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In Vitro Antibacterial, Antifungal, and Antioxidant Activities of the Essential Oil and Methanol Extracts of Herbal Parts and Callus Cultures of *Satureja hortensis* L.

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The present study was designated to evaluate the antimicrobial and antioxidant activities of the essential oil, obtained by using a Clevenger distillation apparatus, water soluble (polar) and water insoluble (nonpolar) subfractions of the methanol extracts from aerial parts of *Satureja hortensis* L. plants, and methanol extract from calli established from the seeds using Gamborg's B5 basal media supplemented with indole-3-butyric acid (1.0 ppm), 6-benzylaminopurine (N^6-benzyladenine) (1.0 ppm), and sucrose (2.5%). The antimicrobial test results showed that the essential oil of *S. hortensis* had great potential antimicrobial activities against all 23 bacteria and 15 fungi and yeast species tested. In contrast, the methanol extract from callus cultures and water soluble subfraction of the methanol extract did not show antimicrobial activities, but the nonpolar subfraction had antibacterial activity against only five out of 23 bacterial species, which were *Bacillus subtilis*, *Enterococcus fecalis*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, and *Streptococcus pyogenes.* Antioxidant studies suggested that the polar subfractions of the methanol extract of intact plant and methanol extract of callus cultures were able to reduce the stable free radical 2,2-diphenyl-1-picrylhydrazyl to the yellow-colored diphenylpicrylhydrazine. In this assay, the strongest effect was observed for the tissue culture extract, with an IC_{50} value of 23.76 ± 0.80 μg/mL, which could be compared with the synthetic antioxidant agent butylated hydroxytoluene. On the other hand, linoleic acid oxidation was 95% inhibited in the presence of the essential oil while the inhibition was 90% with the chloroform subfraction of the intact plant. The chemical composition of a hydrodistilled essential oil of *S. hortensis* was analyzed by gas chromatography (GC)/flame ionization detection (FID) and a GC-mass spectrometry system. A total 22 constituents representing 99.9% of the essential oil were identified by GC-FID analaysis. Thymol (29.0%), carvacrol (26.5%), γ-terpinene (22.6%), and *p*-cymene (9.3%) were the main components.

KEYWORDS: Antimicrobial activity; antioxidant activity; *Satureja hortensis*; GC-FID analysis; callus cultures

INTRODUCTION

Satureja hortensis L. (summer savory) is a well-known aromatic and medicinal plant, which is widely distributed in the Anatolia region of Turkey. Leaves, flowers, and stems of *S. hortensis* are frequently used as tea or additives in commercial spice mixtures for many foods to offer aroma and flavor and called "sater, ank, or anuğ" by the people from different regions in Turkey (*1*). *S. hortensis* has also been used as a folk remedy to treat various ailments such as cramps, muscle pains, nausea, indigestion, diarrhea, and infectious diseases. It has shown antispasmodic, antidiarrheal, antioxidant, sedative, and antimi-

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crobial properties (*2*−*6*). In previous studies, it was demonstrated that the contents of essential oils, in particular, and extracts of medicinal plants containing antimicrobial, antioxidant, and other biological activities may be subject to change, based on the variations in the chemical composition of an essential oil that may be observed due to the origin, the locality, the environmental conditions, and the stage of development of the collected plant material. Thus, it is necessary that the evaluation of the antimicrobial activity of an essential oil should be accompanied by the determination of its chemical composition (*2*, *4*, *7*−*9*) since long time essential oils have been regarded as natural antimicrobial systems. The biological activity of an essential oil is attributed mainly to its major components, although the synergistic or antagonistic effect of one compound in a minor percentage of the mixture has to be considered (*10*).

