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Antioxidant and antimicrobial properties of a new chemotype of woodland sage (*Salvia nemorosa* L. subsp. *nemorosa*, Lamiaceae) essential oil

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Summary. Essential oil of woodland sage (*Salvia nemorosa* subsp. *nemorosa* L., Lamiaceae) was analyzed using gas chromatography–mass spectrometry, and assayed for antimicrobial and antioxidant activities. Antioxidant activity was evaluated by measuring both free radical scavenging capacity (RSC) and lipid peroxidation (LP) effects. Specifically, RSC was assessed by measuring the scavenging activity of essential oils on 2,2-diphenyl-1-picrylhydrazil (DPPH) and hydroxyl radicals; while LP effects were evaluated by monitoring the activities of essential oil in Fe^{2+}/ascorbate and Fe^{2+}/H_2O_2 induction systems. The investigated essential oil reduced DPPH (IC_{50} = 0.98 μL/mL) and OH radical formation (IC_{50} = 0.11 μL/mL) in a dose-dependent manner. Strong LP inhibition in both induction systems was also observed. Antimicrobial activity was tested against 13 bacterial strains and 6 fungi, including *Candida albicans* and 5 dermatomycetes. Although only weak antifungal activity was found; significant antibacterial activity was observed against *Salmonella typhi*, *Shigella sonei* and *Staphylococcus epidermidis*.

Keywords: antimicrobial activity, antioxidants, essential oil, GC-MS, Lamiaceae, *Salvia nemorosa*.

Introduction

Aromatic plants and essential oils have been highly valued since prehistoric times, not only because of their flavour, but also for their medicinal properties and food-preserving power. More recently, aromatic plants and essential oils have been shown to be associated with a wide-range of pharmacological effects, such as: spasmolytic, carminative, anti-inflammatory, hepatoprotective, antimicrobial and antioxidant effects (Blumenthal 1999; Mimica-Dukic and Bozin 2007; Samojlik et al. 2010). In fact, aromatic plants and essential oils are increasingly used by the pharmaceutical, food and cosmetic industries. Thus, because of their medicinal and economic importance, continued systematic studies of medicinal plants are necessary to identify new active compounds.

Many of aromatic plants are characterized by great morphological variation, as reflected in the high number of different taxonomic rank names attributed by botanists over the past 200 years. In addition, frequent hybridization and different ploidy levels in related taxa contribute to the complex variation pattern characterizing most species. Thus, in general, aromatic plants of a single species or hybrid can have different smells and exhibit different biological and pharmacological activities; while plants belonging to different taxa can belong to the same chemotype, have a similar smell and exhibit similar activities (Kokkini 1992; Mimica-Dukic and Bozin 2007; Bozin et al. 2008; Anackov et al. 2009). Similarly, differences in essential oil composition are also reflected in a number of commercial oils.

Woodland sage (*Salvia nemorosa* L. 1762 subsp. *nemorosa*, Lamiaceae) is a hardy herbaceous perennial plant native to a wide area of central Europe and western Asia (Diklić 1974). In addition, because it is

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an attractive plant that is easy to grow and propagate, woodland sage has been passed around by gardeners for many years (Clebsch 2003). Although different species of the genus *Salvia* (especially *S. officinalis* and *S. triloba*) and their essential oils are widely used in food flavouring, pharmaceuticals and perfumes (Bisset and Wichtl 2001), relatively few studies have been published on the biomedical potential of *S. nemorosa*. Moreover, these studies only considered the activity of leaf extracts (Milovanovic et al. 1996; Zupko et al. 2001; Tosun et al. 2009; Janicsak et al. 2010). Furthermore, there is no data in the literature on the biological properties of woodland sage essential oil, and reports on the chemical composition suggest the presence of several chemotypes (Mirza and Sefidkon 1999; Mimica-Dukic et al. 2002; Chalchat et al. 2004). In these studies, caryophyllene oxide was found to be one of the main essential oil components, while differences were found in the content of spathulenol, β-caryophyllene, germacrene B and germacrene D as a second major component (Mirza and Sefidkon 1999; Mimica-Dukic et al. 2002; Chalchat et al. 2004; Malencic et al. 2004). Although not used in traditional medicine, leaves from *S. nemorosa* can be found as contaminants in crude sage leaves, leading to changes in chemical composition and pharmacological effects.

