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Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten.

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ABSTRACT

Satureja cuneifolia Ten. is a well-known aromatic plant which is frequently used as a spice and herbal tea in Anatolia. *S. cuneifolia* oil was analyzed by gas chromatography/mass spectrometry (GC/MS). The major components of *S. cuneifolia* oil were carvacrol (44.99%) and *p*-cymene (21.61%). The essential oil of *S. cuneifolia* exhibited antimicrobial activity against all of the tested foodborne and spoilage bacteria. The minimum inhibitory concentration (MIC) values for test bacteria which were sensitive to the essential oil of *S. cuneifolia* were in the range of 600–1400 μg/ml. Antioxidant activities of the essential oil and the methanolic extract from *S. cuneifolia* were evaluated by using DPPH radical scavenging, β-carotene–linoleic acid bleaching and metal chelating activity assays. In addition, the amounts of total phenol components in the plant methanolic extract (222.5 ± 0.5 μg/mg) and the oil (185.5 ± 0.5 μg/mg) were determined.

1. Introduction

Turkey is regarded as an important gene-centre for the family *Lamiaceae*. *Satureja* L. is a genus of the well-known aromatic plant belonging to the *Lamiaceae* family and comprises numerous species growing wild in the Mediterranean area. The aerial parts of these species have distinctive tastes and can be added to stuffing, meat, chicken, pies and sausages as a seasoning. *S. cuneifolia* Ten. is used to produce essential oil and aromatic water. This plant is also used as a condiment and herbal tea, owing to its stimulating, tonic and carminative effect (Eminagaoglu et al., 2007; Milos, Radonic, Bezic, & Dunkic, 2001).

The exploration of naturally occurring antimicrobials for food preservation is of increased attention due to consumer awareness of natural food products and a growing concern of microbial resistance towards conventional preservatives (Schuenzel & Harrison, 2002). Also, there is a growing interest in organically produced foods that the general public associate with healthier food. Another problem is the use of animal waste as organic fertilizer, whether in organic or non-organic agriculture, that gives rise to concerns about possible contamination of agricultural produce with pathogens (especially *Escherichia coli* O157:H7) and the possible contamination of ground and surface water (Moreira, Ponce, del Valle, & Roura, 2005).

Reactive oxygen and nitrogen species, ROS/RNS are continuously produced in the human body and they are controlled by endogenous enzymes (superoxide dismutase, glutathione peroxidase, catalase). When there is an over-production of these species, an exposure to external oxidant substances or a failure in the defense mechanisms, damage to valuable biomolecules (DNA, lipids, proteins) may occur (Aruoma, 1998). Antioxidants have been reported to prevent oxidative damage by ROS/RNS and may prevent the occurrence of certain diseases such as cancer and the aging process. They can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals and also by acting as oxygen scavengers (Shahidi & Wanasundara, 1992). Recent research is now directed towards finding naturally occurring antioxidants of plant origin. Interest in natural antioxidants has increased dramatically in recent times due to: (1) the possible carcinogenic effects of synthetic antioxidants in foods (Shahidi, 2008), (2) the antioxidative efficacy of a variety of phytochemicals, (3) the consensus that foods rich in certain phytochemicals can affect the aetiology and pathology of chronic diseases and the aging process (Dorman & Hiltunen, 2004).

The main objectives of this study were (i) to investigate the antimicrobial activity of the essential oil of *S. cuneifolia* by broth microdilution, agar well diffusion and disc diffusion methods against some food pathogen bacteria and (ii) to determine the chemical composition of its hydro-distilled essential oil by GC/MS. Besides, this study was also designed to evaluate the antioxidant capacity of the plant methanolic extract and the essential oil. The usefulness of the findings of the present study in the protection of foods against contamination especially *E. coli* O157:H7 was also discussed.

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2. Materials and methods

2.1. Chemicals

Nutrient Broth (NB) was obtained from Oxoid Ltd. (Basingstoke, Hampshire, United Kingdom). Tryptic Soy Broth (TSB), Tween 40, chloroform, anhydrous sodium carbonate, Folin–Ciocalteu's phenol reagent, ethanol, methanol, glycerol and hexane were purchased from Merck (Darmstadt, Germany). Erythromycin and cefoxitin were acquired from Bioanalyse Co., Ltd. (Ankara, Turkey). Ethylenediaminetetraacetic acid (EDTA), β-carotene, dimethylsulphoxide (DMSO), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulphonic acid)-1,2,4-triazine (ferrozine), iron (II) chloride ($FeCl_2$), gallic acid, 2,6-di-*tert*-butyl-4-methylphenol (BHT) and linoleic acid were purchased from Sigma–Aldrich GmbH. (Steinheim, Germany).

