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Evaluation of antimicrobial activities of *Satureja hortensis* L.

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Abstract

The present study was designated to evaluate the antimicrobial activities of methonol and hexane extracts of *Satureja hortensis* L. which is an annual herb used as traditional folk medicine in Eastern Anatolia region of Turkey for the treatment of different infectious diseases and disorders. The antimicrobial activities of the extracts against 147 laboratory strains belong to 55 bacterial species, and 31 isolates of 1 yeast and 4 fungi species were tested by using disc diffusion assay. The results showed that hexane extract of *Satureja hortensis* had no antifungal, but antibacterial activity against four strains of three *Bacillus* species whereas methanol extract of *Satureja hortensis* had both anticandidal and antibacterial effects. It inhibited the growth of 23 strains of 11 bacterial species and 6 isolates of *Candida albicans*, at the concentration of 300 μg/ml. *Satureja hortensis* did not show antimicrobial activity against the remaining microorganisms (83%) tested including most and all of the clinic and plant pathogenic microorganisms, respectively. Methanol extract showed stronger and broader spectrum of antimicrobial activity as compared to hexane extract.

Keywords: Antimicrobial activity; *Satureja hortensis* L.; Medicinal plant

1. Introduction

Satureja hortensis L. (summer savory) is well known aromatic and medicinal plant, which is widely distributed in the Eastern Anatolia region of Turkey, and locally named as 'Koc Out.' Leaves, flowers and stem of *Satureja hortensis* are frequently used as tea or additive in commercial spice mixtures for many foods to offer aroma and flavor. *Satureja hortensis* has been also used as folk remedies to treat various ailments such as cramps, muscle pains, nausea, indigestion, diarrhea, and infectious diseases. It has showed antispasmodic, anti-diarrhoeal, antioxidant, sedative as well as antimicrobial properties (Deans and Svoboda, 1989; Hajhashemi et al., 2000; Leung and Foster, 1996; Madsen et al., 1996; Zargari, 1990). However, so far there have been no attempts to study the potential of *Satureja hortensis* antimicrobial activity against a wide range of clinic and plant-associated microorganisms such as bacteria, yeast, and fungi species. In recent years, multiple drug/chemical resistance in both human and plant pathogenic microorganisms have been developed due to indiscriminate use of commercial antimicrobial drugs/chemical commonly used in the treatment of infectious diseases (Davis, 1994; Loper et al., 1991; Service, 1995). This situation forced the scientists to the searching of new antimicrobial substances from various sources like medicinal plants (Clark, 1996; Cordell, 2000).

The present study was conducted to investigate antimicrobial properties of methanol and hexane extracts of *Satureja hortensis* against both clinical and plant-associated microorganisms (bacteria, fungi, and yeast) which have not been evaluated in previous studies.

2. Material and methods

2.1. Plant materials and extraction procedure

Aerial parts (leaves, flowers, and stems) of wild summer savory (*Satureja hortensis* L., Lamiaceae) plants were collected from Gaziler valley of Senkaya in the Eastern Anatolia region of Turkey. The taxonomic identification of

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plant materials was confirmed by a senior plant taxonomist, Meryem Sengul, in the Department of Biology, Ataturk University, Erzurum, Turkey. Collected plant material was dried in the shade and ground in a grinder with a 2 mm in diameter mesh. The dried and powdered plant materials (500 g) were extracted successively with 1 l of hexane and methanol by using Soxhlet extractor for 72 h at a temperature not exceeding the boiling point of the solvent (Lin et al., 1999). The extracts were filtered using Whatman filter paper (no. 1) and then concentrated in vacuo at 40 °C using a Rotary evaporator. The residues obtained were stored in a freezer at −80 °C until further tests.

2.2. Microorganisms

A total 178 microbial cultures belonging to 55 bacteria, 1 yeast and 4 fungi species, were used in this study. The list of microorganisms used is given in Tables 1 and 2. Microorganisms were provided by the Department of Clinical Microbiology, Faculty of Medicine; and Plant Diagnostic Laboratory, Faculty of Agriculture at Ataturk University, Erzurum, Turkey. Identity of the microorganisms used in this study was confirmed by Microbial Identification System in Biotechnology Application and Research Center at Ataturk University.

