Characterization of the Volatile Composition of Essential Oils of Some Lamiaceae Spices and the Antimicrobial and Antioxidant Activities of the Entire Oils

BILJANA BOZIN,[†] NEDA MIMICA-DUKIC,*,[‡] NATASA SIMIN,[‡] AND
GORAN ANACKOV[§]

Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, and
Departments of Chemistry and Biology and Ecology, Faculty of Natural Sciences and Mathematics,
University of Novi Sad, Trg D. Obradovica 3, 21000 Novi Sad, Serbia and Montenegro

The essential oils of *Ocimum basilicum* L., *Origanum vulgare* L., and *Thymus vulgaris* L. were analyzed by means of gas chromatography−mass spectrometry and assayed for their antioxidant and antimicrobial activities. The antioxidant activity was evaluated as a free radical scavenging capacity (RSC), together with effects on lipid peroxidation (LP). RSC was assessed measuring the scavenging activity of the essential oils on 2,2-diphenyl-1-picrylhydrazil (DPPH•) and OH• 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. Essential oils exhibited very strong RSCs, reducing the DPPH radical formation (IC_{50}) in the range from 0.17 (oregano) to 0.39 μg/mL (basil). The essential oil of *T. vulgaris* exhibited the highest OH radical scavenging activity, although none of the examined essential oils reached 50% of neutralization (IC_{50}). All of the tested essential oils strongly inhibited LP, induced either by Fe^{2+}/ascorbate or by Fe^{2+}/H_2O_2. The antimicrobial activity was tested against 13 bacterial strains and six fungi. The most effective antibacterial activity was expressed by the essential oil of oregano, even on multiresistant strains of *Pseudomonas aeruginosa* and *Escherichia coli*. A significant rate of antifungal activity of all of the examined essential oils was also exhibited.

KEYWORDS: *Ocimum basilicum*; *Origanum vulgare*; *Thymus vulgaris*; essential oils; GC-MS; antimicrobial activity; antioxidant activity

INTRODUCTION

It is well-known that most spices, especially those belonging to the Lamiaceae family, possess a wide range of biological and pharmacological activities. Since ancient times, they have been used to improve the flavor and the organoleptic properties of different types of food. Furthermore, the use of aromatic plants and spices in phytotherapy is mostly related to different activities of their essential oils, such as antimicrobial, spasmolytic, carminative, hepatoprotective, antiviral, anticarcinogenic, etc. activities (*1*, *2*). Particularly, the antimicrobial activity has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine, and natural therapies (*3*−*5*). This aspect assumes a particular relevance due to an increased resistance of some bacterial strains to the most common antibiotics (*6*) and antimicrobial agents for food preservation. Besides these activities, many essential oils were recently qualified as natural antioxidants (*7*−*10*) and proposed as potential substitutes for synthetic antioxidants.

The antioxidant potential of different plant extracts and pure compounds can be measured using numerous in vitro assays. Each of these tests is based on one feature of the antioxidant activity, such as the ability to scavenge free radicals, the inhibition of lipid peroxidation (LP), etc. However, single methods are not recommended for the evaluation of the antioxidant activities of different plant products, due to their complex composition (*11*, *12*). Therefore, the antioxidant effects of plant products must be evaluated combining two or more different in vitro assays to get relevant data.

With respect to this, in the present study, the antioxidant activity in three different model systems and the antimicrobial effects of essential oils extracted from the aerial parts of *Ocimum basilicum* L. and *Origanum vulgare* L. and the leaves of *Thymus vulgaris* L. (Lamiaceae) are reported. The chemical characterization of the investigated essential oils was done by means of gas chromatography−mass spectrometry (GC-MS).

MATERIALS AND METHODS

Plant Material. Aerial parts of cultivated flowering plants of basil (*O. basilicum* L.) and oregano (*O. vulgare* L.) and leaves of thyme (*T.*

---

* To whom correspondence should be addressed. Tel: +381-21-6350-122/978. Fax: +381-21-554-065. E-mail: mimica@ih.ns.ac.yu.
[†] Department of Pharmacy.
[‡] Department of Chemistry.
[§] Department of Biology and Ecology.

Activities of Lamiaceae Spices Essential Oils

J. Agric. Food Chem., Vol. 54, No. 5, 2006 **1823**

vulgaris L.) were collected in July of 2000 in Vojvodina province, Serbia and Montenegro. Voucher specimens of collected plants (*O. basilicum* no. 2165, *O. vulgare* no. 2178, and *T. vulgaris* no. 2206) were confirmed and deposited at the Herbarium of the Department of Biology and Ecology (BUNS), Faculty of Natural Sciences, University of Novi Sad.

