JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Maturation on the Composition and Biological Activity of the Essential Oil of a Commercially Important *Satureja* Species from Turkey: *Satureja cuneifolia* Ten. (Lamiaceae)

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The essential oil obtained by hydrodistillation from aerial parts of *Satureja cuneifolia* Ten., collected in three different maturation stages such as preflowering, flowering, and postflowering, were analyzed simultaneously by gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS). Thymol (42.5–45.2%), *p*-cymene (19.4–24.3%), and carvacrol (8.5–13.2%) were identified as the main constituent in all stages. At the same time, the essential oils and main components were evaluated for their antimicrobial activity using a microdilution assay resulting in the inhibition of a number of common human pathogenic bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and the yeasts *Candida albicans* and *Candida tropicalis*. The minimum inhibitory concentrations (MIC) varied between 62.5 and 250 μg/mL within a moderate antimicrobial activity range. Furthermore, the antioxidant capacity of the essential oils and major components thymol and carvacrol were examined *in vitro*. The essential oils obtained from *S. cuneifolia* in three different stages and its main components were interacted with 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) as a nitrogen-centered stable radical, resulting in IC_{50} = 1.6–2.1 mg/mL. In addition, the effects on inhibition of lipid peroxidation of the essential oils were assayed using the β-carotene bleaching method. All of the tested oils inhibited the linoleic acid peroxidation at almost the same level as butylated hydroxytoluene (BHT) (93.54–94.65%). BHT and ascorbic acid were used as positive controls in the antioxidant assays.

KEYWORDS: ***Satureja cuneifolia;*** **Lamiaceae; essential oil composition; antimicrobial activity; antioxidant activity**

INTRODUCTION

Spices and their products have been used in the food industry for their flavoring and biological activities since ancient times. The shelf life and stability of food products are important issues for the industry. Oxidation, deterioration, and microbial reactions occurring in food products may cause economic loss. Deterioration of the lipids is catalyzed by different internal and external factors such as free radicals, metal ions, light, and temperature (*1–3*). Commonly used synthetic antioxidants such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), and *tert*-butylated hydroquinone (TBHQ) are used to protect the lipids against oxidation. However, in the past decade, scientists have focused on the use of natural sources as antioxidant agents due to harmful effects of the synthetics (*4, 5*).

Antibacterial, antifungal, and antioxidant activities of the aromatic plants and their essential oils are known and well studied. Antioxidant effects of aromatic plants are mainly due to the presence of hydroxyl groups in their phenolic compounds, which may protect the unsaturated lipids present in both foods and the body. Antimicrobial activities of essential oils have been recognized, and bioactive essential oils find more applications also in the pharmaceutical industry (*1, 2, 6*).

The Lamiaceae family is well-known because of the antioxidant properties of its taxa (*1*). Rosemary and sage are used as natural antioxidants both in food and in the pharmaceutical industries. Turkey is an important gene center for the species belonging to the Lamiaceae family. The endemism rate within the family is quite high (44.2%). *Satureja* belongs to the Lamiaceae family and is known and used in Turkey as "kekik", which is the equivalent of oregano. *Satureja* species contain antioxidant and antimicrobial components similar to those in rosemary and sage. The essential oils of *Satureja* species have been recently investigated for their chemical composition and antioxidant and antimicrobial properties as well as some other physiological activities (*2−4, 7, 8*). Dried herbal parts of wildcrafted *Satureja cuneifolia* are exported from Turkey as part of kekik (oregano). It does not have a harmonized system code for export yet.

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10.1021/jf0732253 CCC: $40.75
Published on Web 02/22/2008

The aim of the present work was to evaluate the changes in the chemical composition and antimicrobial and antioxidant activities of *S. cuneifolia* essential oils isolated from preflowering, flowering, and postflowering period collected plant material as well as the main components.

MATERIALS AND METHODS

Materials. The aerial parts of *S. cuneifolia* were collected in three different seasons (preflowering, flowering, and postflowering stages) in April, June, and August 2002, respectively, from Kiraz-Izmir in Turkey. Voucher specimens are kept at the Herbarium of the Faculty of Pharmacy of Anadolu University in Eskişehir, Turkey (ESSE 14424, 14425, and 14426, respectively). All chemicals, standard substances, and solvents of high purity (>99%) were purchased from Sigma, Aldrich, or Merck.

