

Antibacterial Activity and Essential Oil Composition of *Satureja spicigera* from Iran

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The aerial parts of *Satureja spicigera* were collected at full flowering stage at Gazvin, Iran. The essential oil was isolated by hydrodistillation and analyzed by a combination of capillary GC and GC-MS. Fourteen compounds were identified, of which carvacrol (53.74%) and thymol (36.03%) were the main constituents, representing 99.12% of the total oil. The *in vitro* antibacterial activity of the essential oil was determined against six ATCC standard bacterial strains (*Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) using disc diffusion as well as measurement of minimum inhibitory concentrations. The disc diffusion results and MIC values indicated high inhibitory activity against the test bacteria. The most susceptible organisms were the Gram-positive *B. subtilis* and *S. aureus* followed by *E. faecalis*, usually resistant to most common antibiotics. Among the Gram-negative bacteria, *E. coli* and *K. pneumoniae* were highly sensitive to the different oil concentrations in the disc diffusion method. Finally, *P. aeruginosa*, a highly resistant organism to most antibiotics, showed moderate susceptibility to *Satureja spicigera* essential oil.

Key words: Antibacterial Activity, Essential Oil Composition, *Satureja spicigera*

Introduction

The genus *Satureja* is a member of the Lamiales, Nepetoideae, distributed mainly in the Mediterranean region. The genus is represented by 14 species in Iran of which 8 are endemic. These aromatic species are mostly found in the north, northwestern and western parts of Iran (Rechinger, 1982). Many species of the genus *Satureja* are reported to have aromatic and medicinal properties. The aerial parts of these species have distinctive tastes and can be added to stuffing and sausages. The leaves, flowers, and stems are used as herbal tea and, in traditional medicine, to treat various ailments such as cramps, muscle pains, nausea and infectious diseases (Baser, 1995; Eminagaoglu *et al.*, 2007).

A literature survey showed several reports on the essential oil composition of *S. spicigera* (C. Koch) Boiss. from Iran and Turkey (Baser, 1994;

Tumen and Baser, 1996; Sefidkon and Jamzad, 2004; Gohari *et al.*, 2006). The aerial parts of *S. spicigera* from five different localities in Turkey were reported to have thymol (19.6%–34.9%), *p*-cymene (9.1%–34.1%), carvacrol (1.9%–26.1%), methyl carvacrol (5.7%–21.2%) and γ -terpinene (3.4%–14.7%) as major constituents (Tumen and Baser, 1996). Reports on the composition of essential oils from the aerial flowering parts of *S. spicigera* from Iran also showed the list of major components as thymol (35.1%), *p*-cymene (22.1%), γ -terpinene (13.75%) and carvacrol (4.0%) (Sefidkon and Jamzad, 2004).

There are a number of reports on the antifungal and antibacterial activities of the essential oils from several species of *Satureja* (Azaza *et al.*, 2001; Baser *et al.*, 2001; Güllüce *et al.*, 2003; Sahin *et al.*, 2003). To our knowledge, no study has shown the antibacterial activity of the essential oil of *S.*

spicigera. We investigated the composition of the essential oil from *S. spicigera* and determined its antibacterial activity against six ATCC laboratory standard bacterial strains.

Material and Methods

Plant material

The aerial parts of *Satureja spicigera* were collected at Gazvin, located in the central part of Iran, at full flowering stage in early fall 2006. A voucher specimen (number AP-86121) has been deposited at the herbarium of Ecology and Systematic Department, Research Institute of Applied Science, Shahid Beheshti University, Tehran, Iran.

Essential oil isolation

The powdered plant parts (250 g) were hydro-distilled using a Clevenger type apparatus for 3 h. The resulting essential oil was dried over anhydrous sodium sulfate and stored at 4 °C until analyzed and tested.

Essential oil analysis and identification procedure

GC-FID analyses of the oil were conducted using a Thermoquest-Finnigan instrument equipped with a DB-5 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm). Nitrogen was used as the carrier gas at the constant flow of 1.1 ml/min. The split ratio was 1/50. The oven temperature was raised from 60 °C to 250 °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 a 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 1.1 ml/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 of authentic compounds or with those reported in the literature (Adams, 2001). Semi-quantitative data was obtained from FID area percentages without the use of correction factors.

Bacterial strains

Six reference bacterial strains were used which included *Bacillus subtilis* (ATCC 465), *Enterococcus faecalis* (ATCC 29737), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 10031), and *Pseudomonas aeruginosa* (ATCC 85327).

