ELSEVIER

Food Chemistry 84 (2004) 519–525

Food Chemistry

www.elsevier.com/locate/foodchem

Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* (Montbret et Aucher ex Benth.) and *Salvia multicaulis* (Vahl)

Bektas Tepe[a,*], Erol Donmez[a], Mehmet Unlu[b], Ferda Candan[c], Dimitra Daferera[d], Gülhan Vardar-Unlu[b], Moschos Polissiou[d], Atalay Sokmen[a]

[a]*Department of Biology, Faculty of Science and Literature, Cumhuriyet University, Sivas 58140, Turkey*
[b]*Department of Chemistry, Faculty of Science and Literature, Cumhuriyet University, Sivas 58140, Turkey*
[c]*Department of Microbiology, Faculty of Medicine, Cumhuriyet University, Sivas 58140, Turkey*
[d]*Laboratory of General Chemistry, Agricultural University of Athens, Iera Odos 75, Athens 118 55, Greece*

Received 5 February 2003; received in revised form 12 May 2003; accepted 12 May 2003

Abstract

The essential oils and methanolic extracts of *Salvia cryptantha* and *Salvia multicaulis* were examined for their potential antimicrobial and radical scavenging activities. No, or slight, activity was observed when the polar and non-polar subfractions of the extracts were tested, whereas essential oils exhibited antimicrobial activity. The essential oils isolated from *S. cryptantha* and *S. multicaulis* were analysed by GC–MS and 53 and 47 constituents were identified, respectively. Antioxidant activities of the polar subfraction and the essential oil were examined using 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl radical-scavenging and lipid peroxidation assays. The essential oils, in particular, and the non-polar subfractions of methanol extracts, showed antioxidant activity. In conclusion, the results indicate that the oils of *S. cryptantha* and *S. multicaulis* have the capacity to scavenge free radicals and to inhibit the growth of pathogenic microorganisms. Therefore they could be suitable for using as antimicrobial and antioxidative agents in the food industry.
© 2003 Elsevier Ltd. All rights reserved.

Keywords: *Salvia cryptantha*; *Salvia multicaulis*; Essential oil; Antimicrobial activity; Antioxidant activity; GC–MS

1. Introduction

Recently, the essential oils and various extracts of plants have provoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases and the preservation of foods from the toxic effects of oxidants. Particularly, the antimicrobial activities of plant oils and extracts have formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Lis-Balchin & Deans, 1997; Reynolds, 1996). Moreover, they offer an effective way to prevent the development of various off-flavours and undesirable compounds that result from lipid peroxidation in foods (Wang et al., 1998). Following questions about the quality and safety of foods, only selected food additives are allowed (Weng & Wang, 2000). Because of the possible toxicities of the synthetic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), increasing attention has been directed toward natural antioxidants (Naimiki, 1990).

As far as our literature survey could ascertain, antimicrobial and/or antioxidant activities of *Salvia cryptantha* and *Salvia multicaulis* have not previously been published, although there are some reports of the compositions of the oils (Demirci, Tabanca, & Baser, 2002; Rustaiyan, Masoudi, Monfared, & Komeilizadeh, 1999) and di and triterpenoids isolated from these species (Ulubelen, Tan, Sonmez, & Topcu, 1998; Ulubelen & Topçu, 2000; Ulubelen, Topçu, & Terem, 1987).

* Corresponding author. Tel.: +90-346-219-1010x2907; fax: +90-346-219 1606.

E-mail address: bektas_tepe@yahoo.com (B. Tepe).

0308-8146/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0308-8146(03)00267-X

In the present study, the antimicrobial and antioxidative capacities of the essential oils and extracts of *S. cryptantha* and *S. multicaulis* were investigated. The antimicrobial activities were determined by using agar well-diffusion, agar disc diffusion and broth microdilution methods. The antioxidant activities were determined by using four complementary in vitro assays, inhibition of DPPH radical and the oxygen radicals, such as lipid peroxides, and hydroxyl radicals. The chemical composition of the essential oils was evaluated by using GS–MS analysis.