Extraction of the Essential Oils. Dried aerial parts of *S. cuneifolia* collected at three different maturation stages (preflowering, flowering, and postflowering stages) were hydrodistilled for 3 h using a Clevenger apparatus.

GC and GC-MS Analysis of the Essential Oils. The essential oils of *S. cuneifolia* were analyzed by GC using a Hewlett-Packard 6890 system (SEM Ltd., Istanbul, Turkey), and a HP Innowax FSC column (60 m × 0.25 mm ∅, with 0.25 μm film thickness) was used with nitrogen at 1 mL/min. Initial oven temperature was 60 °C for 10 min and increased at 4 °C/min to 220 °C, then kept constant at 220 °C for 10 min and increased at 1 °C/min to 240 °C. Injector temperature was set at 250 °C. Percentage composition of the individual components was obtained from electronic integration using flame ionization detection (FID, 250 °C). *n*-Alkanes were used as reference points in the calculation of relative retention indices (RRI). Three injections were done, and the results of analysis are expressed as mean ± standard deviation (SD) ($n = 3$) as seen in **Table 1**.

GC-MS analyses were performed using a Hewlett-Packard GCD, system (SEM Ltd.), and an Innowax FSC column (60 m × 0.25 mm ∅, 0.25 μm film thickness) was used with helium. GC oven temperature conditions were as described above, the split flow was adjusted at 50 mL/min, and the injector temperature was at 250 °C. Mass spectra were recorded at 70 eV, and the mass range was from *m*/*z* 35 to 425.

Identification of the essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to the series of *n*-alkanes. Computer matching against commercial libraries (Wiley and MassFinder 2.1) (*9, 10*) and the in-house "Baser Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data (*11–14*), was also used for the identification.

Antimicrobial Activity. A microdilution broth susceptibility assay was used (*15, 16*). All microorganisms were obtained from various culture collections (ATCC, NRRL) or clinical isolates (Eskişehir Osmangazi University, Eskişehir) and stored at 85 °C. Prior to use in the assay, microorganisms were inoculated on Mueller−Hinton agar (MHA; Merck, Darmstadt, Germany) in Petri dishes for purity check. Stock solutions of the essential oils, pure substances such as carvacrol and thymol, and the antimicrobial agents (chloramphenicol and ketoconazole) (Sigma-Aldrich, Taufkirchen, Germany) were prepared in 25% (*v*/*v*) dimethyl sulfoxide (DMSO; Carlo Erba, Milan, Italy). Serial dilutions from 2000 to 1.94 μg/mL were prepared using sterile distilled water in a 96-well microtiter plate. Microbial suspensions were grown overnight in double-strength Mueller−Hinton broth (MHB; Merck) and were standardized to 10^8 colony-forming units (CFU) mL^{-1} (McFarland no. 0.5). One hundred microliters of each microbial suspension was then added to the appropriate well. DMSO was also applied as another control to eliminate solvent effects. The last row, which contained only the serial dilutions of the essential oil without microorganism, was used as a negative control. After incubation at 37 °C for 24 h, the first well without turbidity was determined as the minimum inhibitory concentration (MIC, μg/mL). Chloramphenicol and ketoconazole were used as antimicrobial positive controls, and the experiment was repeated in triplicate. Average MIC results are given in **Table 2**.

Antioxidant Activity. *1,1-Diphenyl-2-picrylhydrazyl Radical Scavenging Assay.* The ability of the fractions to scavenge DPPH• radicals was determined according to the method of Gyamfi et al. (*17*). A 50 μL aliquot of each fraction, in 50 mM Tris-HCl buffer (pH 7.4), was mixed with 450 μL of Tris-HCl buffer and 1.0 mL of 0.1 mM DPPH in MeOH. After 30 min of incubation in darkness and at ambient temperature, the resultant absorbance was recorded at 517 nm. The percentage inhibition was calculated by the difference of absorbance of control and the sample versus the absorbance of control multiplied by a factor of 100. BHT and ascorbic acid were used as positive controls at a concentration between 0.00 and 1.00 mg/mL. The resulting IC_{50} values are presented as the average of quadruplicate analyses.