2.3. Antimicrobial activity

2.3.1. Disc diffusion assay

The dried plant extracts were dissolved in the same solvent (hexane and methanol) to a final concentration of 30 mg/ml and sterilized by filtration by 0.45 μm Millipore filters. Antimicrobial tests were then carried out by 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), sabourand dextrose agar (SDA), and potato dextrose agar (PDA) medium, respectively. The discs (6 mm in diameter) were impregnated with 10 μl of the 30 mg/ml extracts (300 μg/disc) placed on the inoculated agar. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. Ofloxacin (10 μg/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 for clinical bacterial strains, 48 h for yeast and 72 h for 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.

2.3.2. Microdilution assays

The minimal inhibition concentration (MIC) values were also studied for the microorganisms which were determined as sensitive to the extracts in disc diffusion assay. The inocula of microorganisms were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The *Satureja hortensis* extracts dissolved in 10% dimethylsulfoxide (DMSO) were first diluted to the highest concentration (600 μg/ml) to be tested, and then serial two-fold dilutions were made in a concentration range from 9.37 to 600 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 and Porter, 2001) with some modifications.

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

3. Results and discussion

The antimicrobial activities of *Satureja hortensis* (hexane and methanol) 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 were given in Tables 1–3. The results showed that the hexane extract of *Satureja hortensis* did not have antimicrobial activity, except four strains belonging to three *Bacillus* species (*Bacillus amyloliquefaciens*, *Bacillus megaterium*, and *Bacillus sphaericus*) (Tables 1 and 2). However, the methanol extract showed inhibition effect of six *Candida albicans* isolates and 23 strains of 11 species belonging to five different bacterial genera including *Bacillus amyloliquefaciens*, *Bacillus atrophaeus*, *Bacillus macerans*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus sphaericus*, *Bacillus substilis*, *Escherichia coli*, *Kocuria varians*, *Micrococcus luteus*, and *Pantoea agglomerans* (Tables 1 and 2). The maximal inhibition zones and MIC values for bacterial strains and

Table 1
Antimicrobial activity of *Satureja hortensis* extracts against the bacterial strains tested based on disc diffusion method

