Screening of Antimicrobial Activity of Essential Oil and Methanol Extract of *Satureja hortensis* on Foodborne Bacteria and Fungi

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Abstract

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The present work reports the *in vitro* antimicrobial activities of the essential oil and methanol extract from *Satureja hortensis* as well as the content of its essential oil. The chemical composition of hydrodistilled essential oil of *Satureja hortensis* was analysed by means of GC-MS. Thirty constituents were identified. The main constituents of the oil were thymol (40.54%), γ-terpinene (18.56%), carvacrol (13.98%), and *p*-cymene (8.97). The essential oil of *Satureja hortensis* exhibited the activity against 25 bacteria, 8 fungi, and a yeast, *C. albicans*; exerting the Minimum Inhibitory Concentration values (MIC) ranging from 15.62 to 250 µl/ml. Similarly, methanol extract of the plant also showed antimicrobial activity.

Keywords: summer savory; *Satureja hortensis*; essential oil; methanol extract; antimicrobial activity

The genus *Satureja,* which belongs to the Lamiaceae, is represented in Turkey by 14 species (Davis 1982). *Satureja hortensis,* one of these species, is an annual plant (10–35 cm tall) and aromatic herb with lilac, purplish or white flowers and linear to linear-oblanceolate leaves. It grows wild on rocky or eroded slopes, screes, gravelly places and coastal dunes, fallow fields and roadsides in Turkey (Davis 1982).

The aerial parts of some *Satureja* plants have been widely used in foods as a flavour component and in folk and traditional medicine (Deans & Svoboda 1990; Zargari 1990; Baytop 1997; Madsen *et al*. 1998; Hajhashemi *et al*. 2000). Within the genus *Satureja*, *S. hortensis* has received the most attention by far.

In earlier investigations, *Satureja* species have been studied with respect to essential oil composition and show to be rich in components such as carvacrol,γ-terpinene, thymol, and *p*-cymene (Dardioti *et al*. 1997; Slavkovska *et al*. 1997; Tumen *et al*. 1998; Akgul *et al*. 1999; Chalchat *et al*. 1999; Sefidkon & Ahmadi 2000; Sefidkon & Jamzad 2000; Baser *et al*. 2001; Kurkcuoglu *et al*. 2001; Ghannadi 2002; Konakchiev & Tsankova 2002; Sajjadi & Baluchi 2002).

The essential oils have been used as flavouring agents in food and beverages and, due to the presence of antimicrobial compounds, they have a potential as natural agents for the food preservation (Helander *et al*. 1998). Recently, many studies have focused on antibacterial and antifungal

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 (Deans & Svoboda 1990; Ciani *et al*. 2000; Baser *et al*. 2001; Ozcan & Erkmen 2001; Azaz *et al*. 2002; Sokovic *et al*. 2002; Gulluce *et al*. 2003) due to the presence of phenolic components such as thymol and carvacrol. In a more recent study, Sahin *et al*. (2003) have found that essential oil and methanol extract of *Satureja hortensis* show a strong inhibition on a wide range of bacteria and fungi.

The above mentioned studies show that there are some published investigations regarding the antimicrobial activity of various *Satureja* species. Unfortunately, so far no reports are known which have included the antimicrobial effect of the essential oil and methanol extract of *Satureja hortensis* on only food-borne bacteria and fungi. To our best knowledge, therefore, this is the first study to determine the antimicrobial activity of the essential oil and the extract of *Satureja hortensis* on only foodborne bacteria and fungi.

MATERIAL AND METHODS

Plant material. *Satureja hortensis* plants at full flowering stage in August 2004 were collected from Yusufeli, Artvin, Turkey. The taxonomic identification of the plant materials was confirmed by a senior plant taxonomist, Meryem Sengul, in the Department of Biology, Ataturk University, Erzurum, Turkey. The collected plant materials were dried in shadow, and the plant leaves were separated from the stems and ground in a grinder with a 2 mm mesh in diameter. The voucher specimen has been deposited at the Herbarium of the Department of Biology, Ataturk University, Erzurum, Turkey.