Antibacterial susceptibility measured by disc diffusion

The antibacterial activity of the essential oil and its main components was determined by the disc diffusion method (NCCLS, 1997). Briefly, 0.1 ml of a suspension of the test microorganism (10⁸ cells/ml) was spread on Mueller-Hinton agar plates, and sterile 6-mm discs, each containing 2.5, 5 and 10 μl of essential oils corresponding to 4, 8 and 16 mg/disc, were placed on the microbial lawns. Discs containing 10 μl of γ-terpinene, *p*-cymene, thymol and carvacrol (0.01 mg/ml) were also used to determine the antibacterial activity of the major oil components. Discs containing the antibiotics penicillin (10 U), chloramphenicol (30 μg), erythromycin (15 μg) and gentamycin (10 μg) were also included. 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.

Determination of minimum inhibitory and bactericidal concentrations

Minimum inhibitory concentrations (MIC) were determined by the broth microdilution assay recommended by the NCCLS (1999). Serial two-fold dilutions of the essential oil were made in Mueller-Hinton broth containing 0.5% Tween 80 in 96-well microtiter plates. Fresh bacterial suspensions prepared from cultures grown overnight in Mueller-Hinton broth were added to give a final concentration of 5 · 10⁵ organisms/ml. Controls of medium with bacteria or the essential oil alone were included. The microplates were incubated at 37 °C for 24 h and the first dilution with

no microbial growth was recorded as MIC. To determine the bactericidal activity of the oil, minimum bactericidal concentrations (MBC) were determined by spreading 100 μ l 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 first well with colony counts of < 5 was considered to be negative for growth and was reported as the MBC.

Results and Discussion

Essential oil composition

The essential oil was obtained by hydrodistillation of the aerial parts of *Satureja spicigera* with the yield of 0.9% (w/w) on dry weight basis. Qualitative and quantitative analytical results are shown in Table I. Fourteen compounds, representing 99.12% of the oil were identified. The essential oil consisted mainly of oxygenated monoterpenes (91.89%), followed by oxygenated sesquiterpenes (6.48%), sesquiterpene hydrocarbons (0.56%) and monoterpene hydrocarbons (0.19%). The major components of the oil were carvacrol (53.74%) and thymol (36.03). Sefidkon and Jamzad (2004)

Table I. Composition of the essential oil of *Satureja spicigera*.

Compound	RI	% of the oil
<i>p</i> -Cymene	1013	0.08
γ -Terpinene	1055	0.11
Linalool	1085	0.12
<i>p</i> -Cymene-8-ol	1161	0.14
4-Terpineol	1164	0.29
α -Fenchyl acetate	1208	0.37
Carvacrol methyl ether	1225	1.2
Thymol	1267	36.03
Carvacrol	1278	53.74
β -Caryophyllene	1425	0.21
β -Bisabolone	1501	0.35
Spathulenol	1569	0.26
Caryophyene oxide	1577	6.05
<i>cis</i> - <i>Z</i> - α -Bisabolone epoxide	1601	0.17
Monoterpene hydrocarbons		0.19
Oxygenated monoterpenes		91.89
Sesquiterpene hydrocarbons		0.56
Oxygenated sesquiterpenes		6.48
Total		99.12

Compounds are listed in order of their elution from a DB-1 column.

RI, retention index relative to *n*-alkanes (C₆–C₂₄).

and Gohari *et al.* (2006) have reported 48 and 46 components, respectively, in the essential oil of *S. spicigera* collected from the north and northwest of Iran. The main constituents identified by the two groups were thymol (35.1% and 37.3%, respectively) followed by *p*-cymene, γ -terpinene and carvacrol. Our sample came from Gazvin, in the central part of Iran, and contained 14 constituents. The content of thymol was very similar to the mentioned reports (36.03%) but roughly 10 times more carvacrol and negligible amounts of *p*-cymene and γ -terpinene were found compared to the two studies. For this reason, the essential oil analysis was repeated and the number of constituents was the same as in the first experiment. We conclude that geological and physiological conditions (locations) are responsible for the differences observed in the oil constituents and their amounts.

Antibacterial activity

The results of the antibacterial activity determination of *Satureja spicigera* essential oil by disc diffusion as well as MIC and MBC values are shown in Table II. According to the disc diffusion method, all concentrations used were inhibitory for all reference bacterial strains. The most susceptible organisms were the Gram-positive *B. subtilis* and *S. aureus* with large inhibition zones even at the lowest oil content tested (4 mg/disc). The MIC and MBC values were also lowest for these bacteria (3 and 6 mg/ml for *B. subtilis* and 1.5 and 6 mg/ml for *S. aureus*, respectively). *E. faecalis*, normally resistant to most common antibiotics was also highly sensitive to the oil at contents of 8 to 16 mg/disc. Remarkably, MIC and MBC values for *E. faecalis* were 6 and 12 mg/ml, respectively. Among the Gram-negative bacteria, *E. coli* and *K. pneumoniae* were highly sensitive to the oil at different concentrations in the disc diffusion method. MIC and MBC values were 6 and 12 mg/ml for *E. coli* and 6 and 6 mg/ml for *K. pneumoniae*. Finally, *P. aeruginosa*, a highly resistant organism to most antibiotics, showed moderate susceptibility to discs containing 8 and 16 mg oil, and MIC and MBC values of 24 mg/ml. The antibacterial activities of the major oil components (carvacrol, thymol) as well as γ -terpinene and *p*-cymene are shown in Table III. The highest antibacterial activity was observed for carvacrol and thymol against all test bacteria, in both, the disc

Table II. Antimicrobial activity of the essential oil of *Satureja spicigera* determined by disc diffusion, MIC and MBC determinations.