2. Materials and methods

2.1. Plant material

The herbal parts of *S. cryptantha* and *S. multicaulis* were collected from the Taşlıdere district and Tecer Mountain, Sivas-Turkey when flowering (July 2001). The voucher specimens were deposited at the Herbarium of the Department of Biology, Cumhuriyet University, Sivas-Turkey (CUFH-Voucher No: ED 5982 and 6206, respectively).

2.2. Preparation of the methanolic extracts

The air-dried and finely ground samples were extracted by using a Soxhlet apparatus for about 6 h (Sokmen, Jones, & Erturk, 1999). The resulting extracts were suspended in water and partitioned with chloroform ($CHCI_3$) to obtain water-soluble (polar) (7.12 and 9.69%, w/w, respectively) and water-insoluble (non-polar, chloroformic) subfractions (4.02 and 6.08%, w/w, respectively), which were then lyophilised and kept in the dark at +4 °C until tested.

2.3. Extraction 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 (yields 0.37 and 0.42% v/w, respectively). The obtained essential oils were dried over anhydrous sodium sulphate and, after filtration, stored at +4 °C until tested and analysed.

2.4. GC–MS analysis conditions

The analysis of the essential oils 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 ionisation system with ionisation energy of 70 eV was used. Helium was the carrier gas, at a flow rate of 1 ml/min. Injector and detector MS transfer line temperatures were set at 220 and 290 °C, respectively. Column temperature was initially kept at 50 °C for 3 min, then gradually increased to 150 °C at 3 °C/min rate, held for 10 min and finally raised to 250 °C at 10 °C/min. Diluted samples (1/100, v/v, in acetone) of 1.0 μl were injected manually and in the splitless mode. The components were identified by comparison of their relative retention times and mass spectra with those of standards (for the main components), NBS75K 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 relative retention indices on non-polar phases reported in the literature (Adams, 2001).

2.5. Antimicrobial activity

2.5.1. Microbial strains

The methanol extracts (both polar and non-polar subfractions) and the essential oils were individually tested against a panel of microorganisms, including *Staphylococcus aureus* ATCC 25923 and ATCC 29213 (for minimum inhibitory concentration test), S*treptococcus pneumoniae* ATCC 49619, *Moraxella catarrhalis* ATCC 49143, *Bacillus cereus* ATCC 11778, *Acinetobacter lwoffii* ATCC 19002, *Enterobacter aerogenes* ATCC 13043, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 7002, *Pseudomonas aeruginosa* ATCC 27853, *Clostridium perfringens* KUKENS-Turkey, mycobacterium; *Mycobacterium smegmatis* CMM 2067, *Candida albicans* ATCC 10239 and *Candida krusei* ATCC 6258. Bacterial strains were cultured overnight at 37 °C in Mueller Hinton agar (MHA), with the exception of *S. pneumoniae* (MHA containing 50 ml citrate blood/l) and *C. perfringens* (in anaerobic conditions). Yeasts were cultured overnight at 30 °C in Sabouraud dextrose agar.

2.5.2. Antimicrobial screening

Two different methods were employed for the determination of antimicrobial activities; an agar well-diffusion method for the methanol extracts and disc diffusion method for the essential oils (NCCLS, 1999). The MICs of the essential oils against the test microorganisms were determined by the broth microdilution method (NCCLS, 1997). The MICs of amikacin, clindamycine and ciprofloxacin were also determined in parallel experiments in order to control the sensitivity of the test microorganisms. All tests were performed in duplicate.

2.5.3. Agar-well diffusion method

The polar subfractions of the methanol extracts were weighed and dissolved in phosphate buffer saline (PBS; pH 7.0–7.2), 10 mg/ml; non-polar ones were dissolved in dimethyl sulphoxide (DMSO), 10 mg/ml. Both subfractions were filter-sterilised using a 0.45-μm membrane filter. Each microorganism was suspended in sterile

saline and diluted at ca. 10^6 colony-forming units (cfu)/ml. They were "flood-inoculated" onto the surface of MHA. The wells (eight mm in diameter) were cut from the agar and 0.06 ml of extract solution was delivered into them. After incubation for 24 h at 37 °C, all plates were examined for any zones of growth inhibition, and the diameters of these zones were measured in millimetres.

