Journal of Applied Botany and Food Quality 90, 330 - 338 (2017), DOI:10.5073/JABFQ.2017.090.041

[1]Department of Morphology and Systematic of Plants, Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade, Serbia
[2]Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia
[3]Institute for Medicinal Plant Research "Dr. Josif Pančić", Belgrade, Serbia
[4,5]Department of Biology, Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University, Institute for Biology and Macedonian Academy of Sciences and Arts, Skopje, Macedonia

Laserpitium ochridanum: antioxidant, antimicrobial and anti-quorum sensing activities against *Pseudomonas aeruginosa*

Ksenija S. Mileski[1*], Ana D. Ćirić[2], Jovana D. Petrović[2], Mihailo S. Ristić[3], Vlado S. Matevski[4,5], Petar D. Marin[1], Ana M. Džamić[1]

(Received June 6, 2016; Accepted August 23, 2017)

Summary

This study shows *Laserpitium ochridanum* essential oil composition, its antifungal potency, and antioxidant, antimicrobial and anti-quorum sensing activities of different extracts. Monoterpene hydrocarbons (40.9%) were the most abundant group of constituents in the oil. Sabinene (22.8%), viridiflorol (14.7%) and α-pinene (11.40%) were the main components of the oil. The ethanolic extract had the highest antioxidant capacity in DPPH and ABTS assays and it was the richest in phenolic contents. Microdilution method revealed the strongest antibacterial activity of ethanolic extracts in comparison to other tested extracts and streptomycin. Essential oil of *L. ochridanum* evidenced the best antifungal potential against used micromycetes. Results of an anti-quorum sensing activity assay indicated high affection of aqueous extract in reduction of PAO1 pyocyanin production (18.07%). Used samples possessed slight reduction of twitching and swimming motility. This study shows for the first time anti-quorum sensing activity of *L. ochridanum* against *Pseudomonas aeruginosa* PAO1, as well as its significant antioxidant potential.

Introduction

The genus *Laserpitium* L. (Apiaceae) comprises about 30 species, mostly biennial and perennial plants, widely distributed from the Canary Islands to Siberia and Iran (Nikolić, 1973). It is characterized by ternate or several times pinnate leaves, white, yellow or pinkish petals (Tutin, 1968). *Laserpitium ochridanum* Micevski is rare and an endemic perennial plant, up to 40-60 cm with white colour of the petals which can be found only at the National park Mt. Galičica (FYROM), at 1600-2000 m a.s.l. (Micevski, 2005). Different parts of some widely distributed *Laserpitium* species (e.g. *L. siler*, *L. latifolium*) have been used as traditional herbal medicines in Europe. They are usually used as tonics for strengthening and refreshing, for treating toothache, as diuretics, for treating gastrointestinal disorders, heart and liver dysfunctions, pulmonary tuberculosis, rheumatism and topically in pruritic dermatomycoses, as well as for sleep disorder and major depression in Taiwan (Popović et al., 2013; Yi-Lin Chen et al., 2015). In earlier studies, sesquiterpene lactones were found as the main secondary metabolites in *Laserpitium* extracts (Appendino et al., 1987, 1993; Đermanović et al., 1996). Recently, it was published that sesquiterpene lactones mainly belong to the class of guajanolides (Popović et al., 2013). However, monoterpene hydrocarbons were predominant compounds in the essential oil (EO) of *Laserpitium* species (Baser and Duman, 1997; Chizzola et al., 1999; Chizzola, 2007; Petrović et al., 2009; Tirillini et al., 2009; Popović et al., 2010, 2013, 2014). Litarature data showed that different *Laserpitium* species possessed antibacterial, antifungal, cytotoxic, anticancer, antinociceptive and antiedematous activities (Petrović et al., 2009; Tirillini et al., 2009; Popović et al., 2010, 2013, 2014). Lately, the interest in studying different pathogens is rapidly incrising, because of their resistance towards synthetic antibiotics or antimycotics. It is known that the pathogenic, gram-negative bacillus *Pseudomonas aeruginosa* is a major cause of nosocomial infections, bronchopneumonia, septic shock and wound infections. This opportunistic bacterium forms populations with distinctive density-dependant behavour. By anti-quorum sensing (anti-QS) agents, growth of *P. aeruginosa* can be weaken and some of its pathologically significant virulence factors, such as production of biofilm, swarming motility, pigment and antibiotic production, can be reduced (Soković et al., 2014; Sepahi et al., 2015). Some medical plants possess anti-QS activity and can be considered as potential anti-quorum agents (Al-Hussaini and Mahasneh, 2009; Koh et al., 2013; Sepahi et al., 2015).

The aim of this study was to define the chemical composition of *L. ochridanum* essential oil and to determined antioxidant, antimicrobial and anti-QS activities of its extracts. To the best of our knowledge *L. ochridanum* crude extracts were assayed for the first time for their antioxidant potency combined with total phenolic and flavonoid contents. Also, no anti-QS activity of this species has been reported to date.

Material and methods

Solvents and chemical reagents

Solvents and chemicals that were provided for performing the experiments were of analytical grade. Organic solvents were procured from Zorka pharma, Šabac, Serbia. Gallic acid, 3-tert-butyl-4-hydroxyanisole (BHA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu phenol reagent, potassium acetate and aluminum trinitrate nonahydrate were obtained from Sigma-Aldrich Co., St Louis, MO, USA. Sodium carbonate anhydrous was purchased from Centrohem d.o.o, Stara Pazova, Serbia. Potassium peroxide sulphate and L(+)-ascorbic acid were obtained from Fisher Scientific UK Ltd., Loughborough, Leicestershire, UK. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and quercetin hydrate were purchased from TCI Europe NV, Binnenveldsweg, Belgium. Malt-broth (MB), tryptic soy broth (TSB), Mueller-Hinton agar (MH), Luria-Bertani medium (LB) (1% w/v NaCl, 1% w/v tryptone, 0.5% w/v yeast extract) and malt agar (MA) were obtained from the Institute of Immunology and Virology, Torlak (Belgrade, Serbia). Streptomycin (Sigma-Aldrich S6501 St. Louis, MQ, USA), ampicillin (Sigma-Aldrich A9393 St. Louis, MQ, USA) and dimethyl sulfoxide (DMSO) (Sigma Aldrich, St. Louis, MQ, USA) were used in these study. Antimicotic diflucan (containing 50 mg fluconazole) was obtained from Pfizer PGM, Pocesur-Cisse, France.

* Corresponding author

Plant material

Plant material was collected during the flowering stage, at Mt. Galičica, the national park in Republic of Macedonia (FYROM) in July, 2013 (GPS: N 40°56′30″, E 20°49′34″). It was determined as *Laserpitium ochridanum* Micevski by one of the authors (prof. Vlado S. Matevski). A voucher specimen (BU16778) is deposited at the herbarium of the Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade, Serbia.

Isolation of the essential oil

The dark blue EO of *L. ochridanum* was obtained from 200 g of dry aerial parts by 3 h of hydrodistilation using a Clevenger type apparatus. The yield of the oil was 0.11% for herbal parts (w/w-dry bases). The essential oil obtained was preserved in sealed vials at 4 ºC prior to further analysis.