Bacterial species	Number of strains/origins	Inhibition zone in diameter (mm/sensitive strains)[a]				Positive controls (mm)[b]
		Satureja hortensis extracts (300 μg/disc)		Negative control		Standard antibiotic discs
		MeOH	Hexane	MeOH	Hexane	
Acinetobacter baumanii	3/Clinic	–	–	–	–	18 (OFX)
Acinetobacter calcoaceticus	2/Clinic	–	–	–	–	16 (OFX)
Acinetobacter lwoffii	1/Clinic	–	–	–	–	27 (OFX)
Acinetobacter johnsonii	1/Clinic	–	–	–	–	25 (OFX)
	1/Plant	–	–	–	–	32 (OFX)
Alcaligenes pacificus	1/Plant	–	–	–	–	NT
Alcaligenes xylosoxydans	2/Clinic	–	–	–	–	12 (SCF)
Agrobacterium radiobacter	1/Plant	–	–	–	–	28 (SCF)
Bacillus amyloliquefaciens	1/Plant	11/1	8/1	–	–	27 (SCF)
Bacillus atrophaeus	2/Plant	8–9/2	–	–	–	21 (SCF)
Bacillus cereus	2/Clinic	–	–	–	–	30 (OFX)
Bacillus licheniformis	1/Plant	–	–	–	–	26 (SCF)
Bacillus macerans	1/Plant	7–9/1	–	–	–	26 (SCF)
Bacillus megaterium	4/Plant	9–13/4	7–9/2	–	–	9 (SCF)
Bacillus pumilus	2/Plant	7–8/2	–	–	–	23 (OFX)
Bacillus sphaericus	1/Plant	12/1	9/1	–	–	18 (OFX)
Bacillus substilis	4/Plant	8–12/4	–	–	–	28 (OFX)
Brevundimonas diminuta	1/Clinic	–	–	–	–	34 (SCF)
Brucella abortus	15/Clinic	–	–	–	–	12 (SCF)
Brucella melitensis	11/Clinic	–	–	–	–	18 (OFX)
Burkholdria cepacia	1/Clinic	–	–	–	–	– (SCF)
Burkholdria gladioli	1/Plant	–	–	–	–	22 (NET)
Citrobacter freundii	1/Clinic	–	–	–	–	23 (SCF)
Clavibacter michiganense	1/Plant	–	–	–	–	25 (SCF)
Curtobacterium flaccumfaciens	2/Plant	–	–	–	–	37 (OFX)
Enterobacter aerogenes	1/Clinic	–	–	–	–	12 (OFX)
Enterobacter cloacae	2/Clinic	–	–	–	–	10 (OFX)
Enterobacter intermedius	2/Plant	–	–	–	–	27 (SCF)
Enterobacter pyrinus	1/Clinic	–	–	–	–	NT
Erwinia amylovora	1/Plant	–	–	–	–	12 (OFX)
Erwinia carotovora	1/Plant	–	–	–	–	30 (OFX)
Erwinia chrysanthemi	1/Plant	–	–	–	–	25 (SCF)
Escherichia coli	7/Clinic	6–8/3	–	–	–	– (OFX)
Flavobacterium blastinum	1/Plant	–	–	–	–	32 (OFX)
Klebsiella pneumoniae	2/Clinic	–	–	–	–	12 (OFX)
Klebsiella trevisanii	1/Clinic	–	–	–	–	23 (OFX)
Kocuria varians	1/Clinic	8/1	–	–	–	18 (OFX)
Leclercia adecarboxylata	1/Clinic	–	–	–	–	NT
Micrococcus luteus	1/Clinic	9/1	–	–	–	– (OFX)
Neisseria spp.	1/Clinic	–	–	–	–	– (OFX)
Pantoea agglomerans	3/Plant	7–8/3	–	–	–	24 (NET)
Plesiomonas shigelloides	1/Clinic	–	–	–	–	– (SCF)
Proteus vulgaris	1/Clinic	–	–	–	–	12 (OFX)
Pseudomonas aeruginosa	15/Clinic	–	–	–	–	22 (NET)
	5/Plant	–	–	–	–	30 (OFX)
Pseudomonas fluorescens	4/Clinic	–	–	–	–	10 (OFX)
	1/Plant	–	–	–	–	24 (SCF)
Pseudomonas huttiensis	2/Plant	–	–	–	–	24 (OFX)
Pseudomonas putida	1/Plant	–	–	–	–	25 (OFX)
Pseudomonas syringae pvs.	5/Plant	–	–	–	–	24 (OFX)
Ralstonia pickettii	1/Plant	–	–	–	–	22 (SCF)
Salmonella typhimurium	1/Clinic	–	–	–	–	27 (SCF)
Staphylococcus aureus	5/Clinic	–	–	–	–	22 (SCF)
Staphylococcus epidermis	5/Clinic	–	–	–	–	– (SCF)
Stenotrophomonas maltophilia	1/Clinic	–	–	–	–	13 (OFX)
	1/Plant	–	–	–	–	NT
Streptococcus pneumoniae	2/Clinic	–	–	–	–	– (OFX)
Streptococcus pyogenes	2/Clinic	–	–	–	–	10 (OFX)
Xanthomonas campestris pvs.	4/Plant	–	–	–	–	20 (SCF)
Total 55 bacterial species	147	6–13/23	7–9/4			

[a] MeOH, methanol extract.

[b] OFX, ofloxacin (10 μg/disc); SCF, sulbactam (30 μg) + cefoperazona (75 μg) (105 μg/disc); NET, netilmicin (30 μg/disc) were used as positive reference standards antibiotic discs (oxoid); NT, not tested.