Because the leaves of Dalmatian sage are widely used as an antiseptic (and recently for the isolation of natural antioxidants), most studies on the genus *Salvia* in the literature have focused on *S. officinalis*. In fact, a literature survey failed to reveal any references to any previous work on the antioxidant and antimicrobial activities of *S. nemorosa* essential oil. Therefore, the aim of the present study was to evaluate the antioxidant and antimicrobial activity of woodland sage (*S. nemorosa*) leaves.

Materials and methods

Plant material

Leaves of wild-growing plants of *Salvia nemorosa* L. subsp. *nemorosa* (Lamiaceae) in full blossom were collected in July of 2009 in Padej, Vojvodina province, Republic of Serbia (UTM 34T DR 1 37). A voucher specimen of the collected plant material (№ 2-1796) was confirmed and deposited at the Herbarium of the Department of Biology and Ecology (BUNS), Faculty of Natural Sciences, University of Novi Sad (Holmgren and Holmgren 2003).

Essential oil isolation and analysis

Air-dried leaves of *S. nemorosa* were submitted to hydro-distillation using *n*-hexane as a collecting solvent (Ph.Eur. IV, 2002). The solvent was removed under vacuum, and the quantity of the essential oil was determined gravimetrically.

Qualitative and quantitative analyses of the essential oil was 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 (FID) (250°C). Helium was the carrier gas (1 mL/min) and an HP 5MS (30 m x 0.25 mm; film thickness 0.25 μm) capillary column was used. Temperature programmes were 60°C to 280°C at a rate of 3°C/min and 60-260°C at a rate of 3°C/min, respectively, with a split ratio of 1:10. Co-elution and MS analysis were based on the identification of individual compounds by comparison of their relative retention times with those of authentic samples. Components for which reference substances were not available (mostly sesquiterpenes and aliphatic compounds) were identified by matching their retention indices and mass spectra with those obtained from authentic samples and/or NIST/NBS, Wiley libraries spectra, as well as by comparison with literature data (Adams 2001).

Antioxidant activity

Antioxidant properties of essential oil samples were evaluated by measuring both free radical scavenging capacity (RSC) and lipid peroxidation (LP) protective effects. RSC was evaluated by measuring the scavenging activity of woodland sage (*S. nemorosa*) essential oil on DPPH and OH radicals. The extent of LP protection was determined by measuring the colour of adduct produced by reaction of TBA with malondyaldehide (MDA) as previously described (Bozin et al. 2007). For this investigation, two systems of induction were used: Fe^{2+}/ascorbate and Fe^{2+}/H_2O_2. A control with *n*-hexane instead of sample was also analyzed for both systems, and displayed no activity.

Free Radical Scavenging Capacity (RSC)

The DPPH assay was performed as previously described (Bozin et al. 2007). Samples containing 3 mL of a methanol solution of the essential oil at different concentrations (from 0.25 to 12.5 μL/mL) were mixed with 1 mL of 90 μM DPPH$^{\bullet}$ solution. The absorbance of the resulting solutions was recorded spectrophotometrically at 515 nm after 1 h at room temperature, against a blank solution containing the same chemicals minus the sample. The same procedure was repeated with *tert*-butylated hydroxytoluene (BHT) as a positive control. Four replicates were recorded for each sample. The percentage of RSC was calculated using the following equation:

RSC(%)=100 × (*A*blank-*A*sample/*A*blank)

The IC_{50} value, which represents the concentration of essential oil that causes 50% neutralisation of DPPH radical, was determined by linear regression analysis from the obtained RSC values.

The scavenging capacity of the investigated essential oil for hydroxyl radicals was evaluated by measuring the degradation of 2-deoxy-D-ribose with OH radicals, generated in a Fenton reaction. The degradation products are thiobarbituric acid-reactive substances (TBARS), and can be determined spectrophotometrically at 532 nm (Bozin et al. 2007). 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 μL/mL), 50% essential oil (1.065 μL/mL) or 20% essential oil (0.425 μL/ mL) in n-hexane were mixed with 0.125 mL of H_2O_2, 0.125 mL of $FeSO_4$, and 0.125 mL of 2-deoxy-D-ribose and diluted with 0.05 M PB pH = 7.4, to a volume of 3 mL. After incubation for1 h at 37 °C, the extent of deoxyribose degradation was measured using the 2-thiobarbituric acid (TBA) reaction. Briefly, 1.5 mL 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 reaction tubes were heated at 100 °C for 20 min. After the mixture was cooled, absorbance at 532 nm was measured against a blank (buffer solution only). n-hexane alone was analyzed as a negative control sample and 0.5 M BHT (220.4 μg/mL) was analyzed as a positive control. Four replicates were recorded for each sample.