Preparation of the methanol extract. The dried and powdered plant leaves (500 g) were extracted successively with 1 l of methanol using Soxhlet extractor for 72 h at a temperature not exceeding the boiling point of the solvent (Lin *et al*. 1999). The methanol extracts were filtered through Whatman filter paper (No. 1) and then concentrated in vacuo at 40°C by means of a Rotary Evaporator. The residues obtained were stored in a freezer at –80°C until further tests.

Isolation of the essential oil. The air-dried and ground aerial parts of the plants collected were submitted to water-distillation for 3 h using a Clevenger-type apparatus (yield 1.13% v/w). The essential oil (EO) obtained was dried over anhydrous sodium sulphate and, after filtration, stored at +4°C until tested and analysed.

GC-MS analysis conditions. The analysis of the essential oil was performed using a Thermofinnigan Trace GC/Trace DSQ/A1300, (E.I Quadrapole) equipped with a SGE-BPX5 MS capillary column (30 m × 0.25 mm i.d., 0.25 μm). For GC-MS detection, an electron ionisation system with ionisation energy of 70 eV was used. Helium was used as the carrier gas at a flow rate of 1 ml/min. The injector and MS transfer line temperatures were set at 220°C and 290°C, respectively. The programme used was 50–150°C at a rate of 3°C/min, held isothermal for 10 minutes and finally raised to 250°C at 10°C/min. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 μl were injected manually and in the splitless mode. The components were identified based on the comparison of their relative retention times and mass spectra with those of standards, Wiley7N library data of the GC-MS system and literature data (Adams 2001). The results were also confirmed by the comparison of the compounds elution order with their relative retention indices on non-polar phases reported in the literature (Adams 2001).

Antimicrobial activity

Microbial strains. The methanol extracts and the essential oil and its fractions were individually tested against a range of 42 microorganisms, among them 32 bacteria, 9 fungi, and 1 yeast species. The list of the microorganisms used is given in Tables 2 and 3. The microorganisms were provided by the Department of Clinical Microbiology, Faculty of Medicine, and Plant Diagnostic Laboratory, and Food Microbiology Laboratory, Faculty of Agriculture at Ataturk University, Erzurum, Turkey. The identity of the microorganisms used in this study was confirmed by Microbial Identification System in Biotechnology Application and Research Center at Ataturk University.

Disc-diffusion assay. The dried plant extracts were dissolved in the same solvent (methanol) to a final concentration of 30 mg/ml and sterilised by filtration through 0.45 μm Millipore filters. Antimicrobial tests were then carried out by the disc diffusion method (Murray *et al*. 1995) using 100 μl of suspension containing 10^8 CFU/ml of bacteria, 10^6 CFU/ml of yeast and 10^4 spore/ml

of fungi spread on nutrient agar (NA), sabouraud dextrose agar (SDA), and potato dextrose agar (PDA) mediums, respectively. The discs (6 mm in diameter) were impregnated with 10 µl of essential oil or 30 mg/ml extracts (300 µg/disc) placed on the inoculated agar. Negative controls were prepared using the same solvents as that employed to dissolve the plant extracts. Ofloxacin (10 µg per disc), sulbactam (30 µg) + cefoperazona (75 µg) (105 µg/disc) and/or netilmicin (30 µg/disc) were used as positive reference standards to determine the sensitivity of one strain/isolate in each microbial species tested. The inoculated plates were incubated at 37°C for 24 h with clinical bacterial strains, 48 h with yeast, and 72 h with fungi isolates. Plant associated microorganisms were incubated at 27°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay in this experiment was repeated twice.

