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Chemical composition and antimicrobial activity of the essential oils of *Geranium columbinum* L. and *G. lucidum* L. *(Geraniaceae)*

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The chemistry of *Geranium columbinum* and *G. lucidum* volatiles has been studied here for the first time. The essential oils were obtained by hydrodistillation, analyzed by GC and GC-MS, and screened for their in vitro antibacterial and antifungal activity in a microdilution assay. In total, 172 constituents were identified, accounting for 88.1%-96.7% of the detected GC peak areas. The essential oils consisted mainly of fatty acids and fatty acid-derived compounds (65.0%-86.3%). The major compounds of the essential oils were hexadecanoic acid, hexahydrofarnesyl acetone, and tetracosane. The antimicrobial assay showed a strong activity of the oil from aerial parts of *G. columbinum* against the pathogenic yeast *C. albicans* (MIC = 0.437, MBC = 1.75 mg/mL).

Key Words: *Geranium columbinum*, *Geranium lucidum*, Geraniaceae, essential oil composition, hexadecanoic acid, hexahydrofarnesyl acetone, tetracosane, antimicrobial activity

Introduction

The genus *Geranium* L. (Geraniaceae) comprises about 250 species, distributed mainly in the temperate region of the northern hemisphere. According to Janković, only 19 *Geranium* species can be found in the Serbian

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flora.[1] *Geranium columbinum* L. (syn. *G. roseo-caeruleis* Gilib.), commonly known as long-stalk crane's-bill ("golubija noga" or "krvavac," in Serbian) is a widely distributed, herbaceous annual-to-biennial plant. It occupies a variety of habitats in foothill and mountain regions, up to 1500 m in altitude. *Geranium lucidum* L., also known as shining crane's-bill ("ilja crvena," in Serbian) is a herbaceous annual plant with a similarly wide distribution. It is usually found in shady, moist but warm habitats, mostly in mountainous regions. In general, the genus *Geranium* has been poorly investigated in respect of its volatile compounds, except for the renowned *Geranium macrorrhizum.*[2] A literature survey revealed few reports regarding phytochemical work done on the taxa investigated in this study, which mainly concerned the study of polyphenols, or to be exact, the flavonoids present in the plant tissues.[3–5] To date, no previous reports dealing with any investigation of the volatiles of these 2 species can been found in the literature.

The aim of this work was to perform a detailed compositional analysis of the volatiles isolated from the mentioned 2 taxa originating from Serbia. We also tested the antimicrobial activity of the essential oils against a panel of microorganisms, as well as against 3 fungal strains that are very common molds in human habitats and responsible for human respiratory allergic diseases.[6–10]

Experimental

Plant material

Plant material was collected from natural populations in June 2006, on the northern slopes of the Suva Planina mountain range, Serbia. *Geranium columbinum* samples (aerial parts consisting of flowers, leaves, and stems, with underground parts separated) were gathered from a locality called Plo$\bar{c}$e, along the road in rocky places. Samples of *G. lucidum* (entire plants that consisted of flowers, leaves, stems, and roots) were collected from rocky places, alongside a railway line, close to the village Dolac, Bela Palanka. The analyzed plant material was dried at room temperature for 2 weeks to a constant weight. Voucher specimens were deposited at the herbarium collection of the Faculty of Science and Mathematics, University of Niš, under the acquisition numbers MD0206 (*G. columbinum*) and MD0306 (*G. lucidum*). The botanical identification was performed by one of the authors (N. Radulović).

Essential oil isolation

Air-dried to a constant weight, plant material (2 × 3 batches of about 500 g for each sample) was subjected to hydrodistillation with approximately 2 L of distilled water for 2.5 h using the original Clevenger-type apparatus.[11] The obtained oils were separated by extraction with freshly distilled diethyl ether (Merck, Germany), dried over anhydrous magnesium sulfate (Aldrich, USA), and immediately analyzed.

Gas chromatography-mass spectrometry

The GC-MS analyses of the oils were carried out, with 3 repetitions for each sample, on a Hewlett-Packard 6890N gas chromatograph equipped with a fused silica capillary column HP-5MS (5% phenylmethylsiloxane, 30 m × 0.25 mm, film thickness 0.25 μm; Agilent Technologies, USA) and coupled with a 5975B mass selective

detector from the same company. The injector and interface were operated at 250 and 280 °C, respectively. The oven temperature was raised from 70 to 225 °C at a heating rate of 5 °C/min and then isothermally held for 10 min. As a carrier gas, helium was used at 1.0 mL/min. The sample, 1 μL of the oil solutions in diethyl ether (1:100), was injected (repeated 3 times) in a pulsed split mode; the flow was 1.5 mL/min for the first 0.5 min and was then set to 1.0 mL/min throughout the remainder of the analysis with a split ratio of 40:1. A mass selective detector was operated in electron impact mode at the ionization energy of 70 eV in the 35-500 amu range, at a scanning speed of 0.34 s.