Preparation of plant extracts

Dried, ground plant material (10 g) was treated with 200 mL of methanol, ethanol and distilled water to obtain different extracts. The ultrasonic extraction procedure was performed during 24 h in the dark; the extracts were exposed to ultrasound for the first and the last hour of extraction and subsequently filtered through a Whatman filter paper No 1. Methanolic and ethanolic extracts were subjected to solvent evaporation under reduced pressure at maximum temperature of 40 ºC. The frozen aqueous extracts were lyophilized, reduced to a fine dried powder and mixed to obtain homogenous samples. The dried and crude extracts were measured, packed in glass bottles, and stored at 4 ºC until subjection to subsequent analysis. Obtained yields of *L. ochridanum* extracts were 0.597 g for methanolic (ME), 1.323 g for ethanolic (EE) and 0.983 g for aqueous extracts (AE).

Gas chromatography–flame ionization detector (GC–FID) and gas chromatography–mass spectrometry (GC–MS)

Qualitative and quantitative analysis of the essential oil was performed using GC and GC-MS methods. The GC analysis of the oil was carried out on a GChP-5890 II apparatus, equipped with split-split less injector, attached to an HP-5 column (25 m × 0.32 mm, 0.52 μm film thickness) and fitted to FID. Carrier gas flow rate (H_2) was 1 mL/min, split ratio 1:30, injector temperature was 250 °C, detector temperature 300 °C, while column temperature was linearly programmed from 40 to 240 °C (at rate of 4 °/min.). The same analytical conditions were employed for GC-MS analysis, where a 1800C Series II GCD system equipped with HP-5MS column (30 m × 0.25 mm, 0.25 μm film thickness) was used. The transfer line was heated at 260 °C. Mass spectra were acquired in EI mode (70 eV), in m/z range 40-400. The identification of the individual EO components was accomplished by comparison of retention times with standard substances and by matching mass spectral data with those of the Wiley 275 mass spectra library. Confirmation was performed using AMDIS software and literature (Adams, 2007). Quantitative analyses were based on area percents obtained by FID.

Analyses of total phenols and total flavonoids

Total phenolic content (TPC)

The spectrophotometric method described by Singleton et al. (1999) with some modifications was applied for recording total TPCs of all tested *L. ochridanum* extracts, using Folin-Ciocalteu reagent and GA as a standard. After preparing a 10% Folin-Ciocalteau reagent, the mixtures of 1000 μL of this solution and 200 μL of extracts solutions (1 mg/mL) were left to react for 6 min. After short incubation, 800 μL of 7.5% sodium carbonate solution was added and thus prepared solution was allowed to stand for 2 h at room temperature in the dark. The absorbance was measured at 736 nm versus a blank sample. Total phenols were calculated from the GA calibration curve (10-100 mg/L). Data were expressed as milligrams of GA equivalents per gram of dry plant extract. The values were presented as means of triplicate analysis.

Total flavonoid content (TFC)

Measurements of TFCs of *L. ochridanum* extracts were based on the method described by Park et al. (1997) with slight modification. An aliquot of each extract solution (1 mL) was mixed with 80% ethanol, 10% aluminium nitrate nonahydrate and 1 M potassium acetate. Absorption readings at 415 nm using a spectrophotometer were taken after 40 min. against a blank sample consisting of a 0.5 mL 96% ethanol instead of the tested sample. The TFCs were determined from the QE standard curve (10-100 mg/L). Results were expressed as mg of QE equivalents/g of dry extract. Generally, All measurements were done in triplicates.

Antioxidant capacity

DPPH assay

Series of EO and extracts solutions in appropriate solvents, with concentrations of 0.25-2 μL/mL for EO and 0.025-0.2 mg/mL for extracts were subjected for examination of free radical scavenging activity by DPPH assay. This spectrophotometric procedure described by Blois (1958), was performed to evaluate the quantity of tested solutions needed to reduce 50% of the initial DPPH radical concentration. 0.2 mL of each dilution was mixed with 1.8 mL of DPPH methanol solution (0.04 mg/mL). The absorbance was recorded at 517 nm after 30 min. of dark incubation at room temperature. BHA and ascorbic acid were used as reference standards and methanol as a blank. The corresponding percentage of inhibitions of each sample was calculated from obtained absorbance values by using following equation:

Percentage (%) of inhibition = (Ac-As)/Ac × 100

Tested concentrations of EO and extracts which decrease absorption of DPPH solution for 50% (IC_{50}) were obtained from the curve dependence of absorption of DPPH solution on 517 nm from concentration for each tested solution and used standards.

ABTS assay

The procedure of Miller and Rice-Evans (1997) with slightly modifications was followed for determination of *in vitro* ABTS radical-scavenging potency. Before usage, 5 mL of the mixture of 2.46 mM potassium persulphate and 19.2 mg of ABTS was allowed to react in the dark for 12-16 h at room temperature to obtain $ABTS^+$ solution. 100-110 mL of distilled water was added to 1 mL of $ABTS^+$ solution to adjust an absorbance of 0.7 ± 0.02 units at 734 nm. The mixtures of 2 mL of diluted $ABTS^{\cdot+}$ solution and 50 μL of each tested extract solution were incubated for 30 min. at 30 ºC and the absorbance was determined spectrophotometrically at 734 nm, using water as a blank. For every experiment a fresh $ABTS^+$ solution was prepared. The results were expressed from an ascorbic acid calibration curve (0-2 mg/L) in mg of ascorbic acid equivalents/g of dry extract. Tests were carried out in triplicate and all measurements were expressed as average of three analyses ± standard deviation.

Evaluation of antimicrobial properties

Microorganisms and culture conditions

The antimicrobial activity of all investigated samples was tested using pure control strains obtained from the mycological laboratory, Department of Plant Physiology, Institute for Biologycal Research

"Siniša Stanković", Belgrade, Serbia. The microorganisms included following bacterial strains: *Bacillus cereus* (food isolate), *Listeria monocytogenes* (NCTC 7973), *Micrococcus flavus* (ATCC 10240) and *Staphylococcus aureus* (ATCC 6538), *Enterobacter cloacae* (human isolate), *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), and *Salmonella typhimurium* (ATCC 13311). The following micromicetes were used: *Aspergillus fumigatus* (ATCC 9197), *Aspergillus niger* (ATCC6275) *Aspergillus ochraceus (*ATCC 12066), *Aspergillus versicolor* (ATCC 11730), *Candida albicans* (ATCC 10231), *Penicillium funiculosum* (ATCC 10509), *Penicillium ochrochloron (*ATCC 9112) and *Trichoderma viride* (IAM 5061). Dilutions of bacterial inocula were cultured on solid MH medium, while micromycetes were maintained on solid MA medium. The cultures were subcultured once a month and stored at +4 °C for further usage (Booth, 1971).

