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Full Length Research Paper

Studies on the antimicrobial activity and chemical composition of the essential oils and alcoholic extracts of *Gentiana asclepiadea* L.

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The present paper describes the chemical composition and antimicrobial activity of the essential oils, methanolic and *n*-butanolic extracts of the *Gentiana asclepiadea* L., collected in Serbia. The essential oils were obtained from underground parts (root and rhizome) and aerial parts (stem, leaves and flowers) of the plant by hydrodistillation and analyzed by GC and GC-MS. The major compounds in the oil from underground part were caryophyllene oxide (7.32%), *β*-damascenone (6.98%) and *β*-ionone (2.79%). The main constituents identified in the aerial part oil of *G. asclepiadea* were toluene (3.79%), tetradecanoic acid (3.37%), linalool (3.17%) and caryophyllene oxide (2.97%). The antimicrobial activity of the essential oils and plant extracts against several pathogenic bacteria and fungi was studied by minimum inhibitory concentration procedures. *Klebsiella pneumoniae* was very sensitive against oil from roots with MIC of 0.62 µl/ml, while the oil from aerial part exhibited maximum activity against *Micrococcus lysodeikticus* and *Candida albicans* with MIC values of 2.5 µl/ml. The methanolic extract of aerial part showed antimicrobial activities on all microorganisms tested at concentrations ranging from 50 to 1600 µg/ml while the *n*-butanolic fraction of methanolic extract of underground part was found to be less effective (MIC values: 312.5 to 2500 µg/ml).

Key words: Antimicrobial, gas chromatography/mass spectrometry (GC/MS), essential oils, *Gentiana asclepiadea* L., minimum inhibitory concentration (MIC).

INTRODUCTION

The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities and low toxicity (Sharma et al., 1992; Vaquero et al., 2010). Antimicrobial activity of herbs has been known and described for several centuries (Begamboula et al., 2003). Many naturally occurring compounds found in edible and medicinal plants, herbs, and spices have been shown to possess antimicrobial functions and could serve as a source of antimicrobial agents against bacteria and fungi (Deans and Ritchie, 1987; Janssen et al., 1985; Kim et al., 1995). Several studies have pointed out the possibility to use essential oils and/or their components in medical and plant pathology as well as in the food industry for the control of microorganisms pathogenic to consumers and/or responsible for food spoilage (Cantore et al., 2009).

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The acceptances of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics have led researchers to investigate the antimicrobial activity of medicinal plants (Maoz et al., 1998; Hammer et al., 1999). During few recent decades and mostly as a result of their diversity, versatility and safety in comparison with the synthetic materials, natural products from plants have attained special interest among academic and industrial scientific communities (Colegate and Molyneux, 2008; Ebrahimabadi et al., 2010). Today we know that the essential oils and various plant extracts have a broad spectrum activity against the Gram-positive and Gram-negative pathogenesis and they also perform the antifungal activity (Kotzekidou et al., 2008; Sartoratto et al., 2004).

Gentiana is the largest genus of the family Gentianaceae (Jensen and Schripsema, 2002). *Gentiana* species are distributed in the North hemisphere, in Europe, Asia and North America; in South America they occur in the Andes. The genus comprises over 300 species (Georgieva et al., 2005; Zhao et al., 2010a). In Europe, 29 species occur and eleven of them are distributed in Serbia (Josifović, 1973). Plants belonging to this genus are best known for their bitter taste that is due to the secoiridoids (e.g. swertiamarin, gentiopicroside, sweroside and amarogentin) (Jiang et al., 2005). These are popular ingredients of many gastric herbal preparations and dietary supplements. As natural sources of food flavouring they are utilized in alcoholic and nonalcoholic beverages (Aberham et al., 2011). The dried root and rhizomes of several *Gentiana* species are widely used throughout the world as hepatoprotective agents (quench the fire of the liver and gallbladder), remedies for poor appetite and digestive problems (Jiang et al., 2005; Szücs et al., 2002).

Gentiana asclepiadea L. is a perennial herb, up to 100 cm, with a strong developed rhizome. The Serbian local name of this plant is a “grass of jaundice” and the root of the *G. asclepiadea* has been traditionally used as medicine for hepatitis A virus infections (Sarić, 1989; Menković et al., 2010). Herb and roots of this plant are also used in the traditional medicine as a bitter tonic and gastric stimulant (Kitanov and Spassov, 1992). The herb is known to contain alkaloids (Southon et al., 1989; Marekov and Popov, 1968), bitter principles – secoiridoid-glycosides (Mpondo and Chulia, 1988; Szücs et al., 2002), flavone- and xanthone-C-glycosydes (Kitanov and Spassov, 1992).

