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The in vitro antioxidative properties of the essential oils and methanol extracts of *Satureja spicigera* (K. Koch.) Boiss. and *Satureja cuneifolia* ten

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Abstract

This study was designed to examine the in vitro antioxidant activities of the essential oil and methanol extracts of *Satureja spicigera* and *S. cuneifolia* from Turkish flora. GC and GC/MS analysis of the essential oils resulted in the identification of 40 and 29 compounds, representing the 99.4% and 99.5% of the oils, respectively. Major constituents of the oils were carvacrol (42.5% and 67.1%), γ-terpinene (21.5% and 15.2%) and *p*-cymene (20.9% and 6.7%), respectively. Methanol extracts were also obtained from the aerial parts of the plants. The samples were subjected to a screening for their possible antioxidant activities by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene–linoleic acid assays. In general, samples obtained from *S. cuneifolia* exerted greater antioxidant activities than did those obtained from *S. spicigera*. In the DPPH test system, free radical-scavenging activity of *S. spicigera* oil was determined to be 127 ± 1.63 μg/ml, whereas IC_{50} value of *S. cuneifolia* was 89.1 ± 2.29 μg/ml. In the β-carotene–linoleic acid test system, antioxidant activities of the oil were 81.7 ± 1.14% and 93.7 ± 1.83%, respectively. Antioxidant activities of the synthetic antioxidant, BHT, ascorbic acid, curcumin and α-tocopherol were also determined in parallel experiments.

Keywords: *Satureja spicigera*, *Satureja cuneifolia*; Antioxidant activity; Essential oil; Methanol extract

1. Introduction

The use of spices and herbs as antioxidants in processed foods is a promising alternative to the use of synthetic antioxidants. Numerous reports of antioxidative activity of spices have appeared, strongly inspired by an increasing consumer interest in ''natural'' food additives. Most investigations have been performed using different model systems, and the spices have been evaluated, either as whole spices or as extract of spices (Madsen & Bertelsen, 1995). Major reasons for increasing interest in natural antioxidants are: doubts on the safety of use of synthetic substances (butylated hydroxytoluene and butylated hydroxyanisole), the antioxidative efficacy of a variety of phytochemicals, the consensus that foods rich in certain phytochemicals can affect the aetiology, and pathology of chronic diseases and the ageing process and the public conception that natural compounds are innately safer than synthetic compounds and are thus more commercially acceptable (Dorman & Hiltunen, 2004).

Many members of the genus *Satureja* have aromatic and medicinal characteristics. The aerial parts of these species have distinctive tastes and can be added to stuffing, meat pies, and sausages as a if seasoning. Fresh sprigs can be

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boiled with pulses, such as peas, beans or lentils, for flavouring or, alternatively, they can be used instead of parsley and chervil as a garnish. The leaves, flowers, and stems are used for herbal tea and, in traditional medicine, to treat various ailments, such as cramps, muscle pains, nausea, indigestion, diarrhoea, and infectious diseases (Gulluce et al., 2003).

The aim of this study was to investigate the in vitro antioxidant activities of the essential oils and methanol extracts of *S. spidgera* and *S. cuneifolia*. A survey of the literature reveals few reports on the antioxidative activity of water extract of *S. cuneifolia*, and none for *S. spicigera* (Dorman, Bachmayer, Kosar, & Hiltunen, 2004).

2. Materials and methods

2.1. Collection of plant material

The aerial parts (leaves and flowers) of *S. spicigera* (Voucher No. OE 3259) and *S. cuneifolia* (Voucher No. AA 3437) were collected from the Kilickaya district, Yusufeli, Artvin-Turkey and Sogutlugol plauteau, Duzici, Osmaniye-Turkey, when flowering (06th September 2004 and 15th July 2004), respectively. The voucher specimens of these plants have been deposited as the herbarium of the Department of Biology, Cumhuriyet University, Sivas-Turkey (CUFH).

