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In vitro antimicrobial activity and chemical composition of some *Satureja* essential oils

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ABSTRACT: Aerial parts of *Satureja hortensis* (1 and 2), *Satureja macrantha*, *Satureja cuneifolia*, *Satureja thymbra* and *Satureja aintabensis* collected from different parts of Turkey were subjected to hydrodistillation to yield essential oils and analysed by GC and GC-MS. The antibacterial and antifungal activity of six essential oils and their major constituents in the gaseous state was evaluated against *Escherichia coli* ATCC 25292, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter aerogenes* NRRL 3567, *Candida albicans* (OGU), *Penicillium clavigerum* (BUB. Czp. 181), *Mucor hiemalis* (BUB. Malt. 163) and *Absidia glauca* ATCC 22752. All tested microorganisms were inhibited by the essential oils of *S. hortensis* 1 and 2, *S. macrantha*, *S. cuneifolia*, *S. thymbra* and *S. aintabensis*. Carvacrol was the main component in the oils of *S. macrantha*, *S. cuneifolia* and *S. thymbra*, respectively (64.4%, 48.7%, 39.0%). The oil of *S. hortensis* (2) contained (43.4%) thymol and the oils of *S. hortensis* (1) 40.6% thymol; *S. aintabensis* contained 59.0% *p*-cymene as the main constituent. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: antibacterial activity; antifungal activity; essential oils; *S. hortensis*; *S. macrantha*; *S. cuneifolia*; *S. thymbra*; *S. aintabensis*

Introduction

The essential oils of many plant species have become popular in recent years. The genus *Satureja* (*Lamiaceae*) is native to the Mediterranean region of Europe, western Asia, North Africa, the Canarian Islands and South America.[1] This genus comprises about 15 species of herbaceous perennials and subshrubs, with five of them endemic to Turkey, including *S. aintabensis*.[2,3] All *Satureja* species are used as herbal tea in various regions of Turkey.[4] Savoury species when compared with thyme and oregano have a similar aroma and, because of this properties, savouries are used as culinary herbs. In addition, these species are used to make thyme oil and thyme juice, and then sold to merchants without processing. Dried herbal parts constitute an important commodity for export.[4]

Essential oils produced by plants have been traditionally used for respiratory tract infections, and are used as ethnic medicines for colds.[5] In the medicinal field, inhalation therapy of essential oils has been used to treat bronchitis and sinusitis.[6] Essential oils are known to possess antimicrobial activity, which has been evaluated mainly in liquid media. Systematic evaluation of the vapour activity was first reported using the Petri dish technique.[7–9]

Antifungal chemotherapy relies heavily on new fungicides and many efforts have been made to standardize test procedures in order to increase reproducibility between laboratories.[10] However, because filamentous fungi do not grow as single cells, standardization is more challenging compared with unicellular yeast and bacteria.[11]

In our previous study chemical composition and antimicrobial activities of *S. boissieri* Hausskn. Ex Boiss., *S. coerulea* Janka, *S. icarica* P. H. Davis and *S. pilosa* Velen. samples have been studied.[12] In the present study, we report on the GC-MS analyses of the essential oils of *S. hortensis* L. (1 and 2), *S. macrantha* G. A. Meyer, *S. cuneifolia* Ten., *S. thymbra* L. and *S. aintabensis* P. H. Davis from different locations.

The essential oils were also evaluated for their antibacterial and antifungal properties against common pathogenic and saprophytic fungi. The antibacterial activity was determined using agar disc diffusion and microdilution methods.

Experimental

Plant material

The aerial parts of the plants collected from various localities are shown in Table 1. Voucher specimens have

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been deposited at Department of Biology, Balikesir University, Balikesir, Turkey.

Extraction of the essential oil

Air-dried aerial parts of plants were hydrodistilled for 3 h using a Clevenger-type apparatus. The percentage yields (%) of the oils calculated on a moisture-free basis are given in Table 1.

