JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2007**, *55*, 7879–7885 **7879**

Antimicrobial and Antioxidant Properties of Rosemary and Sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L., Lamiaceae) Essential Oils

BILJANA BOZIN,*,† NEDA MIMICA-DUKIC,‡ ISIDORA SAMOJLIK,§ AND EMILIJA JOVIN‡

Faculty of Medicine, Department of Pharmacy, University of Novi Sad, Hajduk Veljkova 3, 21000 Novi Sad, Serbia, Faculty of Sciences, Deprtment of Chemistry, University of Novi Sad, Trg D. Obradovica 3, Novi Sad, Serbia, and Faculty of Medicine, Department of Pharmacology and Toxicology, University of Novi Sad, Hajduk Veljkova 3, Novi Sad, Serbia

The essential oils of rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia officinalis* L.) were analyzed by means of gas chromatography–mass spectrometry and assayed for their antimicrobial and antioxidant activities. Antimicrobial activity was tested against 13 bacterial strains and 6 fungi, including *Candida albicans* and 5 dermatomycetes. The most important antibacterial activity of both essential oils was expressed on *Escherichia coli*, *Salmonella typhi*, *S. enteritidis*, and *Shigella sonei*. A significant rate of antifungal activity, especially of essential oil of rosemary, was also exhibited. Antioxidant activity was evaluated as a free radical scavenging capacity (RSC), together with the effect on lipid peroxidation (LP). RSC was assessed by measuring the scavenging activity of essential oils on 2,2-diphenyl-1-picrylhydrazil (DPPH) and hydroxyl radicals. Effects on LP were evaluated following the activities of essential oils in Fe^{2+}/ascorbate and Fe^{2+}/H_2O_2 systems of induction. Investigated essential oils reduced the DPPH radical formation ($IC_{50} = 3.82$ μg/mL for rosemary and 1.78 μg/mL for sage) in a dose-dependent manner. Strong inhibition of LP in both systems of induction was especially observed for the essential oil of rosemary.

KEYWORDS: ***Rosmarinus officinalis*; *Salvia officinalis*; essential oil; chemical composition; antimicrobials; antioxidants**

INTRODUCTION

The oxidation of lipids in food, cosmetic, and pharmaceutical products, together with the growth of undesirable microorganisms results in the development of spoilage, off-flavor, rancidity, and deterioration, rendering such products unacceptable for human consumption. It has been estimated that each year as many as 30% of people in industrialized countries suffer from a foodborne disease. Thus, in the year 2000, at least two million people died from diarrheal disease worldwide (*1*). Furthermore, overproduction of free radicals in organisms and lipid peroxidation in cell membranes has been implicated in various pathophysiological dissorders, including cardiovascular diseases, mutagenesis, diabetes, ischemia-reperfusion injury, coronary atherosclerosis, Alzheimer's disease, and cancerogenesis, as well as the aging process (*2, 3*).

Since ancient times, aromatic herbs and spices have been added to different types of food to improve the flavor and organoleptic properties. Also, they have great potential in the emerging nutritious industry, because these materials are often considered as food and medicines, as well, and are used in prevention and curative treatments throughout the world (*4*). Their use in phytotherapy is mostly related to different activities of secondary biomolecules, especially essential oils, which are antimicrobial, spasmolytic, carminative, hepatoprotective, antiviral, anticarcinogenic, etc. (*5, 6*). Besides, many essential oils and isolated compounds were recently qualified as very strong natural antioxidants (*7–10*) and proposed as potential substitutes for synthetic antioxidants.

Thus, many aromatic plants are today considered as the most important sources for the extraction of compounds with strong antioxidant activity. Rosemary (*Rosmarinus officinalis* L., Lamiaceae) and sage (*Salvia officinalis* L., Lamiaceae) are two spices widely used in folk medicine, cosmetics, phytopharmacy, and the flavoring of food products (*6*). Furthermore, they are defined as very powerful aromatic plants. Many studies have been focused on various biological activities of the secondary metabolites in these two spices (*5, 7, 11–13*). It was found that they possess the best antioxidant activity among the wide range of herbs and spices tested (*14–17*). Furthermore, rosemary is the only spice

* To whom correspondence should be addressed. Tel/Fax: +39121 422 760. E-mail: bbozin2003@yahoo.co.uk.
† Department of Pharmacy.
‡ Deprtment of Chemistry.
§ Department of Pharmacology and Toxicology.

10.1021/jf0715323 CCC: $37.00 © 2007 American Chemical Society
Published on Web 08/21/2007

commercially available for use as an antioxidant in Europe and the United States, marketed in an oil soluble form, in dry powder, and also in water dispersible or water miscible formulations. Phenolic compounds such as carnosol, carnosoic acid, rosmanol, rosmadial, epirosmanol, rosmadiphenol, rosmarinic acid, etc. (*14, 16–18*) are considered to have antioxidant ability. However, data regarding this activity of essential oils of rosemary and sage, are not abundant and the methods for determination are different (*11, 15*). On the contrary, the antimicrobial activity of the rosemary and sage essential oils is well documented (*12, 15, 19–22*). But, these investigations are not so often performed with a precisely defined chemical composition of the essential oil in question. Furthermore, new examinations of antimicrobial activity on a wider spectrum of microorganisms, including some new multiresistant strains of bacteria and fungi, could help the pharmaceutical industry in synthesis or semisynthesis of new antibiotics (*23*).

In this study, the antioxidant activity and antimicrobial effects of essential oils of rosemary and sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L., Lamiaceae) against some pathogen strains of bacteria and fungi are reported. The chemical characterization of the investigated essential oils was performed by gas chromatography–mass spectrometry (GC-MS).