The essential oils from different *Satureja* species have been found to differ both qualitatively and quantitatively (*8*, *11*, *12*). The essential oil of cultivated summer *S. hortensis* has been found to be rich in γ-terpinene and carvacrol (*13*). So far, there have been no attempts to study the chemical composition and biological activities of essential oils and extracts from *S. hortensis* plants collected from the Anatolia region of Turkey, although several papers on the antimicrobial and antioxidant properties of this species collected from elsewhere have been published (*14*, *15*). In recent years, multiple drug/chemical resistance in both human and plant pathogenic microorganisms has developed, due to indiscriminate use of commercial antimicrobial drugs/chemicals commonly used in the treatment of infectious diseases (*16*−*18*). This situation forced the scientists to search for new antimicrobial substances from various sources such as medicinal plants (*19*, *20*).

The introduction of synthetic antioxidants, e.g., butylated hydroxyanisole and butylated hydroxytoluene (BHT), as potential inhibitors of lipid peroxidation in the food industry has also caused some problems due to their highly volatile nature, instability at high temperatures, and strict law restrictions, addressing consumer and thereby manufacturer preferences to the natural antioxidant sources (*21*). Many edible plant species such as tea, fruits, juices, spices, and vegetables throughout the history of mankind have been attractive to scientists as natural sources of compounds that are safer than the synthetic ones. Many other plants have also been screened for their antioxidant capacities, and attempts led to the introduction of natural antioxidants such as those from rosemary and sage (*22*). Antioxidants display different behaviors. One of these is related to the prevention of the reactive oxygen species (ROS). The ROS are formed during normal cell aerobic respiration, and they are the main cause of cell damage, mainly related to cancer, cardiovascular diseases, and other cell damage. Antioxidants play an important role in the protection of cells against oxidative damage caused by ROS (*23*, *24*). For example, enzymatic oxidation of linoleic acid produces a range of products that may cause cancer (*25*). This kind of inhibition is also very important in processed food protection. In our study, antioxidative properties of methanol extracts of *S. hortensis* from intact plant and its callus cultures are reported for the first time.

Plant cell cultures are, on the other hand, useful systems for the production of antimicrobial or antioxidant principles (*26*). These may be considered a de novo production of the needed secondary metabolites through cell culture. To the best of our knowledge, antimicrobial and/or antioxidant compound production in cell cultures of *S. hortensis* has not yet been reported.

The aims of this study were (i) to investigate some biological properties of the callus culture, essential oil, and extracts from *S. hortensis* plants collected from Turkey and (ii) to determine the chemical composition of a hydrodistilled essential oil of *S. hortensis* plants of the same origin by gas chromatography (GC)/flame ionization detection (FID) and GC-mass spectrometry (MS) analaysis.

MATERIALS AND METHODS

Plan Material. *S. hortensis* plants were collected in Beypınarı, ca. 45 km south of Zara-Sivas, Turkey, when flowering (September, 2002). The voucher specimens were deposited at the Herbarium of the Department of Biology, Cumhuriyet University, Sivas, Turkey (CUFH, voucher no. ED 6487).

Preparation of the Methanol Extracts. The air-dried and finely ground sample was extracted by using the method described previously (*27*). The resulting extract (15.35%, w/w) was suspended in water and partitioned with chloroform ($CHCl_3$) to obtain water soluble (polar) (9.15%, w/w) and water insoluble (nonpolar, chloroformic) subfractions (6.20%, w/w), which were then lyophilized and kept in the dark at 4 °C until tested.

The lyophilized material from callus cultures was extracted with MeOH using the same extraction procedure given above, except for subfractionation. The extract yield was 6.11% (w/w).

Isolation of the Essential Oil. The air-dried and ground aerial parts of plants collected were submitted to water distillation for 3 h using a Clevenger type apparatus (yield 1.98%, v/w). The obtained essential oil was dried over anhydrous sodium sulfate and, after it was filtered, stored at 4 °C until tested and analyzed.

GC-FID Analysis Conditions. The essential oil was analyzed using Hewlett-Packard 5890 II GC equipped with FID detector and HP-5 MS capillary column (30 m × 0.25 mm; film thickness, 0.25 μm). Injector and detector temperatures were set at 220 and 290 °C, respectively. The oven temperature was programmed from 50 °C for 3 min, raised to 150 °C at a rate of 3 °C/min, held isothermal for 10 min, and finally raised to 250 °C at 10 °C/min. Helium was the carrier gas, at a flow rate of 1 mL/min. Diluted samples (1/100 in acetone, v/v) of 1.0 μL were injected manually and in the splitless mode.