Essential Oils Isolation. Air-dried plant materials were submitted to hydrodistillation according to Eur. Pharm. 4 (*13*), using *n*-hexane (Merck, Darmstadt, Germany) as a collecting solvent. The solvent was removed under vacuum, and the quantities of the essential oils were determined gravimetrically.

Essential Oils Analysis. Qualitative and quantitative analyses of the essential oils were carried out using a Hewlett-Packard 5973-6890 GC-MS system, operating in 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 column was a 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; split ratio, 1:10. Coelution and MS analysis were based on the identification of individual compounds on comparison of their relative retention times with those of authentic samples (Carl Roth GmbH, Karlsruhe, Germany). For the components, mostly sesquiterpenes and aliphatic compounds, for which reference substances were not available, the identification was performed by matching their retention indices (RIs) and mass spectra with those obtained from authentic samples and/or the NIST/NBS and Wiley libraries spectra as well as with literature data (*14*).

Antioxidant Activity. *Free Radical Scavenging Capacity (RSC).* The RSC was evaluated by measuring the scavenging activity of examined essential oils on 2,2-diphenyl-1-picrylhydrazil (DPPH) and OH radicals.

The DPPH assay was performed as described before (*15*). The samples (from 0.012 to 6.25 μg/mL) were mixed with 1 mL of 90 μM DPPH• solution (Sigma, St. Louis, MO) and filled up with 95% MeOH to a final volume of 4 mL. The absorbance of the resulting solutions and the blank (with same chemicals, except sample) were recorded after 1 h at room temperature, against *tert*-butylated hydroxytoluene (BHT) (Fluka AG, Buchs, Switzerland) as a positive control. For each sample, four replicates were recorded. The disappearance of DPPH• was read spectrophotometrically at 515 nm using a Beckman DU-65 spectrophotometer. The RSC in percent was calculated by following equation:

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

From the obtained RSC values, the IC_{50} values, which represented the concentrations of the essential oils that caused 50% neutralization, were determined by linear regression analysis.

The scavenging capacity of essential oils for hydroxyl radicals was evaluated measuring the degradation of 2-deoxyribose (Fluka AG) with OH radicals, generated in a Fenton reaction. The degradation products are the 2-thiobarbituric acid (TBA) (Sigma) reactive substances, which could be determined spectrophotometrically at 532 nm (*10*). All solutions and reagents were dissolved in 0.05 M KH_2PO_4−K_2HPO_4 phosphate buffer and prepared freshly. In a test tube, 10 μL of pure essential oils (2.13 μg/mL) and 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 TBA reaction. A 1.5 mL amount of TBA reagent and 0.2 mL of 0.1 M ethylenediaminetetraacetic acid (EDTA) (Sigma) were added to the reaction mixture, and the tubes were heated at 100 °C for 20 min. After the mixtures were cooled, the absorbance was read against a blank (containing buffer solution instead sample) at 532 nm. A control with *n*-hexane instead of sample was also tested and expressed no activity.

The absorbance read at the end of the experiment was used for the calculation of the percentage inhibition of deoxyribose degradation by the essential oil:

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

For each sample, four replicates were recorded. A 0.1 M concentration of BHT 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} aluminum plates (Merck) stained with the free radical DPPH• was used (*15, 16*). Appropriate amounts of essential oils (5 μL) were placed on silica gel plates and chromatographed in the solvent system benzene:EtOAc (95: 5). After the mobile phase was dried, the staining of the silica layer was carried out by spraying the layer with 0.4 mM solution of DPPH• in MeOH using a Desaga Spray-Gun. This method is based on the monitoring of purple-colored DPPH• transformation into its reduced yellow-colored form diphenylpicrylhydrazine (DPPH-H). The stained silica layer gave a purple background with yellow spots at the location of those compounds on TLC that possess high RSC. Essential oil compounds responsible for scavenging activity were identified comparing the DPPH TLC chromatogram with a control. The control plate was made visible by spraying with vanillin−sulfuric acid reagent (*17*).

Determination of 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 (*15*). For investigations, two systems of induction, Fe^{2+}/ascorbate and Fe^{2+}/H_2O_2, were used. In both systems of induction, a control with *n*-hexane instead of sample was also analyzed and 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. For the experiment, three concentrations of essential oils were prepared as follows: 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, a 60 μL 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 or 0.1 mL of EDTA, heated at 100 °C for 20 min. After precipitated proteins were cooled and centrifuged (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 of the reactions were carried out in triplicate.

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

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

where A_o 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.