Micro-well dilution assay. The minimal inhibition concentration (MIC) values were determined with the bacterial strains which were sensitive to the essential oil in the disc diffusion assay. The inocula of the bacterial strains were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The essential oils and extracts of *Satureja hortensis,* dissolved in 10% dimethylsulfoxide (DMSO), were first diluted to the highest concentration (500 µg/ml) to be tested, and then serial two-fold dilutions were made in order to obtain the concentration range from 7.8 to 500 µg/ml in 10 ml sterile test tubes containing nutrient broth. MIC values of *Satureja hortensis* extracts against bacterial strains and *Candida albicans* isolates were determined, based on a micro-well dilution method (Zgoda & Porter 2001; Gulluce *et al*. 2004a) with some modifications.

The 96-well plates were prepared by dispensing 95 µl of nutrient broth and 5 µl of the inoculum into each well. 100 µl of the stock solution of *Satureja hortensis* essential oil prepared at the concentration of 500 µg/ml was added into the first wells. Then, 100 µl of their serial dilutions was transferred into six consecutive wells. The last well containing 195 µl of nutrient broth without the compound and 5 µl of the inoculum from each strip was used as negative control. The final volume in each well was 200 µl. Maxipime (Bristol-Myers Squibb) in the concentration range of 500–7.8 µg/ml was prepared in the nutrient broth and used as the standard drug for the positive control. The plate was covered with a sterile plate sealer. The contents of each well were mixed on a plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth in each medium was determined by reading the respective absorbance (Abs) at 600 nm using the ELx 800 universal microplate reader (Biotek Instrument Inc, Highland Park, Vermont, USA), and was confirmed by plating 5 µl samples from clear wells on the nutrient agar medium. The oil tested in this study was screened two times against each organism. The MIC was defined as the lowest concentration of the respective compound able to inhibit the growth of microorganisms.

MIC agar dilution assay. MIC values of the fungi isolates were studied based on the agar dilution method as described previously by Gul *et al*. (2002). The essential oils of *Satureja hortensis* were added aseptically to sterile molten PDA medium, containing Tween 20 (Sigma 0.5%, v/v), in the volume appropriate to produce the concentration range of 7.8–500 µg/ml. The resulting PDA solutions were immediately poured into Petri plates after vortexing. The plates were spot inoculated with 5 µl (10^4 spore/ml) of each fungal isolate. Amphotericin B (Sigma A 4888) was used as a reference antifungal drug. The inoculated plates were incubated at 27°C and 37°C for 72 h with plant and clinical fungi isolates, respectively. At the end of the incubation period, the plates were evaluated for the presence or absence of growth. MIC values were determined as the lowest concentrations of the essential oil where the absence of growth was recorded. Each test was repeated at least twice.

RESULTS AND DISCUSSION

Table 1 indicates the chemical components of the essential oil obtained from the aerial parts of *Satureja hortensis* collected from North-Eastern Anatolia, Turkey. The constituents are listed in order of their elution from the SGE-BPX5 MS capillary column. Thirty components were identified, representing 95.60% of the total oil fraction. The major constituents of the oil were thymol (40.54%), γ-terpinene (18.56%), carvacrol (13.98%), and *p*-cymene (8.97%).

The composition of the essential oil isolated from the aerial parts of *Satureja hortensis* has previously been reported. Our result is in agreement with the literature reports on the essential oils *of Satureja hortensis* and other *Satureja* species (Tumen *et al.*

Table 1. Chemical composition of the essential oil of *Satureja hortensis* growing in North-Eastern Anatolia Region of Turkey