MATERIALS AND METHODS

Plant Material. Leaves of cultivated plants of rosemary (*Rosmarinus officinalis* L., Lamiaceae) were collected in July of 2005 in the Vojvodina province, Republic of Serbia. Leaves of wild-growing sage (*Salvia officinalis* L., Lamiaceae) were collected also in July of 2005 in the Sićevo gorge, in south Serbia. Voucher specimens of collected plants (rosemary no. 1980 and sage no. 1979) were confirmed and deposited at the Herbarium of the Department of Biology and Ecology (BUNS), Faculty of Natural Sciences, University of Novi Sad.

Essential Oil Isolation. Air-dried leaves of rosemary and sage were submitted to hydrodistillation according to ref *24*, using *n*-hexane as a collecting solvent. The solvent was removed under vacuum, and the quantities of the essential oils were determined gravimetrically.

Essential Oil Analysis. Qualitative and quantitative analysis of the investigated essential oils was carried out using a Hewlett-Packard 5973–6890 GC-MS system, operating in electron ionization (EI) mode at 70 eV, equipped with a split-splitless injector (200 °C) and a flame ionization detector (250 °C). Helium was used as carrier gas (1 mL/min), and the capillary columns used were HP 5MS (30 m × 0.25 mm; film thickness 0.25 μm). The temperature programs were 60–280 °C at a rate of 3 °C/min and 60–260 °C at a rate of 3 °C/min, respectively; the split ratio was 1:10. Coelution and MS analysis based the identification of individual compounds on comparison of their relative retention times with those of authentic samples (Carl Roth GmbH, Karlsruhe, Germany). For the other components, mostly sesquiterpenes and aliphatic compounds, for which authentic substances were not available, the identification was performed by matching their retention indices and mass spectra with NIST/NBS, Wiley libraries spectra, and literature data (*25*).

Antimicrobial Activity. *Evaluation of Antifungal Activity.* A collection of five test organisms of dermatomyceta and *Candida albicans*, shown in **Table 2** was used for the bioassay. Micromycetes were isolated directly from patients at the Centre for Preventive Medicine, Military Medical Academy (MMA), Belgrade, Serbia, and maintained on Sabourand agar (SBA) (Torlak, Belgrade, Serbia). Cultures were stored at +4 °C and subcultured once a month.

In order to investigate the antifungal activity of essential oils, the microdilution technique was performed as described before (*8*). The fungal spores were washed from the surface of agar plates with sterile 0.85% saline water containing 0.1% Tween 80 (v/v) (Torlak). The spore suspension was adjusted with sterile saline to a concentration approximately 1.0×10^5 in a final volume of 100 μL per well. A density of the fungal suspensions was determined by McFarland nefelometer (Dalynn Biological Inc.; Calgary, Canada). The inocula were stored at +4 °C for further investigations. Dilutions of the inocula were cultured on solid Malt agar (MA) (Torlak) to verify the absence of contamination and to check the viability of the inoculum. The minimal inhibitory concentration (MIC) determination was performed by a serial dilution technique using 96-well microtiter plates. Investigated samples were dissolved in Malt medium broth (MB) agar (Torlak) with fungal inoculum to achieve concentrations of 1.0–5.0 μL/mL. The microplates were incubated for 72 h at 28 °C. The lowest concentrations without visible growth at the binocular microscope were defined as the concentrations that completely inhibited the fungal growth (MIC). The minimal fungicidal concentrations (MFC) were determined by a serial subcultivation of 2 μL into microtiter plates containing 100 μL of MB per well and further incubation for 72 h at 28 °C. The lowest concentration with no visible growth was defined as the MFC, indicating 99.50% killing of the original inoculum. Bifonazole (in dilution of 1 g/100 mL of ethanol) was used as a synthetic antimycotic for a positive control.

Evaluation of Antibacterial Activity. A collection of 13 test organisms, 5 Gram-positive and 8 Gram-negative bacterial strains, was used. Eight microorganisms of the American type of Culture Collection (ATCC) and five microorganisms of the Institute of Public Health, Faculty of Medicine, University of Novi Sad, Serbia, were isolated directly from the patients (IPH), including four multiresistant strains (IPH-MR) were included in those groups. The source of the bacterial strains is shown in **Table 3**. All test organisms were stored at +4 °C on Mueller-Hinton (MH) agar (Torlak) slants, subcultured every two weeks, and checked for purity.

For the evaluation of the antibacterial activities of the essential oils, the hole–plate agar diffusion method was used as described before (*9*). The bacterial strains were grown on MH slants overnight at 37 °C and checked for purity. After incubation, the bacterial cells were washed from the surface of agar and suspended in sterile 0.1 M phosphate buffer (PB) containing 167 mM NaCl (167 mM NaCl–PB; pH = 7.4). A density of the bacterial suspensions was determined by the McFarland nefelometer (Dalynn Biological Inc.). The samples contained about 2×10^7 colony-forming units (CFU)/mL. Spreading of 0.1 mL of bacterial suspension seeded the surfaces of MH agar plates. The holes 5 mm in diameter on the agar surface were punched, and 15 μL of the tested essential oils (50% and 20% solutions in *n*-hexane) was put in them. The plates were incubated overnight at 37 °C, and the diameter of the resulting zone of inhibition was measured. The evaluation of the antibacterial activities of the essential oils was carried out in five repetitions. Penicillin (500 and 1000 μg/mL) was used as a positive antibiotic control. The effect of the solvent (*n*-hexane) on the microbial growth was also analysed.

Antioxidant Activity. Antioxidant properties of the rosemary and sage essential oils were evaluated as both free radical scavenging capacity (RSC) and protective effect on the lipid peroxidation (LP).

Free Radical Scavenging Capacity (RSC). RSC was evaluated measuring the scavenging activity of the examined essential oils on the DPPH and OH radicals.