2.2. Extraction of the essential oil

The air-dried and finely ground aerial parts of the plants were submitted, for 3 h, to steam distillation, using a Clevenger apparatus (British type) to produce the essential oils in a yield of 2.05% and 1.90% (v/w), based on the dry weight of the samples from *S. spicigera* and *S. cuneifolia*, respectively.

2.3. Gas chromatography (GC) analysis

The essential oil was analyzed using a Hewlett Packard 5890 II GC equipped with a FID detector and HP-5ms capillary column (30 m × 0.25 mm, film thickness 0.25 μm). Injector and detector temperatures were set at 220 and 290 °C, respectively. Oven temperature was raised from 50 to 240 °C by a rate of 3 °C/min. Flow rate of Helium was 1 ml/min. Diluted samples (1/100 in acetone, v/v) of 1.0 μl were injected manually and in the splitless mode. Quantitative data were obtained electronically from FID area percent data without the use of correction factors.

2.4. Gas chromatography/mass spectrometry (GC/MS) analysis

GC/MS analysis of the essential oils was performed under the same conditions with a GC (column, oven temperature, flow rate of the carrier gas) using a Hewlett Packard 5890 II GC equipped with a Hewlett Packard 5972 mass selective detector in the electron impact mode (70 eV). Injector and MS transfer line temperatures were set at 220 and 290 °C, respectively. The components were identified, based on the comparison of their relative retention time and mass spectra with those of standards and/or NIST98 and Wiley275 library data of the GC-MS system and literature data (Adams, 2001). The results were also confirmed by the comparison of the compound elution orders with their Kovats indices on non-polar phases reported in the literature (Adams, 2001).

2.5. Preparation of the methanol extracts

The air-dried and finely ground samples were extracted by using the method described elsewhere (Sokmen, Jones & Erturk, 1999). Briefly, the samples, weighing about 100 g, were extracted in a Soxhlet apparatus with methanol (MeOH) at 60 °C for 6 h. The extracts were then filtered and concentrated in vacuo at 45 °C, yielding a waxy material (15.42 and 14.35% w/w, respectively). Finally, the extracts were lyophilized and kept in the dark at +4 °C until tested.

2.6. Antioxidant activity

2.6.1. DPPH assay

The hydrogen atom or electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of purple-coloured methanol solution of DPPH. This spectrophotometric assay uses the stable radical 2,2′-diphenylpicrylhydrazyl (DPPH) as a reagent (Burits & Bucar, 2000; Cuendet, Hostettmann, & Potterat, 1997). Fifty microlitres of various concentrations of the extracts in methanol were 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 of free radical, DPPH, in percent (*I*%) was calculated in following way:

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

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.

2.6.2. 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 arising from linoleic acid oxidation (Dapkevicius, Venskutonis, Van Beek, & Linssen, 1998). 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 using a vacuum evaporator. Then, 100 ml of distilled water saturated with oxygen (30 min, 100 ml/min.) were added with vigorous shaking. Two thousand five hundred microlitres of this reaction mixture were dispensed to test tubes and 350 μl portions of the extracts prepared at 2 g l^{-1} concentrations were added and the emulsion system was incubated for up to 48 h at room temperature. The same procedure was repeated with the synthetic antioxidant, butylated hydroxytoluene (BHT) as positive control, and a blank. After this incubation period, absorbances of the mixtures were measured at 490 nm. Antioxidative capacities of the extracts were compared with those of BHT and blank.

3. Results and discussion

The oils isolated by hydro-distillation from the aerial parts of *S. spicigera* and *S. cuneifolia* were found to be yellow liquids and obtained in yields of 2.05% and 1.90% (v/w), based on dry weights, respectively.

Forty and twenty-nine components were identified in the essential oils of the plants studied. The major components were carvacrol (42.5% and 67.1%), γ-terpinene (21.5% and 15.2%) and *p*-cymene (20.9% and 6.7%), respectively. The chemical composition of the oils can be seen in Table 1.