Only one phytochemical study on the genus *Gentiana* has been reported, which describes the analysis of the volatiles from essential oils obtained from dried aerial parts (flowers and leaves) of *G. asclepiadea* from Bulgaria (Georgieva et al., 2005). A literature survey showed no previous reports of the analysis of the volatile compound from root of *G. asclepiadea*. According to the best of our knowledge, there are no published reports on antimicrobial activity of the essential oils and extracts of *G. asclepiadea*. Previous studies on the Gentianaceae species revealed the dominant presence of xanthones and secoiridoids, two classes of compounds with interesting biological activities. Secoiridoid glycosides have a variety of biological effects, such as anti-tumor (Isiguro et al., 1986), antibacterial, antifungal (Siler et al., 2010; van der Sluis et al., 1983) and hepatoprotective activities (Kondo et al., 1994; Jaishree and Badami, 2010). According to the literature, xanthone compounds often exhibit a wide range of biological and pharmacological activities, e.g, antioxidative (Zhao et al., 2010b; Gonda et al., 2000; Ashida et al., 1994), hypoglycemic (Bajpai et al., 1991; Basnet et al., 1994), anti-viral (Chen et al., 1996), anti-bacterial (Finnegan et al., 1973; Xin et al., 2010), and hepatoprotective (Hase et al., 1997). Many studies have reported that the *n*-butanol soluble parts of the methanol extracts of Gentianaceae species (genera *Gentiana, Swertia* and *Gentianella*) are rich in secoiridoids and xanthone-C-glycosydes (Krstić et al., 2004; Janković et al., 2005; Hajimehdipoor et al., 2008).

For these reasons, we focused our study on the chemical composition and antimicrobial properties of the essential oils, as well as antibacterial and antifungal activity of methanolic extract of aerial part and *n*-butanolic fraction of methanolic extract of underground part of wild-growing *G. asclepiadea* from Serbia.

MATERIALS AND METHODS

Plant material

Plant material was collected from Jadovnik Mountain (Gostun-Kumanica, at the Serbia–Montenegro border), during the flowering season (October 2008). Voucher specimen (No. 16337) was prepared and deposited in the Herbarium of the Department of Botany, Faculty of Biology, University of Belgrade, Belgrade, Serbia.

Isolation of the essential oils

Air-dried underground and aerial parts of *G. asclepiadea* were subjected to hydrodistillation for 3 h, using a Clevenger-type apparatus to produce oils. Anhydrous sodium sulphate was used to absorb the little water that the essential oils contained. The oils were then stored at +4°C until tested. The yield based on dry weight of the sample was calculated.

Preparation of the extracts

The air-dried aerial parts (68 g) and roots (105 g) of *G. asclepiadea* were separately extracted with methanol by using Soxhlet apparatus at 60°C for 12 h. The extracts were filtered and concentrated under vacuum at 40°C by using a rotary evaporator, yielding brown residues (15.9 and 21.9 g, respectively). Methanol extract of root (16.3 g) was suspended in water and re-extracted

with solvents of increasing polarity (chloroform, ethyl acetate and *n*-butanol) to obtain 0.69 g *n*-butanol extract. The extracts were stored in darkness at 4°C until used.

Gas chromatography (GC)/Mass spectrometry (MS)

Analyses were carried out in an Agilent Technologies (Santa Clara, CA) model 6890N gas chromatograph fitted with an HP-5MS fused silica column (5% phenylmethyl polysiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 µm, Agilent Technologies), interfaced with an Agilent Technologies mass-selective detector model 5975B operated by HP Enhanced ChemStation software (Agilent Technologies). The analytical conditions were as follows: injector and transfer line temperatures of 250 and 280°C, respectively; oven temperature programmed 60°C (isothermal for 5 min) to 130°C at a rate of 4°C/min (isothermal for 10 min) and then at 4°C/min to 240°C; carrier gas, helium at 1 ml/min; injection of 2 µl (10% hexane solution), split ratio 1:50 when the split flow was 50 ml/min, standard electronic impact MS source temperature 230°C, MS quadruple temperature 150°C, mass scan range, 35 to 500 amu at 70 eV, scan velocity: 3.12 scans/s and resulting electron multiplier voltage, 1200 V.