β-Carotene−Linoleic Acid Co-oxidation Inhibition Assay. The antioxidant activities of the essential oils of *S. cuneifolia* and its main components were determined according to β-carotene bleaching methods (*18, 19*). Briefly, 1 mL of β-carotene (0.2 mg/mL dissolved in chloroform) was added to a flask containing linoleic acid (40 mg) and Tween 80 (400 mg). Chloroform was evaporated under a stream of nitrogen. Fifty milliliters of distilled water was added and shaken vigorously. Control was prepared without sample or standards according to the same procedure. Blanks of control and sample were also prepared without β-carotene. Their absorbances were measured on a spectrophotometer at 470 nm. The samples were then subjected to thermal autoxidation by keeping them in a constant-temperature water bath at 50 °C for 2 h. The rate of bleaching of β-carotene was monitored by taking the absorbance at 15 min intervals. Antioxidative activity (AA), oxidation rate ratio (ORR), and antioxidant activity coefficient (AAC) were calculated according to the method of Oomah and Mazza (*18*). BHT and ascorbic acid were used as positive controls at a final concentration of 0.3 mg/mL. The formulas used are

$$AA\% = [1 - (Ab_s^0 - Ab_s^{120})/(Ab_c^0 - Ab_c^{120})] \times 100$$

$$ORR = R_s/R_c$$

$$AAC = [Abs^{120} - Abc^{120})/(Abc^0 - Abc^{120})] \times 100$$

where Ab_s^0 is the absorbance of the sample at $t = 0$ min, Ab_s^{120} is the absorbance of the sample at $t = 120$ min, Ab_c^0 is the absorbance of the control at $t = 0$ min, Ab_c^{120} is the absorbance of the control at $t = 120$ min, and R_c and R_s are the bleaching rates of β-carotene in reactant mix without antioxidant and with plant extract, respectively.

RESULTS AND DISCUSSION

Essential Oil Composition. The essential oils of *S. cuneifolia* samples collected in three different maturation stages (preflowering, flowering, and postflowering stages) were isolated by using Clevenger-type water distillation. The essential oils were obtained in 1.1–1.3% yields.

Ninety-seven compounds were identified in all of the oils analyzed, and the results are given in **Table 1**. Thymol (42.47–45.20%), carvacrol (8.50–13.20%), and *p*-cymene (19.43–24.30%) were found as main components in all samples of the three maturation stages. The essential oil of the preflowering sample was rich in monoterpene hydrocarbons (36.15%), whereas the oil obtained from the flowering sample was rich in oxygenated monoterpenes (66.43%), according to **Table 1** and **Figure 1**. The amount of monoterpenes decreased during the flowering stages, whereas the total amounts of oxygenated monoterpenes increased. After the flowering stage, these amounts were reversed as seen in **Figure 1**. The main components (γ-terpinene, *p*-cymene, thymol, and carvacrol) in the essential oils obtained from different maturation stages were statistically analyzed. The amounts of thymol were found to be statistically the same in all stages ($p > 0.05$), whereas γ-terpinene was statistically decreased during maturation ($p > 0.05$). The amount of *p*-cymene was statistically ($p > 0.05$) decreased during the flowering stage, whereas the carvacrol amount was increased ($p > 0.05$).