RI	Components	Composition (%)	Identification
910	α-thujene	0.67	GC-MS, RI
921	β-pinene	1.15	GC-MS, RI
981	sabinene	1.32	GC-MS, RI
988	1-octen-3-ol	0.34	GC-MS, RI
994	myrcene	1.21	GC-MS, RI
1015	α-phellandrene	0.27	GC-MS, RI
1026	α-terpinene	2.30	GC-MS, RI
1037	*p*-cymene	8.97	GC-MS, RI
1042	β-phellandrene	0.17	GC-MS, RI
1044	1,8-cineole	0.30	GC-MS, RI
1071	γ-terpinene	18.56	GC-MS, RI
1094	α-terpinolene	0.07	GC-MS, RI
1102	dimethyl styrene	0.03	GC-MS, RI
1109	linalool	0.05	GC-MS, RI
1151	camphor	0.96	GC-MS, RI
1162	pinocarvone	1.13	GC-MS, RI
1168	borneol	0.30	GC-MS, RI
1172	4-terpineol	0.63	GC-MS, RI
1243	thymol	40.54	GC-MS, RI
1247	carvacrol	13.98	GC-MS, RI
1266	piperitenone	0.35	GC-MS, RI
1278	nepetalactone 1	0.30	GC-MS, RI
1282	β-bourbonene	0.04	GC-MS, RI
1291	nepetalactone 2	0.48	GC-MS, RI
1320	γ-muurolene	0.15	GC-MS, RI
1332	β-bisabolene	0.26	GC-MS, RI
1335	γ-cadinene	0.10	GC-MS, RI
1336	δ-cadinene	0.22	GC-MS, RI
1362	spathulenol	0.33	GC-MS, RI
1364	caryophyllene oxide	0.42	GC-MS, RI
Total		95.6	

RI = retention index; Compounds listed in order of elution from a BPX5 MS column

1998; Akgul *et al*. 1999; Chalchat *et al*. 1999; Sefidkon & Jamzad 2000; Kurkcuoglu *et al*. 2001; Ghannadi 2002; Konakchiev & Tsankova 2002; Sajjadi & Baluchi 2002; Baher *et al*. 2002; Baser *et al*. 2004).

Antimicrobial activity. The antimicrobial activities of *Satureja hortensis* essential oil and extracts against microorganisms examined in the present study and their potency were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and zone diameter, and MIC values. The results are given in Tables 2 and 3. The data of the study clearly indicated that the essential oil of *Satureja hortensis* plant has a strong

Table 2. Antimicrobial activities of *Satureja hortensis* methanol extract and essential oil against the food borne bacterial strains tested

Test microorganisms	Plant extract (MeOH)		Essential oil		Antibiotics	
	DD[b]	MIC[d]	DD[c]	MIC[d]	DD[a]	MIC[e] (max)
Acinetobacter baumanii A8	8	250	38	15.62	18 mm (OFX)	31.25
Acinetobacter lwoffi F1	14	62.50	12	125	19 mm (OFX)	15.62
Bacillus macerans A199	12	62.50	18	62.50	9 mm (SCF)	15.62
Bacillus megaterium A59	–	–	24	62.50	28 mm (OFX)	62.50
Bacillus subtilis ATCC-6633	8	125	–	–	28 mm (OFX)	125
Bacillus subtilis A57	10	62.50	10	250	12 mm (SCF)	62.50
Brucella abortus A77	–	–	10	250	22 mm (SCF)	125
Burkholdria cepacia A225	–	–	20	62.50	25 mm (SCF)	16.62
Cedecea davisae F2	–	–	16	125	20 mm (NET)	31.25
Enterobacter cloacae A135	–	–	25	62.50	18 mm (SCF)	31.25
Enterococcus faecalis ATCC-29122	–	–	–	–	20 mm (OFX)	62.50
Escherichia coli A1	–	–	–	–	12 mm (OFX)	125
Klebsiella pneumoniae F3	14	31.25	18	62.50	12 mm (OFX)	125
Klebsiella pneumoniae A137	–	–	10	125	13 mm (OFX)	125
Morgonella morgani F4	–	–	–	–	22 mm (NET)	31.25
Proteus vulgaris A161	8	125	–	–	22 mm (NET)	15.62
Proteus vulgaris KUKEM1329	–	–	–	–	24 mm (OFX)	125
Pseudomonas aeruginosa ATCC-9027	–	–	14	250	27 mm (SCF)	62.50
Pseudomonas aeruginosa ATCC-27859	–	–	20	31.25	22 mm (SCF)	31.25
Pseudomonas aeruginosa F5	–	–	18	125	22 mm (SCF)	62.50
Pseudomonas pseudoalkaligenes F6	–	–	22	62.50	12 mm (SCF)	15.62
Salmonella cholerasuis arizonae F7	–	–	20	62.50	10 mm (OFX)	62.50
Salmonella enteritidis ATCC-13076	–	–	22	31.25	13 mm (OFX)	31.25
Serratia plymuthica F8	–	–	18	125	20 mm (SCF)	31.25
Shigella sonnei F9	–	–	22	62.50	18 mm (OFX)	31.25
Staphylococcus aureus A215	–	–	32	15.62	19 mm (OFX)	15.62
Staphylococcus aureus ATCC-29213	–	–	24	62.50	9 mm (SCF)	15.62
Staphylococcus epidermis A233	10	62.50	18	125	28 mm (OFX)	62.50
Staphylococcus hominis F10	–	–	20	62.50	28 mm (OFX)	125
Streptococcus pyogenes ATCC-176	–	–	–	–	12 mm (SCF)	62.50
Streptococcus pyogenes KUKEM-676	–	–	22	62.50	22 mm (SCF)	125
Yersinia enterocolitica F11	–	–	24	62.50	25 mm (SCF)	16.62