Antimicrobial Activity. *Evaluation of Antifungal Activity.* For the bioassay, a collection of five test organisms of dermatomyceta and *Candida albicans*, shown in **Table 5**, was used. Micromycetes were isolated directly from patients at the Centre for Preventive Medicine, Military Medical Academy (MMA) (Belgrade, Yugoslavia) and maintained on Sabourand agar (SBA) (Torlak, Belgrade, Serbia and Montenegro). Cultures were stored at +4 °C and subcultured once a month.

To investigate the antifungal activity of essential oils, the microdilution technique was performed as described before (*15*). 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 of

1824 *J. Agric. Food Chem.,* Vol. 54, No. 5, 2006

Bozin et al.

approximately 1.0×10^5 in a final volume of 100 μL per well. 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 validity of the inoculum. The minimal inhibitory concentrations (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, including five Gram-positive and eight Gram-negative bacterial strains, was used. The groups included eight organisms of American Type of Culture Collection (ATCC) and five organisms of Institute of Public Health, Faculty of Medicine, University of Novi Sad, isolated directly from the patients (IPH), including four multiresistant strains (IPH-MR). The source of the bacterial strains is shown in **Table 4**. All test organisms were stored at +4 °C on Mueller–Hinton (MH) agar (Torlak) slants, subcultured every 2 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 (*10*, *18*). 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 McFarland nefelometer (Dalynn Biological Inc., Calgary, Canada). 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. On the surface of the agar, the 5 mm holes in diameter were punched. Fifteen microliters of the tested essential oils (50 and 20% solutions in *n*-hexane was applied to the holes in Petri plates. 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/cm^3) was used as a positive antibiotic control. The effect of the solvent (*n*-hexane) on the microbial growth was also analyzed.

RESULTS AND DISCUSSION

The content of the essential oils expressed in percentages was as follows: *O. basilicum*, 0.37%; *O. vulgare*, 1.45%; and *T. vulgaris*, 1.80%. **Table 1** lists the chemical components of the investigated essential oils. The main constituents of *O. basilicum* essential oil were methyl chavicole (45.8%) and linalool (24.2%). In the essential oil of *O. vulgare*, the major compounds were oxygenated phenolic monoterpenes carvacrol (61.3%) and thymol (13.9%). The dominant components in the essential oil of *T. vulgaris* were also carvacrol and thymol but in different ratios (**Table 1**), together with γ-terpinene (8.3%). These results are in accordance with the previously published data on basil (*19*), oregano (*20*, *21*), and thyme (*22*) essential oil compositions.

Antioxidant Activity. The antioxidant activity of basil, oregano, and thyme essential oils has been evaluated in a series of in vitro tests. In the DPPH assay, the ability of the investigated essential oils to act as donors of hydrogen atoms or electrons in transformation of DPPH$^\bullet$ into its reduced form DPPH-H was investigated. All of the assessed essential oils were able to reduce the stable, purple-colored radical DPPH into yellow-colored DPPH-H reaching 50% of reduction with an IC$_{50}$ as follows: 0.17 μg/mL for oregano, 0.19 μg/mL for thyme, and 0.39 μg/mL for basil (**Table 2**). Comparison of the DPPH scavenging activity of the investigated essential oils with those expressed by BHT (5.37 μg/mL) showed that all of the examined essential oils expressed stronger antioxidant effects. The identification of the constituents most responsible for RSC was accomplished by comparing the control TLC analysis with the results of GC-MS (**Table 1**) and the TLC-DPPH method. For the neutralization of DPPH radicals, the most responsible compounds were the oxygenated phenolic monoterpenes (carvacrol, thymol, and methyl chavicole) and the mixture of mono- and sesquiterpene hydrocarbons (**Table 3**). These findings are in correlation with the earlier published data on the antioxidant activities of the investigated essential oils and selected essential oils components (*7*, *8*, *20*, *23*, *24*). The obtained results also confirm the previously published data on stronger antioxidant activity of carvacrol as compared to the thymol (*8*).

Furthermore, in **Figure 1**, the hydroxyl RSC of the examined essential oils, measured by the deoxyribose assay, is shown. The protective effects of the essential oils on 2-deoxy-D-ribose were assessed as their ability to remove hydroxyl radicals, formed in Fenton reaction, from the test solution and prevent its degradation. Generally, all of the examined essential oils inhibited the degradation of deoxyribose more than BHT (18.71%), used as a positive control. The highest activity was shown by the essential oils of thyme (in a range from 23.4 to 34.38% of inhibition) and basil (21.95–26.83%).

The protective effects of the essential oils on LP have been evaluated using two systems of induction, Fe^{2+}/ascorbate and Fe^{2+}/H$_2$O$_2$. The inhibition of LP was determined by measuring the formation of MDA, using liposomes as an oxidizable substrate.