Gas chromatography

GC (FID) analyses were carried out under the same experimental conditions using the same column and same gas chromatograph type as described for the GC-MS. The percentage composition was computed from the GC peak areas without the use of correction factors.

Identification procedure

Qualitative analysis of the essential oil constituents was based on the comparison of their linear retention indices, relative to retention times of C_7-C_{26} n-alkanes on the HP-5MS column,[12] with those reported in the literature.[13] Their mass spectra were also compared with those of authentic standards, as well as those from Wiley 6, NIST02, MassFinder 2.3, and a homemade MS library with the spectra corresponding to pure substances and components of known essential oils. They were further compared, wherever possible, by coinjection with an authentic sample (the n-alkanes, some of the terpenoids, and aromatics), as indicated in Table 1 (column: method of identification).

Antimicrobial screening

The in vitro antimicrobial activity of the essential oils was tested against a panel of microorganisms, including gram-positive *Staphylococcus aureus* ATCC 25923, *S. aureus* (clinical isolate), *Clostridium perfringens* ATCC 19574, *C. sporogenes* ATCC 19404, *Bacillus subtilis* ATCC 6633, *Sarcina lutea* ATCC 9341, and *Micrococcus flavus* ATCC 10240; gram-negative *Escherichia coli* ATCC 25922, *E. coli* (Torlak 95), *E. coli* ATCC 8739, *E. coli* (clinical isolate), *Klebsiella pneumoniae* (clinical isolate), *K. pneumoniae* ATCC 10031, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427, and *Salmonella enterica* ATCC 13076; and yeasts *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 9763. The gram-negative bacterium *E. coli* 95 was obtained from the Institute of Immunology and Virology "Torlak" Belgrade, Serbia. Bacterial isolates were clinical isolates from the Institute of Public Health, Kragujevac, and are stored in the microbiological collection of the Microbiology Laboratory, Department of Biology, Faculty of Science and Mathematics, University of Niš. Fungal strains *Aspergillus restrictus*, *A. fumigatus*, and *Penicillium chrysogenum* were isolated from mattress dust and identified by Dr. B. Ranković, Department of Biology, Faculty of Science, University of Kragujevac, Serbia. Cultures of isolated molds were maintained on potato dextrose agar (PDA).

Table 1. Chemical composition of *Geranium columbinum* and *Geranium lucidum* essential oils.

RI calc.	Component	Aerial parts of *G. columbinum* (sample A)	Roots of *G. columbinum* (sample B)	*G. lucidum* (entire plants, sample C)	Method of identification
		(%)[a]			
802	Hexanal	0.1	tr	tr	RI, MS, Co
851	(*Z*)-3-Hexen-1-ol	tr	-	tr	RI, MS
852	3-Methyl-2-hexanone	tr	-	tr	RI, MS
860	1-Hexanol	tr	-	tr	RI, MS, Co
887	2-Butylfuran	tr	-	tr	RI, MS
900	Nonane	tr	-	-	RI, MS, Co
901	Pentanoic acid	tr	-	-	RI, MS, Co
902	Heptanal	0.2	-	-	RI, MS, Co
929	$\alpha-$Thujene	-	-	tr	RI, MS
937	$\alpha-$Pinene	-	-	0.9	RI, MS, Co
952	6-Methyl-2-heptanone	tr	-	-	RI, MS
963	Benzaldehyde	tr	-	tr	RI, MS, Co
965	1-Heptanol	tr	-	-	RI, MS, Co
968	Hexanoic acid	tr	-	0.6	RI, MS, Co
976	Phenol	-	tr	-	RI, MS, Co
977	Sabinene	-	-	0.3	RI, MS, Co
981	2,5-Octadione	tr	-	-	RI, MS
983	$\beta-$Pinene	-	-	tr	RI, MS, Co
991	$\beta-$Myrcene	-	-	0.3	RI, MS, Co
993	2-Pentylfuran	tr	-	-	RI, MS
994	(*E*,*Z*)-2,4-Heptadienal	-	-	tr	RI, MS
1000	Decane	tr	-	-	RI, MS, Co
1004	Octanal	0.2	-	-	RI, MS, Co
1009	$\alpha-$Phellandrene	-	-	tr	RI, MS, Co
1020	$\alpha-$Terpinene	-	-	tr	RI, MS, Co
1020	3-Ethyl-4-methyl-1-pentanol	0.4	-	-	RI, MS
1027	*p*-Cymene	tr	-	0.2	RI, MS, Co
1032	Limonene	-	-	0.2	RI, MS, Co
1033	$\beta-$Phellandrene	-	-	tr	RI, MS
1035	1,8-Cineole	-	-	1.4	RI, MS, Co
1035	Benzyl alcohol	0.3	tr	-	RI, MS, Co
1043	Lavender lactone	tr	-	0.3	RI, MS
1046	Phenylacetaldehyde	0.4	-	tr	RI, MS, Co
1050	4-Oxopentanoic acid	tr	-	0.5	RI, MS