[a]DD = diameter of disc diffusion (mm); OFX = Ofloxacin (10 µg/disc); SCF = sulbactam (30 µg) + cefperazone (75 µg) (105 µg/disc) and NET = Netilmicin (30 µg/disc) were used as positive reference standards antibiotic discs (Oxoid)

[b]DD = inhibition zone in diameter (mm) around the discs (6 mm) impregnated with 300 µg/disc of methanol extract

[c]DD =inhibition zone in diameter (mm) around the discs (6 mm) impregnated with 10 µl of essential oil

[d]minimal inhibitory concentrations as (µg/ml)

[e]MIC= maxipine (µg/ml) was used as reference antibiotic in micro well dilution assay (Sigma)

– no inhibition zone and/or MIC value measured

antimicrobial activity against both bacteria and fungi, i.e. 25 bacteria, 8 fungi, and 1 yeast species. Regarding the bacteria tested, the oil could not inhibit the growth of some bacteria including *Bacillus subtilis*, *Enterococcus feacalis*, *Escherichia coli*, *Morgonella morganii*, *Proteus vulgaris, Staphylococcus aureus* and *Streptococcus pyogenes*. On the other hand, methanol extract from the aerial parts of *S. hortensis* plants showed antimicrobial activity on 8 bacteria and 3 fungi (Tables 2 and 3). This observation supports the hypothesis that the essential oil of plants contains more antimicrobial substances than their extracts including water, methanol, ethanol, and hexane extracts (Ahmad *et al*. 1998; Eloff 1998; Gulluce *et al*. 2004b; Ozer *et al*. 2006).

The maximal inhibition zones and MIC values for bacterial strains, which were sensitive to the essential oil of *Satureja hortensis*, were in the range of 10–38 mm, and 15.62–250 µl/ml (Table 2). The antimicrobial action of the essential oil of *S. hortensis* is probably related to the fact that essential oils may disrupt the permeability barrier of cell membranes and inhibit respiration (Cox *et al*. 2000). In general, the gram-positive bacteria strains tested seem to be affected by the essential oil to the same extent as the gram-negative bacteria strains, while previous works showed that the gram-positive bacteria are more sensitive to plant oil and extracts than the gram-negative ones (Cosentino *et al*. 1999; Karaman *et al*. 2003). As with antifungal and anticandidal efficacy, the maximal inhibition zones and MIC values of the fungi and yeast species sensitive to the essential oil of *S. hortensis* were 24–41 mm and 15.62–62.50 µl/ml, respectively (Table 3). Several studies have been performed concerning the antimicrobial activity of essential oils or extracts of other *Satureja* species. Many of the previous studies demonstrated that the members of the genus *Satureja* show a high antimicrobial activity due to the presence of thymol, carvacrol, and their precursors (Azaz *et al*. 2002; Gulluce *et al*. 2003; Sahin *et al*. 2003).