In **Figure 2**, the results of the antioxidant activities of the examined essential oils and BHT as a positive control in the Fe^{2+}/ascorbate system of induction are presented. All of the essential oils expressed stronger antioxidant capacity as compared to BHT (37.04%). In particular, the essential oils of oregano (from 74.54 to 85.46% of inhibition of LP) and basil (from 66.91 to 86.03%) exhibited very high activity.

In LP induced by Fenton reaction (Fe^{2+}/H$_2$O$_2$), all of the tested essential oils, together with BHT, exhibited very similar antioxidant activities (ranging from 44.12 to 67.14%) (**Figure 3**).

In both systems of induction, the assessed essential oils reached 50% of LP inhibition. However, only in the Fe^{2+}/H$_2$O$_2$-induced LP, the essential oil of *T. vulgaris* expressed dose-dependent activity.

Antimicrobial Activity. The antibacterial activity of the essential oils of *O. basilicum*, *O. vulgare*, and *Thymus vulgaris* against five strains of Gram-positive and eight strains of Gram-negative bacteria is shown in **Table 4**. Generally, the Gram-positive strains of bacteria tested seemed to be more sensitive to the examined essential oils. These results are in accordance with the literature data (*10*, *15*, *24*, *25*). However, this study also recorded a notable susceptibility of the examined Gram-negative pathogenic bacteria. High sensitivity of all of the tested strains of *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella enteritidis*, *Salmonella typhi*, and *Shigella*, including the multiresistant strains, on the essential oil of *O. vulgare* is of particular interest.

The antifungal activity of the tested essential oils against the five examined dermatomycetes and *C. albicans* is shown in **Table 5**. Generally, all of the examined essential oils exhibited

Activities of Lamiaceae Spices Essential Oils

J. Agric. Food Chem., Vol. 54, No. 5, 2006 **1825**

Table 1. Chemical Composition of Essential Oils of *O. basilicum*, *O. vulgare*, and *T. vulgaris*

components	RI[a]	O. basilicum	O. vulgare	T. vulgaris	identification method[b]
α-pinene	935	0.2	0.4	0.2	GC-MS
camphene	951	0.1	0.2	0.4	MS
E-pinane	972	0.2			MS
sabinene	974	0.1			GC-MS
3-octenol	976	0.1			MS
β-pinene	977	0.4	0.5	0.2	GC-MS
E-isolimonene	981	2.0			MS
p-cimene	1026	0.1	0.5	0.8	MS
limonene	1032	1.9	0.4	0.2	GC-MS
1,8-cineole	1036	3.4	1.6	1.9	GC-MS
Z-β-ocimene	1043	0.6			MS
γ-terpinene	1060		3.1	8.3	GC-MS
E-linalool oxide	1067	0.1			GC-MS
2-nonanone	1091			0.8	MS
E-camphenone	1093	0.2			MS
E-sabinene hydrate	1097		0.3		MS
linalool	1100	24.2	0.5	2.2	GC-MS
α-thujone	1104	0.5	0.9	1.0	GC-MS
β-thujone	1114			0.2	GC-MS
allo-ocimene	1132	0.1			MS
camphor	1144	0.3	1.0	1.7	GC-MS
menthone	1157	0.6	0.5	2.2	GC-MS
1,4-dimethoxy-benzene	1165	0.3			MS
borneole	1167	0.1	1.3	2.6	GC-MS
menthofuran	1169	0.1			GC-MS
neomenthol	1170			2.8	MS
menthol	1172		1.1	1.3	GC-MS
terpinen-4-ol	1177		0.8	1.0	GC-MS
1-α-terpineol	1188		0.5	0.6	GC-MS
methyl chavicole (estragole)	1193	45.8			GC-MS
pulegone	1203	0.1		1.1	GC-MS
thymol methyl ether	1235			0.3	MS
piperitone	1248		0.3	1.4	GC-MS
bornyl acetate	1288			0.4	GC-MS
dihydroedulan II	1290	0.4			MS
thymol	1291		13.9	47.9	GC-MS
carvacrol	1299	0.3	61.3	5.9	GC-MS
α-copaene	1375	0.3			MS
geranyl acetate	1380			0.3	GC-MS
3-dodecanone	1387	0.2			MS
β-cubebene	1390	0.6		2.4	MS
β-elemene	1391	1.9			MS
Z-caryophyllene	1405	0.1			MS
E-caryophyllene	1418	4.5	0.8	0.7	GC-MS
α-guaiene	1438	0.7			MS
aromadendrene	1440	0.3			MS
α-humulene	1452	0.6	0.2		GC-MS
β-selinene	1486	0.8	0.4		MS
germacrene D	1490		0.1		MS
α-selinene	1495		0.7	0.3	MS
β-bisabolene	1508		1.0		MS
γ-cadinene	1514		0.3	0.5	MS
myristicin	1518			0.7	MS
Z-calamenene	1521			0.7	MS
δ-cadinene	1524	3.6	0.5		MS
Z-β-farnesene	1526	0.2			MS
ledol	1563	0.2		1.4	MS
caryophyllenol	1567	0.3			MS
spathulenol	1578	0.6	0.8	1.0	MS
caryophyllene-oxide	1582	0.1			MS
viridiflorol	1591	0.1	0.2		MS
vulgarone B	1647	0.2			MS
β-eudesmol	1649		0.3		MS
cadalene	1675			1.8	MS
apiole	1681			0.4	MS
E,E-farnesol	1724	0.1			GC-MS
nonadecene	1900	0.1			MS
E-1-eicosene	1996	0.2			MS
eicosane	2000	0.2	0.1		MS
monoterpene hydrocarbons		5.7	5.1	10.1	
aromatic monoterpene hydrocarbons		0.1	0.5	0.8	
oxygenated monoterpenes		76.0	84.0	75.9	
aromatic oxygenated monoterpenes		46.4	75.2	55.2	
sesquiterpene hydrocarbons		13.6	4.0	6.5	
oxygenated sesquiterpenes		1.6	1.3	2.4	
aliphatic components		1.2	0.1	0.8	
amount of identified compounds (%)		98.2	95.0	96.5	