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

I(%) = 100 × (*A*blank-*A*sample/*A*blank)

Determination of Lipid Peroxidation (LP)

A commercial preparation of liposomes, "PRO-LIPO S" 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 deionized water (1:10), in an ultrasonic bath. Three concentrations of essential oil were prepared for the experiment: pure essential oil (2.13 μL/mL), 50% (1.065 μL/mL) and 20% (0.425 μL/mL) solutions in *n*-hexane.

For Fe^{2+}/ascorbate induction of LP, 60 μL suspensions of liposomes were incubated with 20 μL of 0.01 M $FeSO_4$, 20 μL of 0.01 M ascorbic acid and appropriate amounts of extract samples in 2.89 mL of 0.05 M KH_2PO_4–K_2HPO4 buffer, pH 7.4 (3 mL final solution).

The reaction mixture for Fe^{2+}/H_2O_2-induced LP contained 30 μL of suspension of liposomes, 0.125 mL of 9 mM $FeSO_4$, 0.125 ml of 0.88 M H_2O_2 and the investigated extract samples at different concentrations in 2.71 mL of 0.05 M KH_2PO_4–K_2HPO4 buffer, pH 7.4 (3 mL final solution).

Samples were incubated at 37 °C for 1 h. LP was terminated by both reaction of 1.5 mL of TBA reagent and 0.2 mL of 0.1 M EDTA, and by heating at 100 °C for 20 min. After cooling and centrifugation (4000 rpm for 10 min; to precipitate proteins), MDA (TBARS) content was determined by measuring adduct absorbance at 532 nm.

Both analyses were compared with a commercial synthetic antioxidant, BHT (0.5 M stock solution, concentration 220.4 μg/mL) as a positive control. For control, *n*-hexane was also analyzed. All reactions were carried out in four replicates. The percentage of LP inhibition was calculated by the following equation:

I(%) = 100 × (*A*blank-*A*sample/*A*blank)

Antimicrobial activity

Evaluation of antifungal activity

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

In order to investigate the antifungal activity of the investigated essential oil, the microdilution technique was performed as previously described (Bozin et al. 2007). 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 approximately 1.0×10^5, in a final volume of 100 µL per well. The density of fungal suspensions was determined using a McFarland nefelometer. Inocula were stored at +4°C for further investigations. Dilutions of the inocula were cultured on solid Malt agar (MA) to verify the absence of contamination and to check the viability of the inoculum. Minimal inhibitory concentration (MIC) determination was performed by serial dilution using 96-well microtiter plates. Investigated samples were dissolved in Malt medium broth (MB) agar with fungal inoculum to achieve concentrations of 1.0–5.0 µL/mL. Microplates were then incubated for 72 h at 28°C. The lowest concentration of essential oil which resulted in no visible growth under a binocular microscope was defined as the MIC (the concentration that completely inhibits fungal growth). Minimal fungicidal concentrations (MFC) were determined by serial subcultivation of 2 µL in microtiter plates containing 100 µL of MB per well, with incubation at 28°C for 72 h. The lowest concentration of essential oil which resulted in no visible growth was defined as the MFC (indicating 99.50% mortality of the original inoculum). As a positive control, Bifonazole (1 g/100 mL ethanol) was used as a synthetic antimycotic.

Evaluation of antibacterial activity

A collection of 13 test organisms (5 Gram-positive and 8 Gram-negative bacterial strains) was used: 8 microorganisms were from the American type of Culture Collection (ATCC); 5 microorganisms were from the Institute of Public Health, Faculty of Medicine, University of Novi Sad, Serbia, and were isolated directly from the patients (IPH), including four multiresistant strains (IPH-MR). Bacterial strain sources are shown in Table 6. All test organisms were stored at +4 °C on Mueller-Hinton (MH) agar slants, subcultured every two weeks, and checked for purity.