2.2. Plant materials

S. cuneifolia plants were collected during the flowering stage in August 2004 on Sogut mountain (elevation 1684 m, 37° 21′ 14N–030° 58′ 38 E) from Sutculer-Isparta in Turkey. The identification of plant materials was confirmed by plant taxonomist, Prof. Dr. Mecit VURAL, in the Department of Biology, Gazi University, Ankara, Turkey.

2.3. Isolation of the essential oil

Collected plant material was dried in the shade and ground in a grinder with a 2 mm in diameter mesh. The ground aerial parts of the plant were submitted, for 3 h, to steam distillation, using a Clevenger apparatus to produce the essential oil in a yield of 2.20% (v/w), based on the dry weight of the sample from *S. cuneifolia*. The oil was stored in the dark at 4 °C until used within a maximum period of one week.

2.4. Preparation of the methanol extract

Thirty grams of the dried and powdered plant materials were extracted with methanol (HPLC grade) by using Soxhlet apparatus at 60 °C for 3 h. The extract was filtered and concentrated under vacuum at 50 °C by using a rotary evaporator (Heidolph, Laborota 4000, Schwabach, Germany), yielding a waxy material (18.20% w/w). Finally, the extract was lyophilized and stored in the dark at 4 °C until used within a maximum period of one week.

2.5. Test bacteria

E. coli O157:H7, *Campylobacter jejuni* ATCC 33291**,** *Shigella sonnei* RSKK 8177, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 7644, *Bacillus cereus* RSKK 867, *Pseudomonas aeruginosa* ATCC 29212, *Salmonella enteritidis* ATCC 40376 were used as test bacteria. NB and TSB were used for culturing of test bacteria. All strains were stored at −20 °C in the appropriate medium containing 10% glycerol and regenerated twice before use in the manipulations.

2.6. Determination of minimum inhibitory concentrations (MIC)

Microdilution broth susceptibility assay was used (Koneman, Allen, Janda, Scherckenberger, & Winn, 1997). Stock solution of the essential oil was prepared in 10% DMSO and then serial dilutions of the essential oil were made in a concentration range from 600 to 1400 μg/ml. The 96-well plates were prepared by dispensing into each well 95 μl of NB, 100 μl of the oil (dissolved in 10% DMSO) and 5 μl of the inoculum. The inoculum of microorganisms was prepared using 24 h cultures and suspensions were adjusted to 5.0 McFarland standard turbidity. The final volume in each well was 200 μl. A positive control (containing 5 μl inoculum and 195 μl NB) and negative control (containing 100 μl of essential oil dissolved in 10% DMSO, 100 μl NB without inoculum) were included on each microplate. The contents of the wells were mixed and the microplates were incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms. The experiment was carried out in triplicate.

2.7. Inhibitory effect by the agar well diffusion method

The determination of the inhibitory effect of the essential oil on test bacteria was carried out by agar well diffusion method (Kalemba & Kunicka, 2003). Bacterial cultures were grown at 37 °C for 24 h in NB. *C. jejuni* ATCC 33291 was cultured overnight at 42 °C, *L. monocytogenes* ATCC 7644 was cultured overnight at 37 °C in TSB. The culture suspensions were adjusted by comparing against 5.0 McFarland. Petri dishes with 20 mL of Nutrient Agar were prepared, previously inoculated with 200 μl of the culture suspension. The wells (7.0 mm) were made and the oil diluted in ethanol to test concentration (1/5 and 1/10) was added to wells (20 μl) and the same volume (20 μl) of ethanol was used as a control. The inoculated plates were incubated for 24 h. After incubation, the diameter of the inhibition zone was measured with calipers. The measurements were done basically from the edge of the zone to the edge of the well.

2.8. Inhibitory effect by the disc diffusion method

The disc diffusion method was employed for the determination of the antimicrobial activity of the essential oil (Murray, Baron, Pfaller, Tenover, & Yolke, 1995). The culture suspensions were adjusted by comparing against 5.0 McFarland. One hundred microlitres of suspension of the test microorganisms were spread on solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 10 μl of the oil and placed on the inoculated plates, which were stored at 4 °C for 2 h and then incubated for 24 h. The diameters (mm) of the inhibition zones were measured. Antibiotic discs of erythromycin (15 μg/disc) and cefoxitin (30 μg/disc) were also used as positive controls.