2.5.4. Disc diffusion method

The agar disc diffusion method was employed for the determination of antimicrobial activities of the essential oils in question (NCCLS, 1997). Briefly, a suspension of the tested microorganism (0.1 ml of 10^8 cells/ml) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were soaked with 15 μl of the oil and placed on the inoculated plates and, after staying at 4 °C for 2 h, were incubated at 37 °C for 24 h for bacteria and at 30 °C for 48 h for the yeasts. The diameters of the inhibition zones were measured in millimeters.

2.5.5. Determination of minimum inhibitory concentration (MIC)

A broth microdilution broth susceptibility assay was used, as recommended by NCCLS, for the determination of the MIC (NCCLS, 1999). All tests were performed in Mueller Hinton broth (MHB; BBL) supplemented with Tween 80 detergent (final concentration of 0.5% (v/v), with the exception of the yeasts (Sabouraud dextrose broth-SDB + Tween 80). Bacterial strains were cultured overnight at 37 °C in MHA and the yeasts were cultured overnight at 30 °C in SDB. Test strains were suspended in MHB to give a final density of 5×10^5 cfu/ml and these were confirmed by viable counts. Geometric dilutions ranging from 0.036 mg/ml to 72.00 mg/ml of the essential oil, were prepared in a 96-well microtitre plate, including one growth control (MHB + Tween 80) and one sterility control (MHB + Tween 80 + test oil). Plates were incubated under normal atmospheric conditions at 37 °C for 24 h for bacteria and at 30 °C for 48 h for the yeasts. The bacterial growth was indicated by the presence of a white "pellet" on the well bottom.

2.6. Antioxidant activity

While polar subfractions of methanol extracts were assayed for their antioxidative properties using hydroxyl radical-scavenging and DPPH assays, the essential oils were tested using the whole complementary assays given below. Non-polar subfractions of methanol extracts could not be tested because of partial solubility in aqueous test media and its interference with the spectroscopic measurements. BHT, curcumin and ascorbic acid were included in experiments as positive controls.

2.6.1. Hydroxyl radical scavenging

Hydroxyl radical scavenging was carried out by measuring the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system. The attack of the hydroxyl radical on deoxyribose leads to TBARS formation (Kunchandy & Rao, 1990). Various concentrations of the extracts were added to the reaction mixture containing 3.0 mM deoxyribose, 0.1 mM $FeCI_3$, 0.1 mM EDTA, 0.1 mM ascorbic acid, 1 mM H_2O_2 and 20 mM phosphate buffer (pH 7.4), making up a final volume of 3.0 ml. The reaction mixture was incubated at 37 °C for 1 h. The formed TBARS were measured by the method given elsewhere (Ohkawa, Ohishi, & Yagi, 1979). One millilitre of thiobarbituric acid TBA (1%) and 1.0 ml trichloroacetic acid, TCA (2.8%) were added to test tubes and incubated at 100 °C for 20 min. After cooling, absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. Reactions were carried out in triplicate. Inhibition (I) of deoxyribose degradation in per cent was calculated in following way:

$$I = (A_0 - A_1/A_0)\times 100$$

where A_0 is the absorbance of the control reaction (containing all reagents except the test compound) and A_1 is the absorbance of the test compound.

2.6.2. DPPH assay

The DPPH assay was carried out as described elsewhere (Cuendet, Hostettmann, & Potterat, 1997). Fifty microlitres of various concentrations of the samples 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. The assay was carried out in triplicate.