The oil of *S. spicigera* from Iran is reported by Sefidkon and Jamzad (2004) to contain thymol (35.1%), *p*-cymene (22.1%), γ-terpinene (13.7%) and carvacrol (4.0%). In the study of Tumen and Baser (1996), a variation to the main components thymol (19.56–34.9%), *p*-cymene (7.77–34.1%), γ-terpinene (3.35–19.4%) and carvacrol (1.90–26.1%) was observed, due to the collection of plant material of five different localities of Turkey. In our study, together with *p*-cymene and γ-terpinene, a high amount of carvacrol was found, instead of thymol. This is in accordance with Tanker (1962), who identified carvacrol as the main component in the oil of *S. spicigera*.

The oil of *S. cuneifolia* of Turkish origin is reported to be rich in carvacrol and γ-terpinene (Baydar, Sagdic, Ozkan, & Karadogan, 2004), while samples collected from Croatia were found to contain a low percentage of carvacrol, but were rich in α-pinene, limonene, *p*-cymene, linalool, borneol, and β-cubebene (Milos, Radonic, Bezic, & Dunkic, 2001; Skoćibušić & Bezicb, 2004). Considerable differences were found to exist in the amounts of these compounds, depending on the locality, the season and the stage of development (Milos et al., 2001).

Our study supports the view that a genuine chemotype for the essential oil of *S. cuneifolia* of Turkish origin is that of carvacrol.

The literature outlines different approaches for determination of the antioxidant activities of the plant extracts. Therefore, different methodological approaches lead to scattered results, which are hardly comparable and often conflicting (Koleva, van Beek, Linssen, de Groot, & Evstatieva, 2002; Mantle et al., 1998; Ruberto & Baratta, 2000; Zygadlo, Lamarque, Maestri, & Grosso, 1995). A plethora of different antioxidant assays is available and, because results rely on different mechanisms, they strictly depend on the oxidant/antioxidant models employed and on lipophilic/hydrophilic balance (Frankel, Huang, Kanner, & German, 1994). A single/substance/single-assay produces relative results and it is perceived as a reductive approach whenever a phytocomplex is involved. Therefore, antioxidant activities of the plant extracts studied here were determined by two complementary test systems, namely DPPH free radical-scavenging and β-carotene–linoleic acid systems.

Table 1
Chemical composition of the essential oils of *S. spicigera* and *S. cuneifolia*

No.	K.I.[a]	Components	Composition (%)	
			S. spicigera	*S. cuneifolia*
1	890	α-Thujene	1.5	0.5
2	898	α-Pinene	1.2	0.8
3	911	Camphene	0.3	0.1
4	935	Sabinene	0.1	–
5	938	β-Pinene	0.1	–
6	945	1-Octene-3-ol	0.6	0.4
7	953	β-Myrcene	2.1	1.8
8	967	α-Phellandrene	0.3	0.2
9	973	*p*-Mentha-1(7), 8-diene	0.1	0.1
10	979	α-Terpinene	2.1	2.1
11	989	*p*-Cymene	20.9	6.7
13	1004	Z-β-Ocimene	0.4	–
14	1016	E-β-Ocimene	0.4	0.1
15	1026	γ-Terpinene	21.5	15.2
16	1040	*cis*-Sabinene hydrate	0.1	tr[b]
17	1062	Terpinolene	0.2	0.2
18	1075	*trans*-Sabinene hydrate	0.1	–
19	1078	Linalool	–	0.1
22	1155	Borneol	0.2	0.1
24	1168	Terpinen-4-ol	0.2	0.3
25	1187	α-Terpineol	0.1	tr
26	1192	*cis*-Dihydro carvone	0.1	tr
27	1201	*trans*-Dihydro carvone	tr	–
29	1246	Carvacrol methyl ether	1.7	0.1
30	1279	Carvone	–	tr
33	1312	Thymol	0.3	0.3
34	1322	Carvacrol	42.5	67.1
38	1399	α-Copaene	0.1	–
39	1411	β-Bourbonene	tr	–
42	1448	β-Caryophyllene	0.6	2.6
44	1459	β-Copaene	tr	–
45	1470	Aromadendrene	tr	0.2
46	1486	α-Caryophyllene	tr	0.1
47	1511	γ-Muurolene	0.1	–
48	1516	Germacrene D	0.4	–
50	1531	Viridiflorene	–	0.2
51	1531	Bicyclogermacrene	0.3	–
52	1544	β-Bisabolene	0.4	–
53	1551	γ-Cadinene	0.1	–
54	1560	δ-Cadinene	0.1	tr
55	1580	*trans*-α-Bisabolene	tr	–
56	1619	Spathulenol	0.2	0.1
57	1624	Caryophyllene oxide	tr	0.1
		Total	99.4	99.5