[a] Retention indices relative to C_9–C_{24} *n*-alkanes on the HP 5MS column. GC, identification based on retention times of authentic compounds on HP 5MS column; MS, tentatively identified on the basis of computer matching of the mass spectra of peaks with the NIST/NBS and Wiley libraries and those reported by Adams (*14*).

1826 *J. Agric. Food Chem.,* Vol. 54, No. 5, 2006

Bozin et al.

Table 2. Neutralization of DPPH• of *O. basilicum*, *O. vulgare*, and *T. vulgaris* Essential Oils and BHT (as a Positive Control) in the DPPH Assay (in Percentage)

source	0.01	0.02	0.05	0.12	0.25	0.50	1.20	2.50	3.12	4.80	6.25	IC_{50}
						concentrations (μg/mL)						
O. basilicum			11.11	22.22	33.43	51.56	74.78	90.45	94.98	98.99	98.99	0.39
O. vulgare		12.00	20.00	24.00	60.00	98.99	98.99	98.99	98.99	98.99	98.99	0.17
T. vulgaris	2.50	7.50	17.50	40.00	59.00	85.00	98.99	98.99	98.99	98.99	98.99	0.19
BHT						4.62	11.56	23.12	30.11	44.71	55.22	5.37

Table 3. DPPH Scavenging Active Compounds Identified by the Means of TLC Dot–Blot Technique

source of essential oil	compound	R_f values
O. basilicum	carvacrol	0.62
	methyl chavicole	0.97
	mixture of mono- and sesquiterpene hydrocarbons	0.98
O. vulgare	carvacrol	0.62
	thymol	0.73
	mixture of mono- and sesquiterpene hydrocarbons	0.98
T. vulgaris	carvacrol	0.62
	thymol	0.73
	mixture of mono- and sesquiterpene hydrocarbons	0.98

Figure 1. Inhibition of degradation of 2-deoxyribose by essential oils of *O. basilicum*, *O. vulgare*, *T. vulgaris*, and BHT (as a positive control) in the deoxyribose assay. All examined essential oils were diluted in *n*-hexane (solvent expressed no scavenging activity).

notable fungistatic and fungicidal activity. The very low MFC expressed by the essential oils of *O. vulgare* and *T. vulgaris* against all of the tested fungi as compared to the bifonazole indicate their extraordinary antifungal effects.

However, in both antibacterial and antifungal bioassays, the essential oils of *O. vulgare* and *T. vulgaris* exhibited similar antimicrobial activity higher than that of the essential oil of *O. basilicum*. This could be due to the similar chemical profiles of oregano and thyme essential oils, in which phenolic oxigenated monoterpenes, especially carvacrol and thymol, were