GC-MS Analysis Conditions. The analysis of the essential oil was performed using a Hewlett-Packard 5890 II GC, equipped with a HP-5 MS capillary column (30 m × 0.25 mm i.d., 0.25 μm) and a HP 5972 mass selective detector. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium was the carrier gas at a flow rate of 1 mL/min. Injector and MS transfer line temperatures were set at 220 and 290 °C, respectively. The oven temperature was programmed as in the GC-FID analysis. Diluted samples (1/100, v/v, in acetone) of 1.0 μL were injected manually and in the splitless mode. The components were identified based on the comparison of their relative retention time and mass spectra with those of commercial standards (for the main components), NBS75K library data of the GC-MS system, and literature data (*28*). The results were also confirmed by the comparison of the compounds elution order with their relative retention indices on nonpolar phases reported in the literature (*28*).

Callus Cultures. Seeds were immersed in 70% aqueous EtOH for 2 min, followed by surface sterilization with a 10% NaOCl solution and a rinse with sterile double-distilled water, and then laid on Gamborg's B5 basal media (*29*), supplemented with indole-3-butyric acid (1.0 ppm), 6-benzylaminopurine (N^6-benzyladenine) (1.0 ppm), and sucrose (2.5%) as a carbon source. The initiated calli were then transferred and subcultured using the aforesaid media by transferring small callus pieces at 28 day intervals. All cultures were maintained at 25 ± 2 °C in the dark.

Microbial Strains. The methanolic extracts (both polar and nonpolar subfractions) and the essential oil and its fractions of *S. hortensis* were individually tested against a range of 38 microorganisms, among them 23 bacteria and 15 fungi and yeast species. The list of microorganisms used is given in **Table 3**. Microorganisms were provided by the Department of Clinical Microbiology, Faculty of Medicine, and Plant Diagnostic Laboratory, Faculty of Agriculture, at Ataturk University,

Erzurum, Turkey. The Identity of the microorganisms used in this study was confirmed by the Microbial Identification System in Biotechnology Application and Research Center at Ataturk University.

Antimicrobial Activity. *Disk Diffusion Assay.* The dried plant extracts were dissolved in the same solvent (methanol) to a final concentration of 30 mg/mL and sterilized by filtration through 0.45 μm Millipore filters. Antimicrobial tests were then carried out by the disk diffusion method (*30*) 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, and potato dextrose agar (PDA) medium, respectively. The disks (6 mm in diameter) were impregnated with 10 μL of essential oil, or the 30 mg/mL extracts (300 μg/disk) were placed on the inoculated agar. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. Ofloxacin (10 μg/disk), sulbactam (30 μg) + cefoperazona (75 μg) (105 μg/disk), and/or netilmicin (30 μg/disk) 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 in comparison to a control of negative and reference standards. Each assay in this experiment was repeated twice.

Microwell Dilution Assay. The minimal inhibition concentration (MIC) values were determined for the bacterial strains, which were sensitive to the essential oil and/or extracts in the disk 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 *S. hortensis*, dissolved in 10% dimethyl sulfoxide, were first diluted to the highest concentration (500 μg/mL) to be tested, and then serial 2-fold dilutions were made in order to obtain a concentration range from 7.8 to 500 μg/mL in 10 mL sterile test tubes containing nutrient broth. MIC values of *S. hortensis* essential oil and extracts against bacterial strains and *Candida albicans* isolates were determined based on a microwell dilution method (*31*) 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 amount from the stock solutions of *S. hortensis* essential oil and extracts initially prepared at the concentration of 500 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 the 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 the standard drug for 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) and confirmed by plating 5 μL samples from clear wells on NA 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.