Microdilution method

For determination of antimicrobial activity of *L. ochridanum* oil and extracts, the modified microdilution technique described by Hanel and Raether (1998) was applied. The assay was performed by sterile 96-well microtiter plates, by adding pure EO or dilutions of tested extracts (in 5% DMSO) into corresponding medium - TSB and MA for bacteria and fungi, respectively. To achieve the concentration of 1.0×10^8 colony forming units (CFU)/mL for bacterial strains, 100 μL of overnight cultures were mixed with 900 μL of medium in eppendorf. Fungal inocula were prepared by washing spores with sterile 0.85% saline solution (containing 0.1% Tween 80 (v/v)). The microbial cell suspensions were adjusted with sterile saline to a concentration of approximately 1.0×10^6 CFU/mL for bacteria and 1.0×10^5 CFU/mL for fungi in a final volume of 100 μL per well. The microplates were incubated for 24 h at 37 °C for bacteria and for 72 h at 28 °C for fungi. The lowest concentrations of tested samples completely inhibiting the growth of used pathogens were defined as minimum inhibitory concentrations (MICs). The minimum bactericidal/fungicidal concentrations (MBCs, MFCs) were determined as the lowest concentrations with no visible growth after serial subcultivation, indicating 99.5% killing of the original inoculums (Hanel and Raether, 1998). In addition, bacterial growth was determined by a colorimetric microbial viability assay, based on reduction of an 0.2% *p*-iodonitrotetrazolium violet color (INT) aqueous solution (I 8377-Sigma Aldrich, St. Louis, MQ, USA) and compared with positive control for each bacterial strain (CLSI, 2009; Tsukatani et al., 2012). Two replicates were done for each sample. The solution of synthetic standard streptomycin with concentration of 1 mg/mL 5% DMSO was used as positive control for bacteria, while the fluconazole solution (antimicotic diflucan containing 50 mg fluconazole) at concentration of 2 mg/mL 5% DMSO was included for fungi. Sterilized distilled water containing 0.02% Tween 80 and 5% DMSO was used as negative control.

Preparation of stock solutions of plant extracts for antimicrobial tests

Different quantities of stock solutions of *L. ochridanum* extracts, dissolved in 5% DMSO (20 mg/mL) were tested against various pathogenic microorganisms.

Anti-quorum sensing activity of extracts

Bacterial strains, growth media and culture conditions

In this study, *Pseudomonas aeruginosa* PAO1 from the Institute for Biological Research "Siniša Stanković", Belgrade, Serbia, was used. Bacteria were routinely grown in Luria-Bertani (LB) medium with shaking (220 rpm) and cultured at 37 °C.

Biofilm formation

Considering the results obtained in antimicrobial assay and low yields of isolated EO and ME, further anti-QS analyzes were continued with ethanolic and aqueous extracts of *L. ochridanum*. The samples (0.5, 0.25, 0.125 of MICs, respectively) were tested on biofilm forming ability on polystyrene flat-bottomed microtitre 96 well plates as described by Spoering and Lewis (2001); Drenkard and Ausubel (2002), with some modifications. In brief, 100 μL of overnight culture of *P. aeruginosa* (1.0×10^8 CFU/mL) was added to each well of the plates in the presence of 100 μL subinhibitory concentrations (subMIC) of *L. ochridanum* samples (0.5, 0.25 and 0.125 MIC) or 100 mL medium (control). After incubation for 24 h at 37 ºC, each well was washed twice with sterile PBS (pH 7.4), dried, stained for 10 min with 0.1% crystal violet in order to determine the biofilm mass. After drying, 200 μL of 95% ethanol (v/v) was added to solubilise the dye that had stained the biofilm cells. The excess stain was washed off with distilled water. After 10 min, the content of the wells was homogenized and the absorbance at $\lambda = 620$ nm was read on a Sunrise™ - TecanELISA reader. The experiment was done in triplicate and repeated two times and values were presented as a mean values ± SE.

Twitching and flagella motility

After growth in the presence or absence of subMICs of *L. ochridanum* ethanolic and aqueous extracts, streptomycin and ampicillin, the cells of *P. aeruginosa* PAO1 were washed twice with sterile PBS and re-suspended in PBS at 1.0×10^8 CFU/mL (OD of 0.1 at 660 nm). In brief, the cells were stabbed into a nutrient agar plate with a sterile toothpick and incubated overnight at 37 °C. The plates were then removed from the incubator and incubated at room temperature for two more days. Colony edges and the zone of motility were measured with a light microscope (O'Toole and Kolter, 1998a, b). SubMICs of extracts (0.5 MICs) were mixed into 10 mL of molten LB medium and poured immediately over the surface of a solidified LB plate as an overlay. The plate was point inoculated with an overnight culture of PAO1 once the overlaid agar had solidified and incubated at 37 °C for 3 days. The extent of swimming was determined by measuring the area of the colony (Sandy and Foong-Yee, 2012). The experiment was done in triplicate and repeated two times. The colony diameters were measured three times in different direction and values were presented as a mean values ± SE.

Inhibition of synthesis of P. aeruginosa PAO1 pyocyanin

The flask assay was used to quantify the inhibitory activity of the *L. ochridanum* against *P. aeruginosa* pyocyanin production. Overnight culture of the bacillus PAO1 was diluted to OD_{600} nm 0.2. Then, 0.5 MICs of tested extracts dissolved in 5% of DMSO (1.25 mg/mL for EE and 12.50 mg/mL for AE), were added to the bacteria (5 mL) and incubated at 37 °C for 24 h. The treated culture was extracted with chloroform (3 mL), followed by mixing the chloroform layer with 0.2 M HCl (1 mL). Absorbance of the extracted organic layer was measured at 520 nm using a Shimadzu UV1601 spectrophotometer (Kyoto, Japan) (Sandy and Foong-Yee 2012). The experiment was done in triplicate and repeated two times. The values were expressed as ratio $(OD_{520}/OD_{600}) \times 100$.

Statistical analysis

Three samples were used and all the assays were carried out in triplicates. The results are expressed as mean values and standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with a = 0.05. This analysis was carried out using SPSS v. 18.0 program.

Results

Essential oil composition

Referring to the results presented in Tab. 1, fifty nine components were identified in *L. ochridanum* EO. Monoterpene hydrocarbons were the most abundant group of compounds (40.87%), followed by oxygenated sesquiterpenes (24.14%), oxygenated monoterpenes (15.27%) and sesquiterpene hydrocarbons (13.17%). The dominant compounds of the EO were sabinene (22.8%), viridiflorol (14.7%) and α-pinene (11.4%) (Tab. 1).

Tab. 1: Chemical composition of EO of *L. ochridanum* aerial parts.