2.9. Gas chromatography/mass spectrometry (GC/MS) analysis conditions

The chemical composition of the essential oil was analyzed using GC–MS technique. The mass spectrometer was Agilent 6890 N GC/5973MSD-SCAN (Agilent Technologies, Palo Alto, CA, USA) in the electron impact (EI) ionisation mode (70 eV) and HP-5MS (bonded and cross-linked 5% phenyl-methylpolysiloxane, 30 mm × 0.25 mm, coating thickness 0.25 μm) capillary column (Restek, Bellefonte, PA). Injector and detector temperatures were set at 220 °C. The oven temperature was held at 50 °C for 30 min, then programmed to 240 °C at rate of 3 °C/min. Helium (99.99%) was the carrier gas at a flow rate of 1 ml/min. Diluted samples (1/100 in hexane, v/v) of 1.0 μl were injected manually. The identification of the components was based on the comparison of their mass spectra with those of Wiley 7 N (contains 392,086 compounds spectra), Nist 2002 (contains 174,948 compounds spectra) and flavor (contains 419 compounds spectra) libraries and as well as by comparison of their retention times.

2.10. Antioxidant activity

2.10.1. DPPH radical scavenging assay

Radical scavenging activity was determined by a spectrophotometric method based on the reduction of a methanol solution of DPPH using the method of Blois (1958). One millilitre of various concentrations of the extract or the oil in methanol was added to 1 ml of a 0.004% methanol solution of DPPH. The mixture was shaken vigorously and left to stand at room temperature for 30 min in the dark. Then the absorbance was measured at 517 nm against a blank by a spectrophotometer (Hitachi, U-1800, Tokyo, Japan). Inhibition of free radical, DPPH, in percent (I%) was calculated according to formula:

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

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 from the graph plotting inhibition percentage against extract concentration. Tests were carried out in triplicate. Butylated hydroxytoluene (BHT) was used as positive control.

2.10.2. β-carotene–linoleic acid bleaching assay

The antioxidant activities of the plant extract and the oil were evaluated by the spectrophotometric β-carotene bleaching test (Miller, 1971). A stock solution of β-carotene–linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade); 25 μl linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated by using a vacuum evaporator. Then, 100 ml of distilled water was added with oxygen (30 min at a flow rate of 100 ml min^{-1}) vigorous shaking. Aliquots (2.5 ml) of this reaction mixture were dispensed to test tubes and 350 μl of the extract or the oil prepared at 2 g/l concentration was added and the emulsion system was incubated for up to 48 h at room temperature. The same procedure was repeated with the synthetic antioxidant, BHT as positive control, and a blank containing only 350 μl of ethanol. After this incubation period, absorbances of the mixtures were measured at 490 nm. Antioxidative capacities of the oil and the extract were compared with those of BHT and blank.

2.10.3. Metal chelating activity on ferrous ions (Fe^{2+})

Metal chelating activity was determined according to the method of Decker and Welch (1990), with some modifications. Briefly, 0.5 ml of the plant extract was mixed with 0.05 ml 2 mM $FeCl_2$ and 0.1 ml 5 mM ferrozine. Total volume was diluted with 2 ml methanol. Then, the mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated using the formula given below:

$$\text{Scavenging effect}(\%) = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

where A_{control} is the absorbance of the ferrozine-Fe^{2+} complex and A_{sample} is the absorbance of the test compound. EDTA was used for comparison.

2.10.4. Determination of total phenolic contents

Total phenolic contents of the extract and the oil were analysed using the Folin–Ciocalteu reagent according to the method of Singleton and Rossi (1965) using gallic acid as standard, with some modifications. The oil or the extract solution (0.1 ml) was mixed with 0.2 ml of 50% Folin–Ciocalteu reagent. The mixture was allowed to react for 3 min and 1 ml aqueous solution of 2% Na_2CO_3 was added. Then, the mixture was vortexed vigorously. At the end of incubation for 45 min at room temperature, absorbance of each mixture was measured at 760 nm. The same procedure was also applied to the standard solutions of gallic acid. Total phenol contents were expressed as μg gallic acid equivalents per mg of the extract or the oil.