MIC Agar Dilution Assay. MIC values of the fungi isolates were studied based on the agar dilution method, as described previously (*32*). The essential oils of *S. hortensis* were added aseptically to sterile molten PDA medium, containing Tween 20 (Sigma 0.5%, v/v), at the appropriate volume 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 and 37 °C for 72 h for 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 concentration of the essential oil where the absence of growth was recorded. Each test was repeated at least twice.

Table 1. Chemical Composition of *S. hortensis* Essential Oil (GC-FID)

no.	KI[a]	t_R (min)[b]	components	composition (%)
1	930	9.444	thujene	0.8
2	939	9.722	α-pinene	2.6
3	979	11.654	β-pinene	2.7
4	991	12.418	β-myrcene	1.7
5	1003	12.983	α-phellandrene	0.2
6	1017	13.607	α-terpinene	2.2
7	1025	14.073	*p*-cymene	9.3
8	1037	15.262	β-ocimene	0.1
9	1060	15.956	γ-terpinene	22.6
10	1089	17.175	terpinolene	0.1
11	1165	21.041	borneol	0.3
12	1177	21.626	terpinen-4-ol	0.3
13	1237	25.769	pulegone	0.2
14	1253	26.908	anethole	0.1
15	1290	27.721	thymol	29.0
16	1299	28.266	carvacrol	26.5
17	1352	30.179	thymol acetate	0.3
18	1373	30.992	carvacrol acetate	0.1
19	1419	33.074	caryophyllene	0.4
20	1441	33.906	aromadendrene	0.1
21	1500	36.394	bicyclogermacrene	0.1
22	1506	36.890	β-bisabolene	0.2
			total	99.9

[a] KI, Kovats index relative to *n*-alkanes on nonpolar DB-5 column (*32*). [b] Retention time (in minutes).

Antioxidant Activity. The methanol extract was tested for their antioxidative capacities using two complementary assays given below. BHT was included in the experiments as a positive control.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay. The DPPH assay was carried out following the same method as reported elsewhere (*15*, *33*−*35*). Fifty microliters of various concentrations of the samples dissolved in methanol was added to 5 mL of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition free radical DPPH in percent (*I*%) was calculated in the following way:

$$I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated form the graph-plotted inhibition percentage against extract concentration. The assay was carried out in triplicate.

β-Carotene−Linoleic Acid Assay. In this assay, antioxidant capacity was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides formation from linoleic acid oxidation (*21*). A stock solution of a β-carotene−linoleic acid mixture was prepared as follows: 0.5 mg of β-carotene was dissolved in 1 mL of chloroform (HPLC grade) and 25 μL of linoleic acid, and 200 mg of Tween 40 was added. The chloroform was completely evaporated using a vacuum evaporator. Then, 100 mL of distilled water saturated with oxygen (30 min at a flow rate of 100 mL/min) was added with vigorous shaking. A 350 μL amount of this reaction mixture and 350 μL portions of the extracts (2 g/L in ethanol) were dispensed in each test tube, which was incubated up to 48 h at room temperature. The same procedure was repeated with positive control BHT and a blank. After this incubation period, the absorbance of the mixtures was measured at 490 nm. Antioxidant capacities of the extracts were compared with those BHT and blank.

RESULTS AND DISCUSSION

Chemical Composition of the Essential Oil. GC-FID and GC-MS analysis of the crude oil isolated from dried aerial parts of *S. hortensis* resulted in the identification of 22 compounds, representing 99.9 and 98.8% of the essential oil, respectively. There were no significant differences in the composition of

