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Antimicrobial and Antioxidant Activities of *Melissa officinalis* L. (Lamiaceae) Essential Oil

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The present study describes antimicrobial and free radical scavenging capacity (RSC) together with the effects on lipid peroxidation (LP) of *Melissa officinalis* essential oil. The chemical profile of essential oil was evaluated by the means of gas chromatography−mass spectrometry (GC-MS) and thin-layer chromatography (TLC). RSC was assessed measuring the scavenging activity of essential oil on the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and OH• radicals. The effect on LP was evaluated following the activities on Fe^{2+}/ascorbate and Fe^{2+}/H_2O_2 systems of induction. The antimicrobial activity was tested against 13 bacterial strains and six fungi. The examined essential oil exhibited very strong RSC, reducing the DPPH radical formation (IC_{50} = 7.58 μg/mL) and OH radical generation (IC_{50} = 1.74 μg/mL) in a dose-dependent manner. According to the GC-MS and TLC (dot-blot techniques), the most powerful scavenging compounds were monoterpene aldehydes and ketones (neral/geranial, citronellal, isomenthone, and menthone) and mono- and sesquiterpene hydrocarbons (*E*-caryophyllene). Very strong inhibition of LP, particularly in the Fe^{2+}/H_2O_2 system of induction (94.59% for 2.13 μg/mL), was observed in both cases, also in a dose-dependent manner. The most effective antibacterial activity was expressed on a multiresistant strain of *Shigella sonei*. A significant rate of antifungal activity was exhibited on *Trichophyton* species.

KEYWORDS: ***Melissa officinalis*; Lamiaceae; essential oil; GC-MS; antimicrobial activity; antioxidant activity; DPPH radical; OH radical; LP**

INTRODUCTION

Since ancient times, herbs and spices have been added to different types of food to improve the flavor and organoleptic properties. Also, herbal medicines have a great potential in the emerging nutrition industry, because these materials are often considered foods as well as medicines and are used in preventive and curative treatments throughout the world (*1*). Especially popular today is the concept of foods that combine nutritional and medicinal benefits, so-called "functional foods". Many natural compounds extracted from plants have demonstrated biological activities. Among these various kinds of natural substances, essential oils from aromatic and medicinal plants receive particular attention as potential natural agents for food preservation. In fact, their effectiveness against a wide range of microorganisms has been repeatedly demonstrated (*2*−*5*). Moreover, essential oils are proved to have various pharmacological effects, such as spasmolytic, carminative, hepatoprotective, antiviral, and anticarcinogenic effects, etc. (*6*). Recently, many essential oils have been qualified as natural antioxidants (*3*, *5*−*8*) and proposed as potential substitutes of synthetic antioxidants in specific sectors of food preservation. Furthermore, biologically active natural compounds are of interest to the pharmaceutical industry for the control of human diseases of microbial origin and for the prevention of lipid peroxidative damage, which has been implicated in several pathological disorders, such as ischemia−reperfusion injury, coronary atherosclerosis, Alzheimer's disease, carcinogenesis, and aging processes (*9*, *10*).

Lemon balm (*Melissa officinalis* L., Lamiaceae) is a well-known herb used to give fragrance to different food and beverage products. It has also been used as a medicinal plant for treatment of headaches, gastrointestinal disorders, nervousness, and rheumatism (*11*). The essential oil is a well-known antibacterial and antifungal agent, and it is also responsible for the mild depressive and spasmolytic properties of the plant (*12*). Some literature data pointed out antioxidant properties of methanolic extracts of *M. officinalis*, which are mostly due to

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the high portion of phenolic acids (*13*, *14*). However, until now, no data about the antioxidant properties of the essential oil of this plant are reported.

With respect to this topic, the antioxidant and antimicrobial effects of the chemically characterized essential oil of lemon balm (*M. officinalis* L., Lamiaceae) are reported in the present study.

