I B (2001). Composition and antimicrobial activity of the essential oils two Origanum species. J. Agric. Food Chem., 49: 4168–4170.

Azaz D, Demirci F, Satı´la F, Kurcuoglu M and Baser K. H. (2002). Antimicrobial activity of some Satureja essential oils. Zeitschrift fu¨ r Naturforschung., 57c: 817–821.

Baser KHC, Ozek T, Kırımer N, Tumen G (2004). Comparative study of the essential oils of wild and cultivated *Satureja hortensis* L. J. Essential Oil Res., 16: 422–424.

Baydar H, Sagdic O, Ozkan G, Karadogan T (2004) . Antibacterial activity and composition of essential oils from Origanum, Thymbra and Satureja species with commercial importance in Turkey. Food Control., 15 : 169–172.

Bezi N, Skoibu M, Dunki V (2005) . Phytochemical composition and antimicrobial activity of Satureja montana L. and Satureja cuneifolia Ten. Essential oils. Acta Bot. Croat ., 64 (2): 313–322.

Ciani M , Menghini L , Mariani F, Pagiotti R , Menghini A, Fatichenti F (2000). Antimicrobial properties of essential oil of *Satureja montana* L. on pathogenic and spoilage yeasts. Biotechnol. Lett., 22: 1007–1010.

Gulluce M, Sokmen M, Daferera D, Agar G, Ozkan H, Kartal N, Polissiou M, Sokmen A, Sahin F (2003). *In vitro* antibacterial, antifungal, and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of *Satureja hortensis* L. J. Agri.Food Chem ., 51: 3958–3965

Hajhashemi V, Ghannadi A , Pezeshkian SK (2002). Antinociceptive and anti-inflammatory effects of *Satureja hortensis* L. extracts and essential oil. J. Ethnopharmacol, 82 : 83-87.

Hajhashemi V, Sadraei H, Ghannadi A.R, Mohseni M (2000) . Antispasmodic and anti-diarrhoel effect of *Satureja hortensis* L. essential oil. J. Ethnopharmacol .,71: 187–192.

Helander I.M, Alakomı H.L, Latva-Kala K, Mattıla-Sandholm T, Pol I, Smıd E J, Gorrıs L.G.M and Von Wrıght A (1998). Characterization of the action of selected essential oil components on gram-negative bacteria. J. Agri and Food Chem., 46: 3590–3595

Leung A.Y, Foster S (1996) . Encyclopaedia of Common Natural Ingredients used in Foods, Drugs, and Cosmetics, 2nd ed. Wiley, New York, pp: 465–466.

Ozcan M, Erkmen O (2001). Antimicrobial activity of the essential oils of Turkish plant spices. Eur.Food Research Technol., 212: 658–660.

Ozcan M, Boyraz N (2000). Antifungal properties of some herb decoctions. Eur. Food Research and Technol ., 212(1): 86–88.

Ozcan M and Erkmen O (2001). Antimicrobial activity of the essential oils of Turkish plant spieces. Eur.Food Res and Technol., 212(6): 658–660.

Ozturk S, Ercisli S (2007). Antibacterial activity and chemical constitutions of *Ziziphora clinopodioides*. Food Control., 18 :535–540.

Sahin F, Karaman I, Gulluce M, Ogutcu H, Sengul M, Adıguzel A, Ozturk S, Kotan R (2003) . Evaluation of antimicrobial activities of *Satureja hortensis* L. J. Ethnopharmacol., 87 : 61–65.

Sahin F, Karaman I, Gulluce M, Ogutcu H, Sengul M, Adiguzel A (2003). Evaluation of antimicrobial activities of *Satureja hortensis* L. J.Ethnopharmacol, 87, 61–65.

Salehi P, Sonboli A, Eftekhar F, Nejad-Ebrahimi S, and Yousefzadi M (2005). Essential Oil Composition, Antibacterial and Antioxidant Activity of the Oil and Various Extracts of *Ziziphora clinopodioides* subsp. *rigida* (BOISS.) RECH. f. from Iran. Biol. Pharm. Bull. 28(10) : 1892-1896.

Skocibusic M, Bezic N and Dunkic V (2006). Phytochemical composition and antimicrobial activities of the essential oils of *Satureja subspicata* vis. growing croatia. Food Chemistry., 96: 20–28.

Vagionas K, Graikou K, Ngassapa O, Runyoro D, Chinou I (2007). Composition and antimicrobial activity of the essential oils of three *Satureja* species growing in Tanzania. Food Chem., 103 : 319–324.

Van den Dool Hand Kratz P D (1963) . A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatography., 11: 463–471.

Zargari A(1990). Medicinal Plants, 4th ed. Tehran University Publications, Iran, pp: 42–45.