Table 2. Chemical Composition of *S. hortensis* Essential Oil (GC-MS)

no.	KI[a]	t_R (min)[b]	components	composition (%)
1	930	9.444	thujene	0.8
2	939	9.722	α-pinene	3.0
3	979	11.654	β-pinene	2.5
4	991	12.418	β-myrcene	1.4
5	1003	12.983	α-phellandrene	0.3
6	1017	13.607	α-terpinene	2.7
7	1025	14.073	*p*-cymene	10.0
8	1037	15.262	β-ocimene	0.1
9	1060	15.956	γ-terpinene	21.5
10	1089	17.175	terpinolene	0.1
11	1165	21.041	borneol	0.3
12	1177	21.626	terpinen-4-ol	0.3
13	1237	25.769	pulegone	0.1
14	1253	26.908	anethole	0.5
15	1290	27.721	thymol	28.9
16	1299	28.266	carvacrol	26.1
17	1352	30.179	thymol acetate	0.3
18	1373	30.992	carvacrol acetate	0.1
19	1419	33.074	caryophyllene	0.4
20	1441	33.906	aromadendrene	0.1
21	1500	36.394	bicyclogermacrene	0.2
22	1506	36.890	β-bisabolene	0.1
			total	98.8

[a] KI, Kovats index relative to *n*-alkanes on nonpolar DB-5 column (*32*). [b] Retention time (in minutes).

essential oil in terms of GC-FID and GC-MS analyses (**Tables 1** and **2**) even though the percentage of chemical composition of *S. hortensis* identified by GC-FID (99.9%) was higher than GC-MS (98.8%). Thymol (29.0%), carvacrol (26.5%), and their biosynthetic precursors γ-terpinene (22.6%) and *p*-cymene (9.3%) were the main components (87.4%) of the oil based on GC-FID analysis (**Table 1**). The presence of thymol and carvacrol in almost equal amounts is characteristic of the essential oil composition. As reported elsewhere, the genus *Satureja* may or may not contain phenols, and the phenol-containing species are divided into "carvacrol type" and "thymol type" (*12*). In agreement with this point, the essential oils obtained from cultivated summer *S. hortensis*, as well as *Satureja pilosa*, *Satureja icarica*, and *Satureja montana*, were characterized by the presence of carvacrol, while thymol was absent or detectable in small amounts and traces (*8*, *12*, *13*). On the other hand, relatively higher amounts of thymol than carvacrol have been observed in the essential oil of *Satureja spicigera* from different localities in Turkey (*11*). In our case, there is not this clear division, but a thymol/carvacrol chemotype is determined, which contains those phenols at a ratio of about 1:1. Carvacrol (59.7%), γ -terpinene (12.8%), and *p*-cymene (9.3%) were reported as major constituents of the oil isolated from the seeds of *S. hortensis* (*36*). As emphasized before, essential oil content can be affected by the influence of several local, climatic, and seasonal factors. For example, severe water stress was reported to alter carvacrol/γ-terpinene contents (*37*). On the other hand, callus cultures of *S. hortensis* were found to be incapable of producing essential oil.

Antimicrobial Activity. The antimicrobial activities of *S. hortensis* essential oil and extracts assayed against the microorganisms considered in the present study were qualitatively and quantitatively assessed evaluating the presence of inhibition zones, zone diameter, and MIC values. As shown in **Tables 3** and **4**, the polar subfraction of the methanol extract or callus extract did not show any activity, whereas the nonpolar (chloroformic) one was found to be effective against five out of 23 bacterial species, which were *Bacillus subtilis*, *Enterococcus fecalis*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, and *Streptococcus pyogenes*. Although no generalization can be made, the nonpolar phase or subfraction exhibits, in many cases, greater activity than the polar one (*27*). The activity observed in the nonpolar phase can be attributed to the presence of the phenolic compounds (*38*). On the other hand, the essential oil of *S. hortensis* had great potential of antimicrobial activities