The DPPH assay was performed as described before (*8*). Samples containing the amount of 3 mL of methanol solution of the essential oil at different concentrations (from 0.25 to 12.5 μg/mL) were mixed with 1 mL of 90 μM $DPPH^•$ solution (Sigma Co., St. Louis, MO). The absorbance of the resulting solutions was recorded spectrophotometrically (Beckman DU-65) at 515 nm after 1 h at room temperature, against the blank (with the same chemicals, except for the sample). The same procedure was repeated with *tert*-butylated hydroxytoluene (BHT; Fluka, AG, Buchs, Switzerland) as a positive control. For each sample four replicates were recorded. The percentage of RSC was calculated using the following equation:

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

The IC_{50} value, which represented the concentrations of the essential oil that caused 50% inhibition of LP, was determined by linear regression analysis from the obtained RSC values.

The scavenging capacity of the essential oils for hydroxyl radicals was evaluated by measuring the degradation of 2-deoxy-D-ribose (Fluka

AG) with OH radicals, generated in a Fenton reaction. The degradation products are the thiobarbituric acid-reactive substances (TBARS), which could be determined spectrophotometrically at 532 nm (*9*). All solutions and reagents were freshly prepared by dissolution in 0.05 M KH_2PO_4–K_2HPO_4 phosphate buffer. In a test tube, 10 μL of pure essential oil (2.13 μg/mL), 50% or 20% solution (1.065 and 0.425 μg/mL) in *n*-hexane with 0.125 mL of H_2O_2, 0.125 mL of $FeSO_4$, and 0.125 mL of 2-deoxy-D-ribose were mixed and filled up with 0.05 M PB, pH = 7.4, to a volume of 3 mL. After an incubation period of 1 h at 37 °C, the extent of deoxyribose degradation was measured by the 2-thiobarbituric acid (TBA) (Sigma) reaction. A 1.5 mL amount of TBA reagent (10.4 mL of 10% $HClO_4$, 3 g of TBA, and 120 g of 20% trichloroacetic acid) and 0.2 mL of 0.1 M EDTA (Sigma) were added to the reaction mixture, and the tubes were heated at 100 °C for 20 min. After the mixture was cooled, the absorbance was measured against a blank (containing buffer solution only) at 532 nm. Control with *n*-hexane instead of sample was also analyzed.

The absorbance at the end of the experiment was used to calculate the inhibition rate of deoxyribose degradation by the essential oil:

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

Four replicates were recorded for each sample. BHT 0.5 M (220.4 μg/mL) was used as a positive control.

Rapid Screening for Scavenging Compounds of Essential Oils. For fast screening of essential oil compounds on RSC, the dot-blot test on thin-layer chromatography (TLC) silica gel F_{254} aluminium plates (Merck, Darmstadt, Germany) stained with the free radical $DPPH^{\bullet}$ was used (*8*). An appropriate amount of pure essential oil (5 μL) was placed on a silica gel plate and eluted with benzene:ethyl acetate (95:5). After drying, the plates were sprayed with a 0.4 mM solution of $DPPH^{\bullet}$ in methanol, using a Desaga Spray Gun. Sprayed plates gave a purple background with yellow spots at the location of those compounds that possessed high RSC. Essential oil compounds responsible for scavenging activity were identified comparing the DPPH-TLC chromatogram with the control treated with vanillin–sulphuric acid spray reagent.

Determination of Lipid Peroxidation (LP). The extent of LP was determined by measuring the color of adduct produced in the reaction between TBA and malondialdehyde (MDA), as an oxidation product in the peroxidation of membrane lipids, by the TBA assay (*26*), with small modifications (*9*). For this investigation, two systems of induction, Fe^{2+}/ascorbate and Fe^{2+}/H_2O_2, were used. In both systems, the control with *n*-hexane instead of the sample was also analyzed and it expressed no activity.

The commercial preparation of liposomes "PRO-LIPO S" (Lucas-Meyer, Hamburg, Germany) pH = 5–7 was used as a model-system of biological membranes. The liposomes, 225–250 nm in diameter, were obtained by dissolving the commercial preparation in demineralized water (1:10), in an ultrasonic bath. Three concentrations of essential oils were prepared for the experiment: pure essential oil (2.13 μg/mL) and 50% and 20% solution in *n*-hexane (1.065 and 0.425 μg/mL).

In the Fe^{2+}/ascorbate induced LP, 60 μL of suspension of liposomes was incubated with 20 μL of 0.01 M $FeSO_4$, 20 μL of 0.01 M ascorbic acid and 10 μL of essential oil samples in 2.89 mL of 0.05 M KH_2PO_4–K_2HPO_4 buffer, pH = 7.4 (3 mL final solution).

The reaction mixture in Fe^{2+}/H_2O_2 induced LP contained 30 μL of a suspension of liposomes, 0.125 mL of 9 mM $FeSO_4$, 0.125 mL of 0.88 M H_2O_2, and 10 μL of tested essential oil samples in 2.71 mL of 0.05 M KH_2PO_4–K_2HPO_4 buffer, pH = 7.4 (3 mL final solution).

Samples were incubated at 37 °C for 1 h. LP was terminated using the reaction with 1.5 mL of TBA reagent and 0.2 mL of 0.1 M EDTA, heating at 100 °C for 20 min. After the cooling of solutions and precipitation of proteins by centrifugation (4000 rpm for 10 min), the content of the MDA (TBARS) was determined by measuring the absorbance of adduct at 532 nm. Both analyses were compared with the commercial synthetic antioxidant BHT (0.5 M stock solution, concentration 220.4 μg/mL) as a positive control. All reactions were carried out in triplicate. The control with *n*-hexane was also analyzed.

The percentage of LP inhibition was calculated by the following equation:

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

where A_0 was the absorbance of the control reaction (full reaction, without the test compound) and A_1 was the absorbance in the presence of the inhibitor.