Gas chromatography (GC)

GC analysis was carried out using a Shimadzu GC-9A with CR4-A integrator. A thermon 600T FSC column (50 m × 0.25 mm i.d. × film thickness 0.2 μm) was used with nitrogen as carrier gas. The oven temperature was kept at 70 °C for 10 min and programmed to 180 °C at a rate of 2 °C/min, and then kept constant at 180 °C for 30 min. The split ratio was adjusted to 60:1. The injector and FID detector temperatures were 250 °C.

GC-MS analysis conditions

A Shimadzu GCMS-QP5050A system, with CP-Sil 5CB column (25 m × 0.25 mm i.d. 0.4 μm film thickness) was used with helium as carrier gas. The GC oven temperature was kept at 60 °C and programmed to 260 °C at a rate of 5 °C/min, and then kept constant at 260 °C for 40 min. The split flow was adjusted at 50 ml/min. The injector temperature was at 250 °C. The mass spectrometry (MS) analysis involved the use of a quadrupole analyser with an electron impact source with the ionization energy set at 70eV. A total ion current (TIC) chromatogram was produced by scanning between *m/z* 30 and 425. From this TIC chromatogram, the mass spectra of the essential oil components were selected and searched using the in-house BASER Library of Essential Oil Constituents. Relative percentage amounts of the separated compounds were calculated from the FID data. The components identified in the oils are listed in Table 1.

Antimicrobial screening

Three different methods were employed for the determination of antimicrobial activities. The agar disc diffusion method, microdilution broth susceptibility assay[13] and single spore culture technique (for filamentous fungi)[11] were used. The minimum inhibitory concentration (MIC) of the essential oils against the test microorganisms were determined using the microdilution broth susceptibility assay. All test were performed in duplicate.

Agar disc diffusion method

The agar disc diffusion method was employed for the determination of antimicrobial activities of essential oils (NCCLS, Wayne, PA, USA). A suspension of the tested microorganism (10^5 CFU/μl) was spread on the solid media plates. Filter paper discs (5 mm in diameter) were soaked with 10 μl of the oils and placed on the inoculated plates. After keeping then at 2 °C for 2 h, they were incubated 37 °C for 3 days to encourage bacterial and yeast growth. The diameters of the inhibition zones were measured in millimetres.

Determination of MIC

Microdilution broth susceptibility assay was used.[13] Stock solutions of essential oils were prepared in dimethylsulphoxide (DMSO). Serial dilutions of essential oils were prepared in sterile distilled water in 96-well microtitre plates. Freshly grown bacterial suspension in double strength Mueller Hinton Broth (Merck) and yeast suspension of *Candida albicans* in Saboraud Dextrose Broth were standardized to 10^8 cfu/μl (McFarland no. 0.5). Microtitre plates were incubated at 37 °C for 3 days. Each test was performed in duplicate. Chloramphenicol and Ketoconazole served as positive controls.

Fungal spore inhibition assay

The fungi were inoculated on Czapex Dox agar (Merck) and Malt Extract Agar media in 9 cm Petri dishes at 25 °C for 7 days. Harvesting was carried out by suspending the conidia in a 1% (w/v) sodium chloride solution containing 5% (w/v) DMSO. All spore suspensions were filtered and transferred in to the tubes and stored at –20 °C.[10] The 1 ml spore suspension was taken and diluted in a loop drop until a single spore could be captured.[14]

One loop drop from the spore suspension was applied onto the centre of the Petri dish containing Czapex Dox agar (Merck) and malt extract agar (Oxoid) medium. A 0.2 ml aliquot of each essential oil was applied to sterile paper discs (5 mm diameter), placed in the Petri dishes and incubated at 25 °C for 72 h. Spore germination during the incubation followed using a microscope (Olympus BX51) over an 8 h interval. The fungi *Penicillium clavigerum* (BUB Czp.181), *Mucor hiemalis* (BUB Malt.163) and *Absidia glauca* ATCC 22752 were used for this assay and were deposited in the Department of Biology (BUB) at Balikesir University.