MATERIALS AND METHODS

Plant Material. Aerial parts of cultivated flowering plant *M. officinalis* L. were collected in July of 2000 in Vojvodina. Voucher specimens (No. 1984) 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 material was submitted to hydrodistillation according to Eur. Pharm. 4 (*15*), 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. The essential oil yield was 0.2%.

Essential Oil Analysis. Qualitative and quantitative analyses of the essential oils were carried out using a Hewlett-Packard 5973-6890 gas chromatography−mass spectrometry (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 a carrier gas (1 mL/min), and the capillary columns used were HP-5 MS (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. The identification of individual compounds was based on comparison of their relative retention times with those of authentic samples (Carl Roth GmbH; Karlsruhe, Germany), by coelution and MS analysis. For the components, mostly sesquiterpenes and aliphatic compounds, for which reference substances were not available, the identification was performed by matching their retention indices and mass spectra with those obtained from authentic samples and/or the NIST/NBS, Wiley libraries spectra, and literature data (*16*).

Antimicrobial Activity: Evaluation of Antibacterial Activity. A collection of 13 test organisms, including both Gram-positive and Gram-negative bacterial strains, was used. Each group included eight organisms of the American Type of Culture Collection (ATCC) and five organisms of the 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 2**. All of the test organisms were stored at +4 °C on Mueller−Hinton (MH) (Torlak; Belgrade, Serbia and Montenegro) agar 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 (*17*) with slight modifications. 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 the agar and suspended in sterile 0.1 M phosphate buffer (PB) containing 167 mM NaCl (167 mM NaCl−PB; pH 7.4). The density of bacterial suspensions was determined by McFarland nephelometer (Dalynn Biological Inc.; Calgary, Canada). 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 agar surface, holes 5 mm in diameter were punched. In the holes, 15 μL of tested essential oil (50 and 20% solutions in *n*-hexane) was applied. Plates were incubated overnight at 37 °C, and the diameter of the resulting zone of inhibition was measured. The evaluation of antibacterial activities of 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.

Evaluation of Antifungal Activity. For the bioassay, a collection of six test organisms of dermatomyceta, shown in **Table 3**, was used. Micromycetes were isolated directly from patients at the Centre for Preventive Medicine, Military Medical Academy (MMA) (Belgrade, Yugoslavia) and maintained on Sabouraud agar (Torlak). Cultures were stored at +4 °C and subcultured once a month.

To investigate the antifungal activity of the essential oil, the modified microdilution technique (*18*) was used. The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v) agar (Torlak). The spore suspension was adjusted with sterile saline to a concentration of 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 (Torlak) to verify the absence of contamination and to check the validity of the inoculum. 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 concentrations that completely inhibited fungal growth (MIC). The minimal fungicidal concentrations (MFC) were determined by 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 control.

Antioxidant Activity: Free Radical Scavenging Capacity (RSC). RSC was evaluated measuring the scavenging activity of examined essential oils on the 2,2-diphenyl-1-picrylhydrazil (DPPH) and OH radicals. The DPPH assay was performed as described before (*19*), with small modifications. The samples (from 0.50 to 12 μg/mL) were mixed with 1 mL of 90 μM DPPH$^\bullet$ solution (Sigma; St. Louis, MO) and filled up with 95% methanol to a final volume of 4 mL. The absorbance of the resulting solutions and the blank (with the same chemicals, except for the 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$^\bullet$ was measured spectrophotometrically at 515 nm using a Beckman DU-65 spectrophotometer. 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, was determined by linear regression analysis from the obtained RSC values.