RESULTS AND DISCUSSION

The content of essential oils (v/w in dry matter) was very similar in rosemary (1.18%) and sage (1.34%). The percentage composition of the essential oils is presented in **Table 1**. The total number of chemical constituents identified in essential oils was for rosemary 38 and for sage 31, representing 98.4% and 93.2% of the total oil content, respectively.

The oil distilled from the *Rosmarinus officinalis* was found to be composed of approximately equal amounts of the oxygenated monoterpenes (46.9%) and monoterpene hydrocarbons (46.7%). In the oil obtained from the *Salvia officinalis*, the oxygenated monoterpenes were found to be the major class of substances (57.8%), followed by the oxygenated hydrocarbons (19.5%). Hydrocarbons limonene (21.7%) and α-pinene (13.5%) and oxygenated monoterpenes camphor (21.6%) and Z-linalool oxide (10.8%) were identified as the main constituents in the rosemary essential oil. Besides, in a considerable amount, monoterpenes borneol (6.2%), camphene (3.9%), sabinene (2.0%), 1,8-cineole (2.1%), and α-terpineol (1.9%) were also detected (**Table 1**). In the essential oil of sage, three compounds were dominant: α-thujone (19.9%), camphor (18.9%), and viridiflorol (17.5%). They were followed by borneol (5.4%), 1,8-cineole (4.2%), β-thujone (3.8%), and bornyl acetate (3.3%).

Earlier data pertaining to rosemary essential oil point out the persistence of two chemotypes, Morocco/Tunisian (containing 38–55% of 1,8-cineole) and Spanish, characterized by a high amount of two monoterpene hydrocarbons α-pinene (18–26%) and camphene (8–12%) and 1,8-cineole (16–25%) (*6*). The investigated essential oil, obtained from the plant material cultivated in the Pannonian plane, has a specific chemical composition and could not be categorized in one of the two previously described chemotypes. On the contrary, the composition of the sage essential oil is in accordance with the earlier published data (*6, 27*). The specificity of this essential oil was a high amount of viridiflorol, a sesquiterpene alcohol that has not been reported yet as one of the major compounds in sage oil.

Antimicrobial Activity. Antimicrobial activity along with the antioxidant effectiveness of essential oils is one of the most examined features, important for both food preservation and control of human and animal diseases of microbial origin. Numerous reports suggest strong antibacterial and antifungal activities of a wide range of essential oils, especially those belonging to the Lamiaceae family (*8–10, 12, 15, 19–23, 28*). However, in order to get more relevant data about the influence of some essential oil compounds on the activity cited, further examinations are necessary.

The antifungal activity of the tested essential oils against the five examined dermatomycetes and *Candida albicans* is presented in **Table 2**. In general, both examined essential oils exhibited notable fungistatic and fungicidal activity. Comparing to the bifonazole, the essential oil of rosemary showed lower MIC especially against *C. albicans*, *Trichophyton tonsurans*, and *T. rubrum* indicating its significant antifungal effect. Strong antifungal activity against *C. albicans* is in accordance with earlier published data (*20, 22*), but no data about the antifungal effect of this oil on *Trichophyton* and *Microsporum* species have been reported yet.

Table 1. Chemical Composition of the Essential Oil of *Rosmarinus officinalis* and *Salvia officinalis*

pick no.	compounds	RI[a]	percentage		identification method[b]
			Rosmarinus officinalis	*Salvia officinalis*	
	Monoterpene Hydrocarbons		**44.7**	**3.0**	
1	α-pinene	939	13.5	0.5	RT GC MS
2	camphene	951	3.9	0.7	RT* MS
3	sabinene	972	2.0		RT GC MS
5	β-pinene	978	1.1	0.8	RT GC MS
6	E-isolimonene	982	0.3		RT* MS
8	*allo*-ocimene	1130	0.1		RT* MS
9	limonene	1032	21.7	1.0	RT GC MS
10	β-phellandrene	1034	0.9		RT* MS
12	γ-terpinene	1061	0.6		RT GC MS
13	α-terpinolene	1088	0.6		RT* MS
	Aromatic Monoterpene Hydrocarbons		**2.0**	**0.3**	
7	*o*-cymene	1020	2.0	0.3	
	Oxygenated Monoterpenes		**46.9**	**57.8**	
11	1,8-cineole	1035	2.1	4.2	RT GC MS
14	Z-linalool oxide	1065	10.8		RT* MS
15	Z-sabinene hydrate	1095	0.1		RT* MS
16	linalool	1101	1.1	0.6	RT GC MS
17	α-thujone	1104		19.9	RT GC MS
18	*endo*-fenchol	1111	0.2		RT* MS
19	β-thujone	1114	0.2	3.8	RT GC MS
20	camphor	1146	21.6	18.9	RT GC MS
21	camphene hydrate	1148	0.1		RT* MS
22	menthone	1156	0.4		RT GC MS
23	borneol	1167	6.2	5.4	RT GC MS
24	neomenthol	1170		0.7	RT* MS
25	terpinen-4-ol	1176	0.7	0.8	RT GC MS
26	α-terpineol	1188	1.9		RT GC MS
27	myrtenol	1196		0.2	RT* MS
28	pulegone	1235	0.1		RT GC MS
29	bornyl acetate	1288	1.4	3.3	RT GC MS
	Aromatic Oxygenated Monoterpenes		**0.4**		
30	carvacrol	1298	0.3		RT GC MS
32	methyl eugenol	1401	0.1		RT* MS
	Sesquiterpene Hydrocarbons		**2.2**	**10.1**	
31	α-jlangene	1372	0.1		RT* MS
33	longifolene	1403		0.8	RT* MS
34	E-caryophyllene	1419	1.0	1.8	RT GC MS
35	α-humulene	1452	0.9	2.6	RT GC MS
36	*allo*-aromadendrene	1462		0.7	RT* MS
37	α-muurolene	1498	0.1		RT* MS
39	Z-α-bisabolene	1504		0.9	RT* MS
40	β-bisabolene	1508	0.1	0.8	RT* MS
41	E,E-α-farnesene	1509		2.5	RT* MS
	Oxygenated Sesquiterpenes		**0.3**	**19.5**	
42	ledol	1563		0.6	RT* MS
43	caryophyllnol	1568	0.2	0.4	RT* MS
44	spathulenol	1576		0.2	RT* MS
45	caryophyllene oxide	1584	0.1	0.7	RT* MS
46	viridiflorol	1593		17.5	RT* MS
51	sclareol	2220		0.1	RT* MS
	Aliphatic Compounds		**1.9**	**2.5**	
4	3-cctenol	976	1.3		RT* MS
38	pentadecane	1501		0.4	RT* MS
47	heptadecane	1700		0.1	RT* MS
48	octadecane	1799	0.2		RT* MS
49	nonadecane	1900	0.3		RT* MS
50	eicosane	2001	0.1	2.0	RT* MS
	Total of Identified Compounds		**98.4**	**93.2**	