Table 1. Continued.

RI calc.	Component	Aerial parts of *G. columbinum* (sample A)	Roots of *G. columbinum* (sample B)	*G. lucidum* (entire plants, sample C)	Method of identification
		(%)[a]			
1061	$\gamma-$Terpinene	-	-	0.3	RI, MS, Co
1068	Heptanoic acid	-	-	tr	RI, MS, Co
1069	1-Octanol	0.4	-	tr	RI, MS, Co
1069	Acetophenone	tr	-	0.3	RI, MS, Co
1075	*cis*-Linalool oxide (furanoid)	-	-	0.3	RI, MS, Co
1094	*p*-Cymenene	-	-	tr	RI, MS
1100	Undecane	tr	-	-	RI, MS, Co
1103	*trans*-Sabinene hydrate	-	-	tr	RI, MS
1105	Nonanal	0.3	tr	-	RI, MS, Co
1106	(*E*)-Hotrienol	-	-	tr	RI, MS
1111	$\alpha-$Thujone	-	-	0.2	RI, MS, Co
1117	2-Phenyl-1-ethanol	tr	-	-	RI, MS, Co
1121	$\beta-$Thujone	-	-	tr	RI, MS, Co
1126	*cis*-*p*-Menth-2-en-1-ol	-	-	tr	RI, MS
1128	Octyl formate	tr	-	-	RI, MS, Co
1130	Chrysanthenone	-	-	tr	RI, MS
1144	*trans*-Pinocarveol	-	-	0.2	RI, MS, Co
1150	Camphor	tr	tr	0.5	RI, MS, Co
1154	(*E*,*Z*)-2,6-Nonadienal	-	-	tr	RI, MS
1166	Pinocamphone	-	-	0.8	RI, MS, Co
1165	Benzoic acid	2.1	tr	0.9	RI, MS, Co
1171	Octanoic acid	tr	tr	tr	RI, MS, Co
1172	Borneol	0.8	-	0.4	RI, MS, Co
1173	*cis*-Linalool oxide (pyranoid)	-	-	tr	RI, MS
1177	*trans*-Linalool oxide (pyranoid)	tr	-	0.3	RI, MS
1180	Isopinocamphone	-	-	0.6	RI, MS, Co
1182	Terpinen-4-ol	-	-	0.5	RI, MS, Co
1188	*p*-Methylacetophenone	tr	-	-	RI, MS
1189	*p*-Cymen-8-ol	tr	-	0.1	RI, MS, Co
1191	(3*E*)-2,6-Dimethyl-3,7-octadiene-2,6-diol	tr	-	tr	RI, MS
1196	$\alpha-$Terpineol	-	-	tr	RI, MS, Co
1200	Dodecane	tr	-	tr	RI, MS, Co
1203	Myrtenol	-	-	tr	RI, MS, Co

Table 1. Continued.