Scavenging capacity of the essential oils for hydroxyl radicals was evaluated measuring the degradation of 2-deoxy-D-ribose (Fluka AG) with OH radicals, generated in a Fenton reaction. The degradation products are the 2-thiobarbituric acid (TBA) (Sigma) reactive substances (TBARS), which could be determined spectrophotometrically at 532 nm (*5*). All solutions and reagents were freshly prepared by dissolution in 0.05 M KH_2PO_4−K_2HPO_4 PB. 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 TBA 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 (Sigma)] and 0.2 mL of 0.1 M EDTA (Sigma) were added to the reaction mixture, and 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. A 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:

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

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} aluminum plates

(Merck) stained with the free radical DPPH• was used (*19*). 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 they were dried, the plates were sprayed with a 0.4 mM solution of DPPH• in methanol, using a Desaga Spray Gun. Sprayed plates gave a purple background with yellow spots at the location of those compounds that possess high RSC. Essential oil compounds responsible for scavenging activity were identified comparing the DPPH-TLC chromatogram with the control, treated with vanillin−sulfuric acid spray reagent.

Determination of Lipid Peroxidation. The extent of LP was determined by measuring the color of the adduct produced in the reaction between TBA and malondialdehyde (MDA), as an oxidation product in the peroxidation of membrane lipids, by the TBA assay (*20*).

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: 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 a 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). A control with *n*-hexane instead of sample was also analyzed for both systems of induction of LP.

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 or 0.1 M EDTA, heating at 100 °C for 20 min. After the solution was cooled and the precipitated proteins were centrifuged (4000 rpm for 10 min), the content of the MDA (TBARS) was determined by measuring the absorbance of the 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 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.

RESULTS AND DISCUSSION

Table 1 lists the chemical components of the essential oil grouped as classes of compounds. The main constituents of the essential oil of *M. officinalis* are citrals (geranial + neral, 39.9%), citronellal (13.7%), limonene (2.2%), geraniol (3.4%), β-caryophyllene (4.6%), β-caryophyllene oxide (1.7%), and germacrene D (2.4%). These results are in accordance with previous published data on *M. officinalis* essential oils (*21*).

Antimicrobial Activity. The antibacterial activity of essential oil against a range of Gram-positive (five strains) and Gram-negative (eight strains) is shown in **Table 2**. Obtained results revealed that essential oil exhibited variable levels of antibacterial activity against all tested bacterial strains. According to the literature data (*2*−*5*), Gram-positive bacteria seemed to be more sensitive to the different examined essential oils than Gram-negative bacteria. However, this study also recorded a notable susceptibility of examined Gram-negative pathogenic bacteria, such are *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella enteritidis*, *Salmonella typhi*, and *Shigella* strains, particularly the multiresistant ones. Especially considerable is that the highest sensitivity to essential oil of *M. officinalis* was observed by *E. coli* ATCC 25922 (30.2 and 39.8 mm) and the multiresistant strain of *Shigella sonei* IPH-MR (37.4 and 38.4 mm).