Table 1. Chemical Composition of the Essential Oils of *Satureja cuneifolia*

RRI[a]	component	preflowering stage[b]	flowering stage[b]	postflowering stage[b]	identification method
1014	tricyclene	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	a
1032	α-pinene	1.57 ± 0.06	1.20 ± 0.00	1.83 ± 0.12	a, b
1035	α-thujene	0.27 ± 0.06	0.20 ± 0.00	0.30 ± 0.00	a
1051	2,5-dimethyltetrahydrofuran	0.10 ± 0.00	tr[c]	0.10 ± 0.00	a
1076	camphene	1.37 ± 0.06	1.10 ± 0.00	1.73 ± 0.12	a, b
1118	β-pinene	0.20 ± 0.00	0.10 ± 0.00	0.20 ± 0.00	a, b
1174	myrcene	0.90 ± 0.26	0.63 ± 0.06	0.70 ± 0.10	a, b
1176	α-phellandrene	0.10 ± 0.10	0.10 ± 0.00	0.10 ± 0.00	a, b
1188	α-terpinene	1.33 ± 0.06	1.17 ± 0.06	1.07 ± 0.06	a, b
1203	limonene	0.67 ± 0.06	0.50 ± 0.00	0.87 ± 0.06	a, b
1213	1,8-cineole	0.53 ± 0.06	0.47 ± 0.06	0.70 ± 0.00	a, b
1218	β-phellandrene	0.20 ± 0.00	0.17 ± 0.06	0.20 ± 0.00	a
1225	(*Z*)-3-hexenal	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	a
1246	(*Z*)-β-ocimene	0.50 ± 0.00	0.40 ± 0.00	0.50 ± 0.00	a
1255	γ-terpinene	4.57 ± 0.15*	4.20 ± 0.26**	2.50 ± 0.10**	a, b
1266	(*E*)-β-ocimene	0.20 ± 0.00	0.20 ± 0.00	0.10 ± 0.00	a
1280	*p*-cymene	24.07 ± 0.97*	19.43 ± 1.08**	24.30 ± 1.15*	a, b
1290	terpinolene	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	a, b
1393	3-octanol	0.20 ± 0.00	0.10 ± 0.00	0.20 ± 0.00	a
1450	*trans*-linalool oxide (furanoid)	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	a
1452	α,*p*-dimethylstyrene	tr	tr	0.07 ± 0.06	a
1452	1-octen-3-ol	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	a
1474	*trans*-sabinene hydrate	0.10 ± 0.00	0.10 ± 0.00	0.30 ± 0.00	a
1478	*cis*-linalool oxide (furanoid)	tr	0.10 ± 0.00	0.10 ± 0.00	a
1553	linalool	1.43 ± 0.06	1.63 ± 0.12	1.93 ± 0.06	a, b
1556	*cis*-sabinene hydrate	tr	tr	0.07 ± 0.06	a
1571	*trans*-*p*-menth-2-en-1-ol	tr	tr	0.10 ± 0.00	a
1604	thymol methyl ether	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	a, b
1611	terpinen-4-ol	0.80 ± 0.00	0.90 ± 0.00	1.17 ± 0.06	a, b
1614	carvacrol methyl ether	0.20 ± 0.00	0.40 ± 0.00	0.20 ± 0.00	a, b
1628	aromadendrene	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	a
1630	terpinen-4-yl acetate	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	a
1638	*cis*-*p*-menth-2-en-1-ol	tr	tr	0.10 ± 0.00	a
1683	*trans*-verbenol	tr	tr	0.07 ± 0.06	a
1704	γ-muurolene	tr	0.07 ± 0.06	0.10 ± 0.00	a
1706	α-terpineol	0.20 ± 0.00	0.20 ± 0.00	0.23 ± 0.06	a, b
1708	ledene	tr	tr	0.07 ± 0.06	a
1719	borneol	2.80 ± 0.00	2.97 ± 0.06	3.57 ± 0.06	a, b
1741	β-bisabolene	0.53 ± 0.06	0.63 ± 0.06	0.63 ± 0.06	a
1773	δ-cadinene	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	a
1776	γ-cadinene	tr	tr	0.10 ± 0.00	a
1802	cumin aldehyde	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	a, b
1864	*p*-cymen-8-ol	0.30 ± 0.00	0.23 ± 0.06	0.30 ± 0.00	a, b
1867	thymol acetate	0.10 ± 0.00	0.13 ± 0.06	0.10 ± 0.00	a, b
1890	carvacryl acetate	tr	0.07 ± 0.06	tr	a, b
1941	4-isopropyl salicylaldehyde	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	a
1942	4-hydroxy-2-methyl acetophenone[d]	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	a
2008	caryophyllene oxide	0.47 ± 0.06	0.47 ± 0.12	0.57 ± 0.06	a, b
2098	globulol	0.07 ± 0.06	0.10 ± 0.00	0.10 ± 0.00	a
2113	cumin alcohol	tr	0.03 ± 0.06	0.07 ± 0.06	a, b
2144	spathulenol	0.33 ± 0.06	0.43 ± 0.06	0.37 ± 0.06	a, b
2181	isothymol	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	a, b
2198	thymol	44.53 ± 1.21*	45.20 ± 0.82 *	42.47 ± 1.45 *	a, b
2221	isocarvacrol	0.20 ± 0.00	0.20 ± 0.00	0.10 ± 0.00	a, b
2239	carvacrol	9.23 ± 0.31**	13.20 ± 0.44*	8.50 ± 0.30**	a, b
2389	caryophylla-2(12),6-dien-5α-ol (= caryophyllenol I)	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	a
2392	caryophylla-2(12),6-dien-5β-ol (= caryophyllenol II)	0.10 ± 0.00	0.07 ± 0.06	0.10 ± 0.00	a
2931	hexadecanoic acid	0.10 ± 0.00	0.13 ± 0.06	0.13 ± 0.06	a, b
	monoterpene hydrocarbons	36.15	29.6	34.6	
	oxygenated monoterpenes	61.02	66.43	60.68	
	sesquiterpene hydrocarbons	0.83	1.0	1.2	
	oxygenated sesquiterpenes	1.07	1.17	1.24	
	others	0.9	0.73	1.0	
	identified compounds (%)	99.97 ± 0.21	98.93 ± 0.12	98.72 ± 0.26	