Table 3. Antimicrobial Activity of *S. hortensis* Extract and Essential Oil against the Bacterial Strains Tested Based on Disk Diffusion Method

	inhibition zone in diameter (mm) around the disks impregnated with 10 μL of essential oil and extracts (300 μg/disk)					
		methanol extracts from plants		MeOH		
bacterial species	essential oil (10 μL/disk)	polar	nonpolar	callus culture[a]	negative control	standard antibiotic disks[b]
Acinetobacter baumanii-A8	19					18 (OFX)
Bacillus amyloliquefaciens-142	24					27 (SCF)
Bacillus cereus-RK75	26					30 (OFX)
Bacillus macerans-M58	26					19 (OFX)
Bacillus megaterium-M3	24					9 (SCF)
B. subtilis-ATCC-6633	21		16			28 (OFX)
Brucella abortus-A77	5					12 (SCF)
Burkholdria cepacia-PR-1217	12					22 (SCF)
Clavibacter michiganense-A227	26					25 (SCF)
Enterobacter cloacae-A135	18					20 (NET)
E. fecalis-ATCC-29122	21		9			18 (SCF)
Escherichia coli-Hak59	13					(OFX)
Klebsiella pneumoniae-A137	23					12 (OFX)
Proteus vulgaris-A161	18					12 (OFX)
P. aeruginosa-ATCC-9027	27		11			22 (NET)
Pseudomonas fluorescens-RK242	29					10 (OFX)
Pseudomonas syringae pv. *Tomato*-A35	17					24 (OFX)
S. enteritidis-IK27	18		16			27 (SCF)
Staphylococcus aureus-ATCC-29213	22					22 (SCF)
Staphylococcus epidermis-A233	18					(SCF)
Streptococcus pneumoniae-IK3	21					(OFX)
S. pyogenes-ATCC-176	16		11			10 (OFX)
Xanthomonas campestris-A235	15					20 (SCF)
total of 23 bacterial species		5–29		9–16		

[a] MeOH = methanol extract. [b] OFX = ofloxacin (10 μg/disk); SCF = sulbactam (30 μg) + cefoperazona (75 μg) (105 μg/disk); and NET = netilmicin (30 μg/disk) were used as positive reference standards antibiotic disks (Oxoid).

Table 4. Antimicrobial Activity of *S. hortensis* Extract and Essential Oil against the Yeast and Fungi Isolates, Tested Based on Disk Diffusion Method

	inhibition zone in diameter (mm) around the disks impregnated with 10 μL of essential oil and extracts (300 μg/disk)					
	essential oil	methanol extracts from plants		MeOH		standard antibiotic
yeast and fungi species	(10 μL/disk)	polar	nonpolar	callus culture[a]	negative control	disks[b]
			yeast			
C. albicans-A117	20					(NET)
			fungi			
Alternaria alternata	27					(NET)
Aspergillus flavus	37					(NET)
Aspergillus variecolor	27					(NET)
Fusarium culmorum	33					(NET)
Fusarium oxysporum	26					(NET)
Penicillium spp.	31					(NET)
Rhizopus spp.	23					(NET)
Rhizoctonia solani	25					(NET)
Moniliania fructicola	30					(NET)
Trichophyton rubrum	19					(NET)
Trichophyton mentagrophytes	17					(NET)
Microsporum canis	21					(NET)
Sclerotinia sclerotiorum	29					(NET)
Sclerotinia minor	25					(NET)
total 15 isolates	17–37					

[a] MeOH = methanol extract. [b] OFX = ofloxacin (10 μg/disk); SCF = sulbactam (30 μg) + cefoperazona (75 μg) (105 μg/disk); and NET = netilmicin (30 μg/disk) were used as positive reference standards antibiotic disks (Oxoid).

Table 5. MIC Values of the Essential Oil and Nonpolar Subfraction of the Methanol Extract of *S. hortensis* Plants against the Bacterial Strains Tested in the Microdilution Assay (μg/mL)