[a] K.I., Kovats index relative to *n*-alkanes on non-polar HP-5ms column.
[b] Tr, traces ⩽ 0.06%.

Table 2
Free radical-scavenging capacities of the extracts measured in DPPH and β-carotene–linoleic acid assays[a]

Extracts	DPPH (μg/ml)	β-Carotene–linoleic acid inhibition (%)
Satureja spicigera (essential oil)	127 ± 1.63	81.7 ± 1.14
Satureja spicigera (methanol extract)	267 ± 1.42	65.9 ± 1.77
Satureja cuneifolia (essential oil)	89.1 ± 2.29	93.7 ± 1.83
Satureja cuneifolia (extract)	68.0 ± 1.76	72.9 ± 2.16
p-Cymene	NA[b]	NA
γ-Terpinene	NA	NA
Carvacrol	245 ± 3.26	54.5 ± 1.42
BHT	18.0 ± 0.40	96.6 ± 1.29
Ascorbic acid	3.80 ± 0.10	94.5 ± 2.14
Curcumin	7.80 ± 0.30	89.3 ± 1.86
α-Tocopherol	6.50 ± 0.70	96.7 ± 1.72

[a] Results are means of three different experiments.
[b] NA, Not active.

In both the test systems, all of the extracts and essential oils exhibited remarkable antioxidant activities. In general, samples obtained from *S. cuneifolia* exerted greater antioxidant activities than did those obtained from *S. spicigera*. In general, essential oils showed greater activity than those of the extracts except for *S. cuneifolia* essential oil in the DPPH system. Results of the antioxidant activity test systems can be seen in Table 2.

In the DPPH test system, free radical-scavenging activity of *S. spicigera* oil was determined to be 127 ± 1.63 μg/ml, whereas IC_{50} value of *S. cuneifolia* was 89.1 ± 2.29 μg/ml. In the second case, antioxidant activities of the oil were 81.7 ± 1.14% and 93.7 ± 1.83%, respectively. When compared, methanol extracts of *S. cuneifolia* exhibited greater antioxidant activity than did *S. spicigera* extracts, in both the test systems.

As far as our literature survey could ascertain, methanol extract of *S. cuneifolia* showed better activity than did the aqueous one measured by the same DPPH method (Dorman et al., 2004).

Lamiaceae species are known to produce a diverse array of secondary metabolites, such as volatile and non-volatile terpenes, hydroxybenzoates, hydroxycinnamates and flavonoids, among others. The cardinal mode of action of natural antioxidants is their ability to scavenge free radicals before they can initiate free radical chain reactions in cellular membranes or lipid-rich matrices in foodstuffs, cosmetics or pharmaceutical preparations (Dorman & Hiltunen, 2004).

When compared with the antioxidative potentials of the standard compounds used in this study (BHT, ascorbic acid, curcumin and α-tocopherol), both essential oils and methanolic extracts of the *Satureja* species studied exerted remarkable antioxidant activity.

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