RI calc.	Component	Aerial parts of *G. columbinum* (sample A)	Roots of *G. columbinum* (sample B)	*G. lucidum* (entire plants, sample C)	Method of identification
		(%)[a]			
1207	Decanal	tr	-	tr	RI, MS, Co
1214	2,6-Dimethylundecane	tr	-	-	RI, MS
1250	Phenylacetic acid	0.3	-	tr	RI, MS, Co
1270	Nonanoic acid	0.6	tr	0.3	RI, MS, Co
1272	1-Decanol	0.2	-	-	RI, MS, Co
1276	2,6-Dimethyl-1,7-octadien-3,6-diol	tr	-	0.3	RI, MS
1295	2-Undecanone	tr	-	-	RI, MS
1300	Tridecane	tr	-	0.2	RI, MS, Co
1309	Undecanal	tr	-	-	RI, MS
1366	Decanoic acid	tr	tr	0.4	RI, MS, Co
1374	1-Undecanol	tr	-	-	RI, MS
1377	Farnesane[b]	tr	-	-	RI, MS
1392	$\beta-$Bourbonene	tr	-	-	RI, MS
1392	1-Tetradecene	tr	-	tr	RI, MS
1400	Tetradecane	0.5	-	tr	RI, MS, Co
1407	Tetrahydrogeranyl acetone	0.3	-	tr	RI, MS
1411	Dodecanal	tr	-	tr	RI, MS, Co
1414	2-Dodecanol	tr	-	-	RI, MS
1430	Carvone hydrate	tr	-	tr	RI, MS
1457	5-Methyltetradecane[b]	tr	-	-	RI, MS
1463	Homofarnesane[b]	1.1	-	0.1	RI, MS
1465	Undecanoic acid	tr	-	tr	RI, MS
1476	1-Dodecanol	0.3	tr	tr	RI, MS, Co
1483	$\gamma-$Muurolene	0.2	-	-	RI, MS
1491	$(E)-\beta$-Ionone-5,6-epoxide	tr	-	-	RI, MS
1492	1-Pentadecene	tr	-	-	RI, MS
1494	$\beta-$Eudesmene	tr	-	-	RI, MS
1497	2-Tridecanone	tr	-	-	RI, MS
1497	10,11-Epoxycalamenene	-	-	tr	RI, MS
1500	Pentadecane	0.6	-	tr	RI, MS, Co
1512	Tridecanal	0.4	-	tr	RI, MS
1523	3,4-Dimethyl-5-pentyl-2(5*H*)-furanone (=dihydrobovolide)[b]	tr	tr	tr	RI, MS

Table 1. Continued.

RI calc.	Component	Aerial parts of *G. columbinum* (sample A)	Roots of *G. columbinum* (sample B)	*G. lucidum* (entire plants, sample C)	Method of identification
		(%)[a]			
1538	Dihydroactinidiolide	0.2	tr	0.2	RI, MS
1550	$\alpha-$Calacorene	tr	-	-	RI, MS
1556	5,5-Dimethyl-4-(3-oxobutyl)dihydro-2(3*H*)-furanone (=homoterpenyl methyl ketone)	-	-	tr	MS
1559	Elemicin	-	-	tr	RI, MS
1563	Dodecanoic acid	tr	tr	1.8	RI, MS, Co
1571	Tetrahydrofarnesol[b]	0.3	-	-	RI, MS
1578	1-Tridecanol	0.5	tr	0.5	RI, MS
1580	6-Methyl-5-(3-methylphenyl)-2-heptanone	-	-	tr	MS
1584	*ar*-Turmerol	0.5	-	-	RI, MS
1592	Caryophyllene oxide	1.1	-	-	RI, MS, Co
1597	5-Guaien-11-ol	-	-	0.9	RI, MS
1599	2-Tetradecanone	tr	-	-	RI, MS
1601	Globulol	0.4	-	-	RI, MS
1602	4(14)-Salvialen-1-one	tr	-	-	RI, MS
1606	Longiborneol	0.5	-	-	RI, MS
1607	3,4,5-Trimethoxybenzaldehyde	-	-	tr	RI, MS, Co
1614	Tetradecanal	1.7	tr	0.2	RI, MS
1642	Hexahydrofarnesol[b]	0.3	-	1.2	RI, MS
1644	Hexahydrofarnesol (isomer II)[b]	0.2	-	-	RI, MS
1659	$\beta-$Eudesmol	-	-	0.3	RI, MS
1662	Tridecanoic acid	tr	tr	0.3	RI, MS
1678	1-Tetradecanol	3.0	tr	1.3	RI, MS, Co
1683	Cadalene	tr	-	0.6	RI, MS
1700	2-Pentadecanone	-	-	tr	RI, MS
1700	Heptadecane	0.5	tr	-	RI, MS, Co
1711	10-*nor*-Calemenen-10-one	tr	-	0.3	RI, MS
1718	Pentadecanal	2.4	tr	0.4	RI, MS
1766	Tetradecanoic acid	1.0	3.2	5.4	RI, MS, Co
1771	Benzyl benzoate	0.3	tr	0.8	RI, MS, Co
1779	1-Pentadecanol	0.4	tr	-	RI, MS, Co

Table 1. Continued.