[a] Retention indices relative to C_9–C_{24} *n*-alkanes on the HP 5MS column. [b] (RT) comparison with pure standard retention time; (GC) gas chromatographic coelution with pure standard; (MS) mass spectrometry; (RT*) comparison of the relative retention time with those obtained from the NIST/NBS, Wiley libraries spectra, and those reported by Adams (*25*).

The antibacterial activity of tested essential oils of rosemary and sage against five strains of Gram-positive and eight strains of Gram-negative bacteria is shown in **Table 3**. In general, tested Gram-positive strains of bacteria tested seemed to be more sensitive to the examined essential oils. These results are in accordance with the earlier published literature data (*12, 15, 20–23*). However, this study also recorded a notable susceptibility of the examined Gram-negative pathogenic bacteria. All tested strains of *E. coli*, including the multiresistant one, showed high sensitivity to the essential oils of rosemary and sage, which is of a particular interest. Furthermore, both essential oils exhibited important activity against *Shigella sonei* and *Salmonella typhi*.

The essential oil of rosemary exhibited antibacterial and antifungal activity as expected although in several cases that effect was stronger than in earlier published data (*12, 15, 20–22*)

Table 2. Antifungal Activity of Essential Oils[a] of *Rosmarinus officinalis*, *Salvia officinalis*, and Bifonazole (as a Positive Control)

		Rosmarinus officinalis		*Salvia officinalis*		bifonazole	
source	organism	MIC	MFC	MIC	MFC	MIC	MFC
MMA	*Candida albicans*	30.2 ± 0.3	60.2 ± 0.3	200.0 ± 0.0	250.0 ± 0.0	20.0 ± 0.0	25.2 ± 0.3
MMA	*Trichophyton mentagrophytes*	15.3 ± 0.6	30.0 ± 0.0	60.0 ± 1.0	100.0 ± 0.0	9.7 ± 0.6	9.7 ± 0.6
MMA	*Trichophyton tonsurans*	15.2 ± 0.3	30.0 ± 0.0	60.0 ± 1.0	100.3 ± 0.6	10.0 ± 0.0	10.0 ± 0.0
MMA	*Trichophyton rubrum*	15.0 ± 0.0	30.2 ± 0.3	60.0 ± 1.0	100.3 ± 0.6	10.3 ± 0.6	10.3 ± 0.6
MMA	*Epidermophyton floccosum*	30.0 ± 0.0	30.0 ± 0.0	100.0 ± 0.0	200.2 ± 0.3	10.0 ± 0.0	10.0 ± 0.0
MMA	*Microsporum canis*	30.2 ± 0.3	30.2 ± 0.3	100.2 ± 0.3	200.2 ± 0.3	15.0 ± 0.0	20.0 ± 0.0

[a] Concentrations shown in the table were expressed in microliters. If they are expressed in real concentrations of active substances (μg), the expressed values would be 10 times higher.

Table 3. Antibacterial Activity[a] of Essential Oils[b] of *Rosmarinus officinalis*, *Salvia officinalis*, and Penicillin (as a Positive Control)

		Rosmarinus officinalis		*Salvia officinalis*		penicillin (μg/mL)	
source	organism	20%	50%	20%	50%	500	1000
ATCC 27853	*Pseudomonas aeruginosa*	0.0	0.0	0.0	0.0	0.0	0.0
IPH-MR	*Pseudomonas aeruginosa*	0.0	0.0	0.0	0.0	0.0	0.0
ATCC 35218	*Escherichia coli*	19.8 ± 0.45	23.0 ± 0.71	20.2 ± 0.45	20.4 ± 0.55	0.0	0.0
ATCC 25922	*Escherichia coli*	22.0 ± 0.71	25.0 ± 0.71	23.8 ± 1.09	24.6 ± 0.55	12.6 ± 0.89	13.4 ± 0.55
IPH-MR	*Escherichia coli*	18.6 ± 0.89	18.2 ± 0.45	13.8 ± 0.84	14.2 ± 0.84	0.0	0.0
IPH-MR	*Salmonella typhi*	18.0 ± 0.00	16.2 ± 0.45	40.2 ± 0.45	50.0 ± 0.00	0.0	0.0
IPH	*Salmonella enteritidis*	26.6 ± 2.07	29.0 ± 0.71	16.6 ± 2.07	10.0 ± 0.71	22.0 ± 1.22	26.2 ± 0.83
IPH-MR	*Shigella sonei*	19.4 ± 0.89	19.6 ± 0.89	21.2 ± 0.83	13.2 ± 0.84	0.0	0.0
ATCC 10240	*Micrococcus flavus*	30.0 ± 0.00	23.6 ± 2.07	56.0 ± 0.71	60.6 ± 0.89	40.0 ± 0.55	40.0 ± 0.89
ATCC 9341	*Sarcina lutea*	16.2 ± 1.48	43.2 ± 1.92	21.0 ± 0.71	21.2 ± 0.84	0.0	0.0
ATCC 6538	*Staphylococcus aureus*	17.2 ± 1.09	29.0 ± 0.71	24.0 ± 1.00	20.0 ± 0.71	48.0 ± 0.00	50.0 ± 0.00
ATCC 12228	*Staphylococcus epidermidis*	18.2 ± 1.48	20.2 ± 1.48	26.0 ± 1.41	49.2 ± 0.84	14.2 ± 0.44	16.8 ± 0.83
ATCC 10707	*Bacillus subtilis*	20.2 ± 0.45	21.4 ± 0.89	41.4 ± 1.67	60.2 ± 0.45	38.2 ± 0.44	38.0 ± 0.70