Table 1. Chemical Composition of *M. officinalis* Essential Oil

pick no.	components[a]	RI[b]	percentage (%)	identification method[c]
1	α-pinene	937	0.3	RT GC MS
2	camphene	952	0.2	RT* MS
3	β-pinene	976	0.3	RT GC MS
4	*E*-isolimonene	983	0.7	RT* MS
5	3-octanol	995	0.4	RT* MS
6	δ-3-carene	1011	0.3	RT* MS
7	limonene	1032	2.2	RT GC MS
8	*Z*-β-ocimene	1042	0.2	RT* MS
9	linalool	1100	0.5	RT GC MS
10	α-thujone	1103	0.2	RT GC MS
11	β-thujone	1116	0.8	RT GC MS
12	*E*-rose oxide	1127	0.3	RT* MS
13	citronellal	1153	13.7	RT GC MS
14	isomenthone	1167	3.0	RT GC MS
15	menthol	1172	2.9	RT GC MS
16	estragole (methyl chavicol)	1195	0.1	RT GC MS
17	nerol	1227	0.5	RT* MS
18	citronellol	1228	0.8	RT GC MS
19	neral (*Z*-citral)	1241	16.5	RT GC MS
20	geraniol	1255	3.4	RT GC MS
21	methyl citronellate	1260	2.7	RT* MS
22	geranial (*E*-citral)	1271	23.4	RT* MS
23	bornyl acetate	1288	0.2	RT GC MS
24	*Z*-limonene oxide	1304	0.4	RT* MS
25	*Z*-carvyl acetate	1362	0.1	RT* MS
26	neryl acetate	1364	1.6	RT* MS
27	α-copaene	1375	0.1	RT* MS
28	geranyl acetate	1380	0.8	RT GC MS
29	*E*-β-damascenone	1381	0.1	RT* MS
30	dodecenal	1407	0.4	RT* MS
31	*E*-caryophyllene	1419	4.6	RT GC MS
32	α-humulene	1452	0.4	RT* MS
33	β-selinene	1486	0.1	RT* MS
34	germacrene D	1490	2.4	RT* MS
35	δ-cadinene	1524	0.2	RT* MS
36	*Z*-β-farnesene	1526	0.1	RT* MS
37	ledol	1565	0.2	RT* MS
38	caryophyllene oxide	1582	1.7	RT* MS
39	nonadecane	1902	0.2	RT* MS
40	1-eicosene	1900	0.1	RT* MS
41	eicosane	2001	0.5	RT* MS
42	*n*-heneicosane	2100	0.3	RT* MS
43	pentacosane		0.5	RT* MS
	total identified		88.4	
	monoterpene hydrocarbons		4.2	
	oxygenated monoterpenes		71.9	
	sesquiterpene hydrocarbons		7.9	
	oxygenated sesquiterpenes		2.0	
	aliphatic compounds		2.4	

[a] Compounds listed in order of elution from a HP-5 MS column. [b] Retention indices relative to C_9–C_{24} *n*-alkanes on the HP-5 MS column. [c] 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 (*16*).

The antifungal activity of the essential oil against dermatomycetes is shown in **Table 3**. Generally, essential oil exhibited notable, but not in all cases, considerable antifungal activity. Notable is the low MIC and MFC against *Trichophyton tonsurans* (both 15 μL/mL) as compared with the synthetic antimycotic bifonazole (both 10 μL/mL).

Antioxidant Activity. The antioxidant activity of the *M. officinalis* essential oil has been evaluated in a series of in vitro test. In the DPPH test, the ability of the essential oil to act as the donor for hydrogen atoms or electrons in transformation of

Table 2. Antibacterial Activity (Inhibition Zone Measured in mm, Including Hole of 5 mm Diameter)[a] of *M. officinalis* Essential Oil[b]

		solution of essential oil[b]		penicillin (μg/cm^3)	
source	organism	20%	50%	500	1000
ATCC 27853	*P. aeruginosa*	13.4 ± 0.89	14.8 ± 1.30	0.0	0.0
IPH-MR	*P. aeruginosa*	15.6 ± 0.55	0.0	0.0	0.0
ATCC 35218	*E. coli*	14.8 ± 0.84	17.2 ± 0.84	0.0	0.0
ATCC 25922	*E. coli*	30.2 ± 0.44	39.8 ± 1.09	12.6 ± 0.89	13.4 ± 0.55
IPH-MR	*E. coli*	18.2 ± 1.22	18.6 ± 0.89	0.0	0.0
IPH	*S. enteritidis*	15.2 ± 0.44	16.2 ± 0.84	22.0 ± 1.22	26.2 ± 0.83
IPH-MR	*S. typhi*	19.8 ± 0.44	24.4 ± 0.89	0.0	0.0
IPH-MR	*S. sonei*	37.4 ± 1.95	38.4 ± 1.67	0.0	0.0
ATCC 9341	*Sarcina lutea*	27.0 ± 1.41	24.6 ± 1.52	0.0	0.0
ATCC 10240	*Micrococcus flavus*	27.4 ± 0.89	30.0 ± 0.00	40.0 ± 0.55	40.0 ± 0.89
ATCC 6538	*Staphylococcus aureus*	24.0 ± 1.22	19.4 ± 0.89	48.0 ± 0.00	50.0 ± 0.00
ATCC 12228	*Staphylococcus epidermidis*	18.2 ± 1.48	26.6 ± 1.67	14.2 ± 0.44	16.8 ± 0.83
ATCC 10707	*Bacillus subtilis*	29.6 ± 0.89	28.2 ± 1.48	38.2 ± 0.44	38.0 ± 0.70

[a] The values shown represent the average of five determinations ± standard deviations. [b] Essential oil was diluted with *n*-hexane (solvent expressed no antimicrobial activity).