RI calc.	Component	Aerial parts of *G. columbinum* (sample A)	Roots of *G. columbinum* (sample B)	*G. lucidum* (entire plants, sample C)	Method of identification
		(%)[a]			
1784	Phenanthrene	-	-	0.7	RI, MS, Co
1785	(*E*)-3-Octadecene	tr	-	-	RI, MS
1800	Octadecane	tr	tr	-	RI, MS, Co
1802	2-Hexadecanone	tr	-	tr	RI, MS
1811	*trans*-6-Hydroxyisocalamenene	-	-	tr	RI, MS
1818	Hexadecanal	1.0	-	tr	RI, MS, Co
1841	Neophytadiene (isomer I)	tr	-	-	RI, MS
1848	Hexahydrofarnesyl acetone	11.6	10.4	5.3	RI, MS, Co
1865	Pentadecanoic acid	2.3	2.5	3.3	RI, MS, Co
1877	Benzyl salicylate	tr	tr	0.8	RI, MS, Co
1883	1-Hexadecanol	0.6	tr	-	RI, MS, Co
1884	Clovane-2,9-diol	-	-	0.2	MS
1895	$\gamma-$Tetradecalactone	tr	tr	tr	RI, MS
1900	Nonadecane	3.2	tr	tr	RI, MS, Co
1905	2-Heptadecanone	tr	-	-	RI, MS
1907	5-(4,8-Dimethylnonyl)-5-methyldihydro-2(3*H*)-furanone[b]	tr	-	tr	MS
1929	2-Methylanthracene	-	-	tr	RI, MS
1951	Isophytol	1.2	tr	0.5	RI, MS
1969	Hexadecanoic acid	17.6	60.8	32.3	RI, MS, Co
1971	3-(4,8,12-Trimethyltridecyl)furan (=phytofuran)[b]	tr	-	-	RI, MS
2000	Eicosane	1.1	tr	tr	RI, MS, Co
2036	Methyl 2-oxohexadecanoate	0.4	-	-	RI, MS
2063	Heptadecanoic acid	tr	tr	0.5	RI, MS
2080	1-Heneicosene	tr	-	-	RI, MS
2086	1-Octadecanol	0.5	tr	1.4	RI, MS, Co
2100	Heneicosane	4.4	tr	0.4	RI, MS, Co
2107	$\gamma-$Hexadecalactone	0.3	tr	0.2	RI, MS
2117	(*E*)-Phytol	tr	-	-	RI, MS, Co
2125	Nonadecanal	tr	-	-	RI, MS
2140	(Z)-9-Octadecenoic acid (=oleic acid)	0.6	tr	0.9	RI, MS, Co

Table 1. Continued.

RI calc.	Component	Aerial parts of *G. columbinum* (sample A)	Roots of *G. columbinum* (sample B)	*G. lucidum* (entire plants, sample C)	Method of identification
		(%)[a]			
2164	Octadecanoic acid	1.2	tr	1.4	RI, MS, Co
2183	1-Nonadecanol	0.8	-	-	RI, MS, Co
2200	Docosane	0.5	tr	0.3	RI, MS, Co
2224	Eicosanal	tr	-	0.9	RI, MS
2261	4-Methyldocosane	1.5	-	-	RI, MS
2287	1-Eicosanol	tr	-	-	RI, MS
2300	Tricosane	5.8	tr	2.1	RI, MS, Co
2309	2-Heneicosanone	tr	-		RI, MS
2327	Methyl eicosanoate	tr	-		RI, MS, Co
2354	5-Methyl-5-(4,8,12-trimethyltridecyl)dihydro-2(3*H*)-furanone[b]	0.6	-	-	MS
2400	Tetracosane	2.7	19.8	8.4	RI, MS, Co
2500	Pentacosane	7.2	tr	6.2	RI, MS, Co
2535	Methyl docosanoate	tr	-	-	RI, MS
	Total Identified	88.1	96.7	90.9	
	Grouped Components				
	Fatty acids and fatty acid-derived compounds	65.0	86.3	69.7	
	Carotenoid-derived compounds	12.1	10.4	5.5	
	Terpenoids	6.6	tr	12.2	
	Monoterpenoids	0.8	tr	8.1	
	Monoterpene hydrocarbons	tr	-	2.2	
	Oxygenated monoterpenes	0.8	tr	5.9	
	Sesquiterpenoids	4.6	-	3.6	
	Sesquiterpene hydrocarbons	1.3	-	0.7	
	Oxygenated sesquiterpenes	3.3	-	2.9	
	Diterpenoids	1.2	tr	0.5	
	Oxygenated diterpenes	1.2	tr	0.5	
	Others[c]	4.4	tr	3.5	