Microorganism	IZ ^a	IZ ^a	IZ ^a	MIC ^b	MBC ^c	IZ ^a			
	4 mg/disc	8 mg/disc	16 mg/disc	[mg/ml]	Pen	Cm	Em	Gm	
<i>B. subtilis</i>	30	38	68	3	6	15	21	23	–
<i>E. faecalis</i>	12	17.5	28	6	12	–	24	19	–
<i>S. aureus</i>	27	29.5	38	1.5	6	21	21	24	23
<i>E. coli</i>	25	27	39	6	12	–	22	–	18
<i>K. pneumoniae</i>	20	25	28	6	6	–	17	–	15
<i>P. aeruginosa</i>	8	12.5	13	24	24	–	–	–	17

^a Inhibition zone (in mm) includes diameter of the disc (6 mm). Pen, penicillin; Cm, chloramphenicol; Em, erythromycin; Gm, gentamycin.

^b Minimum inhibitory concentration.

^c Minimum bactericidal concentration.

–, Inactive; 7–14, moderately active; >14, highly active.

Table III. Antimicrobial activity of the main compounds of the essential oil of *Satureja spicigera*.

Microorganism	γ -Terpinene		<i>p</i> -Cymene		Thymol		Carvacrol	
	IZ ^a	MIC ^b	IZ ^a	MIC ^b	IZ ^a	MIC ^b	IZ ^a	MIC ^b
<i>B. subtilis</i>	19 ± 0.6	3.75 (27.5)	17 ± 0.6	3.75 (27.9)	46 ± 0.3	0.2 (1.3)	40 ± 0.3	0.2 (1.3)
<i>E. faecalis</i>	12 ± 0.4	7.5 (55.1)	–	nt	25 ± 0.4	0.8 (5.2)	23 ± 0.2	0.8 (5.2)
<i>S. aureus</i>	9 ± 0.1	>15 (110.3)	10 ± 0.2	15 (55.9)	34 ± 0.5	0.4 (2.6)	36 ± 0.4	0.4 (2.6)
<i>E. coli</i>	12 ± 0.1	7.5 (55.1)	12 ± 0.3	15 (55.9)	32 ± 0.4	0.4 (2.6)	35 ± 0.5	0.4 (2.6)
<i>K. pneumoniae</i>	–	nt	11 ± 0.2	>15 (55.9)	24 ± 0.4	1.6 (10.4)	28 ± 0.3	0.8 (5.2)
<i>P. aeruginosa</i>	–	nt	–	nt	12	1.6 (10.4)	25 ± 0.2	0.8 (5.2)

Main compounds tested at 10 μ l/disc. Values are given as means ± standard deviation.

^a Inhibition zone (in mm) includes diameter of the disc (6 mm).

^b Minimum inhibitory concentration in mg/ml (mm).

–, Inactive; 7–13, moderately active; >14, highly active; nt, not determined.

diffusion method and MIC determinations (zones of 23–40 and 12–46 mm, and MIC values of 0.2–0.8 and 0.2–1.6 mg/ml, respectively). γ -Terpinene and *p*-cymene also showed good antibacterial activity against *B. subtilis*, but were moderately active against the other organisms tested (Table III). These results may indicate that thymol and carvacrol are the most active antibacterial compounds among the four major oil constituents reported for *S. spicigera*. The antimicrobial activity of the aerial parts of several *Satureja* species from Turkey including *S. pilosa*, *S. icarica*, *S. boissieri*, *S. coerulea*, *S. hortensis*, *S. thymbra* and *S. wiedemanni* have been investigated (Azaza *et al.*, 2001; Baser *et al.*, 2001; Goren, 2004; Güllüce *et al.*, 2003;

Sahin *et al.*, 2003). However, we believe that this is the first report on the antimicrobial activity of the essential oil from *Satureja spicigera*. Interestingly, the effective antimicrobial compounds reported in most of these studies included thymol and carvacrol. We conclude that, compared to the antibiotics tested, *Satureja spicigera* essential oil has good antibacterial activity due to the presence of thymol and carvacrol, perhaps similar to some other *Satureja* species.

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