Compounds	KIE	KIL	%
α-Thujene	919.1	924	0.32
α-**Pinene**	924.8	932	**11.36**
Thuja-2,4(10)-diene	944.9	953	0.17
Sabinene	965.6	969	**22.76**
β-Pinene	974.7	974	0.80
Myrcene	985.7	988	0.69
n-Octanal	997.2	998	0.39
α-Terpinene	1009.4	1014	1.02
p-Cymene	1018.0	1020	0.35
β-Phellandrene	1020.9	1025	0.96
γ-Terpinene	1051.3	1054	1.93
cis-Sabinene hydrate	1057.3	1065	0.56
n-Octanol	1069.3	1063	3.86
Terpinolene	1080.7	1086	0.51
6-Camphenone	1088.1	1095	0.59
trans-Sabinene hydrate	1097.0	1098	0.29
6-Camphenol	1113.5	1111	0.43
α-Campholenal	1119.3	1122	0.56
trans-Pinocarveol	1132.4	1135	0.85
trans-Sabinol	1135.5	1137	0.40
trans-Verbenol	1139.6	1140	1.79
Terpinen-4-ol	1171.0	1174	3.86
Thuj-3-en-10-al	1178.9	1181	0.31
α-Terpineol	1186.8	1186	0.28
Myrtenal	1188.8	1193	0.37
Myrtenol	1192.1	1194	0.54
Verbenone	1204.0	1204	0.19
Octanyl acetate	1207.5	1211	1.26
Isobornyl acetate	1277.8	1283	0.50
α-Terpinyl acetate	1343.0	1346	0.21
Cyclosativene	1366.2	1369	0.27
α-Copaene	1370.2	1374	1.12
Daucene	1376.8	1380	0.69
β-Cubebene	1381.1	1387	0.23
β-Elemene	1383.4	1389	0.43
(*E*)-Caryophyllene	1409.0	1417	1.62
trans-α-Bergamotene	1427.0	1432	0.18
α-humulene	1443.5	1452	0.66
(*E*)-β-Farnesene	1450.2	1454	0.19
cis-Muurola-4(14),5-diene	1460.6	1465	0.20
γ-Himachalene	1467.9	1468	0.81
Dauca-5,8-diene	1471.4	1471	1.96
ar-Curcumene	1475.5	1479	0.19
β-Selinene	1487.0	1489	1.73
cis-Eudesma-6,11-diene	1488.0	1489	0.83
Bicyclogermacrene	1494.9	1500	0.44
β-Bisabolene	1500.6	1505	0.12
δ-Cadinene	1514.3	1522	0.70
α-Calacorene	1546.1	1544	0.80
Spathulenol	1570.3	1577	2.65
Caryophyllene oxide	1573.4	1582	1.16
Viridiflorol	1588.9	1592	**14.71**
1-*epi*-Cubenol	1627.5	1627	0.54
β-Cedren-9-one	1631.6	1630	2.44
β-Eudesmol	1649.4	1649	0.44
α-Bisabolol	1679.3	1685	2.20
Chamazulene	1721.9	1730	3.04
Neophytadiene (isomer II)	1829.5	1830	1.02
Incensole acetate	2181.9	2184	0.52
Total			**100.00**
Number of constituents			59
Monoterpene hydrocarbons			40.87%
Oxygenated monoterpenes			15.27%
Sesquiterpene hydrocarbons			13.17%
Oxygenated sesquiterpenes			24.14%
Others			6.55%

KIE = Kovats (retention) index experimentally determined (AMDIS)
KIL = Kovats (retention) index - literature data (ADAMS, 2007)

Total phenolic contents

According to the results obtained for TPC in *L. ochridanum* extracts (Tab. 2), phenols were present from 111.28 to 141.30 mg GA/g of dry extract for methanolic and ethanolic extract, respectively. In tested extracts, TFC ranged from 21.38 to 67.69 mg QE/of dry extract for aqueous and ethanolic extract, respectively. In general, greater variation in tested extracts was recorded in flavonoid contents. The highest phenolic and flavonoid concentrations were measured in EE of *L. ochridanum* (Tab. 2).

Antioxidant activity

The results of obtained antioxidant activity for *L. ochridanim* are listed in Tab. 2. In DPPH test, used extracts exhibited similar antioxidant activity, stronger than BHA, but lower activity compared to ascorbic acid. Still, the strongest radical scavenging activity was recorded for EE (0.113 ± 0.002 mg/mL), which was in accordance with the highest measured total phenolic and flavonoid contents. EO of *L. ochridanum* showed the lowest antioxidant potency compared to all other samples. Results obtained by the ABTS test showed that the AE was the most effective agent in concentration of 2.172 ± 0.005 mg ascorbic acid/g of dry extract. According to the obtained results this sample had slightly lower antioxidant capacity than standard QE (2.749 ± 0.004 mg ascorbic acid/g of dry extract).

Antimicrobial properties

Antibacterial activity

The results presented in Tab. 3 indicate that *L. ochridanum* extracts exhibited moderate antibacterial activity. The EE was the strongest in bactericidal activity (MBCs = 1.00-5.00 mg/mL), while the lowest potency had AE (MBCs = 11.00-14.00 mg/mL). Both, methanolic and ethanolic extracts were more effective compared to streptomycin, but all extracts, including aqueous, showed stronger inhibitory activity on *L. monocytogenes* and *E. cloacae* than used antibiotic.

Tab. 2: TPC, TFC and antioxidant activity of *L. ochridanum* extracts and EO (means ± SD).

***L. ochridanum* extracts/ EO**	**Total phenolic contents**		**Antioxidant activity**	
	TPC 1 mg/mL (mg GA/g of DE)	**TFC 1 mg/mL (mg QE/g of DE)**	**DPPH (IC_{50} = mg/mL)**	**ABTS 1 mg/mL (mg ascorbic acid/g of DE)**
ME	111.28 ± 0.005^{c}	31.31 ± 0.010^{b}	0.12 ± 0.011^{b}	1.63 ± 0.009^{b}
EE	141.30 ± 0.013^{a}	67.69 ± 0.018^{a}	0.11 ± 0.002^{b}	1.56 ± 0.004^{b}
AE	125.30 ± 0.010^{b}	21.38 ± 0.004^{c}	0.12 ± 0.000^{b}	2.17 ± 0.005^{a}
EO	/	/	1.88 ± 0.009^{c}	/
Standards	/	/	BHA 0.13 ±0.012^{b} ascorbic acid 0.03 ± 0.008^{a}	QE 2.75 ± 0.004^{a}

Indicated letters mean significant difference ($p < 0.05$)

B. cereus and *S. aureus* (MBCs = 1.00-11.00 mg/mL) were the most sensitive bacteria, while *E. coli* and *M. flavus* (MBCs = 5.00->14.00 mg/mL) proved to be the most resistant strains.

Antifungal activity

The results obtained for the antifungal activity of investigated samples are presented in Tab. 4. The EO of this species had the strongest activity in inhibition of micromycetes growth (MFCs = 0.55-2.20 mg/mL) and it was similar to the activity of applied fluconazole (MFCs = 0.03-1.50 mg/mL). Among the investigated extracts, the EE showed the highest activity (MFCs = 5.00 mg/mL) on all used fungal strains, exept for *A. niger* (MFCs =18.00 mg/mL) (Tab. 4). The most resistant micromycetes were *A. niger* and *A. fumigatus,* while the most sensitive strains were *A. versicolor*, *P. ochrochloron* and *P. funiculosum*. Fungi *T. viride* and *P. ochrocloron* were more sensitive to *L. ochridanum* oil (MFC = 1.10 mg/mL), than to fluconazole (MFC = 1.50 mg/mL) (Tab. 4).

Tab. 3: Antibacterial activity of *L. ochridanum* extracts in mg/mL (means ± SD).