2.6.3. Inhibition of lipid peroxide formation

The reaction mixture contained 0.1 ml of 25% (w/v) rat liver homogenate in 40 mM Tris–HCl buffer, pH 7.0, 30 mM KCI, 0.16 mM ferrous iron, various concentrations of the extract and positive controls and 0.06 mM ascorbic acid in a final volume of 0.5 ml. It should be noted that extract and positive controls have their own control reactions (containing all related reagents except the test compounds). The mixture was then incubated at 37 °C for 1 h (Bishayee & Balasubramanian, 1971). The lipid peroxide formation was measured by the method given elsewhere (Ohkawa et al., 1979). For this, 0.4 ml of the reaction mixture was treated with 0.2 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml 0.8% thiobarbituric acid and 1.5 ml of 20% acetic acid solution adjusted to pH 3.5 with NaOH. The total volume was then made up to 4 ml by adding distilled water and kept in a water bath at 95 °C for 1 h. After cooling, 1ml of distilled water

and 5 ml of the mixture of *n*-butanol and pyridine (15:1 v/v) were added and shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and its absorbance at 532 nm was measured. The percentage inhibition of lipid peroxidation was determined by comparing the results of the test compounds with those of controls not treated with the extracts. Calculations were done as mentioned for the hydroxyl radical scavenging method.

3. Results and discussion

3.1. Chemical composition of the essential oils

Except for some negligible differences between the essential oils studied, both plants have highly similar chemical compositions. About 53 (90.9% of the total oil) and 47 (96.1% of the total oil) constituents were identified from *S. cryptantha* and *S. multicaulis* essential oils, respectively. GC–MS analysis revealed that the major constituents of the *S. cryptantha* and *S. multicaulis* oils were α-pinene (18.1 and 21.9%), eucalyptol (15.3 and 20.1%), camphor (7.7 and 11.0%), camphene (6.4 and 7.8%) and borneol (4.8 and 7.3%), respectively, as listed in Table 1.

GC–MS analysis of the oils demonstrated the abundance of monoterpene hydrocarbons and oxygenated monoterpenes. The findings on the major components of *S. cryptantha* oil were in agreement with the previous report (Baser, Beis, & Özek, 1995) except for borneol. According to Ahmadi and Mirza (1999) limonene was one of the major components of *S. multicaulis*, whereas it was not detected in the present study. These differences might have been derived from local, climatic and seasonal factors (Perry et al., 1999).

3.2. Antimicrobial activity

The non-polar subfraction of the methanol extract of *S. cryptantha* was found to possess moderate activity against *St. pneumoniae* and *C. albicans*, whereas that of *S. multicaulis* had broader activity against tested microorganisms, including *S. pneumoniae*, *B. cereus*, *S. aureus*, *M. catarrhalis*, *C. perfringens* and *C. albicans*. On the other hand, no activity was exhibited by the polar subfractions of either plant extract (Table 2). When compared to the methanol extracts, the essential oils exhibited stronger and broader activity. No significant differences were evident between *S. cryptantha* and *S. multicaulis* essential oils in terms of the antimicrobial spectrum. *C. albicans*, *C. krusei*, *M. smegmatis*, *A. lwoffii*, *S. pneumoniae* and *C. perfringens* were the most sensitive microorganisms to the *S. cryptantha* essential oil (having MIC values ranging from 2.25 to 18 mg/ml), whereas *S. multicaulis* essential oil had pronounced activity against *S. pneumoniae*, *C. krusei*, *C. perfringens*, *M. smegmatis*, *C. albicans* and *S. aureus* (having MIC values ranging from 2.25 to 36 mg/ml).

Eucalyptol (1,8-cineole) and camphor are well-known chemicals having antimicrobial potentials (Pattnaik, Subramanyam, Bapaji, & Kole, 1997; Tzakou, Pitarokili, Chinou, & Harvala, 2001). Based on a report, α-pinene (monoterpene hydrocarbon) and borneol (oxygenated monoterpene) had slight activity against a panel of microorganisms (Dorman & Deans, 2000). Preliminary results on commercial substances indicate that α-pinene has remarkable activity against only the yeasts and *C. perfringens* among the strains used in this study (unpublished data). The antimicrobial effects of borneol were also reported elsewhere (Knobloch, Pauli, Iberi, Wegand, & Weis, 1989; Tabanca, Kırımer, Demirci, Demirci, & Baser, 2001; Vardar-Unlu et al., 2003). As a result of these findings, antimicrobial activities of *S. cryptantha* and *S. multicaulis* oils could be attributed to eucalyptol, camphor and derivatives and borneol. The synergistic effects of these chemicals with each other and minor constituents of the essential oil should be taken into consideration for the activity. The mechanism of action of terpenes is not fully understood but it is thought to involve membrane disruption by the lipophilic compounds (Cowan, 1999).