	MeOH extract		
bacteria species	essential oil	nonpolar subfraction	standard drug (Maxipime)
A. baumanii-A8	125		31.25
B. amyloliquefaciens-142	62.5		15.62
B. cereus-RK75	125		31.25
B. macerans-M58	125		15.62
B. megaterium-M3	125		15.62
B. substilis-ATCC-6633	31.25	250	7.80
B. abortus-A77	500		62.5
B. cepacia PR-1217	500		125
C. michiganense-A227	62.5		15.62
E. cloacae-A135	250		31.25
E. faecalis-ATCC-29122	125	500	31.25
E. coli-Hak59	250		62.5
K. pneumoniae-A137	62.5		125
P. vulgaris-A161	250		125
P. aeruginosa-ATCC-9027	31.25	250	31.25
P. fluorescens-RK242	62.5		31.25
P. syringae pv. *Tomato*-A35	250		125
S. enteritidis-IK27	125	500	62.5
S. aureus-ATCC-29213	125		31.25
S. epidermis-A233	125		15.62
S. pneumoniae-IK3	62.5		31.25
S. pyogenes-ATCC-176	250	500	62.5
X. campestris-A235	125		31.25
	31.25–500	250–500	7.80–125

Table 6. MIC Values of *S. hortensis* Essential Oil against the Yeast and Fungi Isolates Tested in Agar Dilution Assay (μg/mL)

bacteria species	essential oil	standard drug (Amphotericin B)
	yeast	
C. albicans-A117	250	31.25
	fungi	
A. alternata	62.5	15.62
A. flavus	31.25	15.62
A. variecolor	125	62.5
F. culmorum	125	31.25
F. oxysporum	250	62.5
Penicillium spp.	125	31.25
Rhizopus spp.	250	
R. solani	125	31.25
M. fructicola	31.25	15.62
T. rubrum	31.25	15.62
T. mentagrophytes	62.5	31.25
M. canis	62.5	15.62
S. sclerotiorum	125	62.5
S. minor	250	125
	31.25–250	15.62–125

against all of the 23 bacteria and 15 fungi and yeast species tested. The maximal inhibition zones and MIC values for bacterial strains, which were sensitive to the essential oil and nonpolar extract of *S. hortensis*, were in the range of 5−29 mm and 31.25−500 μL/mL and 9−16 mm and 250−500 μL/mL, respectively (**Tables 3** and **5**). The maximal inhibition zones and MIC values of the yeast and fungi species sensitive to the essential oil of *S. hortensis* were 17−37 mm and 31.25−250 μL/mL, respectively (**Tables 4** and **6**). Findings in this study supported the observations of some other researchers about *S. hortensis* containing some substances with antibacterial properties (*2*, *4*, *6*). On the basis of these results, it is possible to conclude that the essential oil of *S. hortensis* has a stronger and broader spectrum of antimicrobial activity as compared to the other crude plant extracts tested. The antifungal activity of *S. hortensis* essential oil in this study seemed to be higher than that of amphotericin B, the positive standard (**Table 6**). Even though *S. hortensis* essential oil has been evaluated in terms of antimicrobial activity against a limited number of microorganisms by Deans and Svoboda (1989), this is the first study confirming that the essential oil from *S. hortensis* possesses a wide antimicrobial spectrum, since it inhibits the growth of all of the human and plant pathogenic and/or food spoilage bacteria, fungi, and yeast species tested. Furthermore, this study shows that *S. hortensis* essential oil has a high content (86.5%) of thymol, carvacrol, γ-terpinene, and *p*-cymene, which were found in the essential oils composition of some medicinal plants including a few *Satureja* species but not *S. hortensis* (*33*, *39*−*44*). The antimicrobial nature of the essential oils is apparently

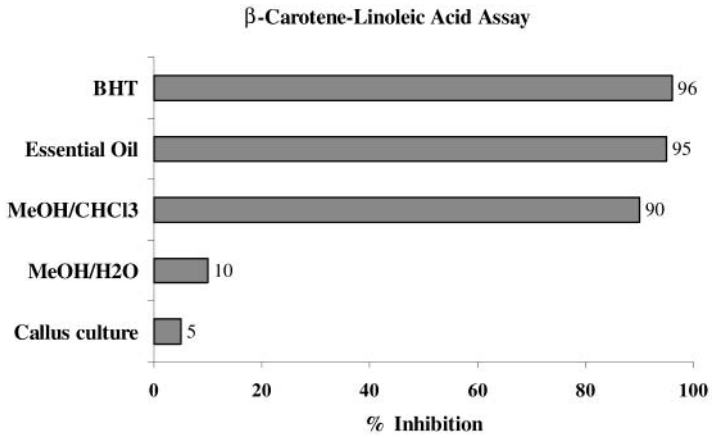

Figure 1. Inhibition of the linoleic acid oxidation in the presence of the essential oil, polar and nonpolar subfractions of the methanol extracts from the aerial parts, and the methanol extract from the callus cultures of *S. hortensis* in β-carotene–linoleic acid assay (BHT was used as the positive control).