***L. ochridanum*/ Bacteria**	**ME**		**EE**		**AE**		**Streptomycin**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
B. cereus	0.40 ± 0.06^{a}	1.00 ± 0.03^{a}	0.50 ± 0.02^{a}	1.00 ± 0.00^{a}	6.00 ± 0.02^{a}	11.00 ± 0.01^{a}	1.50 ± 0.01^{a}	2.50 ± 0.06^{a}
M. flavus	4.00 ± 0.02^{c}	5.00 ± 0.02^{b}	2.00 ± 0.01^{ab}	5.00 ± 0.00^{b}	10.00 ± 0.01^{b}	>14.00 ± 0.03^{b}	2.50 ± 0.00^{a}	5.00 ± 0.00^{b}
L. monocytogenes	3.00 ± 0.04^{c}	4.00 ± 0.07^{b}	0.50 ± 0.00^{a}	2.00 ± 0.10^{a}	5.00 ± 0.02^{a}	11.00 ± 0.01^{a}	15.00 ± 0.10^{c}	20.00 ± 0.02^{c}
P. aeruginosa	3.00 ± 0.00^{c}	4.00 ± 0.05^{b}	0.50 ± 0.02^{a}	2.00 ± 0.01^{a}	5.00 ± 0.00^{a}	11.00 ± 0.02^{a}	2.50 ± 0.07^{a}	5.00 ± 0.01^{b}
E. coli	4.00 ± 0.01^{c}	8.00 ± 0.03^{c}	4.00 ± 0.02^{b}	5.00 ± 0.01^{b}	10.00 ± 0.00^{b}	>14.00 ± 0.01^{b}	2.50 ± 0.04^{a}	5.00 ± 0.03^{b}
E. cloacae	2.00 ± 0.01^{b}	4.00 ± 0.01^{b}	1.00 ± 0.10^{a}	4.00 ± 0.02^{b}	5.00 ± 0.10^{a}	11.00 ± 0.01^{a}	10.00 ± 0.02^{b}	20.00 ± 0.00^{c}
S. typhymurium	2.00 ± 0.00^{b}	3.00 ± 0.05^{b}	1.00 ± 0.06^{a}	2.00 ± 0·05^{a}	10.00 ± 0.01^{b}	>14.00 ± 0.02^{b}	2.50 ± 0.01^{a}	5.00 ± 0.01^{b}
S. aureus	0.40 ± 0.10^{a}	1.00 ± 0.07^{b}	1.00 ± 0.05^{a}	2.00 ± 0.03^{a}	5.00 ± 0.07^{a}	11.00 ± 0.00^{a}	2.50 ± 0.05^{a}	5.00 ± 0.00^{b}

Indicated letters mean significant difference ($p < 0.05$)

Tab. 4: Antifungal activity of *L. ochridanum* extracts and EO in mg/mL (means ± SD).

***L. ochridanum*/ Fungi**	**ME**		**EE**		**AE**		**EO**		**Fluconazole**	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
C. albicans	8.00 ± 0.02^{c}	12.00 ± 0.01^{b}	6.00 ± 0.10^{c}	12.00 ± 0.05^{b}	12.00 ± 0.01^{b}	14.00 ± 0.03^{a}	0.55 ± 0.00^{b}	1.10 ± 0.02^{b}	0.02 ± 0.05^{a}	0.03 ± 0.03^{a}
T. viride	3.00 ± 0.00^{a}	8.00 ± 0.02^{a}	5.00 ± 0.05^{b}	14.00 ± 0.07^{b}	10.00 ± 0.01^{a}	12.00 ± 0.02^{a}	0.55 ± 0.07^{b}	1.10± 0.03^{b}	1.00 ± 0.02^{d}	1.50± 0.02^{d}
P. ochrochloron	6.00 ± 0.03^{b}	10.00 ±0.10^{a}	4.00 ± 0.02^{a}	5.00 ± 0.05^{a}	10.00 ± 0.00^{a}	14.00 ± 0.00^{a}	0.28 ± 0.05^{a}	1.10 ± 0.01^{b}	1.00 ± 0.00^{d}	1.50 ± 0.02^{d}
P. funiculosum	3.00 ± 0.01^{a}	8.00 ± 0.05^{a}	4.00 ± 0.00^{a}	5.00 ± 0.00^{a}	12.00 ± 0.07^{b}	14.00 ± 0.02^{a}	0.55 ± 0.05^{b}	2.20 ± 0.01^{c}	0.25 ± 0.01^{b}	0.50 ± 0.00^{b}
A. fumigatus	6.00 ± 0.00^{b}	14.00 ± 0.01^{b}	6.00 ± 0.10^{c}	14.00 ± 0.20^{b}	12.00 ± 0.10^{b}	14.00 ± 0.02^{a}	0.55 ± 0.10^{b}	2.20 ± 0.07^{c}	0.50 ± 0.02^{c}	1.00 ± 0.00^{c}
A. versicolor	6.00 ± 0.03^{b}	8.00 ± 0.20^{a}	4.00 ± 0.01^{a}	5.00 ± 0.03^{a}	10.00 ± 0.20^{a}	12.00 ± 0.07^{a}	0.28 ± 0.02^{a}	0.55± 0.00^{a}	0.13 ± 0.00^{a}	0.50 ± 0.01^{b}
A. ochraceus	6.00 ± 0.02^{b}	19.00 ± 0.03^{c}	4.00 ± 0.03^{a}	5.00 ± 0.02^{a}	10.00 ± 0.00^{a}	14.00 ± 0.05^{a}	0.55 ± 0.10^{b}	1.10 ± 0.02^{b}	0.50 ± 0.05^{c}	1.00 ± 0.03^{c}
A. niger	8·00 ± 0.01^{c}	19.00 ± 0.05^{c}	6.00 ± 0.00^{c}	18.00 ± 0.05^{c}	12.00 ± 0.01^{b}	18.00 ±0.10^{b}	1.10 ±0.07^{c}	2.20 ± 0.00^{c}	0.25 ± 0.03^{b}	1.00 ± 0.10^{c}

Indicated letters mean significant difference ($p < 0.05$)

Anti-QS activity evaluation

Biofilm formation

Tab. 5 presents the effects of *L. ochridanum* extracts on *P. aeruginosa* PAO1 biofilm formation. The samples were tested at 0.5, 0.25 and 0.125 of MIC values. *L. ochridanum* extracts showed significant difference in terms of anti-biofilm formation activity. *L. ochridanum* EE showed dose dependant inhibitory activity, reducing from 8.63% to 63.88% of biofilm formation, where the best result was obtained in the presence of 0.5 MIC of the extract. Results revealed that *L. ochridanum* EE reduced biofilm formation more effectively than both antibiotics allowing formation of PAO1 in the range from 36.12% to 91.37%. Contrary, the tested subMIC concentrations of AE did not show any suppression of *P. aeruginosa* biofilm formation (Tab. 5). In the presence of commercial antibiotics streptomycin and ampicillin, biofilm formation occurred in narrower range, with slightly stronger biofilm inhibition recorded for streptomycin.

Tab. 5: Effects of *L. ochridanum* extracts on biofilm formation of *P. aeruginosa* PAO1 (%).