Table 3. Antifungal Activity of Essential Oil[a] of *M. officinalis*

		essential oil		bifonazole	
source	organism	MIC	MFC	MIC	MFC
MMA	*Trichophyton mentagrophytes* var. *mentagrophytes*	15	30	10	10
MMA	*Trichophyton rubrum*	15	30	10	10
MMA	*T. tonsurans*	15	15	10	10
MMA	*Microsporum canis*	30	30	15	20
MMA	*Epidermophyton floccosum*	30	60	10	10
MMA	*Candida albicans*	30	60	20	25

[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.

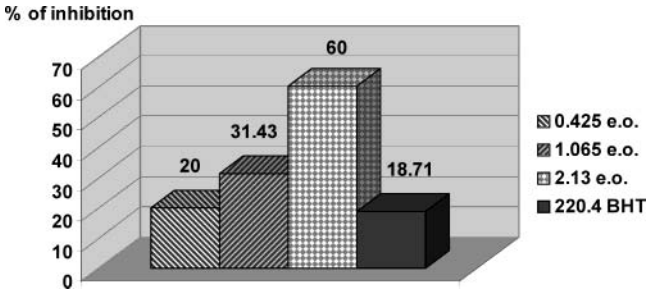

Figure 1. Inhibition of degradation of 2-deoxyribose by the essential oil of *M. officinalis* and BHT (as a positive control) in the deoxyribose assay. Concentrations shown are expressed in μg/mL. Essential oil and BHT were diluted in *n*-hexane (solvent expressed no antioxidant activity).

DPPH$^\bullet$ into its reduced form DPPH−H was measured spectrophotometrically (**Table 4**). Assessed essential oil was able to reduce the stable radical DPPH to the yellow-colored DPPH−H reaching 50% of reduction with an IC_{50} of 7.58 μg/mL. Comparison of DPPH scavenging activity of essential oil to those expressed by BHT pointed out very similar IC_{50} values (for BHT, IC_{50} = 5.37 μg/mL).

The hydroxyl RSC of the essential oil (**Figure 1**) was measured by the deoxyribose assay. Hydroxyl radicals formed in Fenton reaction were detected by their ability to degrade 2-deoxy-D-ribose into fragments that on heating with TBA at low pH, form a pink adduct. Protective effects of the essential oil were followed as its ability to remove hydroxyl radicals from the test solution and prevent the degradation. High inhibition of degradation was observed for all of the tested essential oil concentrations, especially 2.13 μg/mL (60%), against BHT as a positive control (18.71%).

Identification of the constituents most responsible for the RSC was accomplished by comparing the control TLC analysis with the results of GC-MS and TLC dot-blot method. It appears that for the neutralization of DPPH radicals the most active compounds were monoterpene aldehydes and ketones (citrals, citronellal, isomenthone, and menthone) and the mixture of mono- and sesquiterpene hydrocarbons (first *E*-caryophyllene). These findings are in some correlation with earlier published data on antioxidant activities of different essential oils and selected essential oil components (*5*).

Protective effects of the essential oil in lipid peroxidation have been evaluated using two systems of induction (Fe^{2+}/ascorbate and Fe^{2+}/H_2O_2), by the TBA assay. Inhibition of LP was determined by measuring the formation of secondary components (MDA) of the oxidative process, using liposomes as an oxidizable substrate.