Results and Discussion

Water distillation of dried parts of *S. hortensis* (1 and 2),

Table 1. Information on *Satureja* spp. and their oil yields and essential oil compositions

Satureja sp.	Collection site and date	Collector number	Oil yields (%)	Main components	Relative percentage from FID
Satureja hortensis L. (1)	Malatya-Yazihan-vicinity of Karacaköy 15 July 1999	FS 1047	0.5	*p*-Cymene	40.6
				Thymol	39.9
				Carvacrol	5.7
				γ-Terpinene	3.7
				α-Pinene	1.2
Satureja hortensis L. (2)	K.Maras-Andirin 26 July 2001	FS 1016	0.7	Thymol	43.4
				p-Cymene	35.9
				Carvacrol	6.0
				γ-Terpinene	3.2
				α-Pinene	1.1
Satureja macrantha C. A. Meyer	Erzurum-Aksar-Senkaya (Taht village) 1 August 1999	FS 1045	1.5	Carvacrol	64.4
				p-Cymene	22.6
				γ-Terpinene	2.1
				β-Caryophyllene	1.5
				α-Terpinene	1.2
				Thymol	1.2
Satureja cuneifolia Ten.	Izmir-Kiraz-banks of Küçük Menderes River August 1995	FS 1010	0.9	Carvacrol	48.7
				p-Cymene	38.1
				α-Terpineol/borneol	1.9
				β-Bisabolene	1.8
				Terpinen-4-ol	1.2
				1,8-Cineole	1.1
				Thymol	0.5
Satureja thymbra L.	Izmir-Kiraz-Sarigöl 20 June 2001	FS 1046	0.9	Carvacrol	39.0
				γ-Terpinene	29.0
				p-Cymene	10.2
				β-Caryophyllene	6.3
				α-Pinene	2.4
				α-Terpinene	2.4
				Myrcene	1.5
				α-Terpineol/borneol	1.0
				Caryophyllene oxide	1.0
				Thymol	0.3
Satureja aintabensis P. H. Davis	Gaziantep: Dülükbaba Forest, dry calcareous place 14 July 2001	FS 1012	2.0	*p*-Cymene	59.0
				Thymol	17.5
				γ-Terpinene	8.4
				Linalool	3.6
				α-Pinene	1.6
				Carvacrol	1.5
				α-Terpinene	1.1
				Myrcene/α-phellandrene	1.1
				Isothymol	1.0

FS, Fatih Satil.

S. macrantha, *S. cuneifolia*, *S. thymbra* and *S. aintabensis* yielded 0.5, 0.7, 1.5, 0.9, 0.9 and 2.0%, respectively. Chemical analysis revealed that all the oils tested in this analysis contained thymol, carvacrol and *p*-cymene. The major constituents of *S. aintabensis* linalool, myrcene/α-phellandrene and isothymol were not found in any of the other oils (Table 1).

Both oil samples of *S. hortensis* contained higher levels of *p*-cymene (40.6 and 43.4%) and thymol (39.9 and 35.9%), respectively. This cannot be regarded as a true chemotype situation since *p*-cymene is a known precursor of thymol. An earlier work reported that the essential oil of *S. hortensis* contained lower percentages of *p*-cymene (8.09, 7.03, 4.18, 2.55, 2.79 and 4.82%) and thymol (0.14, 0.16, 0.13, 0.12, 0.13 and 38.70%).[15] These differences can probably be attributed to the different genotypes or different environmental conditions of the plant materials.

E. coli ATCC 25292 showed the highest sensitivity to *S. hortensis* (1), *S. macrantha* and *S. thymbra*. *Pseudomonas aeruginosa* ATCC 27853 showed the highest sensitivity to *S. thymbra* and *S. aintabensis*. On the other hand, *Enterobacter aerogenes* NRRL 3567 showed the same sensitivity to *S. hortensis* (2) and *S. thymbra*. *Staphylococcus aureus* ATCC 6538 displayed lower sensitivity than the other microorganisms (Table 2). In addition, Azaz *et al.*[12] reported that all tested oils were also active against the microorganisms employed in this study using various inhibitory concentrations. *Enterobacter aerogenes* was also inhibited by *S. pilosa* and *S. icarica* essential oils more strongly than by the standard chloramphenicol.[12]