Table 2
Antimicrobial activity of *Satureja hortensis* extracts against yeast and fungi isolates tested based on disc diffusion method

Yeast and fungi species	Number of isolates/origins	Inhibition zone in diameter (mm/sensitive strains)[a]				Positive control[b]
		Satureja hortensis extracts (300 μg/disc)		Negative control		Standard antibiotic discs
		MeOH	Hexane	MeOH	Hexane	
Yeast						
Candida albicans	23/Clinic	7–9/6	–	–	–	– (NET)
Fungi						
Alternaria alternata	2/Plant	–	–	–	–	– (NET)
Aspergillus flavus	2/Clinic	–	–	–	–	– (NET)
Fusarium oxysporum	2/Plant	–	–	–	–	– (NET)
Penicillium spp.	2/Clinic	–	–	–	–	– (NET)
Total	31 isolates	7–9/6 isolates	–	–	–	–

[a] MeOH, methanol extract.
[b] OFX, ofloxacin (10 μg/disc); SCF, sulbactam (30 μg) + cefoperazona (75 μg) (105 μg/disc); NET, netilmicin (30 μg/disc) were used as positive reference standards antibiotic discs (oxoid); NT, not tested.

Table 3
The MIC values of *Satureja hortensis* extracts against the microorganisms tested in microdilution assay (MIC in μg/ml)

Microorganisms	Number of strains and isolates/origins	*Satureja hortensis* extracts		Standard drug (Maxipime)
		MeOH	Hexane	
Bacillus amyloliquefaciens	1/Plant	31.25	31.25	15.60
Bacillus atrophaeus	2/Plant	31.25–62.50	–	31.25
Bacillus macerans	1/Plant	125	–	62.50
Bacillus megaterium	4/Plant	31.25–62.50	62.50–125	15.60–31.25
Bacillus pumilus	2/Plant	62.50	–	15.60
Bacillus sphaericus	1/Plant	15.60	31.25	31.25
Bacillus substilis	4/Plant	31.25–125	–	31.25–62.50
Escherichia coli	3/Clinic	250–500	–	125
Kocuria varians	1/Clinic	250	–	250
Micrococcus luteus	1/Clinic	125	–	250
Pantoea agglomerans	3/Plant	125–250	–	125
Candida albicans	6/Clinic	125–250	–	250–500
Total	29			

Candida albicans isolates, which were sensitive to *Satureja hortensis* methanol extract, were in the range of 6–13 mm, and 15.60–250 μl/ml; and 7–9 mm and 125–250 μl/ml, respectively (Tables 1–3). In the case of the hexane extract, the maximal inhibition zones and MIC values of the bacterial strains sensitive to the hexane extract were 7–9 mm and 31.25–125 μl/ml, respectively (Tables 1 and 3). Based on these results, it is possible to conclude that methanol extract has stronger and broader spectrum of antimicrobial activity as compared to hexane extract. This observation confirmed the evidence in a previous study reported that methanol is a better solvent for extraction of antimicrobial substances from medicinal plants than water, ethanol, and hexane (Ahmad et al., 1998; Eloff, 1998). Findings in this study supported the observations of some other researchers about *Satureja hortensis* containing some substances with antibacterial properties (Deans and Svoboda, 1989; Leung and Foster, 1996; Zargari, 1990). Since only the essential oils from *Satureja hortensis* have been evaluated in terms of antimicrobial activity against a limited number of microorganisms up to now (Deans and Svoboda, 1989), this is the first study to provide data that the extracts of *Satureja hortensis* plants evaluated against a wide range of microorganisms possess potential antibacterial and anticandidal activities.

4. Conclusions

The clinical strains/isolates in the species of *Candida albicans*, *Escherichia coli*, *Kocuria varians*, and *Micrococcus luteus* were found to be sensitive to *Satureja hortensis* extracts. However, the plant-associated microorganisms sensitive to *Satureja hortensis* extracts were not economically important plant pathogens in the genus of *Clavibacter*, *Pseudomonas*, and *Xanthomonas*. Therefore, this result may suggest that *Satureja hortensis* extracts possess compounds with antimicrobial properties which can be used as antimicrobial agents in new drugs for therapy of

infectious diseases in human, but not for management of plant diseases.

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