***L. ochridanum*/ Standards**	**Biofilm formation***		
	0.5 MIC (% ± SE)	**0.25 MIC (%± SE)**	**0.125 MIC (% ± SE)**
EE	36.12 ± 1.73	60.82 ± 1.05	91.37 ± 0.42
AE	n.d.	n.d.	n.d.
Ampicillin	69.16 ± 0.65	56.46 ± 0.46	92.16 ± 0.37
Streptomycin	49.40 ± 0.46	70.97 ± 0.36	88.36 ± 0.42

*Biofilm formation values were calculated as: ((mean A_{620} control well-mean A_{620} treated well)/mean A_{620} control well) × 100.
– Values are expressed as means ± SE.
– n.d. not determinate

Twitching and flagella motility

In addition, the ethanolic and aqueous extracts of *L. ochridanum* reduced the twitching and flagella motility activity of *P. aeruginosa* (Tab. 6 and Fig. 1). As presented in Tab. 6, the color of the colony ranged from white, through light green to green. Used extracts changed the color and diameter of treated colonies to a certain extent. In the presence of extracts, colonies where white and larger (14.00 mm and 17.67 mm for EE and AE, respectively) in comparison to colonies treated with antibiotics (Tab. 6). The green colony of *P. aeruginosa* with streptomycin, had minimal growth (11.00 mm) and completely reduces protrusions. Also, the most reduced flagella in size, shape and number were in colony with streptomycin (Fig. 1).

Inhibition of synthesis of P. aeruginosa PAO1 pyocyanin

SubMICs of *L. ochridanum* samples were tested for inhibition of *P. aeruginosa* pigment production and both extracts showed substantial activity in pigment synthesis inhibition. The affection was observed by the reduction of the green pigmentation of the samples,

Tab. 6: Effects of *L. ochridanum* extracts on twitching and flagella motility of *P. aeruginosa* (PAO1).

***L. ochridanum* extracts/ Standards**	**Colony diameter (mm ± SE)**	**Flagella diameter (µm)**	**Colony colour**	**Colony edge**
EE	14.00 ± 2.65	40-160	White	Rare flagella
AE	17.67 ± 5.51	16-80	White	Tiny flagella
Streptomycin	11.00 ± 1.00	24-56	Green	Tiny flagella
Ampicillin	13.33 ± 5.03	16-56	Green	Regular flagella
***P. aeruginosa* (PAO1)**	12.00 ± 1.00	56-80	Light green	Regular flagella

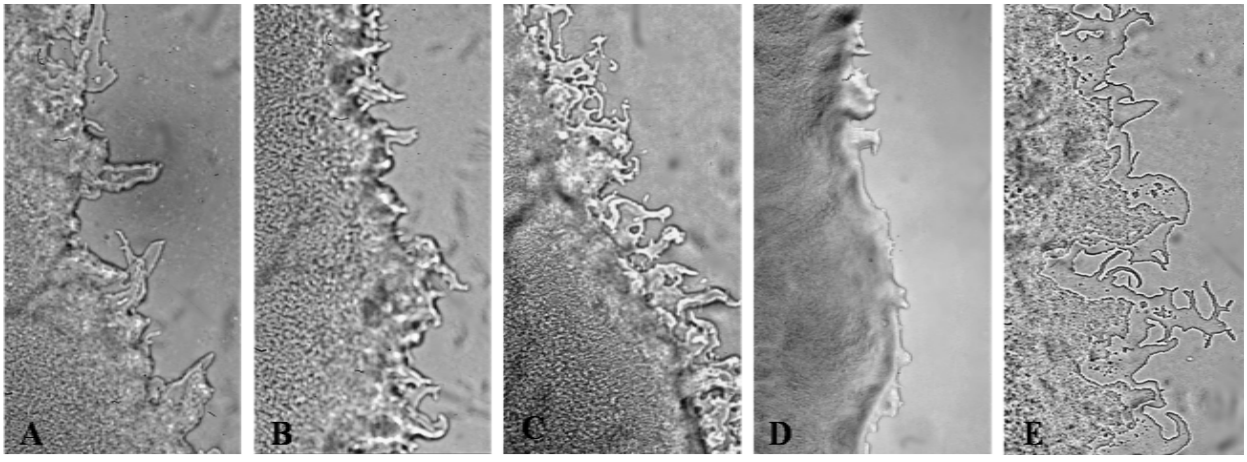

Fig. 1: Light microscopy of colony edges of *P. aeruginosa* in twitching motility, grown in the presence or absence of *L. ochridanum* extracts and commercial antibiotics. The colonies from the bacteria grown with extracts in concentration of 0.5 MIC (**A-B**). The colony with EE was with moderately reduced protrusions (**A**); In the presence of AE colony formed almost regular protrusions (**B**); *P. aeruginosa* colony in the presence of streptomycin (0.5 MIC) with reduced protrusion (**C**); *P. aeruginosa* colony in the presence of ampicillin with regularly formed protrusions (**D**); *P. aeruginosa* produced a flat, widely spread, irregularly shaped colony in the absence of extracts and commercial antibiotics (**E**); Magnification: (**A-D**) × 100.

compared to the coloration of the control PAO1 sample (Fig. 2). The strongest inhibition of pigment's production was detected for *L. ochridanum* AE. In the presence of tested concentrations of extracts, pyocyanin was less produced (23.46% and 18.07% for ethanolic and aqueous extracts, respectively), than by control strain (141.55%). All extracts were better in prevention of pigment production regarding to applied antibiotics (Fig. 2).

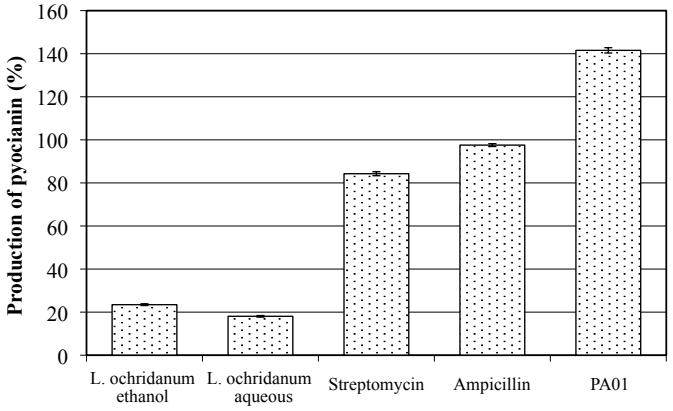

Fig. 2: Reduction of pyocyanin production of *P. aeruginosa* PAO1 by *L. ochridanum* extracts streptomycin and ampicillin tested at subMICs (mg/mL).

Discussion

The chemical compositions of EOs of different *Laserpitium* species have been previously reported (Baser and Duman, 1997; Chizzola et al., 1999; Chizzola, 2007; Petrović et al., 2009; Tirillini et al., 2009; Popović et al., 2010, 2014). Results obtained for *L. ochridanum* EO revealed sabinene, viridiflorol and α-pinene as the most dominant compounds (Tab. 1). Recent studies on *L. ochridanum* herb oil showed sabinene and α-pinene as the major components, which is in accordance with our results. In contrary, limonene, which was the main constituent of fruit oil, was not detected in our sample (Popović et al., 2014, 2015). Our results revealed that EE of *L. ochridanum* was the richest in phenols and flavonoids among all extracts. Also, EE had the highest DPPH radical scavenging activity, while AE was the strongest agent in ABTS radical scavenging assay. In general, all extracts possessed comparable activity to synthetic antioxidants. In contrary, EO showed low antioxidant capacity.