[a] Relative retention indices calculated against *n*-alkanes on the HP Innowax column. [b] Mean percent calculated from FID data ± SD ($n = 3$). Values are presented as means ± 95% confidence intervals. Numbers with the same symbols (*, **, ***) are not significantly ($p > 0.05$) different. [c] Trace (<0.1%). [d] From Wiley library: a, comparison of mass spectra with the Wiley and Mass Finder libraries and retention times; b, comparison with genuine compounds on the HP Innowax column.

In all investigated samples of the essential oils of *S. cuneifolia* 39 compounds were identified in trace amounts.

Different compounds have been found and reported previously as main constituents in *S. cuneifolia* essential oils. Generally, thymol, carvacrol, *p*-cymene, γ-terpinene, limonene, and β-cubebene were reported as major compounds in *Satureja* essential oils (*2–8, 20, 21*). The *Satureja* species of Turkey were reported to be rich in either thymol or carvacrol, with a high

Table 2. Minimal Inhibitory Concentration (MIC, μg/mL) of *Satureja cuneifolia* Essential Oils[a]

microorganism	culture	A	B	C	Car	Thy	Chl	Keto
Escherichia coli, Gr (−)	NRRL B-3008	250	500	250	250	250	3.9	nt[b]
Pseudomonas aeruginosa, Gr (−)	ATCC 27853	500	500	500	250	500	7.8	nt
Enterobacter aerogenes, Gr (−)	NRRL 3567	500	250	250	250	500	1.9	nt
Proteus vulgaris, Gr (−)	NRRL B-123	250	250	250	250	250	nt	nt
Salmonella typhimurium, Gr (−)	ATCC 13311	500	250	250	250	250	7.8	nt
Bacillus cereus, Gr (+)	NRRL B-3711	500	250	250	250	250	nt	nt
MRSA, Gr (+)[c]	clinical isol[d]	500	250	250	250	250	31.3	nt
Candida albicans, yeast	clinical isol	250	125	500	125	125	nt	62.5
Candida tropicalis, yeast	NRRLY-12968	250	250	62.5	125	62.5	nt	250

[a] A, preflowering stage; B, flowering stage; C, postflowering stage; Car, carvacrol; Thy, thymol; Chl, chloramphenicol; Keto, ketoconazole. [b] Not tested. [c] Methycillin-resistant *Staphylococcus aureus*. [d] Eskişehir Osmangazi University, Faculty of Medicine, Eskisehir.