Gas chromatography (GC)

GC/FID analysis was performed on a 6890N gas chromatograph fitted with an HP-5MS fused silica column, and FID, was employed. All chromatographic conditions were the same with GC/MS analysis.

Component identification and quantification

The constituents of the volatile oils were identified by comparison of their mass spectra with those in the MS library (Wiley7Nist) incorporated in the HP Enhanced ChemStation software and those in literature (Jennings and Shibamoto, 1980; Adams, 2007) as well as by comparison of their retention indices with literature data (Adams, 2007). Retention indices were calculated for all components, using a homologous series of *n*-alkanes injected in conditions equal to the sample one. Some commercially available components of the essential oil were also co-injected for further confirmation of their identification. For quantification purposes, relative area percentages obtained by FID were used.

Microbial strains

The tests with oils from *G. asclepiadea* were performed on *Escherichia coli* (ATCC 25922), *Micrococcus lysodeikticus* (ATCC 4698), and fungus *Candida albicans* (ATCC 10259); the clinically isolated strains were *Bacillus subtilis* (FSB 2), *Klebsiella pneumoniae* (FSB 26) and *Staphylococcus aureus* (FSB 30). The extracts of *G. asclepiadea* were individually tested against a panel of 19 microorganisms. Following microbial strains were used in this research: *E. coli* (ATCC 25922), *M. lysodeikticus* (ATCC 4698), *S. aureus* (ATCC 25923), *S. aureus* (FSB 30), *Enterococcus faecalis* (ATCC 29212), *B. subtilis* (FSB 2), *K. pneumoniae* (FSB 26); test fungi were *C. albicans* (ATCC 10259), *Penicillium verrucosum* (FSB 21), *Penicillium chrysogenum* (FSB 22), *Penicillium cyclopium* (FSB 23), *Penicillium canescens* (FSB 24), *Phialophora fastigiata* (FSB 81), *Aspergillus glaucus* (FSB 32), *Aureobasidium pullulans* (FSB 61), *Alternaria alternata* (FSB 51), *Trichoderma viride* (FSB 11), *Trichoderma harzianum* (FSB 12) and *Trichoderma longibrachiatum* (FSB 13).

All test microbial strains were obtained from the Faculty of Biochemistry and Chemistry, University of Belgrade and Laboratory for Microbiology, Department of Biology, Faculty of Science, Kragujevac, University of Kragujevac, Serbia. Bacterial strains were cultured overnight at 37°C in nutrient agar (NA) and fungi were cultured on Sabouraud dextrose agar (SDA) at 28°C for 5 days. The material obtained was suspended in sterile water with a concentration of 5×10^5 CFU/ml for bacterial strains and 3×10^4 CFU/ml for fungi.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MIC) of the essential oils and extracts of *G. asclepiadea* against tested microorganisms were determined based on a microdilution method in 96 multi-well microtiter plates (Sarker et al., 2007). All tests with bacterial strains were performed in Muller–Hinton broth (MHB) with the exception of the fungi when Sabouraud dextrose broth (SDB) was used. A volume of 100 µl stock solutions of oils (in methanol, 10 µl/ml), methanol extract (in 0.9% NaCl solution, 3.2 mg/ml) and *n*-butanol extract (in 0.9% NaCl solution, 5 mg/ml) was added into the first row of the plate. To all other wells 50 µl of MHB or SDB (supplemented with Tween 80 at a final concentration of 0.5% (v/v) for analysis of oil) was added. A volume of 50 µl from first test well was pipetted into the second well of each microtiter line, and then 50 µl of scalar dilution was transferred from the second to the twelfth well. To each well, 10 µl of resazurin indicator solution (prepared by dissolving 270 mg in 40 ml of sterile distilled water) and 30 µl of nutrient broth were added.

Finally, 10 µl of bacterial suspension (5×10^5 CFU/ml) and yeast spore suspension (3×10^4 CFU /ml) was added to each well. A 10 µl of SDB was added in tests with fungi instead of resazurin indicator solution. For each strain, the growth conditions and the sterility of the medium were checked. Standard antibiotic amracin (tetracycline) was used to control the sensitivity of the tested bacteria, whereas nystatin was used as control against the tested fungi. Plates were placed in an incubator at 37°C for 24 h for the bacteria and at 28°C for 48 h for the fungi. Color change was then assessed visually. Any color change from purple to pink or colorless was recorded as positive. The lowest concentration at which no observed color change was taken as the MICs value for bacterial strains and the lowest concentrations without visible growth, were defined as concentrations that completely inhibited fungal growth (MICs). All tests were repeated in triplicate.