3.3. Antioxidant activity

The antioxidative capacities of *S. cryptantha* and *S. multicaulis* extracts were determined by comparing with the activities of known antioxidants, such as BHT, curcumin and ascorbic acid by the following three in vitro complementary assays; DPPH, inhibition of hydroxyl radicals and lipid peroxidation. Since the non-polar subfractions of the extracts are partly soluble in aqueous test media and their colour interfered the spectroscopic measurements, only polar subfractions and essential oils could be tested for their antioxidative capacities.

Effects of *S. cryptantha* and *S. multicaulis* essential oils, methanol extracts (polar subfractions) and positive controls on the in vitro free radical (dpph and hydroxyl) and lipid peroxidation generation are given in Table 3.

The results of in vitro assays show that the methanolic extract and essential oil of *S. multicaulis* possessed slightly greater antioxidative properties than those of *S. cryptantha*. The essential oils of both plants had stronger activity than the methanolic extracts. When compared to the positive controls, curcumin, ascorbic acid and BHT, the essential oils had greater activity, whereas the methanolic extracts were found to be less efficient in radical scavenging and lipid peroxidation inhibition. Hydroxyl radical scavenging and lipid peroxidation

Table 1
Chemical compositions of *S. cryptantha* and *S. multicaulis* essential oils (%)

	Compounds[a]	Rt.[b] (min)	KI[c]	*S. cryptantha*	*S. multicaulis*
1	Tricyclene	9.106	927	0.1	0.2
2	Thujene	9.383	930	0.2	0.2
3	α-Pinene	9.760	939	18.1	21.9
4	Camphene	10.354	954	6.4	7.8
5	β-Pinene	11.613	979	4.6	4.7
6	1-Octen-3-ol	11.870	979	–	0.8
7	β-Myrcene	12.357	991	1.4	1.3
8	α-Phellandrene	12.922	1003	0.1	0.2
9	α-Terpinene	13.516	1017	0.2	0.2
10	Eucalyptol	14.438	1031	15.3	20.1
11	(*Z*)-β-Ocimene	14.665	1037	0.9	1.0
12	(*E*)-β-Ocimene	15.142	1050	0.2	0.2
13	γ-Terpinene	15.617	1060	0.4	0.4
14	*cis*-Sabinene hydrate	16.063	1070	0.3	0.3
15	Terpinolene	17.075	1089	0.2	0.2
16	Linalool	17.709	1097	0.1	0.1
17	Octen-1-ol, acetate	18.363	–	0.1	0.1
18	Camphor	20.058	1146	7.7	11.0
19	*trans*-Pinocamphone	20.702	1163	0.2	0.1
20	Pinocarvone	20.781	1165	–	0.1
21	Borneol	21.168	1169	4.8	7.3
22	*cis*-Pinocamphone	21.436	1175	*tr*[d]	–
23	Terpinen-4-ol	21.604	1177	0.5	0.8
24	α-Terpineol	22.268	1189	0.1	0.1
25	Myrtenol	22.685	1196	3.2	2.2
26	*cis*-Piperitol	23.070	1196	0.1	0.1
27	Piperitone	25.311	1253	0.1	0.1
28	Bornyl acetate	26.927	1289	3.7	3.3
29	*trans*-Sabinyl acetate	27.284	1291	3.4	–
30	α-Cubebene	29.762	1351	0.1	0.1
31	α-Copaene	30.971	1377	0.4	0.2
32	Sesquithujene (7-epi)	32.338	1391	–	0.1
33	Caryophyllene	33.032	1419	3.4	4.2
34	β-Copaene	33.350	1432	0.1	–
35	γ-Elemene	33.548	1437	0.2	0.1
36	α-Guaiene + Aromadendrene	33.874	1440–1441	0.5	0.5
37	α-Caryophyllene	34.460	1455	1.1	1.1
38	Isobornyl n-butanoate	34.935	1475	–	0.1
39	γ-Muurolene	35.431	1480	0.2	0.2
40	Germacrene D	35.629	1485	0.5	0.3
41	β-Selinene	35.857	1490	0.2	–
42	Bicyclogermacrene	36.283	1500	0.5	0.5
43	β-Curcumene	36.907	1516	–	0.1
44	γ-Cadinene	37.037	1514	0.2	–
45	δ-Cadinene	37.463	1523	0.4	0.2
46	Elemol	38.702	1550	0.2	–
47	Germacrene B	39.079	1561	0.5	–
48	(*E*)-Nerolidol	39.435	1563	0.4	0.2
49	(-)-Spathulenol	40.258	1578	0.5	0.4
50	Caryophyllene oxide	40.555	1583	0.8	0.2
51	Viridiflorol	41.041	1593	0.6	–
52	γ-Eudesmol	43.499	1624	0.4	–
53	Caryophylla-4(14), 8(15)-dien-5-ol	43.866	1632	0.5	–
54	β-Eudesmol	44.946	1651	2.4	1.0
55	α-Eudesmol	45.164	1654	1.4	0.5
56	Valeranone	46.621	1675	2.5	0.9
57	α-Bisabolol	47.236	1686	0.4	0.4
58	Manool (epi-13)	56.156	1695	0.1	–
	Total			90.9	96.1