Table 4. Antibacterial Activity (Inhibition Zone Measured in mm, Including Hole 5 mm in Diameter)[a] of Essential Oils[b] of *O. basilicum*, *O. vulgare*, and *T. vulgaris*
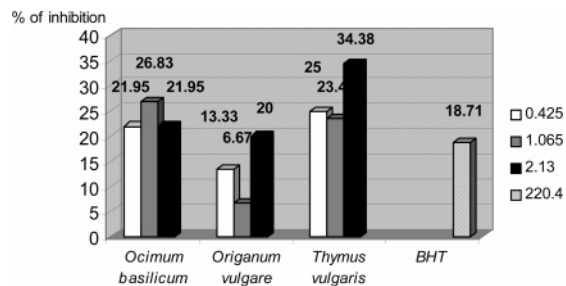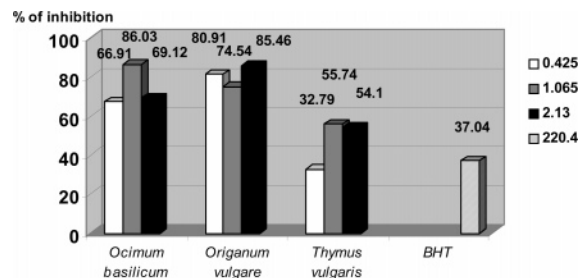
source	organism	*O. basilicum* (%) 20	50	*O. vulgare* (%) 20	50	*T. vulgaris* (%) 20	50	penicillin (μg/cm^3) 500	1000
ATTC 27853	*P. aeruginosa*	0.0	0.0	20.0 ± 0.71	19.4 ± 0.89	12.0 ± 1.41	12.2 ± 0.45	0.0	0.0
IPH-MR	*P. aeruginosa*	0.0	0.0	14.2 ± 0.45	20.0 ± 0.71	0.0	0.0	0.0	0.0
ATTC 35218	*E. coli*	18.0 ± 0.00	19.4 ± 0.89	38.2 ± 1.48	42.6 ± 1.67	29.4 ± 0.89	25.2 ± 0.84	0.0	0.0
ATTC 25922	*E. coli*	19.4 ± 0.89	18.2 ± 1.48	50.0 ± 0.71	50.2 ± 0.45	29.0 ± 0.71	25.2 ± 0.84	12.6 ± 0.89	13.4 ± 0.55
IPH-MR	*E. coli*	11.0 ± 0.71	17.8 ± 2.28	30.2 ± 0.45	28.0 ± 1.87	21.0 ± 1.00	29.4 ± 0.89	0.0	0.0
IPH-MR	*S. typhi*	29.0 ± 0.71	28.0 ± 2.12	49.4 ± 0.89	40.2 ± 0.45	28.2 ± 1.09	25.4 ± 0.54	0.0	0.0
IPH	*S. enteritidis*	10.0 ± 0.70	16.6 ± 1.34	44.0 ± 0.71	40.2 ± 0.45	35.4 ± 0.55	29.8 ± 0.45	22.0 ± 1.22	26.2 ± 0.83
IPH-MR	*Shigella sonei*	34.8 ± 3.63	23.6 ± 1.34	40.0 ± 0.1	44.6 ± 0.55	30.4 ± 0.89	30.6 ± 0.89	0.0	0.0
ATTC 10240	*Micrococcus flavus*	34.6 ± 0.89	50.0 ± 0.00	58.0 ± 0.71	60.0 ± 0.00	48.2 ± 1.48	50.0 ± 0.00	40.0 ± 0.55	40.0 ± 0.89
ATTC 9341	*Sarcina lutea*	20.8 ± 0.84	19.4 ± 0.89	37.6 ± 1.52	45.4 ± 0.89	25.6 ± 0.89	32.4 ± 1.82	0.0	0.0
ATTC 6538	*Staphylococcus aureus*	20.0 ± 0.71	28.0 ± 1.41	38.0 ± 1.58	40.2 ± 0.45	26.2 ± 0.44	39.8 ± 0.40	48.0 ± 0.00	50.0 ± 0.00
ATTC 12228	*Staphylococcus epidermidis*	22.6 ± 0.89	22.0 ± 0.71	63.8 ± 2.17	50.2 ± 1.4	48.0 ± 1.41	40.6 ± 0.89	14.2 ± 0.44	16.8 ± 0.83
ATTC 10707	*Bacillus subtilis*	22.2 ± 1.48	59.0 ± 1.00	57.2 ± 0.84	57.4 ± 0.89	40.6 ± 0.89	48.8 ± 1.79	38.2 ± 0.44	38.0 ± 0.70

[a] The values shown represent the average of five determinations ± standard deviations. [b] All essential oils were diluted in *n*-hexane (solvent expressed no activity on bacterial growth).