[a] Percentage present mean values of 9 individual analyses; [b] Correct isomer not defined; [c] Unclassified constituents and/or compounds of possible anthropogenic origin; RI calc.–experimentally determined linear retention indices on an HP-5MS column; tr–trace amounts (w < 0.05%); RI–retention indices matching with literature data;[13] MS–mass spectra matching; Co–coinjection with an authentic sample.

The broth microdilution method employed for the determination of antimicrobial activities of the essential oils was according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS).[14] Minimum inhibitory concentration determination was performed by a serial dilution method in 96-well microtiter plates. Bacterial species were cultured at 37 °C in Mueller Hinton agar and fungi were cultured in Sabouraud dextrose agar at 30 °C. After 18 h of cultivation, bacterial suspensions were made in Mueller Hinton broth and their turbidity was standardized to 0.5 McFarland. Optical density of every suspension was confirmed on a spectrophotometer (UVProbe, Shimadzu). The final density of the bacterial and yeast inocula was 5×10^5. Suspensions of the molds were made in Sabouraud dextrose broth and their turbidity was confirmed by viable counting in a Thoma chamber. The final size of the fungal inoculum was 1×10^4. The starting concentrations of the essential oil solutions were 14.0 mg/mL (aerial parts of *G. columbinum*), 12.0 mg/mL (underground parts of *G. columbinum*), and 13.4 mg/mL (*G. lucidum*). Further stock solutions of the essential oils were prepared in 10% aqueous dimethylsulfoxide (DMSO) and then double serial dilutions of the oils were made. The inoculum was added to all wells and the plates were incubated at 37 °C for 24 h (bacteria) or at 30 °C for 48 h (fungal strains). The bacterial growth was visualized by adding 20 μL of 0.5% 2,3,5-triphenyltetrazolium chloride (TTC) aqueous solution.[15] Additionally, chloramphenicol and nystatin were tested in the same way and used as positive controls (the obtained results concerning these antibiotics are included in Table 2). Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the oils that inhibited visible growth (red-colored pellet at the bottom of the wells after the addition of TTC), while the minimum bactericidal concentration (MBC) was defined as the lowest oil concentration that killed 99.9% of bacterial cells. To determine MBC/MFC (minimum fungicidal concentration), broth was taken from each well without visible growth and inoculated on Mueller Hinton agar for 24 h at 37 °C for bacteria or on Sabouraud dextrose agar for 48 h at 28 °C (molds) or 30 °C (yeasts). The experiments were done in quintuplicate.

Statistical analysis

In order to statistically evaluate any significant differences among mean values, a one-way ANOVA test was used. In all tests the significance level at which we evaluated critical values differences was 5%.

Results and discussion

Hydrodistillation of the aerial parts (stems, flowers, and leaves) and roots of *G. columbinum* and entire plants of *G. lucidum* all gave bright yellow, semiliquid essential oils in mean yields of 0.01% and 0.09% for the aerial parts and the roots of *G. columbinum*, respectively, and 0.03% for *G. lucidum*. In total, GC-MS analyses allowed the identification of 184 volatile compounds, accounting for 88.1%-96.7% of the detected GC peak areas. The list of the identified volatile constituents and their grouping into 3 classes, namely fatty acids and fatty acid-derived (FAD) compounds, terpenoids, and unclassified constituents, are given in Table 1.

All oil samples were dominated by fatty acids and fatty acid-derived compounds (65.0%-86.3%), with hexadecanoic and tetradecanoic acids, hexahydrofarnesyl acetone, and tetracosane as their main constituents. The terpenoid fractions of both taxa (6.6% and 12.2% in the oils from aerial parts and roots of *G. columbinum*, respectively) were dominated by oxygen-containing compounds and made up of mono- and sesquiterpenoids. The most abundant terpenoids in the oil from the aerial parts of *G. columbinum* were borneol (0.8%), homofarnesane

Table 2. Minimum inhibitory concentrations (MICs, mg/mL) and minimum bactericidal and fungicidal concentrations (MBC/MFCs, mg/mL) of the investigated essential oils.