Results obtained in a microdilution assay, indicated that gram-positive and gram-negative bacteria showed similar sensitivity to *L. ochridanum* extracts. According to the literature data, *L. ochridanum* chloroform extract of roots and rhizomes exhibited antimicrobial activity against some food spoilage microorganisms (Popović et al., 2015). In comparison with those results, it can be concluded that methanolic and ethanolic extracts of *L. ochridanum* in our study showed higher antimicrobial potency. We found that extracts indicated stronger antibacterial than antifungal potential (Tab. 3 and 4). EO of *L. ochridanum* showed similar inhibitory effect as fluconazole (Tab. 4). Also, *L. ochridanum* EO was more effective than *L. garganicum* EO in growth inhibition of the most resistant micromycete *A. niger* (Tirillini et al., 2009).

Inhibition of bacterial QS offers new strategies for the treatment of bacterial infections. Anti-QS agents can interfere with the bacterial communication system and can disrupt the pathogenicity process of bacteria (Sepahi et al., 2015). Therefore, *L. ochridanum* extracts were submitted for anti-QS screening for the first time since it is of great importance to discover new potential anti-QS agents. The screening results indicated that EE and AE samples showed some potential of anti-QS activity. *L. ochridanum* EE demonstrated an inhibition at the initial stage of biofilm formation in the manner of different tested concentrations. That is of great importance since bacteria form biofilms as a protection against host's immune system and as a factor of antibiotic resistance (Soković et al., 2014). It was clearly indicated that tested concentrations of *L. ochridanum* extracts were more effective on *P. aeruginosa* pigment production than those of applied antibiotics. This green, toxic pigment acts as a virulence factor in bacteria, so reduction of its production is crucial for increasing the effectiveness of host defense. While AE notably demonstrated the best activity in suppression of pyocyanin synthesis, it did not show any reduction of colony formation at the tested subMICs. Opposite results obtained for AE in these two assays could be associated with possible different mechanisms responsible for its activity. At this point, there is no sufficient data to highlight the exact method of QS inhibition. A few potential modes of action have been proposed, many of them to interfere with the QS system such as inhibition of biosynthesis of auto-inducer molecules, inactivation or degradation of the auto-inducer, interference with the signal receptor and inhibition of the genetic regulation system. Due to complex phytochemistry of plant extracts, different compounds could be associated with specific effects linked to the QS system, so the difference in the activities of *L. ochridanum* aqueous sample suggest less polar nature of *L. ochridanum* active compounds in reduction of *P. aeruginosa* biofilm formation. The importance of plant extracts preparation should be also considered (Adonizio et al., 2006; Adonizio, 2008: Glamočlija et al., 2015). In the research of Adonizio et al. (2008), where aqueous extract of *Schefflera actinophylla*, with no anti-QS activity, was used as a negative control, it was concluded that plant extracts differentially affect biofilm formation. Inhibition of swarming and twitching motility of PAO1 by *L. ochridanum* samples was achieved in moderate extent with better results obtained for EE. Both types of motilities are important in the initial stages of biofilm formation of *P. aeruginosa* (O'Toole and Kolter, 1998b).

Conclusions

This study established the chemical characterization of *L. ochridanum* EO and provided new data concerning antioxidant and anti-QS activities of crude extracts/EO. The presence of phenolic compounds and previously reported sesquiterpene lactones mostly contribute to biological potency of *L. ochridanum* extracts. Among all extracts, EE possessed the best potency in evaluated antioxidant and antibacterial activities. The EO revealed strong antifungal potential. *L. ochridanum* showed promising anti-QS effectiveness that was sufficient for the reduction of biofilm formation and pyocyanin production. To establish the application of this species in various pharmaceutical, dietary or alternative medicine branches, further research is needed especially concerning that the exact mechanistic interactions with the QS system should be resolved.

Acknowledgements

The authors are grateful to the Ministry of Education, Science and Technological Development of the Republic of Serbia for financial support Grants No. 173029 and 173032.

References

Adams, R.P., 2007: Identification of essential oil components by gas chromatography/mass spectrometry, 4th ed. Carol Stream, IL, USA: Allured Publishing Corp.

Adonizio, A.L., Downum, K., Bennett, B.C., Mathe, K., 2006: Anti-

quorum sensing activity of medicinal plants in Southern Florida. J. Ethnopharmacol. 105, 427-435. DOI: 10.1016/j.jep.2005.11.025

Adonizio, A.L., 2008: Anti-quorum sensing agents from South Florida medicinal plants and their attenuation of *Pseudomonas aeruginosa* pathogenicity. FIU Electronic Theses and Dissertations 13, 14-21.

Al-Hussaini, R., Mahasneh, A.M., 2009: Microbial growth and quorum sensing antagonist activities of herbal plants extracts. Molecules 14, 3425-3435. DOI: 10.3390/molecules14093425

Appendino, G., Cravotto, G., Nano, G.M., 1993: Sesquiterpene lactones from *Laserpitium gallicum*. Phytochemistry 33, 883-886.

Appendino, G., Valle, G.M., Gariboldi, P., 1987: Sesquiterpene lactols from *Laserpitium halleri*. Phytochemistry 26, 1755-1757.

Baser, K.H.C., Duman, H., 2011: Composition of the essential oil of *Laserpitium petrophilum* Boiss. et Heldr. J. Essent. Oil Res. 9, 707-708. DOI: 10.1080/10412905.1997.9700818

Blois, M.S., 1958: Antioxidant determinations by the use of a stable free radical. Nature 181, 1199-1200. DOI: 10.1038/1811199a0

Booth, C., 1971: Fungal culture media. In: Norris, J.R., Ribbons, D.W. (eds), Methods in microbiology, 4th ed. London, UK: Academic Press.

Chen, Y.L., Lee, C.Y., Huang, K.H., Kuan, Y.H., Chen, M., 2015: Prescription patterns of Chinese herbal products for patients with sleep disorder and major depressive disorder in Taiwan. J. Ethnopharmacol. 171, 307-316. DOI: 10.1016/j.jep.2015.05.045

Chizzola, R., 2007: Composition of the essential oil from *Laserpitium gallicum* grown in the wild in Southern France. Pharm. Biol. 45, 182-184. DOI: 10.1080/13880200701213054

Chizzola, R., Johannes, N., Chlodwig, F., 1999: Fruit oil of *Laserpitium siler* L. grown in France. J. Essent. Oil Res. 11, 197-198. DOI: 10.1080/10412905.1999.9701109

Clinical and laboratory standards institute methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically, 2009: Aproved standards. 8th ed. Clinically and laboratory standards Institute, M07-A8, Wayne: CLSI publication.