Evaluation of the antibacterial activity of the essential oil was performed using the hole–plate agar diffusion method as previously described (Bozin et al. 2007). Bacterial strains were grown on MH slants overnight at 37°C and checked for purity. After incubation, bacterial cells were washed from the agar surface and re-suspended in sterile 0.1 M phosphate buffer (PB) containing 167 mM NaCl (167 mM NaCl–PB; pH 7.4). Bacterial suspension density was determined using a McFarland nefelometer. Samples contained ~2×10^7 colony-forming units (CFU)/mL. MH agar plate surfaces were seeded by spreading 0.1 mL of bacterial suspension. Holes, 5 mm in diameter, were punched out of the agar surface and filled with 15 µL of the tested essential oils (50% and 20% solutions in n-hexane). Plates were incubated overnight at 37°C, and the diameter of the resulting zone of inhibition was measured. Evaluation of antibacterial activity was conducted in five repetitions. Penicillin (500 and 1000 µg/mL) was used as a positive control. As a control, the effect of solvent (n-hexane) on microbial growth was also analysed.

Statistical analysis

Data are reported as mean values ± standard deviation (SD). Values representing the concentrations of investigated essential oils that cause 50% neutralization or inhibition (IC_{50}) were determined by regression analysis of RSC (%) and LP inhibition results (Microsoft Excel program for Windows, v. 2000).

Results and Discussion

Chemical composition

The essential oil was 0.11% (v/w in dry matter), similar to earlier published data (Mimica-Dukic et al. 2002; Chalchat et al. 2004; Malencic et al. 2004). How-

ever, the percentage yield of woodland sage essential oil was significantly lower than that usually obtained from Dalamtian sage (*Salvia officinalis*), and other cultivated or wild growing *Salvia* species (Mimica-Dukic et al. 2002; Bozin et al. 2007; Anačkov et al. 2009).

The percent composition of the extracted essential oil is shown in Table 1. The total number of chemical constituents identified in the essential oil was 48 (88.37%). Sesquiterpene hydrocarbons (39.19%) and oxygenated sesquiterpenes (26.15%) comprised a total of 65.34% of all components; while monoterpene hydrocarbons made up 16.61% of the sample. Minor components included oxygenated monoterpenes (5.29%) and aliphatic compounds (1.39%). Sesquiterpenes E-caryophyllene (22.95%) and caryophyllene oxide (17.98%) were identified as the main components, followed by the monoterpene hydrocarbons sabinene (7.69%) and limonene (3.07%). In addition, significant amounts of sesquiterpene hydrocarbons β-cubebene (2.22%), α-humulene (2.17%) and γ-muurolene (5.23%) were found, as well as oxygenated sesquiterpene alcohols (caryophyllenol, 2.97%; and spathulenol, 3.39%) (see Table 1).

It is well-known that the major class of compounds found in essential oils extracts of some representatives of the genus *Salvia* (*S. nemorosa* and *S. pratensis*) are sesquiterpene hydrocarbons (mainly Z- or E-caryophyllene). Thus, our results are generally in agreement with those published in the literature (Mathe et al. 1993; Mimica-Dukić et al. 2002). However, earlier data on woodland sage essential oil reported the presence of several chemotypes, of which caryophyllene oxide was the major compound, followed by either pathulenol, β-caryophyllene, germacrene B or germacrene D (Mirza and Sefidkon 1999; Mimica-Dukić et al. 2002; Chalchat et al. 2004; Malenčić et al. 2004). The investigated essential oil, which was obtained from plant material collected in the northern part of the Pannonian plane, has a specific chemical composition which could not be categorized in any previously described chemotype. Major compounds in this essential oil are E-caryophyllene (22.95), followed by caryophyllene oxide (17.98%). Based on these results, it is obvious that caryophyllane class sesquiterpenes (E-caryophyllene, caryophyllene oxide and caryophyllenol) are the dominant components of the investigated essential oil.

Antioxidant activity

Recently, several synthetic industrial antioxidants were reported to be possibly mutagenic and toxic to humans (Namiki 1990), putting renewed focus on the antioxidant potential of plant products and isolated plant compounds. Antioxidant activity can be evaluated using numerous assays. The first step in this process is screening for potential activity using a series of different in vitro tests. Each test is based on one aspect of antioxidant activity, such as: free radical scavenging ability; lipid peroxidation inhibition; and transition metal ion (TMI) chelation. However, due to their complex composition, use of a single method for testing the antioxidant activity of plant products is not recommended. Therefore, in the present study, the antioxidant activity of the investigated essential oil was evaluated using a series of in vitro tests.