[a] Compounds listed in order of elution from a HP-5 MS column.
[b] Retention time (as min).
[c] Kovats index on DB-5 column in reference to *n*-alkanes (Adams, 2001).
[d] *tr:* trace (<0.05%).

Table 2
Antimicrobial activity of the essential oils and the methanol extracts of *S. cyptantha and S. multicaulis*, using agar disc diffusion, agar well diffusion and minimum inhibitory concentration methods

Microorganisms	Essential oils				MeOH extracts[a]				The MIC of antibiotics[b]		
	S. cryptantha		*S. multicaulis*		*S. cryptantha*		*S. multicaulis*				
	DD[c]	MIC[d]	DD	MIC	H_2O	$CHCl_3$	H_2O	$CHCl_3$	AK	CF	CM
Staphylococcus aureus	9	72.0	11	36.0	na[e]	na	na	12	2.00	0.25	nt
Streptococcus pneumoniae	11	2.25	20	4.50	na	14	na	13	nt[f]	nt	0.125
Moraxella catarrhalis	9	72.0	9	72.0	na	na	na	11	nt	nt	nt
Bacillus cereus	na	> 72.0	10	36.0	na	na	na	12	nt	nt	nt
Acinetobacter lwoffii	15	18.0	10	36.0	na	na	na	na	nt	nt	nt
Enterobacter aerogenes	na	> 72.0	na	> 72.0	na	na	na	na	nt	nt	nt
Escherichia coli	na	> 72.0	8	72.0	na	na	na	na	2.00	0.015	nt
Klebsiella pneumoniae	9	72.0	9	72.0	na	na	na	na	nt	nt	nt
Proteus mirabilis	na	> 72.0	8	> 72.0	na	na	na	na	nt	nt	nt
Pseudomonas aeruginosa	na	> 72.00	na	> 72.0	na	na	na	na	1.00	0.25	nt
Clostridium perfringens	11	2.25	15	2.25	na	na	na	10	nt	nt	0.25
Mycobacterium smegmatis	18	2.25	14	9.00	na	na	na	na	nt	nt	nt
Candida albicans	25	2.25	12	18.0	na	13	na	10	nt	nt	nt
Candida krusei	20	4.50	15	9.00	na	na	na	na	nt	nt	nt

[a] Diameter of inhibition zone (mm) including well diameter of 8 mm.
[b] AK, amikacin; CF, Agar well diffusion method ciprofloxacine; CM, clindamycine.
[c] DD, agar disc diffusion method. Diameter of inhibition zone (mm) including disk diameter of 6 mm.
[d] MIC, minimum inhibitory concentration; values given as mg/ml for the essential oils and as μg/ml for antibiotics.
[e] NA, not active.
[f] NT, not tested.