Bacterial strains	*G. columbinum* aerial parts' oil		*G. columbinum* underground parts' oil		*G. lucidum* oil		Chloramphenicol
	MIC	MBC	MIC	MBC	MIC	MBC	MIC
	mg/mL						mg/mL
E. coli (clinical isolate)	0.875	0.875	12.0	> 12.0	> 13.4	> 13.4	0.031
E. coli ATCC 25922	> 14.0	> 14.0	> 12.0	> 12.0	> 13.4	> 13.4	0.062
E. coli ATCC 8379	> 14.0	> 14.0	> 12.0	> 12.0	> 13.4	> 13.4	0.062
E. coli (Torlak 95)	14.0	14.0	> 12.0	> 12.0	13.4	> 13.4	0.062
K. pneumoniae ATCC 10031	14.0	> 14.0	> 12.0	> 12.0	13.4	> 13.4	0.062
K. pneumoniae (clinical isolate)	1.750	1.75	0.750	> 12.0	0.873	1.675	0.062
P. aeruginosa ATCC 27853	0.875	7.00	6.00	12.0	0.837	0.837	0.250
S. aureus ATCC 25923	1.750	1.75	12.0	> 12.0	3.35	3.35	0.015
S. aureus (clinical isolate)	3.50	14.0	6.00	> 12.0	1.675	13.4	0.062
C. perfringens ATCC 19414	0.437	3.50	6.00	12.0	1.675	> 13.4	0.062
C. sporogenes ATCC 19404	3.50	7.00	12.0	> 12.0	6.70	> 13.4	0.250
P. vulgaris ATCC 8427	7.00	> 14.0	12.0	> 12.0	13.4	> 13.4	0.125
S. enterica ATCC 13076	14.0	> 14.0	> 12.0	> 12.0	13.4	> 13.4	0.125
S. lutea ATCC 9341	7.00	> 14.0	> 12.0	> 12.0	13.4	> 13.4	0.125
M. flavus ATCC 10240	7.00	> 14.0	6.00	> 12.0	13.4	13.4	0.031
B. subtilis ATCC 6633	14.0	> 14.0	12.0	> 12.0	13.4	> 13.4	0.015
Fungal strains							Nystatine
P. chrysogenum (clinical isolate)	7.00	14.0	12.0	> 12.0	> 13.4	> 13.4	0.039
A. restrictus (clinical isolate)	7.00	14.0	12.0	> 12.0	13.4	> 13.4	0.078
A. fumigatus (clinical isolate)	0.109	7.00	0.375	> 12.0	0.837	1.675	0.039
C. albicans ATCC 10231	0.437	1.75	1.75	3.50	0.837	3.350	2.50
S. cerevisiae ATCC 9763	0.437	0.437	> 12.0	> 12.0	6.70	> 13.4	1.75

(1.1%), and caryophyllene oxide (1.1%). α-Pinene (0.9%), 1,8-cineole (1.4%), and hexahydrofarnesol (1.2%) were the main terpenoids of the *G. lucidum* essential oil. The essential oil isolated from the roots of *G. columbinum* consisted almost entirely of FAD compounds, while terpenoids were detected only in trace amounts (summarized data in Table 1); this fact indicates the localized production/accumulation of the mentioned group of volatile compounds in the above-ground plant organs of *G. columbinum*. The distribution of dominant compounds to the mentioned constituent classes of the investigated oils corroborates the conclusions of Radulović et al., who put forward a hypothesis concerning the link between oil yield and composition. Namely, due to the lack of a substantial production of volatile secondary metabolites (e.g. terpenoids or phenylpropanoids),

species poor in essential oils (oil yields lower than 0.1%) are dominated by FAD and/or carotenoid-derived compounds.[16]