Table 5. Antifungal Activity of Essential Oils[a] of *O. basilicum*, *O. vulgare*, and *T. vulgaris*

source	organism	*O. basilicum* MIC	MFC	*O. vulgare* MIC	MFC	*T. vulgaris* MIC	MFC	bifonazole MIC	MFC
MMA	*C. albicans*	30.0 ± 0.0	30.0 ± 0.0	2.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	20.0 ± 0.0	25.2 ± 0.3
MMA	*Trichophyton mentagrophytes*	8.3 ± 0.6	15.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	2.2 ± 0.3	4.0 ± 0.0	9.7 ± 0.6	9.7 ± 0.6
MMA	*Trichophyton tonsurans*	8.0 ± 0.0	15.0 ± 0.0	1.0 ± 0.0	2.0 ± 0.0	2.2 ± 0.3	4.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0
MMA	*Trichophyton rubrum*	8.3 ± 0.6	15.2 ± 0.3	1.2 ± 0.3	2.2 ± 0.3	2.0 ± 0.0	4.2 ± 0.3	10.3 ± 0.6	10.3 ± 0.6
MMA	*Epidermophyton floccosum*	15.0 ± 0.0	30.0 ± 0.0	2.0 ± 0.0	2.3 ± 0.6	4.0 ± 0.0	4.2 ± 0.3	10.0 ± 0.0	10.0 ± 0.0
MMA	*Microsporum canis*	15.2 ± 0.8	30.2 ± 0.3	2.0 ± 0.0	2.2 ± 0.3	2.2 ± 0.3	4.0 ± 0.0	15.0 ± 0.0	20.0 ± 0.0

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

Activities of Lamiaceae Spices Essential Oils

J. Agric. Food Chem., Vol. 54, No. 5, 2006 **1827**

Figure 2. Inhibition of LP in Fe^{2+}/ascorbate system of induction by essential oils of *O. basilicum*, *O. vulgare*, *T. vulgaris*, and BHT (as a positive control) in the TBA assay. Essential oils and BHT were diluted in *n*-hexane (solvent expressed no antioxidant activity).
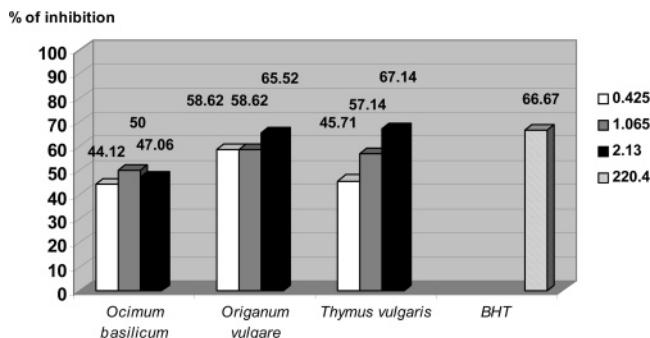

Figure 3. Inhibition of LP in Fe^{2+}/H_2O_2 system of induction by essential oils of *O. basilicum*, *O. vulgare*, *T. vulgaris*, and BHT (as a positive control) in the TBA assay. Essential oils and BHT were diluted in *n*-hexane (solvent expressed no antioxidant activity).

found in the ratio of 76.24% for *O. vulgare* and 55.17% for *T. vulgaris* (**Table 1**). Furthermore, a higher antimicrobial activity of *O. vulgare* essential oil could be explained by a significant amount of carvacrol (61.32%). On the other hand, a low amount of carvacrol (0.33%) and the absence of thymol in the oil of *O. basilicum* could be responsible for its weaker antimicrobial activity. It is obvious that methyl chavicole, although it belongs to oxygenated phenolic monoterpenes, has no significant role in antimicrobial activity of the essential oil of basil.

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 free radical scavengers and LP inhibitors. In particular, the investigated essential oils exhibited high RSC against DPPH radical, which was found to be in correlation to the content of oxygenated phenolic monoterpenes, especially carvacrol and thymol. Also, a very strong protective activity of the essential oils in LP processess was recorded. Furthermore, the study reveals significant antibacterial and antifungal activity of the essential oils of the investigated Lamiaceae spices, particularly the essential oil of oregano. The antimicrobial activity of the examined essential oils is also related to the content of carvacrol and thymol. Thus, the use of investigated essential oils or its aromatic monterpene constituents could be useful in food production. Furthermore, the use of phytopharmaceuticals based on investigated essential oils in the prevention and treatments of various human diseases seems to be reasonable.

LITERATURE CITED

(1) Blumenthal, M., Ed. *The Complete German Commission E Monographs*; American Botanical Council: Austin, Texas, 1999.

(2) Bruneton, J. *Pharmacognosy, Phytochemistry, Medicinal Plants*, 2nd ed.; Intercept Ltd.: London, Paris, New York, 1999.

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

(4) Reddy, M. V. B.; Angers, P.; Gosselin, A.; Arul, J. Characterization and use of essential oil from *Thymus vulgaris* against *Botrytis cinerea* and *Rhyzopus stolonifer* in strawberry fruits. *Phytochemistry* **1998**, *47* (8), 1515−1520.