RESULTS AND DISCUSSION

Chemical compositions of the essential oils

The oil yields were calculated on a dry weight basis as 0.3% (v/w) for underground and 1.1% (v/w) for aerial part. The complex mixtures of compounds from essential oils characterized in this study are given in Table 1, in which they are classified based on their chemical structures in 14 classes. The GC/MC and GC/RI data showed differences in the profiles of compounds between underground (root) and aerial (stem, leaves and flowers) part of *G. asclepiadea*. In the oil of the underground part 140 constituents were identified, representing 95.9% of

Table 1. Chemical composition of *Gentiana asclepiadea* essential oils determined by GC and GC-MS.

No.	Compound	RI[a]	Percentage, %	
			Underground part	Aerial part
	Aldehydes			
1	2-Hexenal	854	0.07	-[c]
2	Heptanal	899	tr[b]	-
3	Hexanal	901	-	0.76
4	2,4-Hexadienal	906	tr	2.47
5	2-Heptenal	956	0.03	1.27
6	1-Methyl-1-cyclohexene-4-carboxaldehyde	982	0.09	1.55
7	Octanal	1001	tr	-
8	2,4-Heptadienal	1005	0.04	-
9	Nonanal	1103	0.24	-
10	2,4-Octadienal	1113	0.02	1.12
11	2,6-Nonadienal	1155	0.14	-
12	2-Nonenal	1162	0.11	0.32
13	Decanal	1205	0.12	-
14	2,6,6-Trimethyl-1-cyclohexene-1-acetaldehyde	1244	0.11	0.98
15	*p*-Anisaldehyde	1251	-	0.16
16	2-Decenal	1260	0.39	0.14
17	2,4-Decadienal	1314	0.47	0.58
18	Dodecenal	1399	1.12	0.28
19	Tetradecanal	1607	0.07	0.29
20	Pentadecanal	1714	2.10	0.94
21	Hexadecanal	1816	2.11	-
	Subtotal		**7.23**	**10.86**
	Alcohols			
22	1-Pentanol	768	-	0.03
23	2-Hexanol	799	0.05	-
24	3-Hexanol	854	0.02	-
25	*cis*-3-Hexen-1-ol	857	0.04	0.95
26	1-Hexanol	867	0.03	2.32
27	1-Heptanol	969	-	2.21
28	1-Octen-3-ol	978	0.21	2.14
29	3-Octanol	993	-	0.69
30	6-Methyl-5-hepten-2-ol	994	-	1.56
31	3,5-Octadien-2-ol	1039	-	0.09
32	2-Octen-1-ol	1169	-	0.16
33	1-Nonanol	1175	-	0.47
34	Hexadecanol	1872	0.21	-
	Subtotal		**0.56**	**10.62**
	Acids			
35	Nonanoic acid	1287	0.02	0.57
36	Dodecanoic acid	1580	0.05	1.12
37	Tridecanoic acid	1663	-	0.32
38	Tetradecanoic acid	1780	2.76	3.37
39	Pentadecanoic acid	1868	1.10	1.11
40	9-Hexadecenoic acid	1953	0.19	-
41	Hexadecanoic acid	1991	0.21	-

Table 1. Continued.