2.11. Statistical analysis

All experiments were done in triplicate, and mean values are presented. Statistical analysis was performed on the data by SPSS 11.0 Bivariate Correlation Analysis (SPSS Inc., Chicago, III) with statistical significance determined at $P < 0.05$. The Pearson rank order correlation test was used for comparisons of broth microdilution, agar well diffusion and disc diffusion methods to the antimicrobial activity of the essential oil.

3. Results and discussion

Recently, there has been considerable interest in essential oils from aromatic plants with antimicrobial activities for controlling pathogens and/or toxin producing microorganisms in foods (Alzoreky & Nakahara, 2003; Valero & Salmeron, 2003). A few preservatives containing essential oils are already commercially available. 'DMC Base Natural' is a food preservative produced by DOMCA S.A., Alhendín, Granada, Spain and comprises 50% essential oils from rosemary, sage and citrus and 50% glycerol. 'Protecta One' and 'Protecta Two' are blended herb extracts produced by Bavaria Corp. Apopka, FL, USA and are classed as generally recognized as safe (GRAS) food additives in the US. Although the precise contents are not made known by the manufacturer, the extracts probably contain one or more essential oils and are dispersed in solutions of sodium citrate and sodium chloride, respectively (Burt, 2004). The aerial parts of the plant were subjected to steam distillation for 3 h using a modified Clevenger type apparatus to yield 2.20% v/w of yellowish oil from *S. cuneifolia*. All the test bacteria were sensitive to the essential oil. However, *B. cereus* RSKK 867, *E. coli* O157:H7 and *S. aureus* ATCC 25923 were more sensitive to the essential oil than the other test bacteria. The inhibition zones of agar well, disc and MIC values for bacterial strains which were sensitive to the essential oil of *S. cuneifolia,* were in the range of 7.0–21.8 mm, 2.3–14.0 mm (1/5 diluted with ethanol) and 600–1400 μg/ml, respectively (Table 1). The highest inhibitory activity was against *B. cereus* RSKK 867 and *E. coli* O157:H7 which showed the lowest MIC (600 μg/ml) and largest growth inhibition halos for agar well diffusion assay (21.8–18.9 mm, respectively) and for disc diffusion assay (14.0–12.2 mm, respectively). The inhibition zones of the essential oil for each assay on test bacteria showed a significant correlation with MIC values ($P < 0.05$). Control treatment (absolute alcohol) did not show an inhibitor effect on any of the bacteria. The sensitivities of studied bacteria against erythromycin and cefoxitin are given in Table 1. This active oil for all test bacteria will be helpful devising antimicrobial formulations with which to protect foods against infection by multiple pathogens.

E. coli O157:H7 is a concern to public health on a global scale and is found in a wide variety of foodstuffs including meat and meat products, milk, yogurt, water, salad vegetables, fruits, fruit juices and cider. Pasteurisation and cooking are adequate methods of ensuring that viable cells are eliminated, but heat treatment is not desirable for all foods and cross-contamination cannot always be prevented (Burt & Reinders, 2003). Controlling the numbers and growth of *E. coli* O157:H7 therefore remains an important objective for sectors of the food production industry. In the present study MIC value of the essential oil of *S. cuneifolia* against *E. coli* O157:H7 was 600 μg/ml. The minimum lethal concentration

Table 1
Antimicrobial activity of the essential oil of *S. cuneifolia*[a]