Table 7. Effects of *S. hortensis* Methanol Extracts and Positive Control on the In Vitro Free Radical (DPPH) Scavenging IC_{50} (μg/mL)

sample	DPPH
the oil	350.00 ± 5.00
water subfraction of MeOH extract	30.89 ± 0.80
chloroform subfraction of MeOH extract	86.26 ± 0.50
callus culture (MeOH extract)	23.76 ± 0.80
BHT (positive control)	19.80 ± 0.50

related to their high phenolic contents, particularly carvacrol and thymol (*33*, *39*−*42*). This claim is further supported by our findings (**Tables 1** and **2**), demonstrating a high content of carvacrol and thymol in the essential oil of *S. hortensis.* Besides, the synergistic effect of these chemicals could also account for the activity of the essential oil (*44*−*46*). Preliminary screening of both standard and isolated compounds (carvacrol, thymol, γ-terpinene, and *p*-cymene) on the same microorganisms, under identical conditions, showed similar results (unpublished data).

Antioxidant Activity. Free radical scavenging activity of various extracts of *S. hortensis* is given in **Table 7** and **Figure 1**. The polar subfraction of the methanol extract of intact plant and methanol extract of in vitro tissues was able to reduce the stable free radical DPPH to the yellow-colored diphenylpicrylhydrazine. The strongest effect was observed for the tissue culture extract, with an IC_{50} value of 23.76 ± 0.80 μg/mL, which could be compared with the synthetic antioxidant agent BHT (19.80 ± 0.50 μg/mL). (The production of antioxidant agents via tissue culture system is especially state of the art. This effect can be improved by changing the culture conditions, and the production of these agents may be promoted.) On the other hand, linoleic acid oxidation was 95% inhibited in the presence of the essential oil while inhibition was 90% with chloroform subfraction of the intact plant (**Figure 1**). Other extracts showed weaker or no activity. In contrast to free radical scavenging, linoleic acid oxidation was not greatly inhibited by the extracts obtained from tissue cultures. Antioxidant properties of thymol, carvacrol, and γ-terpinene were reported previously (*47*−*49*). Therefore, the activity of the essential oil is considered related to high contents of these components in the oil. (The polar subfraction as well as the callus culture were found to expose high activity.) The presence of phenolic acids such as rosmarinic acid derivatives in *S. hortensis* has been reported (*50*), and these polyphenols proved to possess an antioxidant activity in different test systems (*51*). The activity observed in DPPH assay can be related to the presence of rosmarinic acid derivatives in the extract. The production of these phenolic acids might be promoted in the callus cultures by changes in media composition and culture conditions, inducing the cells to overproduce the targeted molecules in vitro.

CONCLUSION

The essential oil and nonpolar subfraction of the methanol extract of *S. hortensis* contains compounds with antimicrobial properties, which can be used as antimicrobial agents in pharmaceuticals and natural therapies of infectious diseases in humans, management of plant diseases, and preservation and/or extension of the shelf-life of raw and processed foods. The results in the present study support the use of *S. hortensis* plants as tea or additives in foods and traditional remedies for the treatment of infectious diseases.

The antioxidant activity of the *S. hortensis* extracts indicates that they have a protective effect against ROS and can therefore be used as a natural preservative ingredient in the food or pharmaceutical industry. The antioxidative activity observed in the in vitro cultured callus is particularly important, since the production of active principles can be provided throughout the year.

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