In the DPPH test, the ability of the investigated essential oil to act as a hydrogen or electron donor during reduction of DPPH radical to DPPH-H was measured spectrophotometrically (Table 2). The tested essential oil was able to reduce stable DPPH radicals to the yellow-coloured DPPH-H, reaching 50% reduction with an IC_{50} of 0.98 μL/mL. Comparison of the DPPH scavenging activity of the tested essential oil with that of BHT revealed that BHT has significantly lower antioxidant activity (IC_{50} = 5.37 μL/mL).

The hydroxyl RSC of the essential oil (Table 3) was measured using the deoxyribose assay. Hydroxyl radicals formed during the 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. The protective effects of the essential oil were evaluated by measuring the ability of the oil to scavenge hydroxyl radicals from the test solution, preventing degradation of 2-deoxy-D-ribose. Significant 2-deoxy-D-ribose degradation inhibition was observed for all tested essential oil concentrations, with an IC_{50} of 0.11 μL/mL, against BHT as a positive control (18.71% for 220.4 μL/mL).

The protective effects of woodland sage essential oil against lipid peroxidation were evaluated by thiobarbituric acid (TBA) assay using two induction systems: Fe^{2+}/ascorbate and Fe^{2+}/H_2O_2. LP inhibition was determined by measuring the formation of MDA,

Table 1. Chemical composition of essential oil of *Salvia nemorosa*.

Pick No.	Components	R.I.[a]	%
Monoterpene Hydrocarbons			16.52
1	α-Thujene	932	1.53
2	α-Pinene	938	0.42
3	α Fenhane	951	0.24
4	Camphene	953	0.11
5	Sabinene	974	7.69
7	β-Pinene	978	0.43
8	β-Myrcene	992	0.29
9	α-Terpinene	1017	0.40
11	Limonene	1035	3.07
12	γ-Terpinene	1063	1.06
13	Terpinolene	1088	0.38
Aromatic Monoterpene Hydrocarbons			0.90
10	π-Cymene	1026	0.90
Oxygenated Monoterpenes			5.12
14	Z-Sabinene hydrate	1067	0.42
15	α-Thujone	1104	0.36
16	β-Thujone	1114	0.13
17	Camphor	1144	0.49
18	Menthone	1156	0.72
19	Menthofuran	1164	0.15
20	Borneol	1167	0.14
21	Menthol	1173	0.47
22	Terpinen-4-ol	1178	1.21
24	Pulegone	1236	0.09
25	Bornyl acetate	1288	0.19
27	Citronelyl acetate	1354	0.28
31	Z-Jasmone	1395	0.19
Aromatic Oxygenated Monoterpenes			0.14
23	π-Cymen-8-ol	1182	0.14
36	Geranyl acetone	1453	0.14
Sesquiterpene Hydrocarbons			39.19
26	α-Cubebene	1352	0.81
28	α-Copaene	1375	0.80
29	β-Bourbonene	1383	1.03
30	β-Cubebene	1389	2.22
32	Longifolene	1403	0.24
33	E-Caryophyllene	1417	22.95
34	γ-Elemene	1432	0.53
35	Aromadendrene	1440	0.34
37	α-Humulene	1454	2.17
38	γ-Muurolene	1475	5.23
39	α-Zingiberene	1496	0.40
40	γ-Cadinene	1514	1.01
41	δ-Cadinene	1524	1.46
Oxygenated Sesquiterpenes			26.15
42	Caryophylenol	1567	2.97
43	Spathulenol	1576	3.39
44	Caryophyllene oxide	1584	17.98
45	Viridiflorol	1593	1.53
46	Vidrol	1597	0.28
Aliphatic Components			1.39
6	3-Octenol	976	1.00
47	Nonadecane	1900	0.16
48	Eicosane	2001	0.23
Total identified			88.37

[a]Retention indices relative to C9-C24 *n*-alkanes on the HP-5 MS column

using liposomes as an oxidizable substrate. However, the TBA assay is non-specific for MDA, and non-lipid substances present in plant extracts, together with peroxidation products other than malondialdehyde, can react positively with TBA. Because these interfering compounds can distort obtained results; all final results were corrected using sample absorbance after the TBA test (without liposomes) (Janero 1990).