It is interesting to note that several identified oil constituents could be formed by autoxidation during the drying of plant material and/or the hydrodistillation procedure. Namely, Rontani et al. recently reported that hexahydrofarnesyl acetone, which accounted for 5.3%-11.6% of the present oils, might be formed by the photodegradation of the chlorophyll phytyl side chain.[17] The same authors also showed that 5-methyl-5-(4,8,12-trimethyltridecyl)dihydro-2(3*H*)-furanone may be formed by autoxidation of α-tocopherol (vitamin E), omnipresent in the plant kingdom.[18] Additionally, α,β-unsaturated-γ-lactone dihydrobovolide, identified in the present oils, according to Horita et al.,[19] may be formed in a light-induced process in dry leaves. 5,5-Dimethyl-4-(3-oxobutyl)dihydro-2(3*H*)-furanone, trivially termed homoterpenyl methyl ketone, a monoterpene lactone identified in the *G. lucidum* oil, could have been formed from α-terpineol by oxidative fission of the double bond followed by lactonization of the resulting γ-hydroxy acid (air oxidation of α-terpineol).[20] This array of oxygenated compounds suggests that autoxidation plays an important role in the formation of plant volatiles isolated from dry plant material. According to a recent study, the oxygenated sesquiterpene clovane diol, identified in the *G. lucidum* oil (0.2%), may arise by the hydrolysis of caryophyllene oxide during hydrodistillation and could represent a possible artifact of the isolation procedure.[21]

A branched alcohol 3-ethyl-4-methyl-1-pentanol has been identified in *Harpegnathos saltator* and *Formica rufa* workers, *Formica polyctena* queens, and, recently, *Polyergus breviceps* queens.[22–24] In the latter case, the (*R*)-enantiomer of the same compound was identified as a sex attractant pheromone that attracts male ants.[25] This compound was also detected in the oil from the aerial parts of *G. columbinum* (0.4%) and several other plant species.[26,27] Considering its role in the attraction of selected ant species, 3-ethyl-4-methyl-1-pentanol may have an ecological role in plant-ant interactions (e.g. pollination or protection against herbivores).

The essential oils were tested in a broth microdilution assay against a panel of microorganisms including 16 bacterial strains and 5 fungal organisms. The obtained results, along with the activity (MIC) for the standard antibiotics, are presented in Table 2, showing a broad spectrum of activity. From these results, higher activity against gram-positive strains can be observed, in agreement with the conclusions of previous screenings of medicinal plants for antimicrobial activity.[28–31] The essential oil isolated from the aerial parts of *G. columbinum* was the most active among the tested essential oils, except against 2 strains of *E. coli* (ATCC 25922 and ATCC 8739) that turned out to be completely resistant to all tested oils. This oil was most active against *C. perfringens* (MIC = 0.437 mg/mL). On the contrary, the oil from the *G. columbinum* roots showed the lowest antimicrobial activity when compared with the other 2 oils. The most susceptible strain to this oil was the clinical isolate of *K. pneumoniae* (MIC = 0.750 mg/mL), but this activity turned out to be only inhibitory (MBC > 12.00 mg/mL), while the additional 6 strains showed resistance at the tested dose (Table 2). It appears that this oil possesses a mostly bacteriostatic effect, with observed bactericidal activity against 2 strains. *Geranium lucidum* oil showed the highest effect on the growth of *P. aeruginosa* (MIC = MBC = 0.837 mg/mL) and the clinical isolate of *K. pneumoniae* (MIC = 0.837, MBC = 1.675 mg/mL). The yeasts and molds showed considerably different responses when treated with these oils. The higher activity of all 3 oils was observed against *C. albicans* and *S. cerevisiae*. Once again, the most active sample was that of the oil from *G. columbinum* aerial parts, with the best result obtained in this work against the pathogenic yeast *C. albicans* (MIC = 0.437, MBC = 1.750 mg/mL).

All 3 essential oils lacked any significant amount of monoterpenoids, which are significant for a high antimicrobial potential, except in the case of 1,8-cineole (1.4%) in *G. lucidum* oil. This could be a reason for the generally observed moderate antimicrobial activity. On the other hand, the noted activity could be attributed to the presence of significantly high percentages of hexadecanoic acid in all 3 oils, which is a confirmed antibacterial and antifungal compound.[32–36] Hexahydrofarnesyl acetone, present as one of the major oil compounds, has also been suggested as a possible antimicrobial principle of essential oils.[37] However, it should be emphasized that the activity of the oils cannot be connected alone to the presence of these compounds, because their amount in the oils and the activity observed for the same oils were not mutually correlated. Hence, we could speculate that the observed activity was the result of a synergistic action of oil components. The oil from aerial parts of *G. columbinum* differed from the other 2 in the presence of the renowned strong antifungal compound caryophyllene oxide in a relatively significant amount,[34] probably resulting in the increased antifungal activity when compared to the other 2 oils.

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