Drenkard, E., Ausubel, F.M., 2002: *Pseudomonas* biofilm formation and antibiotic resistance are linked to phenotypic variation. Nature 416, 740-743. DOI :10.1038/416740a

Đermanović, M., Stefanović, M., *Đermanović*, V., Milovanović, M., 1996: Structure elucidation of the sesquiterpene lactones from plant species *Laserpitium latifolium* L. J. Herbs Spices Med. Plants. 3, 3-10. DOI: 10.1300/J044v03n02_02

Glamočlija, J., Ćirić, A., Nikolić, M., Fernandes, Â., Barros, L., Calhelh, R.C., Ferreira, I.C., Soković, M., van Griensven, L.J., 2015: Chemical characterization and biological activity of Chaga (*Inonotus obliquus*), a medicinal "mushroom". J. Ethnopharmacol. 162, 323-332. DOI: 10.1016/j.jep.2014.12.069

Hanel, H., Raether, W., 1998: A more sophisticated method of determining the fungicidal effect of water-insoluble preparations with a cell harvester, using miconazole as an example. Mycoses 31, 148-154. DOI: 10.1111/j.1439-0507.1988.tb03718.x

Koh, C.L., Sam, C.K., Yin, W.F., Tan, L.Y., Krishnan, T., Chong, Y.M., Chan, K.G., 2013: Plant-derived natural products as sources of anti-quorum sensing compounds. Sensors 13, 6217-6228. DOI: 10.3390/s130506217

Micevski, K., 2005: *Laserpitium* L. In: Matevski, V. (ed.), Flora of Republic of Macedonia, 6th ed. Skopje, Macedonian Academy of Sciences and Arts, 1647-1651.

Miller, N., Rice-Evans, C., 1997: Factors influencing the antioxidant activity determined by the ABTS radical action assay. Free Radic. Res. 26, 195-199.

Nikolić, V., 1973: *Laserpitium* L. In: Josifivić, M. (ed.), Flora of Republic of Serbia, 5th ed. Belgrade, Serbian Academy of Sciences and Arts, 308-313.

O'Toole, G.A., Kolter, R., 1998a: Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds *via* multiple convergent signalling pathways: a genetic analysis. Mol. Microbiol. 28, 449-461. DOI: 10.1046/j.1365-2958.1998.00797.x

O'Toole, G.A., Kolter, R., 1998b: Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. Mol. Microbiol. 30, 295-304. DOI: 10.1046/j.1365-2958.1998.01062.x

Park, Y.K., Koo, M.H., Ikegaki, M., Contado, J.L., 1997: Comparison of the flavonoid aglycone contents of *Apis mellifera* propolis from various regions of Brazil. Arq. Biol. Tecnol. 40, 97-106.

Petrović, S., Pavlović, M., Popović, V., Couladis, M., Tzakou, O., Milenković, M., Vučićević, D., Niketić, M., 2009: Composition of antimicrobial activity of essential oils from flower and leaf of *Laserpitium zernyi* Hayek. J. Essent. Oil Res. 21, 467-470. DOI: 10.1080/10412905.2009.9700220

Popović, V., Heyerick, A., Petrović, S., Calenbergh, S.V., Karalic, I., Niketić, M., Deforce, D., 2013: Sesquiterpene lactones from the extracts of two Balkan endemic *Laserpitium* species and their cytotoxic activity. Phytochemistry 87, 102-111. DOI: 10.1016/j.phytochem.2012.11.011

Popović, V., Heyerick, A., Petrović, S., Calenbergh, S.V., Karalić, I., Niketić, M., Deforce, D., 2013: Cytotoxic activity of *Laserpitium latifolium* L. extract and its daucane and phenylpropanoid constituents. Rec. Nat. Prod. 7, 245-249.

Popović, V., Petrović, S., Pavlović, M., Milenković, M., Couladis, M., Tzakou, O., Duraki, S., Niketić, M., 2010: Essential oil from the underground parts of *Laserpitium zernyi*: potential source of alpha-bisabolol and its antimicrobial activity. Nat. Prod. Commun. 5, 307-310.

Popović, V., Petrović, S., Tomić, M., Stepanović-Petrović, R., Micov, A., Pavlović-Drobac, M., Couladis, M., Niketić, M., 2014: Antinociceptive and anti-edematous activities of the essential oils of two Balkan endemic *Laserpitium* species. Nat. Prod. Commun. 9, 125-128.

Popović, V., Petrović, D.S., Milenković, T.M., Drobac., M., Couladis, A.M., Niketić, S.M., 2015: Composition and antimicrobial activity of the essential oils of *Laserpitium latifolium* L. and *L. ochridanum* Micevski (Apiaceae). Chem. Biodivers. 12, 170-177. DOI: 10.1002/cbdv.201400127

Popović, V., Stojković, D., Nikolić, M., Heyerick, A., Petrović, S., Soković, M., Niketić, M., 2015: Extracts of three *Laserpitium* L. species and their principal components laserpitine and sesquiterpene lactones inhibit microbial growth and biofilm formation by oral *Candida* isolates. Food Funct. 6, 1205-1211. DOI: 10.1039/c5fo00066a

Sandy, S.M., Foong-Yee, T., 2012: Anti-quorum sensing and antimicrobial activities of some traditional chinese medicinal plants commonly used in South-East Asia. Malays. J. Microbiol. 8, 11-20. DOI: 10.21161/mjm.34911

Sepahi, E., Tarighi, S., Ahmadi, F.S., Bagheri, A., 2015: Inhibition of quorum sensing in *Pseudomonas aeruginosa* by two herbal essential oils from Apiaceae family. J. Microbiol. 53, 176-180. DOI: 10.1007/s12275-015-4203-8

Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M., 1999: Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Method Enzymol. 299, 152-178. DOI: 10.1016/S0076-6879(99)99017-1

Soković, M., Ćirić, A., Glamočlija, J., Nikolić, M., van Griensven, J.L.D.L., 2014: *Agaricus blazei* hot water extract shows anti-quorum sensing activity in the nosocomial human pathogen *Pseudomonas aeruginosa*. Molecules 19, 4189-4199. DOI: 10.3390/molecules19044189

Spoering, A.L., Lewis, K., 2001: Biofilms and planktonic cells of *Pseudomonas aeruginosa* have similar resistance to killing of antimicrobials. J. Bacteriol. 183, 6746-6751. DOI: 10.1128/JB.183.23.6746-6751.2001

Tirillini, B., Pagiotti, R., Angelini, P., Pintore, G., Chessa, M., Menghini, L., 2009: Chemical composition and fungicidal activity of the essential oil of *Laserpitium garganicum* from Italy. Chem. Nat. Compd. 45, 103-10. DOI: 10.1007/s10600-009-9237-x

Tsukatani, T., Suenaga, H., Shiga, M., Noguchi, K., Ishiyama, M., Ezoe, T., Matsumoto, K., 2012: Comparison of the WST-8 colorimetric me-

thod and the CLSI broth microdilution method for susceptibility testing against drug-resistant bacteria. J. Microbiol. Meth. 90, 160-166. DOI: 10.1016/j.mimet.2012.05.001

TUTIN, T.G., 1968: *Laserpitium* L. In: Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (eds.), Flora Europaea, 2nd ed, 368-370. Cambridge, Cambridge University Press.

Address of corresponding author:
Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia
E-mail: ksenija.mileski@bio.bg.ac.rs