[a] The inhibition zone is measured in millimeters, including the hole of 5 mm in diameter. The values shown represent the average of five determinations ± standard deviations. [b] All essential oils were diluted in *n*-hexane.

Table 4. Neutralization of DPPH• by *Rosmarinus officinalis* and *Salvia officinalis* Essential Oils and BHT (as a Positive Control) in the DPPH Assay (in Percentage)

	concentrations (μL/mL)											
source	0.25	0.50	1.20	2.50	3.12	4.80	6.25	7.20	9.60	12.00	12.50	IC_{50}
Rosmarinus officinalis		13.89	19.44	36.11	47.56	58.33	64.42	66.67	80.55	88.89	92.24	3.82
Salvia officinalis	11.76	23.53	38.23	70.59	87.54	98.99	>100	>100	>100	>100	>100	1.78
BHT		4.62	11.56	23.12	30.11	44.71	55.22	65.07	67.51	70.12	74.22	5.37

possibly due to the specific chemical profile of the essential oil, in which limonene and camphor are the main compounds, followed with high amounts of α-pinene, Z-linalool oxide, and borneol (**Table 1**).

Antioxidant Activity. The antioxidant potential of plant products and pure compounds can be evaluated using numerous assays. The first step in these examinations is the screening of the potential activity by different in vitro tests. Each of those is based on one feature of the antioxidant activity, such as the ability of scavenging free radicals, the inhibition of lipid peroxidation, the chelating of transition metal ions (TMI), etc. However, in order to get relevant data, a single method for testing antioxidant activities of plant products is not recommended due to their complex composition (*29*). Therefore, the antioxidant activity of the tested essential oils of rosemary and sage has been evaluated in a series of in vitro tests.

In the DPPH assay, the ability of essential oils of interest to act as donors of hydrogen atoms or electrons in transformation of DPPH• into its reduced form DPPH–-H was investigated. Both examined essential oils were able to reduce the stable, purple-colored radical DPPH into yellow-colored DPPH–H, reaching 50% of reduction with IC_{50} values of 3.82 for rosemary and 1.78 μL/mL for sage (**Table 4**). Comparison of the DPPH scavenging activity of the investigated essential oils and those expressed by BHT (5.37 μL/mL) showed that both examined essential oils possessed stronger antioxidant effects than BHT. These findings are in correlation with the earlier published data on the notable antiradical activity of both essential oils (*11*). The comparison of control TLC analysis with the results of GC-MS (**Table 1**) and TLC-DPPH methods accomplished the identification of the constituents most responsible for RSC. For the neutralization of DPPH radicals, the most responsible compounds were the oxygenated monoterpenes (α- and β-thujone, bornyl acetate, camphor, and menthone) and the mixture of mono- and sesquiterpene hydrocarbons (**Table 5**). Although found in a small amount in the essential oil of rosemary, carvacrol (**Table 1**) exhibited notable scavenging activity, too. These findings confirm the earlier obtained data on the antioxidant activities of the selected components of essential oils (*7–10*). A higher RSC was gained by the sage essential oil ($IC_{50} = 1.78$ μL/mL) compared to that expressed by the essential oil of rosemary ($IC_{50} = 3.82$ μL/mL). This could be explained partially by the presence of a higher amount of compounds responsible for this activity, such as bornyl acetate, camphor, and sesquiterpene hydrocarbons recorded in the sage essential oil (**Table 1**).

Furthermore, in **Figure 1**, the hydroxyl RSC of the examined essential oils measured by the deoxyribose assay is shown. Protective effects of the essential oils on 2-deoxy-D-ribose were assessed through their ability to remove hydroxyl radicals

Table 5. DPPH Scavenging Active Compounds Identified by the TLC Dot-Blot Technique

source of essential oil	compound	Rf values
Rosmarinus officinalis	carvacrol	0.65
	menthone	0.67
	bornyl acetate	0.69
	camphor	0.80
	mixture of mono- and sesquiterpene hydrocarbons	0.97
Salvia officinalis	α- and β-thujone	0.64–0.68
	bornyl acetate	0.69
	camphor	0.80
	mixture of mono- and sesquiterpene hydrocarbons	0.98

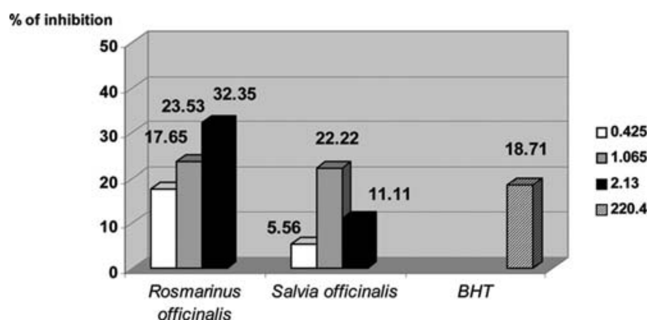

Figure 1. Inhibition of degradation of 2-deoxyribose by essential oils (all examined essential oils were diluted in *n*-hexane (the solvent expressed no scavenging activity)) of *Rosmarinus officinalis*, *Salvia officinalis*, and BHT (as a positive control) in the deoxyribose assay. The numbers and labels on the right show concentrations expressed in microliters per milliliter.

formed in Fenton reaction from the test solution and to prevent its degradation. However, this feature of the antioxidant activity of investigated essential oils has not been investigated yet. Generally, both essential oils inhibited the degradation of deoxyribose more than BHT (18.71%), used as a positive control. Higher activity was shown by the essential oil of rosemary (in a range from 17.65 to 32.35% inhibition), compared to that exhibited by the sage essential oil (5.56–22.22%).