Antioxidant activity results of the examined essential oil vs. BHT (positive control) are shown in Table 4. The investigated essential oil displayed stronger antioxidant capacity than BHT (37.04%) in the Fe^{2+}/ascorbate induction system, and exhibited very high activity (IC_{50} = 0.58 μL/mL). Similarly, the investigated essential oil also displayed strong antioxidant activity against LP induced by the Fenton reaction (Fe^{2+}/H_2O_2), (IC_{50} = 0.01 μL/mL).

Comparison of antioxidant activity (IC_{50} values) of the investigated essential oil (Fig. 1) revealed variable effects, depending on the model system used for evaluation. Generally, the strongest activity (with the small variation) was observed for LP protection. In contrast, significant variation was observed during RSC testing. Although woodland sage essential oil contains a relatively small amount of oxygenated monoterpenes (Table 1), it still exhibited stronger scavenging activity versus Dalmatian sage essential oil (Bozin et al. 2007). However, comparison of the obtained results with those reported for a methanol extract isolated from *S. nemorosa* leaves (Tosun et al. 2009) shows that leaf extracts contain stronger RSC properties.

Antimicrobial activity

In addition to antioxidant capacity, antimicrobial activity is one of the most examined features of essential oils, and is important for both food preservation and control of human and animal diseases of microbial origin. However, to obtain useful data concerning the influence of some essential oil compounds on the activity cited, further examinations are necessary, particularly when followed by a well-defined chemical composition.

The antifungal activity of the investigated essential oil was tested against five dermatomycetes and *Candida albicans* (see Table 5). In general, the essential

Table 2. Percentage of neutralization of DPPH of essential oil of *Salvia nemorosa* and BHT (as a positive control) in the DPPH assay.

Samples	Concentrations (μL/mL)										IC_{50}
	0.48	1.20	2.40	3.12	4.80	6.25	7.20	9.60	12.00	12.50	
Salvia nemorosa	20.59	53.22	71.95	77.76	88.23	94.38	98.99				0.98
BHT	4.62	11.56	23.12	30.11	44.71	55.22	59.07	67.51	72.12	74.22	5.37

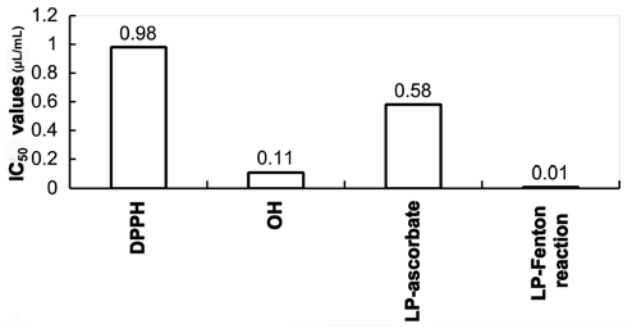

Figure 1. Comparison of antioxidant activity (IC_{50} values) exhibited by examined essential oil of *Salvia nemorosa* in different model systems.

oil displayed weak fungistatic and fungicidal activity. Although there is no data available in the literature on the antimicrobial activity of woodland sage essential oil for comparison, the obtained results are at least partially similar to those obtained for Dalmatian sage (Bozin et al. 2007).

The antibacterial activity of the investigated essential oil against 5 strains of Gram-positive and 8 strains of Gram-negative bacteria is shown in Table 6. In general, the Gram-positive bacterial strains appear to be more sensitive to the essential oil. These results are in agreement with previously published literature data (Mimica-Dukic and Bozin 2007). Similar to the antifungal results presented earlier, essential oil of *S. nemorosa* essential oil displayed low antibacterial activity. One possible explanation for this weak antimicrobial effect could the specific chemical composition of the investigated essential oil, which contains a significant amount of mono- and sesquiterpene hydrocarbons, which clearly do not possess notable antimicrobial activity.

However, because this is the first documented data on the antimicrobial activity of S. *nemorosa* essential oil, it is important to point out that significant antibacterial activity was observed against multi-resistant strains of *Salmonella typhi* IPH-MR and *Shigella sonei* IPH-MR, as well as against the Gram-positive bacterium *Staphylococcus epidermidis* (ATCC 12228).