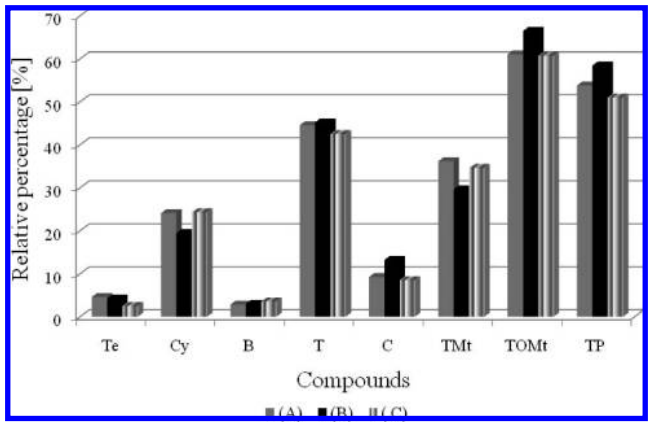

Figure 1. Seasonal variation of the major components in essential oils of *S. cuneifolia* [Te, γ-terpinene; Cy, *p*-cymene; B, borneol; T, thymol; C, carvacrol; TMt, total monoterpenes; TOMt, total oxygenated momoterpenes; TP, total phenolics (carvacrol and thymol); A, preflowering; B, flowering; C, postflowering].

amount of *p*-cymene. Major components were identified in those papers as thymol (17.5–45.2%), carvacrol (18.9–64.4%), *p*-cymene (10.2–59.0%), and γ-tepinene (13.40–29.0%) (*7*). The characterization of essential oils of *Satureja* species may show variations within the same species, and these changes seem to depend on the genetic variations and geographical differences (*4, 22*).

Antimicrobial Activity. The antimicrobial activities of essential oils of *S. cuneifolia* samples collected in three different maturation periods as well as two of the major compounds, carvacrol and thymol, were evaluated against seven human pathogenic bacteria and two yeasts in the present study. The results are given in **Table 2** as minimal inhibitory concentration (MIC, μg/mL). Chloramphenicol and ketoconazole were used in an adapted microdilution broth susceptibility assay (*16*) as positive controls against the bacteria and *Candida* species, respectively. Overall, the essential oils of *S. cuneifolia* exhibited relatively good antifungal activity (62.5–500 μg/mL) against *Candida* species rather than antibacterial compared to the assayed Gram-positive and Gram-negative bacteria (125–500 μg/mL). Also, an increase of the antimicrobial activity during the maturation period was observed. Good inhibitions were observed using thymol, carvacrol, and the postflowering sample (C) against the pathogenic yeast *C. tropicalis* (62.5, 125, and 62.5 μg/mL, respectively), comparably a better inhibition than the antifungal standard agent (MIC, 250 μg/mL). The antibacterial activity of the oils was rather moderate (250–500 μg/mL).

In previous activity works, the antimicrobial activity level of the essential oils from different *Satureja* species showed variations, which were associated with the composition of the essential oils (*5–7, 21, 23*). In general *S. cuneifolia* essential oils containing thymol and carvacrol as major constituents showed higher antimicrobial activity against Gram-postive and Gram-negative bacteria and fungi when compared to the β-cubebene- and limonene-rich oils (*7, 21*). Skočibušić et al. (*3*) reported the antimicrobial importance of the presence of other constituents such as β-cubebene, limonene, α-pinene, spathulenol, and β-caryophyllene within the essential oils other than carvacrol and its isomer thymol. Cosentino et al. (*22*) investigated the inhibitory effects of thymol, carvacrol, and *p*-cymene among other monoterpenes against a large number of bacterial strains and reported that high relative amounts of *p*-cymene may exert an antagonistic antimicrobial effect in the presence of phenolic constituents resulting in weaker inhibitions. Güllüce et al. (*5*) also demonstrated that *Satureja hortensis* growing in Turkey with thymol, carvacrol, γ-terpinene, and *p*-cymene as major constituents in the oil showed stronger inhibitory effects than its methanol extract against a wide spectrum of microorganisms. A recent study on the influence of the harvesting time on essential oil and antibacterial activity was performed on *Satureja thymbra* and *Satureja parnassica*; phytochemical variation was present and affected the activity potency with synergy in respect to the presence of the phenolic terpenes carvacrol and thymol. In another study, *S. cuneifolia*, which contained β-cubebene (8.7%), limonene (8.3%), and α-pinene (6.9%) as major constituents, was found to be active against the bacteria *Staphylococcus aureus* and *Escherichia coli* and MRSA with MICs of 0.5−6% (*3*).

In our present results, it was also observed that the same antagonism may be due to the *p*-cymene level and monoterpene phenols such as thymol and carvacrol, rather than a synergy which would be expected. The analysis of the investigated essential oil of *S. cuneifolia* showed the presence of relatively high amounts of *p*-cymene (19.43–24.30%) and comparably lower amounts of carvacrol (8.50–13.20%), which may support the relatively weak antimicrobial and, in particular, antibacterial activities of the essential oils in this study. However, the selective antifungal activity toward *Candida* species is worth pursuing.