42	Oleic acid	2141	-	1.26
43	Octadec-9-enoic acid	2152	0.27	0.54
	Subtotal		**4.60**	**8.29**
	Aromatic compounds			
44	Toluene	773	0.56	3.79
45	Ethylbenzene	868	0.02	0.17
46	*m*-Xylene	869	0.10	-
47	*o*-Xylene	894	tr	0.01
48	Benzaldehyde	961	0.11	0.89
49	Phenylacetaldehyde	1044	0.23	0.79
50	Benzyl acetate	1165	0.08	0.97
51	Ethyl-2-phenyl acetate	1256	-	0.15
52	1,2-Dihydro-1,1,6-trimethyl-naphthalene	1354	0.52	0.33
53	Eugenol	1356	0.11	0.57
54	Elemicin	1555	-	0.45
55	*β*-Asarone	1598	0.23	-
56	Eupatoriochromene	1759	3.47	2.12
57	4-Isopropyl-1,6-dimethylnaphthalene	1676	**-**	0.69
	Subtotal		**5.43**	**10.93**
	Esters			
58	Butyl acetate	812	0.04	1.16
59	Butyl butanoate	996	-	0.86
60	Ethyl dodecanoate	1597	1.41	-
61	Isopropyl myristate	1824	0.57	-
62	Methyl hexadecanoate	1926	0.35	1.69
63	Methyl linoleate	2093	1.54	-
64	Methyl ester 8,11-octadecadienoic acid	2112	0.27	-
65	Methyl ester 9,12,15-octadecatrienoic acid	2113	0.56	-
	Subtotal		**4.74**	**3.71**
	Furanes			
66	2-Furfural, 2-furancarboxaldehyde	830	tr	0.12
67	2,4-Dimethylfuran	921	-	2.18
68	2-Pentylfurane	992	0.11	-
69	2-(2-Pentenyl)furan	1008	0.04	-
	Subtotal		**0.15**	**2.30**
	Ketones			
70	3-Hexanone	782	0.03	0.01
71	2-hexanone	788	0.03	tr
72	6-Methyl-5-hepten-2-one	985	0.02	-
73	2,2,6-Trimethyl-cyclohexanone	1029	tr	-
74	3,5-Octadien-2-one	1081	-	0.62
75	Neryl acetone	1429	-	1.27
76	Geranyl acetone	1440	1.21	-
77	Hexahydrofarnesyl acetone	1843	0.18	-
78	6,10,14-Trimethyl-2-pentadecanone	1845	1.76	-
79	2-Hydroxy-cyclopentadecanone	1853	0.79	-

Table 1. Continued.

80	2-Heptadecanone	1903	-	1.56
	Subtotal		**4.02**	**3.55**
	Aliphatic hydrocarbons			
81	4-Methyl-pentadecane	1495	0.93	-
82	Heptadecane	1690	0.76	0.56
83	Octadecane	1800	0.43	0.11
84	1-Nonadecene	1892	0.25	-
85	Nonadecane	1900	0.31	-
86	1-Eicosene	1995	-	1.10
87	Eicosane	2000	-	0.95
88	Heneicosane	2122	1.67	1.89
89	Docosane	2225	1.05	2.05
90	Tricosane	2300	1.93	2.26
91	Tetracosane	2400	0.29	0.53
92	Pentacosane	2500	0.39	0.64
93	Hexacosane	2600	0.21	0.47
94	Heptacosane	2700	0.12	0.15
95	Octaacosane	2800	0.06	0.09
	Subtotal		**8.40**	**10.8**
	Monoterpene hydrocarbons			
96	*α*-Pinene	939	0.12	0.03
97	*p*-Cymene	1026	0.06	-
98	Limonene	1028	0.10	-
99	neo-allo-Ocimene	1141	-	0.53
	Subtotal		**0.28**	**0.56**
	Oxygenated monoterpenes			
100	*p*-Cymenene	1087	0.22	-
101	Linalool	1099	0.80	3.17
102	*cis*-Rose oxide	1114	tr	-
103	Nerol oxide	1157	0.10	-
104	*α*-Terpineol	1188	0.34	1.87
105	Myrtenal	1193	0.08	-
106	Safranal	1201	0.06	-
107	Verbenone	1204	tr	-
108	*β*-Cyclocitrale	1220	0.34	2.39
109	Nerol	1227	-	0.99
110	Geraniol	1255	0.29	1.54
111	Geranial	1270	0.11	-
112	Vitispirane	1286	0.24	-
113	Bornyl acetate	1288	0.12	-
114	Carvacrol	1301	tr	-
115	*α*-Ionene	1335	0.09	-
116	*β*-Damascenone	1381	6.98	1.97
117	*cis*-Linalool oxide	1074	-	1.47
118	*α*-Ionone	1426	1.73	-
119	*trans*-Linalool oxide	1438	-	0.25
120	Megastigma-3,5-diene-9-ol	1467	0.21	-

Table 1. Continued.