Test bacteria	MIC (μg/ml)	Inhibition zone diameter (mm)					
		Agar well diffusion		Disc diffusion		Antibiotics	
		1/5	1/10	1/5	1/10	*Erythromycin*	*Cefoxitin*
E. coli O157:H7	600[a]	18.9 ± 0.3[g]	11.6 ± 0.4[d]	12.6 ± 0.2[f]	5.5 ± 0.3[d]	R[b]	16.4 ± 0.2
C. jejuni ATCC 33291	800[b]	11.1 ± 0.1[b]	8.2 ± 0.0[b]	4.4 ± 0.2[b]	2.8 ± 0.2[b]	13.9 ± 0.3	18.4 ± 0.2
S. sonnei RSKK 8177	800[b]	13.2 ± 0.2[d]	9.8 ± 0.2[c]	5.4 ± 0.2[c]	2.5 ± 0.1[b]	R[b]	11.2 ± 0.2
S. aureus ATCC 25923	600[a]	16.6 ± 0.2[f]	14.8 ± 0.2[e]	12.2 ± 0.2[f]	4.8 ± 0.0[c]	2.1 ± 0.7	18.4 ± 0.2
L. monocytogenes ATCC 7644	600[a]	14.0 ± 0.0[e]	11.4 ± 0.3[d]	7.7 ± 0.1[d]	1.5 ± 0.1[a]	16.8 ± 0.2	4.0 ± 0.2
B. cereus RSKK 867	600[a]	21.8 ± 0.2[h]	16.9 ± 0.1[f]	14.0 ± 0.4[g]	8.8 ± 0.4[e]	10.7 ± 0.1	8.6 ± 0.2
P. aeruginosa ATCC 29212	1400[c]	7.0 ± 0.2[a]	5.1 ± 0.3[a]	2.3 ± 0.1[a]	1.5 ± 0.1[a]	R[b]	R[b]
S. enteritidis ATCC 40376	800[b]	12.3 ± 0.1[c]	9.2 ± 0.2[c]	10.2 ± 0.2[e]	4.4 ± 0.0[c]	R[b]	R[b]

[a] Values represent averages ± standard deviations for triplicate experiments. Values in the same column with different superscripts are significantly ($P < 0.05$) different.
[b] No inhibition zone.

(MLC: the lowest concentration at which no growth occured) was determined as 700 μg/ml. *S. cuneifolia* is frequently used as a spice in Anatolia and may be used as a natural preservative against *E. coli* O157:H7 for the food production industry.

Thirty components were identified in *S. cuneifolia* 99.89% of the oil (Table 2). Carvacrol (44.99%) and *p*-cymene (21.61%) were found as the major compounds. Other important compounds were thymol (9.01%), γ-terpinene (4.35%), borneol (2.51%) and terpinen-4-ol (2.04%). Carvacrol, γ-terpinene and *p*-cymene were determined as major components of the essential oil obtained from Turkish *S. cuneifolia* (Baydar, Sagdıc, Ozkan, & Karadogan, 2004). Also, Azaz, Kurkcuoglu, Satil, Baser, and Tumen (2005); Biavati, Ozcan, and Piccaglia (2004); Eminagaoglu et al. (2007); Tumen, Kirimer, Ermin, and Baser (1998) determined carvacrol as a major component of the oil belonging to *S. cuneifolia* collected from Turkey. On the other hand, carvacrol percentage of the oil belonging to *S. cuneifolia* collected from Croatia was determined low (Milos et al., 2001; Skoćibušić & Bezić, 2004). The differences in chemical composition of essential oils depend on climatic, seasonal, and geographic conditions (Baydar et al., 2004). Our study supports the view that carvacrol is a major component for the essential oil of *S. cuneifolia* of Turkish origin. Essential oils rich in phenolic compounds such as carvacrol and thymol are widely reported to possess high levels of antimicrobial activity (Aligiannis, Kalpoutzakis, Mitaku, & Chinou, 2001; Bagamboula, Uyttendaele, & Debevere, 2004; Baydar et al., 2004; Kalemba & Kunicka, 2003), which has been confirmed and extended in the present study. Low activity was observed with components containing only an aromatic ring with alkyl substituents as in *p*-cymene (Bagamboula et al., 2004). *p*-Cymene is not an effective antibacterial when used alone, but when combined with carvacrol, synergism has been observed against *B. cereus in vitro* and in rice (Ultee, Slump, Steging, & Smid, 2000). Essential oils rich in 1,8-cineole demonstrated activity against Gram-positive and Gram-negative bacteria, including *L. monocytogenes*. Aliphatic alcohols (e.g. linalool) were reported to possess strong to moderate activities several bacteria. The position of the alcohol functional group was found to affect molecular properties of the component, such as a hydrogen-bonding capacity, and hence terpinen-4-ol was active against *P. aeruginosa*, while α-terpineol was inactive (Koroch, Juliani, & Zygadlo, 2007). The antimicrobial effects of borneol (Vardar-Ünlü et al., 2003), geraniol (Prashar, Hilli, Veness, & Evans, 2003), linalool and caryophyllene oxide (Sibanda et al., 2004) were also reported. In addition, no antimicrobial activity has been reported for γ-terpinene (Aligiannis et al., 2001; Sivropoulou et al., 1996). The essential oil of savory contains antioxidative compounds, namely carvacrol, thymol, β-caryophyllene, γ-terpinene, *p*-cymene, together with linalool, which was reported to possess a strong antioxidant activity (Ruberto & Baratta, 2000).