As a conclusion, with respect to the relatively potent antioxidant and antimicrobial effects, *Satureja* oils are also a good alternative for *Origanum* species used as oregano. These may especially be used in foods, food systems, beverages, and herbal teas as microbial preventives and shelf life prolongers.

Antioxidant Activity. Generally, DPPH• radical scavenging and β-carotene−linoleic acid co-oxidation assay are used to determine the antioxidant capacity of essential oils. Because the activity of the tested compound depends on the polarity of the reaction media, both polar and nonpolar activity tests can be used to determine *in vitro* activity.

The DPPH• radical scavenging assay is one of the most used model reaction systems to determine the radical scavenging

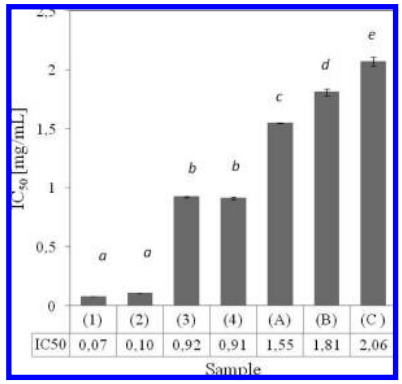

Figure 2. Radical scavenging activities of *S. cuneifolia* essential oils and standards on DPPH• (1, BHT; 2, ascorbic acid; 3, thymol; 4, carvacrol; A, preflowering; B, flowering; C, postflowering). Values are presented as means ± 95% confidence intervals. Bars with the same lowercase letter (a−e) are not significantly ($p > 0.05$) different.

activity of the samples. In this assay, the antioxidant compounds donate the electron or hydrogen to the DPPH• molecule and convert it to the nonradical hydrazine form; the pink color of the radical form fades away, and the absorbance at 517 nm is decreased. The radical scavenging activity of the essential oil of *S. cuneifolia* obtained in three different maturation stages and the major compounds (thymol and carvacrol) were analyzed using the DPPH• free radical, and the cumulative results are given in **Figure 2**. All of the oils scavenged the DPPH• radicals at physiological pH and did so in a concentration-dependent fashion (data not shown). IC_{50} values, defined as the concentration required to scavenge 50% of the available free radicals, estimated by nonlinear regression for all of the extracts, are presented also in **Figure 2**. The high IC_{50} value indicates the low free radical scavenging activity. According to **Figure 2**, when compared the essential oil obtained from the preflowering sample was the most active free radical scavenger. However, none of the oils was as active as the positive controls, BHT and ascorbic acid, and this was also the case for the tested main constituents thymol and carvacrol. As seen in **Figure 2**, free radical scavenging activity was decreased during maturation stages.

The activity of essential oils of *Satureja* species on DPPH• free radical has previously been reported. *S. cuneifolia* from Artvin, northern Turkey, was found to be active as a free radical scavenger and against linoleic acid peroxidation (*2*). The essential oil studied in the said paper contained carvacrol (67.1%), γ-terpinene (15.2%), and *p*-cymene (6.7%) as main constituents. The results of the β-carotene bleaching assay are in agreement with ours, but DPPH• radical scavenging activity results were found to be different as reported by Eminagaoglu et al. (*2*). In the same study, the IC_{50} value of 89.1 μg/mL of was obtained from *S. cuneifolia* essential oil, whereas 1.6–2.1 mg/mL was obtained from our samples as seen in **Figure 2**. Carvacrol showed comparably better activity than the essential oils used in our study, having IC_{50} values of 0.8 and 1.6–2.1 mg/mL, respectively. The difference was shown between the IC_{50} values of carvacrol in the literature and our results. The DPPH radical scavenging assay protocols in all reports used (*2, 5, 24*) for essential oils were different from the protocol used in this work. Thus, the results were not comparable.