Table 3. Anticandidal and antifungal activities of *Satureja hortensis* methanol extract and essential oil against yeast and fungi isolates tested

Tested yeast and fungi	Plant extract (MeOH)		Essential oil		Antibiotics[a]	
	DD[b]	MIC[d]	DD[c]	MIC[d]	DD[a]	MIC[e] (Amp B)
Yeast						
Candida albicans-A117	20	31.25	–	–	(NET)	31.25
Fungi						
Alternaria alternate	37	15.62	–	–	(NET)	31.25
Aspergillus flavus	41	15.62	18	62.50	(NET)	15.62
Aspergillus variecolor	32	62.50	–	–	(NET)	15.62
Fusarium acuminatum	–	–	–	–	(NET)	62.50
Fusarium oxysporum	24	62.50	–	–	(NET)	62.50
Fusarium solani	35	62.50	20	125	(NET)	62.50
Fusarium tabacinum	35	31.25	–	–	(NET)	62.50
Penicillum spp.	42	62.50	24	62.50	(NET)	31.25
Rhizopus spp.	30	62.50	–	–	(NET)	125

[a]DD = diameter of disc diffusion (mm); NET = Netilmicin (30 µg/disc) were used as positive reference standards antibiotic discs (Oxoid)

[b]DD = inhibition zone in diameter (mm) around the discs (6 mm) impregnated with 300 µg/disc of methanol extract

[c]DD = inhibition zone in diameter (mm) around the discs (6 mm) impregnated with 10 µl of essential oil

[d]minimal inhibitory concentrations as (µg/ml)

[e]MIC = Amphotericin B (µg/ml) was used as reference antibiotic in MIC agar dilution (Sigma)

– no inhibition zone and/or MIC value measured

From the results of the present study, it becomes evident that a relationship exists between the high activity of *S. hortensis* and the presence of phenolic components, such as thymol (40.54%), carvacrol (13.98%), and their precursors, γ-terpinene (18.56%) and *p*-cymene (8.97%). The high antimicrobial activity of *S. hortensis* essential oil could be explained by the higher percentage of thymol and carvacrol which are well known to have antibacterial activity (Kim *et al*. 1994; Muller *et al*. 1995; Adam *et al*. 1998). Furthermore, the antimicrobial activity of essential oils may be altered by synergistic and antagonistic effects between some components (Dorman & Deans 2000). The synergistic activity of carvacrol and thymol was also reported by Didry *et al*. (1993) and Sivropoulou *et al*. (1996).

The mechanism of the action of the oil is probably related to the outer membrane disintegrating properties of thymol and carvacrol (Helander *et al*. 1998). In the literature, some investigations suggest that these compounds penetrate inside the cell, where they interfere with cellular metabolism (Marino *et al*. 2001). Other studies indicate that they disturb the structure of the cellular membrane and react with the active sites of enzymes or act as a H^+ carrier, depleting adenosine triphosphate pool (Farag *et al*. 1989; Ultee *et al*. 2002).

Due to the potential for antimicrobial and antioxidant properties (Bandoniene *et al*. 2002; Exarchou *et al*. 2002; Gulluce *et al*. 2003; Sahin *et al*. 2003; Dorman & Hiltunen 2004), *Satureja hortensis* is becoming increasingly important in food studies. To our knowledge, this is the first report of the antimicrobial properties of *Satureja hortensis* oil and extract active towards only foodborne bacteria and fungi. The present study clearly demonstrates that the essential oil and extract of S*atureja hortensis* contain compounds possessing antimicrobial properties. On the basis of the results of this and previous studies, *S. hortensis* can be added as a protective agent to various food products. However, further studies including other important food pathogens, such as *Listeria monocytogenes*, C*lostridium perfinges*, *Clostridium botilinum*, *Bacillus cereus*, *Vibrio parahaemolyticus*, *Campylobacter jejuni*, are needed to improve our understanding of the influence of *S. hortensis* on food born bacteria and fungi. More importantly, there is a need for more information on the results of the use of *S. hortensis* essential oil and extracts in different foods.

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