Table 2
Chemical composition of *S. cuneifolia* essential oil

Compounds		RT[a]	Composition (%)
Satureja cuneifolia			
1.	Carvacrol	25.038	44.99
2.	*p*-Cymene	12.273	21.61
3.	Thymol	24.275	9.01
4.	γ-Terpinene	13.808	4.35
5.	Borneol	18.662	2.51
6.	Terpinen-4-ol	19.223	2.04
7.	Carvacrol methyl ether	22.340	1.83
8.	Geraniol	22.848	1.73
9.	1.8-Cineole	12.522	1.66
10.	α-Terpinene	11.937	0.97
11.	Linalool	15.724	0.97
12.	Caryophyllene	29.878	0.95
13.	2,6-Octadienal	22.203	0.83
14.	α-Phellandrene	8.256	0.69
15.	α-Pinene	8.501	0.60
16.	Camphene	9.014	0.57
17.	β-Bisabolene	33.589	0.56
18.	*p*-Cymenly	15.183	0.55
19.	β-Myrcene	10.884	0.53
20.	α-Terpineol	19.854	0.47
21.	Caryophyllene oxide	36.395	0.38
22.	α-Ocimene	12.941	0.37
23.	Spathulenol	36.197	0.28
24.	δ-Cadinene	34.149	0.27
25.	*p*-Cymene-8-ol	19.609	0.26
26.	α-Amorphene	32.261	0.25
27.	β-Ocimene	13.393	0.23
28.	Ledene	32.995	0.15
29.	Nerol	21.629	0.15
30.	Dihydrocarvove	20.146	0.13
	Total		99.89

[a] Retention time.

Antioxidant activities of the essential oil and the methanolic extract from *S. cuneifolia* were tested by the DPPH radical scavenging, the β-carotene–linoleic acid bleaching and the metal chelating activity assays. The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability or radical scavenging activity. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form diphenypicrylhydrazine (non radical) with the loss of this violet colour (Molyneux, 2004). Free radical scavenging properties of the methanolic extract and the essential oil are presented in Table 3. Lower IC_{50} value indicates higher antioxidant activity. The extract and the essential oil of *S. cuneifolia* exhibited remarkable antioxidant activities. The methanolic extract (IC_{50} = 26.0 ± 1.2 μg/ml) showed higher scavenging ability on DPPH radicals than the essential oil (IC_{50} = 65.1 ± 2.2

Table 3
Antioxidant activities of *S. cuneifolia* essential oil and methanolic extract[a]

Material	DPPH IC_{50} (μg/ml)	β-carotene bleaching (RAA) (%)	Metal chelating activity IC_{50} (mg/ml)	Total phenol contents (μg/mg)
Methanolic extract	26.0 ± 1.2	95.2 ± 0.2	3.79 ± 0.04	222.5 ± 0.5
Essential oil	65.1 ± 2.2	84.5 ± 0.3	Ns[b]	185.5 ± 0.5
BHT	23.1 ± 1.4	100	Ns[b]	Ns[b]

[a] Values represent averages ± standard deviations for triplicate experiments.
[b] Not studied.

μg/ml). Also, DPPH scavenging abilities of the methanolic extract and the oil were lower than that of synthetic antioxidant BHT (IC_{50} = 23.1 ± 1.4 μg/ml). Kosar, Demirci, Demirci, and Baser (2008) determined DPPH radical scavenging activity of the essential oil (IC_{50} = 1.6–2.1 mg/ml) of *S. cuneifolia* in three different stages. Eminagaoglu et al. (2007) also determined DPPH radical scavenging activity of the methanolic extract (IC_{50} = 68.0 ± 1.76 μg/ml) and the essential oil (IC_{50} = 89.1 ± 2.29 μg/ml) of *S. cuneifolia.* In this study, DPPH radical scavenging activity of test samples was in the order BHT > Methanolic extract > Essential oil. Also, Eminagaoglu et al. (2007) observed similar order in their study.