Free radical chains are broken with the donation of hydrogen atom of phenolic compounds. The free radical scavenging activity of essential oils depends on the presence of phenolic compounds for reduction (*4*). The oils studied in the present work were found to be rich in phenolic volatile compounds such as thymol and carvacrol. The sums of these two phenolic compounds were 53.76, 58.40, and 50.97% in three different harvesting periods. The radical scavenging activity of thymol and carvacrol is well-known (*2, 5, 24*). In the present study, the relative amounts of thymol and carvacrol increased before the flowering period and then decreased after the flowering stage, as shown in **Table 1**. However, radical scavenging activities of the oils continuously increased during all three periods in the test system employed (see **Figure 2**). The composition of essential oils of *S. cuneifolia* showed big differences according to collecting places and times. Therefore, the antioxidant and antiradical activities of these essential oils showed variations. In the literature, the relationship between phenolic/oxygenated compounds and the antioxidant/antiradical activities of the oils was associated with possible synergism. According to these reports, the activity can also be affected by the ratio of the amounts of thymol and carvacrol and the presence of other minor oxygenated compounds as well (*2, 4, 24*).

Table 3. Effect of *Satureja cuneifolia* Essential Oils on β-Carotene−Linoleic Acid Co-oxidation

sample[a]	oxidant rate ratio (ORR)[b]	antioxidant activity coefficient (AAC)[b]	inhibition (%)
BHT	0.26 ± 0.01	845.62 ± 3.20	98.74 ± 0.09
AscAs	nd[c]	61.78 ± 1.80	nd
thymol	0.75 ± 0.02	151.70 ± 5.30	92.54 ± 1.15
carvacrol	0.69 ± 0.03	220.36 ± 3.75	94.61 ± 0.82
A	0.54 ± 0.01	153.17 ± 1.36	93.54 ± 0.41
B	0.55 ± 0.01	157.08 ± 0.74	93.56 ± 0.46
C	0.55 ± 0.01	194.57 ± 1.27	94.65 ± 0.40

[a] BHT, butylated hydroxytoluene; AscAs, ascorbic acid; A, preflowering stage; B, flowering stage; C, postflowering stage. [b] Mean ± SD ($n = 6$). [c] Not detected.

Food lipids and the cell membranes contain unsaturated fatty acids, linoleic and arachidonic acids, which can easily be oxidized with oxidative agents. Therefore, the used unsaturated fatty acid–base medium in antioxidant activity tests is important to determine the respective activities of test samples (*18, 19*). The β-carotene−linoleic acid bleaching assay is such a model widely used to investigate the oxidation of unsaturated fatty acids, especially in the cell wall and food products. In the present study, the essential oils obtained from *S. cuneifolia* at three different maturation periods were also tested in the β-carotene−linoleic acid bleaching assay, using BHT and ascorbic acid as positive controls and references including the major constituents, thymol and carvacrol (the results are shown in **Table 3**). As seen in **Table 3**, BHT showed the highest inhibitory activity in the β-carotene−linoleic acid emulsion system. The essential oils demonstrated the same inhibitory activity level as thymol and carvacrol. The inhibition percentages of both oils and the main compounds of *S. cuneifolia* on the linoleic acid oxidation were comparable to the standard BHT. These results were in agreement with previous works (*4, 5*). Also, it was found in the present study that ascorbic acid displayed a pro-oxidant effect in this assay, which was also in agreement with the work of Siddhuraju and Becker (*25*). It is well-known that nonpolar antioxidants are concentrated at the lipid/air interface and demonstrate high protection in emulsions against polar antioxidants such as ascorbic acid presented in aqueous phase (*25*).

In conclusion, *Satureja* species show great variability in chemical composition, especially with regard to the major volatile components related to geographical location and genetic variations (*4, 5, 7*). Thus, the antimicrobial activity of the essential oils of *Satureja* species is related to the chemical composition and in particular to its main components depending also on the tested bacteria and fungi strains. The essential oil

of *S. cuneifolia* investigated in this study inhibited linoleic acid peroxidation at the same level as the commercial synthetic antioxidant BHT during maturation stages. Therefore, the essential oil of *S. cuneifolia* can be used as a food preservative, especially in lipoid media.

ACKNOWLEDGMENT

We thank Prof. Dr. Gülendam Tümen and Dr. Fatih Satil for collection and determination of the plant specimen.

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Received for review November 5, 2007. Revised manuscript received January 7, 2008. Accepted January 12, 2008.

JF0732253