Table 3
Effects of *S. cryptantha* and *S. multicaulis* essential oils, methanol extracts (polar subfractions) and positive controls on the in vitro free radical (dpph and hydroxyl) and lipid peroxidation generation Ic_{50} (μg/ml)

	Essential oils		MeOH Extracts		Controls		
	S. cryptantha	*S. multicaulis*	*S. cryptantha*	*S. multicaulis*	BHT	Curcumin	Ascorbic acid
DPPH	3.9±0.03	2.4±0.05	18.1±0.07	16.3±0.1	19.8±0.4	7.8±0.3	3.76±0.1
Hydroxyl	1.9±0.05	1.6±0.05	391±0.09	249±0.2	32.0±1.2	13.2±0.07	nt[a]
Lipid peroxidation	17.8±0.03	14.5±0.1	nt	nt	7.8±0.04	38.4±0.1	nt

[a] nt, not tested.

assays were not performed with ascorbic acid since this compound was already present in the test medium.

The findings above show the presence of natural antioxidant phenolic compounds in these plants, with better performance than BHT known as a very efficient synthetic antioxidant agent and widely used in food technology (Potterat, 1997). Indeed, the major components of the both essential oils, α-pinene, eucalyptol and camphor, have been reported to have high antioxidative activity (Ruberto & Baratta, 2000). The slight quantitative differences in the amounts of these components might also explain the minor differences between the activities of the two oils.

The results reported here can be considered as the first information on the antimicrobial and antioxidant properties of *S. multicaulis* and *S. cryptantha*. Further studies are needed to evaluate the in vivo potential of these oils in animal models.

Acknowledgements

Financial support from the Research Council of Cumhuriyet University, Sivas-Turkey (Project No: F-88) is gratefully acknowledged.

References

Adams, R. P. (2001). *Identification of essential oils components by gas chromatography/quadrupole mass spectroscopy*. Illinois, USA: Allured Publishing Corporation.

Ahmadi, L., & Mirza, M. (1999). Essential oil of Salvia multicaulis Vahl from Iran. *Journal of Essential Oil Research*, *11*, 289–290.

Baser, K. H. C., Beis, S. H., & Özek, T. (1995). Composition of the essential oil of *Salvia cryptantha* Montbret et Aucher ex Benth. from Turkey. *Journal of Essential Oil Research*, *7*, 113–114.

Bishayee, S., & Balasubramanian, A. S. (1971). Lipid peroxide formation in rat brain. *Journal of Neurochemistry*, *18*, 909–920.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, *12*, 564–582.

Cuendet, M., Hostettmann, K., & Potterat, O. (1997). Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. *Helvetica Chimica Act*, *80*, 1144–1152.

Demirci, B., Tabanca, N., & Baser, K. H. C. (2002). Enantiomeric distribution of some monoterpenes in the essential oils of some Salvia species. *Flavour and Fragrance Journal*, *17*, 54–58.

Dorman, H. J. D., & Deans, S. G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, *88*, 308–316.

Knobloch, K., Pauli, A., Iberi, B., Wegand, H., & Weis, N. (1989). Antibacterial and antifungal properties of essential oil components. *Journal of Essential Oil Research*, *1*, 119–128.

Kunchandy, E., & Rao, M. N. A. (1990). Oxygen radical scavenging activity of curcumin. *International Journal of Pharmaceutics*, *58*, 237–240.

Lis-Balchin, M., & Deans, S. G. (1997). Bioactivity of selected plant essential oils against *Listeria monocytogenes*. *Journal of Applied Bacteriology*, *82*, 759–762.