The protective effects of the rosemary and sage essential oils on LP have been evaluated using two systems of induction, Fe^{2+}/ascorbate and Fe^{2+}/H_2O_2. The inhibition of LP was determined by measuring the formation of MDA, using liposomes as an oxidizable substrate.

In **Figure 2**, results of antioxidant activities of the examined essential oils and BHT as a positive control in the Fe^{2+}/ascorbate system of induction are presented. Both essential oils expressed stronger antioxidant capacity than BHT (37.04%). In particular, the essential oil of rosemary exhibited very high activity (from 56.29 to 75.79% inhibition). In LP induced by the Fenton reaction (Fe^{2+}/H_2O_2), both examined essential oils, together with BHT, exhibited very similar antioxidant activities (ranging from 33.33 to 66.67%) (**Figure 3**). Essential oil obtained from rosemary exhibited a little bit of a stronger effect on the LP (33.33–58.33%) than sage essential oil (33.33–40%). In both systems of induction, the essential oil of rosemary reached 50% of LP inhibition and also showed dose-dependent activity. These findings are in accordance with earlier published data about antioxidant activities of these two essential oils (*15*).

In conclusion, the results of the antioxidant effects of the investigated essential oils, obtained with different methods of assessment, point out very strong protective activities, both as

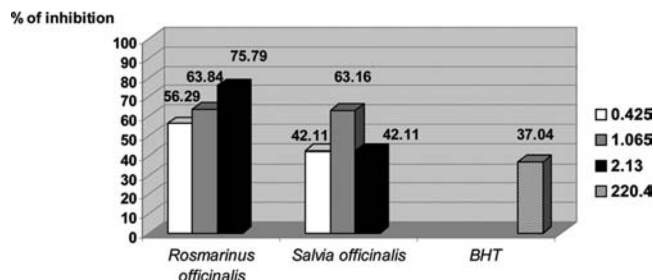

Figure 2. Inhibition of lipid peroxidation (LP) in Fe^{2+}/ascorbate system of induction by the essential oils of *Rosmarinus officinalis*, *Salvia officinalis*, and BHT (as a positive control; the essential oils and BHT were diluted in *n*-hexane (the solvent expressed no antioxidant activity)) in the TBA assay. The numbers and labels on the right show concentrations expressed in microliters per milliliter.

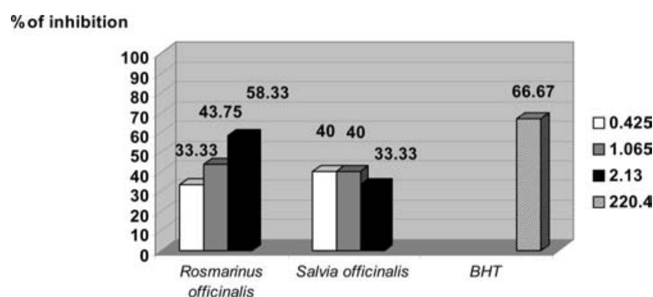

Figure 3. Inhibition of lipid peroxidation (LP) in Fe^{2+}/H_2O_2 system of induction by the essential oils of *Rosmarinus officinalis*, *Salvia officinalis*, and BHT (as a positive control; the essential oils and BHT were diluted in *n*-hexane (the solvent expressed no antioxidant activity)) in the TBA assay. The numbers and labels on the right show concentrations expressed in microliters per milliliter.

free radical scavengers and LP inhibitors. However, from the obtained results, it is obvious that the assayed essential oils, together with the compounds present in them, have different modes of action. Thus, the essential oil of sage exhibited stronger RSC against DPPH radical, which was found to be in correlation to the content of some oxygenated monoterpene compounds and sesquiterpene hydrocarbons. The essential oil of rosemary expressed a better effect against hydroxyl radical and in the processes of the peroxidation of lipids. Furthermore, the study confirmed the earlier published data about significant antibacterial and antifungal activity of the investigated essential oils against a wide spectrum of bacteria and fungi. The use of investigated essential oils and spices could be useful not only in food and cosmetics production but also as important functional food in the prevention and treatment of various human diseases.

LITERATURE CITED

(1) WHO. Food safety and food borne illness. In *World health Organization Fact sheet*; World Health Organization: Geneva, Switzerland, 2002; pp 237.

(2) Halliwell, B.; Gutteridge, J. M. C. *Free radicals in biology and medicine*; Clarendon Press: Oxford, UK, 1989; pp 23–30.

(3) Smith, M. A.; Perry, G.; Pryor, W. A. Causes and consequences of oxidative stress in Alzheimer's disease (1,2). *Free Radical Biol. Med.* **2002**, *32* (11), 1049.

(4) Diplock, A. T.; Charleux, J. L.; Crozier-Willi, G.; Kok, F. J.; Rice-Evans, C.; Roberfroid, M.; Stahl, W.; Vina-Ribes, J. Functional food science and defence against reactive oxidative species. *Brit. J. Nutr.* **1998**, *80*, S77–S112.

(5) Buchbauer, G.; Jirovetz, L. Aromatherapy-Use of Fragrances and Essential Oils as Medicaments. *Flavour Fragrance J.* **1994**, *9*, 217–222.