Figure 2 shows the antioxidant activity of *M. officinalis* essential oil against BHT as a positive control in the first system of induction (Fe^{2+}/ascorbate). In general, the examined essential oil expressed strong antioxidant capacity. In particular, 2.13 μg/mL of the oil showed higher activity than the stock solution of BHT (66.67 against 37.4%).

In the Fe^{2+}/H_2O_2 system of induction (Fenton reaction) inhibitory activities, all of the tested concentrations of essential oil and BHT were considerably higher (**Figure 3**). Furthermore, lemon balm essential oil proved to be more active even at the lowest concentration (0.425 μg/mL) than the standard (84.68 as compared to 66.67%).

Table 4. Percentage of Neutralization of DPPH of Essential Oil of *M. officinalis* and BHT (as Positive Control) in the DPPH Assay

	concentrations (μg/mL)										
source	0.48	1.20	2.40	3.12	4.80	6.25	7.20	9.60	12.00	12.50	IC_{50}
M. officinalis	11.11	18.52	33.33	43.40	44.44	45.82	48.15	55.55	62.96	65.58	7.58
BHT	4.62	11.56	23.12	30.11	44.71	58.22	67.07	67.51	70.12	74.22	5.37

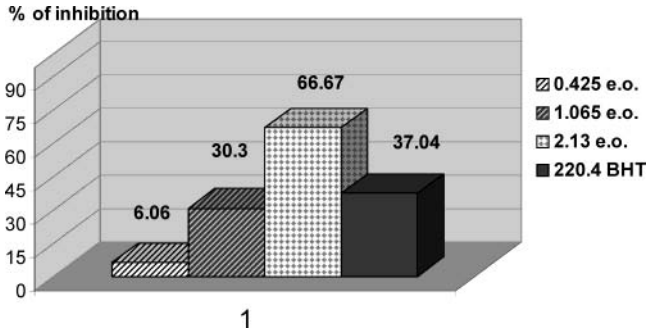

Figure 2. Inhibition of lipid peroxidation (LP) in Fe^{2+}/ascorbate system of induction by the essential oil of *M. officinalis* and BHT (as a positive control) in the TBA assay. Concentrations shown are expressed in μg/mL. Essential oil and BHT were diluted in *n*-hexane (solvent expressed no antioxidant activity).

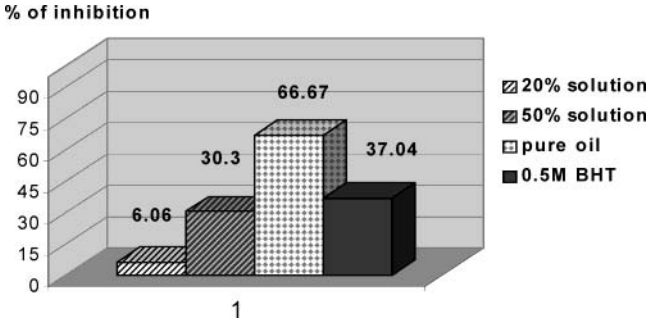

Figure 3. Inhibition of lipid peroxidation (LP) in Fe^{2+}/H_2O_2 system of induction by the essential oil of *M. officinalis* and BHT (as a positive control) in the TBA assay. Concentrations shown are expressed in μg/mL. Essential oil and BHT were diluted in *n*-hexane (solvent expressed no antioxidant activity).

In conclusion, the study revealed significant antimicrobial, particularly antibacterial, activity of the investigated essential oil. The examined oil exhibited high RSC, which was found to be in correlation to the content of mainly monoterpene ketones and aldehydes. Also, a very strong protective activity of the essential oil in lipid peroxidation processes, especially against hydroxyl radicals formed in the Fenton reaction, was recorded. These results indicate that essential oils could serve not only as flavor agents but also as safe antioxidant and antiseptic supplements in preventing deterioration of foodstuff and beverage products and pharmaceuticals. Also, consumption of food produced with natural essential oils or aromatic plant extracts (functional foods) is expected to prevent the risk of free radical-dependent diseases.

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