Table 2. Antimicrobial activity (MIC) of *Satureja* spp. essential oils

Microorganisms	Sources	*S. hortensis* (1)	*S. hortensis* (2)	*S. macrantha*	*S. cuneifolia*	*S. thymbra*	*S. aintabensis*	Control
Escherichia coli	ATCC 25292	31.25	62.5	31.25	62.5	31.25	25	Chloramphenicol
Staphylococcus aureus	ATCC 6538	125	62.5	125	62.25	62.5	62.5	Chloramphenicol
Pseudomonas aeruginosa	ATCC 27853	62.5	62.5	62.5	62.5	31.25	31.25	Chloramphenicol
Enterobacter aerogenes	NRRL 3567	125	31.25	125	125	31.25	125	Chloramphenicol
Candida albicans	OGU	125	125	125	25	125	25	Ketoconazole

Table 3. Inhibition zones according to the agar disc diffusion method (mm)

Microorganisms	Serial dilution (100 µl stock +µl H_2O)				
	Collector number	Stock solution	100	200	300
Staphylococcus aureus	FS1046	17	10	7	
ATCC 6538	Chloramphenicol				23
	Streptomycin				13
Pseudomonas aeruginosa	FS1046	12	10	8	
ATCC 27853	Chloramphenicol				24
	Streptomycin				13
Enterobacter aerogenes	FS1046	11	9	9	
NRRL 3567	Chloramphenicol				21
	Streptomycin				13
Escherichia coli	FS1046	12	9	9	
ATCC 25292	Chloramphenicol				23
	Streptomycin				13

FS, Fatih Satil.
FS 1046, *Satureja thymbra* L.
Stock solution: 4 mg essential oil + 2 ml DMSO. As the same concentration of each essential oils from each of the six FS revealed close zone values for each microorganism tested, only one FS per microorganism was used in the table.

Table 4. Fungal spore inhibition

Microorganisms	Collector number	Serial dilution (100 µl stock +µl H_2O)			
		Stock solution	100	200	300
P. clavigerum	FS1045	–	+	+	+
	FS1010	+	+	+	+
Mucor hiemalis	FS1045	–	–	–	+
	FS1010	–	–	–	+
Absidia glauca	FS1047	–	–	+	+
ATCC 22752	FS1045	–	+	+	+

(+) Spore germination. (–) Spore inhibition.

The results of antibacterial activity according to the agar disc diffusion method indicated that all the tested oils have a broad spectrum of inhibitory activity (Table 3). In general, Gram-positive bacteria seemed to be more sensitive to the oils than Gram-negative bacteria. This is in agreement with observations by other authors that Gram-positive bacteria are more susceptible to essential oils than Gram-negative bacteria.[16] Monoterpenic phenols in essential oils are also responsible for the antimicrobial activity.[17]

Interestingly, all of the essential oils (25 µg/ml), except for the *S. thymbra* oil, had the same MIC value of 125 µg/ml against *Candida albicans* (Table 2). When the filamentous fungal spore inhibition assay was applied to the oils (Table 4), observation during the 72 h. incubation period showed that *Mucor hiemalis* spores were inhibited by the *S. macrantha* and *S. cuneifolia* (25 µg/ml) oils. *Absidia glauca* spores were inhibited by *S. hortensis* (1) (50 µg/ml) and *S. macrantha* (stock solution), while *Penicillium clavigerum* spores displayed a lower sensitivity to *S. macrantha* (stock solution). The other concentrations of these essential oils did not inhibit these microfungi.

The essential oils with the strongest antibacterial action are also active on fungi. However, treatment must be continued over a longer period. Fundamental studies have revealed the antifungal activity of alcohols and sesquiterpenic lactones. If the inhibition zone measures

between 2 and 3 mm, then the essential oil has a good bactericidal action. If the inhibition zone is more than 3 mm across, then it is considered very effective, but if there is no inhibition zone then the essential oil has no activity on the bacterium, and will not be retained for treatment.[17]

All the oils tested demonstrated some antibacterial activity, although to differing extents. The higher concentrations of oils showed greater spore inhibition activity. Finally, it is important to note that these essential oils were active against food spoilage microorganisms.

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