In conclusion, evaluation of the antioxidant effects of the investigated essential oil revealed very strong protective activities, both as free radical scavengers and LP inhibitors. However, from the obtained results, the assayed essential oil and the compounds present in it clearly have different modes of action. Furthermore, this study found significant antimicrobial activity against some of the tested bacteria and fungi. However, the results obtained indicate the necessity for further research related to the potential bio-

Table 3. Percentage of inhibition of degradation of 2-deoxyribose by essential oil[a] of *Salvia nemorosa* and BHT (as a positive control) in the deoxyribose assay.

Samples	Concentrations (μL/mL)				IC_{50}
	0.425	1.065	2.13	220.4	
Salvia nemorosa	67.6	85.04	89.89	-	0.11
BHT	-	-	-	18.71	n.r.[b]

[a]examined essential oil was diluted in *n*-hexane (the solvent expressed no scavenging activity).
[b]n.r. – not reached.

Table 4. Inhibition of lipid peroxidation (LP) in Fe^{2+}/ascorbate and Fe^{2+}/H_2O_2 system of induction by the essential oil[a] and BHT (as a positive control) in the TBA assay.

System of induction	Samples	Concentrations (μL/mL)				IC_{50}
		0.425	1.065	2.13	220.4	
Fe^{2+}/ascorbate	*S. nemorosa*	48.38	54.84	65.58	-	0.58
	BHT	-	-	-	37.04	n.r. [b]
Fe^{2+}/H_2O_2	*S. nemorosa*	84.69	91.84	93.88	-	0.01
	BHT	-	-	-	66.67	n.r.[b]

[a]examined essential oil was diluted in *n*-hexane (the solvent expressed no scavenging activity).
[b]n.r. – not reached.

Table 5. Antifungal activity of essential oil and Bifonazole (as a positive control).[a]

Fungal strain	Essential oil		Bifonazole	
	MIC	MFC	MIC	MFC
Trichophyton mentagrophytes	100.3 ± 0.6	250.7 ± 0.6	9.7 ± 0.6	9.7 ± 0.6
T. rubrum	100.0 ± 1.0	200.2 ± 0.3	10.3 ± 0.6	10.3 ± 0.6
T. tonsurans	100.0± 0.0	200.0± 0.0	10.0 ± 0.0	10.0 ± 0.0
Microsporum canis	200.0± 0.0	200.2 ± 0.3	15.0 ± 0.0	20.0 ± 0.0
Epidermophyton floccosum	200.0± 0.0	250.2 ± 0.3	10.0 ± 0.0	10.0 ± 0.0
Candida albicans	200.2 ± 0.3	400.2 ± 0.3	20.0 ± 0.0	25.2 ± 0.3

[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.

medical effects of the investigated essential oil, as well as on the safety of its possible use.

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Table 6. Antibacterial activity of essential oil and Penicillin (as a positive control).[a]

Microorganism	Essential oil[b]		Penicillin	
	20%	50%	500	1000
Gram negative strains				
Pseudomonas aeruginosa (ATCC 27853)	0,0	0,0	0,0	0,0
P. aeruginosa (IPH-MR)	0,0	0,0	0,0	0,0
Escherichia coli (ATCC 35218)	0,0	0,0	0,0	0,0
E. coli (ATCC 25922)	0,0	0,0	12,6 ± 0,89	13,4 ± 0,55
E. coli (IPH-MR)	0,0	0,0	0,0	0,0
Salmonella enteritidis (IPH)	0,0	0,0	22,0 ± 1,22	26,2 ± 0,83
S. typhi (IPH-MR)	15,2 ±0,84	19,8 ±0,45	0,0	0,0
Shigella sonei (IPH-MR)	16,6 ±1,14	17,6 ±1,67	0,0	0,0
Gram positive strains				
Sarcina lutea (ATCC 9341)	15,8 ±0,45	19,0 ±0,71	0,0	0,0
Micrococcus flavus (ATCC 10240)	25,6 ±0,55	20,0 ±0,71	40,0 ± 0,55	40,0 ± 0,89
Staphylococcus aureus (ATCC 6538)	19,4 ±0,89	19,6 ±0,89	48,0 ± 0,00	50,0 ± 0,00
S. epidermidis (ATCC 12228)	17,8 ±0,45	18,2 ±0,45	14,2 ± 0,44	16,8 ± 0,83
Bacillus subtilis (ATCC 10707)	24,0 ±0,00	23,2 ±0,84	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 (exhibited no activity).

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