Naimiki, M. (1990). Antioxidants/antimutagens in foods. *CRC Critical Reviews in Food Science and Nutrition*, *29*, 273–300.

NCCLS (National Committee for Clinical Laboratory Standards). (1997). *Performance standards for antimicrobial disk susceptibility test* (6th ed.). Wayne PA: Approved Standard. M2-A6.

NCCLS (National Committee for Clinical Laboratory Standards). (1999). *Performance standards for antimicrobial susceptibility testing. 9th International Supplement. M100-S9*.

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, *95*, 351–358.

Pattnaik, S., Subramanyam, V. R., Bapaji, M., & Kole, C. R. (1997). Antibacterial and antifungal activity of aromatic constituents of essential oils. *Microbios*, *89*, 39–46.

Perry, N. B., Anderson, R. E., Brennan, N. J., Douglas, M. H., Heaney, A. J., McGimpsey, J. A., & Smallfield, B. M. (1999). Essential oils from Dalmation Sage (*Salvia officinalis* L.): variations among individuals, plant parts, seasons and sites. *Journal of Agricultural and Food Chemistry*, *47*, 2048–2054.

Potterat, O. (1997). Antioxidants and free radical scavengers of natural origin. *Current Organic Chemistry*, *1*, 415.

Rustaiyan, A., Masoudi, S., Monfared, A., & Komeilizadeh, H. (1999). Volatile constituents of three salvia species grown wild in Iran. *Flavour and Fragrance Journal*, *14*, 276–278.

Reynolds, J. E. F. (1996). *Martindale—the extra pharmacopeia* (31st ed.). London: Royal Pharmaceutical Society of Great Britain.

Ruberto, G., & Baratta, M. T. (2000). Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chemistry*, *69*, 167–174.

Sokmen, A., Jones, B. M., & Erturk, M. (1999). The in vitro antibacterial activity of Turkish plants. *Journal of Ethnopharmacology*, *67*, 79–86.

Tabanca, N., Kırımer, N., Demirci, B., Demirci, F., & Baser, K. H. C. (2001). Composition and antimicrobial activity of the essential oils of *Micromeria cristata* subsp. *phyrgia* and the enantiomeric distribution of borneol. *Journal of Agricultural and Food Chemistry*, *49*, 4300–4303.

Tzakou, O., Pitarokili, D., Chinou, I. B., & Harvala, C. (2001). Composition and antimicrobial activity of the essential oil of *Salvia ringens*. *Planta Medica*, *67*, 81–83.

Ulubelen, A., Tan, N., Sonmez, U., & Topcu, G. (1998). Diterpenoids and triterpenoids from *Salvia multicaulis*. *Phytochemistry*, *47*, 899–901.

Ulubelen, A., Topçu, G., & Terem, B. (1987). Abietane diterpenoids from the roots of *Salvia cryptantha*. *Phytochemýstry*, *26*, 1534–1535.

Ulubelen, A., & Topcu, G. (2000). Salvimultine, a new noricetexane diterpene from the roots of Salvia multicaulis. *Journal of Natural Production*, *63*, 879–880.

Vardar-Unlu, G., Candan, F., Sokmen, A., Daferera, D., Polissiou, M., Sokmen, M., Donmez, E., & Tepe, B. (2003). Antimicrobial and antioxidant activity of the essential oil and methanol extracts of *Thymus pectinatus* Fisch. et Mey. Var. pectinatus (Lamiaceae). *Journal of Agricultural and Food Chemistry*, *51*, 63–67.

Weng, X. C., & Wang, W. (2000). Antioxidant activity of compounds isolated from *Salvia plebeia*. *Food Chemistry*, *71*, 489–493.

Wang, M., Li, J., Rangarajan, M., Shao, Y., LaVoie, E. J., Huang, T. C., & Ho, C. T. (1998). Antioxidative phenolic compounds from sage (*Salvia officinalis*). *Jornal of Agricultural and Food Chemistry*, *46*, 4869–4873.