β-Carotene bleaching method is based on the loss of the yellow colour of β-carotene due to its reaction with radicals formed by linoleic acid oxidation in an emulsion. The rate of β-carotene bleaching can be slowed down in the presence of antioxidants (Kulisic, Radonic, Katalinic, & Milos, 2004). The relative antioxidative activities (RAAs) of the extracts were calculated from the equation, $RAA = A_{sample}/A_{BHT}$, where A_{BHT} is the absorbance of the control (BHT) and A_{sample} is the absorbance of the extract or the oil. The calculated RAAs of the extract and the oil are given in Table 3. The inhibition values of linoleic acid oxidation were estimated as 95.2 ± 0.2% and 84.5 ± 0.3% in the presence of the methanol extract and the essential oil, respectively. A relationship between the DPPH scavenging ability and β-carotene bleaching extent was found. In both assays, the methanolic extract showed better antioxidative capacity than the oil. On the other hand, Eminagaoglu et al. (2007) reported β-carotene bleaching of the oil was higher than that of the methanolic extract from *S. cuneifolia*.

The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have a metal chelation potential (Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995). Among the transition metals, iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. The ferrous state of iron accelerates lipid oxidation by breaking down hydrogen peroxide and lipid peroxides to reactive free radicals via the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\cdot}OH + {}^{-}OH$). Fe^{3+} ion also produces radicals from peroxides, although the rate is tenfold less than that of Fe^{2+} ion (Miller, Sampson, Candeias, Bramley, & Rice-Evans, 1996). The production of these radicals can lead to lipid peroxidation, protein modification and DNA damage. Chelating agents may inactivate metal ions and potentially inhibit the metal-dependent processes (Finefrock, Bush, & Doraiswamy, 2003). Chelating activity of the extract was determined by the ferrozine assay. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of the rate of color reduction therefore allows estimation of the chelating activity of the coexisting chelator (Yamaguchi, Ariga, Yoshimira, & Nakazawa, 2000). Effect of the extract on inhibition of ferrylbipyridyl formation is presented in Table 3. The extract inhibited the formation in a dose dependent variable with the IC_{50} value of 3.79 ± 0.04 mg/ml. At 5 mg/ml, chelating ability of methanolic extract from *S. cuneifolia* was 66.1%. However, EDTA showed an excellent chelating ability of 93.7% at 5 mg/ml.

The amounts of total phenolics in the extract and the oil were determined spectrometrically according to the Folin–Ciocalteu procedure and calculated as gallic acid equivalents. Gallic acid is a water soluble polyhydroxyphenolic compound which can be found in various natural plants, such as grapes, strawberries, bananas and many other fruits (Sun, Chu, Wu, & Liu, 2002). The standard curve equation is, y (absorbance) = 0.0067 × (μg gallic acid) + 0.3005, R^2 = 0.9999. The absorbance value was inserted in the above equation and the total amount of phenolic compound was calculated. The amounts of total phenols found in the plant methanolic extract and the oil were very high. As seen in Table 3, the total phenolic contents of the methanolic extract and the essential oil of *S. cuneifolia* are 222.5 ± 0.5 μg/mg and 185.5 ± 0.5 μg/mg, respectively. The results indicated that the methanolic extract has higher total phenolic compounds than the oil. Also, according to these results, there is a relationship between total phenol contents and antioxidant activity. Phenolic compounds, biologically active components, are the main agents that can donate hydrogen to free radicals and thus break the chain reaction of lipid oxidation at the first initiation step. This high potential of phenolic compounds to scavenge radicals may be explained by their phenolic hydroxyl groups (Sawa, Nakao, Akaike, Ono, & Maeda, 1999). In the present study, carvacrol (44.99%) which is a phenolic component was found as the major compound therefore the oil of *S. cunelifolia* showed high antioxidant and antimicrobial activity.

As a result of the present study, the methanolic extract and the oil were found to be effective antioxidants in different *in vitro* assays including β-carotene bleaching, DPPH radical scavenging and metal chelating activities.

4. Conclusions

The essential oils as antimicrobial agents present two main characters: the first is their natural origin which means more safety to the people and the environment, the second is that they have been considered at low risk for resistance development by pathogenic microorganisms. The growth of foodborne pathogens especially *E. coli* O157:H7 can be inhibited when *S. cuneifolia* is added as a spice in food products. Also, the sufficient amounts of this plant necessary for good protection against oxidation are easy to reach in a normal diet. In this respect, *S. cuneifolia* is highly recommended for using as a spice in meat, chicken and food products. According to the results of this study, the essential oil or the methanolic extract of *S. cuneifolia* may be suggested as a new potential source of natural antioxidant and antimicrobial for food industry.

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