(5) Tsigarida, E.; Skandamis, P.; Nychas, G. J. E. Behaviour of *Lysteria monocytogenes* and autochthonous flora on meat stored under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of oregano essential oil at 5 °C. *J. Appl. Microbiol.* **2000**, *89*, 901−909.

(6) Adam, D. Global antibiotic resistance in *Streptococcus pneumoniae*. *J. Antimicrob. Chemother.* **2002**, *50* (1), 1−5.

(7) Lagouri, V.; Boskou, D. Screening for antioxidant activity of essential oils obtained from spices. In *Food Flavours: Generation, Analysis and Process Influence*; Charalambous, G., Ed.; Elsevier Science B.V.: Amsterdam, London, New York, Oxford, Paris, Shannon, Tokyo, 1995; pp 969−879.

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

(9) Botsoglou, N. A,; Govaris, A.; Botsoglou, E. N.; Grigoropoulou, S. H.; Papageorgiou, G. Antioxidant activity of dietary oregano essential oil and α-tocopheryl acetate supplementation in long-term frozen stored turkey meat. *J. Agric. Food Chem.* **2003**, *51*, 2930−2936.

(10) 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.

(11) Chu, Y. H.; Chang, C. L.; Hsu, H. F. Flavonoid content of several vegetables and their antioxidant activity. *J. Sci. Food Agric.* **2000**, *80*, 561−566.

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

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

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

(15) 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.

(16) Espin, J. C.; Soler-Rivas, C.; Wichers, H. J. Characterisation of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-diphenyl-1-pirylhydrazyl radical. *J. Agric. Food Chem.* **2000**, *48* (3), 648−656.

(17) Wagner, H.; Bladt, S. *Plant Drug Analysis*; Springer-Verlag: Berlin, Heidelberg, New York, 2001.

(18) Van den Berghe, D. A.; Vlietinck, A. J. Screening methods for antibacterial and antiviral agents from higher plants, assays for bioactivity. In *Methods in Plant Biochemistry*; Hostettman, K., Ed.; Academic Press: London, San Diego, New York, Boston, Sydney, Tokyo, Toronto, 1991; Vol. 6, pp 47−69.

(19) Lewinsohn, E.; Ziv-Raz, I.; Dudai, N.; Tadmor, Y.; Lastochkin, E.; Larkov, O.; Chaimovitsh, D.; Ravid, U.; Putievsky, E.; Pichersky, E.; Shoham, Y. Biosynthesis of estragole and methyl eugenol in sweet basil (*Ocimum basilicum* L.). Developmental and chemotypic association of allylphenol *O*-methyltransferase activities. *Plant Sci.* **2000**, *160*, 27−35.

(20) Milos, M.; Mastelic, J.; Jerkovic, I. Chemical composition and antioxidant effect of glycosidally bound volatile compounds from oregano (*Origanum vulgare* L. ssp. *hirtum*). *Food Chem.* **2000**, *71*, 79−83.

(21) Mockute, D.; Bernotiene, G.; Judzentiene, A. The essential oil of *Origanum vulgare* L. ssp. *vulgare* growing wild in Vilnius district (Lithuania). *Phytochemistry* **2001**, *57*, 65−69.

1828 *J. Agric. Food Chem.,* Vol. 54, No. 5, 2006

Bozin et al.

(22) Hudaib, M.; Speroni, E.; Di Pietra, A. M.; Cavrini, V. GC/MS evaluation of thyme (*Thymus vulgaris* L.) oil composition and variations during the vegetative cycle. *J. Pharm. Biomed. Anal.* **2002**, *29*, 691−700.

(23) Lee, B. M.; Lee, S. K.; Kim, H. S. Inhibition of oxidative DNA damage, 8-OhdG, and carbonyl contents in smokers treated with antioxidants (vitamin E, vitamin C, β-carotene and red ginseng). *Cancer Lett.* **1998**, *132*, 219−227.

(24) Baratta, T. M.; Dorman, D. J. H.; Deans, G. S.; Biondi, D. M.; Ruberto, G. Chemical composition, antimicrobial and antioxidant activity of laurel, sage, rosemary, oregano and coriander essential oils. *J. Essent. Oil Res.* **1998**, *10*, 618−627.

(25) Deans, S. G. Evaluation of antimicrobial activity of essential (volatile) oils. In *Modern Method of Plant Analysis. Essential Oils and Waxes*; Linskens, H. F., Jackson, J. F., Eds.; Springer-Verlag: Berlin, Germany, 1991; Vol. 12, pp 309−320.