(6) Bruneton, J. *Pharmacognosy, Phytochemistry, Medicinal Plants*, 2nd ed.; Intercept Ltd.: London, 1999; pp 539–540.

(7) Ruberto, G.; Baratta, M. T. Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chem.* **2000**, *68*, 167–174.

(8) Mimica-Dukic, N.; Bozin, B.; Sokovic, M.; Mihajlovic, B.; Matavulj, M. Antimicrobial and Antioxidant Activities of Three Mentha Species Essential Oils. *Planta Med.* **2003**, *69*, 413–419.

(9) Mimica-Dukic, N.; Bozin, B.; Sokovic, M.; Simin, N. Antimicrobial and Antioxidant Activities of *Melissa officinalis* L. (Lamiaceae) Essential Oil. *J. Agric. Food Chem.* **2004**, *52*, 2485–2489.

(10) Bozin, B.; Mimica-Dukic, N.; Simin, N.; Anackov, G. Characterization of the Volatile Composition of Essential Oils of Some Lamiaceae Species and the Antimicrobial and Antioxidant Activities of the Entire Oils. *J. Agric. Food Chem.* **2006**, *54*, 1822–1828.

(11) Lee, S. E.; Lee, H. S.; Ahn, Y. J. Scavenging Effect of Plant-Derived Materials on Free Radicals and Active Oxygen Species. *Agric. Chem. Biotechnol.* **1999**, *42* (1), 40–44.

(12) Daferera, D. J.; Ziogas, B. N.; Polissiou, M. G. GC-MS analysis of essential oils from some greek aromatic plants and their fungitoxicity on *Penicillium digitatum*. *J. Agric. Food Chem.* **2000**, *48*, 2576–2581.

(13) Kosar, M.; Dorman, H. J. D.; Hiltunen, R. Effect of an acid treatment on the phytochemical and antioxidant characteristics of extracts from selected Lamiaceae species. *Food Chem.* **2005**, *91*, 525–533.

(14) Madsen, H. L.; Bertelsen, G. Spices as antioxidants. *Trends Food Sci. Technol.* **1995**, *6*, 271–277.

(15) Baratta, M. T.; Dorman, H. J. D.; Deans, S. G.; Biondi, D. M.; Ruberto, G. Chemical Composition, Antimicrobial and Antioxidative Activity of Laurel, Sage, Rosemary, Oregano and Coriander Essential Oils. *J. Ess. Oil. Res.* **1998**, *10*, 618–627.

(16) Bicchi, C.; Binello, A.; Rubiolo, P. Determination of Phenolic Diterpene Antioxidants in Rosemary (*Rosmarinus officinalis* L.) with Different Methods of Extraction and Analysis. *Phytochem. Anal.* **2000**, *11*, 236–242.

(17) Yanishlieva, N. V.; Marinova, E.; Pokorny, J. Natural antioxidants from herbs and spices. *Eur. J. Lipid Sci. Technol.* **2006**, *108*, 776–793.

(18) Wijeratne, S. K.; Cuppett, S. L. Potential of Rosemary (*Rosmarinus officinalis* L.) Diterpenes in Preventing Lipid Hydroperoxide-Mediated Oxidative Stress in Caco-2 Cells. *J. Agric. Food Chem.* **2007**, *55*, 1193–1199.

(19) Lis-Balchin, M.; Deans, S. G. Bioactivity of selected plant essential oils against *Listeria monocytogenes*. *J. Appl. Bacteriol.* **1997**, *82*, 759–762.

(20) Hammer, K. A.; Carson, C. F.; Riley, T. V. Antimicrobial activity of essential oils and other plant extracts. *J. Appl. Microbiol.* **1999**, *86*, 985–990.

(21) Managena, T.; Muyima, N. O. Comparative evaluation of the antimicrobial activities of essential oils of *Artemisia afra*, *Pteronia incana* and *Rosmarinus officinalis* on selected bacteria and yeast strains. *Lett. Appl. Microbiol.* **1999**, *28*, 291–296.

(22) Yesil Celiktas, O.; Hames Kocabas, E. E.; Bedir, E.; Vardar Sukan, F.; Ozek, T.; Baser, K. H. C. Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. *Food Chem.* **2007**, *100* (2), 553–559.

(23) Cowan, M. M. Plant Products as Antimicrobial Agents. *Clin. Microbiol. Rev.* **1999**, *12* (4), 564–582.

(24) *European Pharmacopeia*, 4th ed.; Council of Europe: Strasbourg Cedex, France, 2002; *Vol. 2.8.12*, pp 183–184.

(25) Adams, R. P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*; Allured Publishing Corp.: Carol Stream, Illinois, 1995.

(26) Afanasev, I. B.; Doroszhko, A. I.; Brodski, A. V.; Kostyuk, V. A.; Potapovitch, A. I. Chelating and free radical scavenging mechanism of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochem. Pharmacol.* **1989**, *38* (11), 1763–1768.

(27) Couladis, M.; Tzakou, O.; Mimica-Dukic, N.; Jancic, R.; Stojanovic, D. Essential oil of *Salvia officinalis* L. from Serbia and Montenegro. *Flav. Fragr. J.* **2002**, *17*, 199–126.

(28) Morris, J. A.; Khettry, A.; Seitz, E. W. Antimicrobial activity of aroma chemicals and essential oils. *J. Am. Oil Chem. Soc.* **1979**, *56*, 598–619.

(29) Nuutila, A. M.; Puupponen-Pimia, R.; Aarni, M.; Oksman-Caldentey, K. M. Comparision of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. *Food Chem.* **2003**, *81*, 485–493.

Received for review May 25, 2007. Revised manuscript received July 18, 2007. Accepted July 21, 2007. The Ministry of Sciences and Environmental Protection, Republic of Serbia, No. 142036, supported this research work.

JF0715323