121	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one	1487	1.57	-
122	*β*-Ionone	1488	2.79	0.33
	Subtotal		**16.07**	**13.98**
	Sesquiterpene hydrocarbons			
123	*α*-Longipinene	1343	0.22	-
124	Aromadendrene	1439	0.24	-
125	*β*-Selinene	1484	0.05	-
126	*α*-Caryophyllene	1489	0.03	-
127	*α*-Muurolene	1496	0.32	-
128	*γ*-Cadinene	1509	0.18	-
129	*γ*-Muurolene	1515	0.11	-
130	*cis*-Calamenene	1517	0.06	-
131	*δ*-Cadinene	1523	0.75	-
132	*α*-Amorphene	1528	0.62	-
133	Cadina-1,4-diene	1532	0.56	-
134	Dehydroaromadendrene	1699	0.42	-
135	*α*-Calacorene	1542	0.39	-
136	Cadalene	1673	0.97	-
	Subtotal		**4.92**	**-**
	Oxygenated sesquiterpenes			
137	Caryophyllene oxide	1581	7.32	2.97
138	Spathulenol	1582	2.54	1.36
139	Viridiflorol	1590	0.58	-
140	*β*-Oplopenone	1608	0.21	-
141	*τ*-Cadinol	1640	0.34	-
142	*τ*-Muurolol	1643	2.53	0.95
143	*α*-Cadinol	1653	1.94	1.11
144	Caryophylla-4(12),8(13)-dien-5-ol	1668	1.57	-
	Subtotal		**20.03**	**6.39**
	Oxygenated diterpenes			
145	Farnesyl acetone	843	0.73	1.67
146	Isophytol	1954	1.26	-
	Subtotal		**1.99**	**1.67**
	Threeterpene hydrocarbons			
147	Squalene	3058	0.24	0.26
	Subtotal		**0.24**	**0.26**

[a] retention indices values relative to C_6 to C_{24} *n*-alkanes calculated on a non-polar HP-5MS capillary column; [b]tr, trace (< 0.10%); [c]Not detected.

the total oil. Oxygenated sesquiterpenes (8 compounds, 20.03%) were the main class and of these, caryophyllene oxide (7.32%) predominated, followed by spathulenol (2.54%) and *τ*-muurolol (2.53%). Other important compounds were the oxygenated monoterpenes *β*-damascenone (6.98%) and *β*-ionone (2.79%).

In the oil from underground part, the class of aliphatic hydrocarbons was represented by 13 compounds (8.4%), of which tricosane (1.93%) and heneicosane (1.67%) were the principal compounds. The essential oil also

Table 2. Antimicrobial activities of *G. asclepiadea* essential oils and extracts.

Test microorganisms	MIC[a] (µg/ml)				
	Underground part		Aerial part		Standard[b]
	Essential oil	*n*-BuOH extract	Essential oil	MeOH extract	
E. coli	2.5	>2500	-[c]	800	2.5
S. aureus (ATCC 25923)	-	312.5	-	200	0.625
S. aureus (FSB 30)	2.5	2500	5.0	800	1.25
M. lysodeikticus	>5	1250	2.5	200	0.625
K. pneumoniae	0.62	1250	5.0	400	0.625
E. faecalis	-	2500	-	200	0.625
B. subtilis	2.5	625	5.0	1600	0.31
C. albicans	5.0	1250	2.5	100	1.25

[a]MIC, minimum inhibitory concentration (as µg/ml); [b]The standard drugs used were amracin for bacteria and nystatin for *C. albicans*; [c]Not tested.

contained smaller percentage of aldehydes (7.23%), with hexadecanal (2.11%) and pentadecanal (2.10%) as the main constituents. Aromatic compounds (11 compounds) are not present in large quantity (5.43%); eupatoriochromene (3.47%) was found to be the major constituent.

The minor chemical compounds were characterized in the essential oil derived from the aerial parts. Ninety compounds, constituting 92.64% of the oil, were identified in the oil from the aerial parts. Oxygenated monoterpenes (9 compounds, 13.98%) are the main class, of which linalool (3.17%) was the major compound followed by *β*-cyclocitrale (2.39%) with lower amounts of *β*-damascenone (1.97%) and *α*-terpineol (1.87%). A higher percentage (10.8%) of aliphatic hydrocarbons (12 compounds), with respect to underground part of *G. asclepiadea,* was detected with tricosane (2.26%), docosane (2.05%) and heneicosane (1.89%) in similar amount. Aromatic compounds (12 compounds) represent a large fraction of the oil (10.93%), with toluene (3.79%) and eupatoriochromene (2.12%) as the main constituents. The essential oil from aerial part also contained alcohols (10.62%) and aldehydes (10.86%) large quantity.

Previous chemical studies on the genus *Gentiana* seemed to indicate that the straight chain aliphatic hydrocarbons, branched aliphatic hydrocarbons and alkylated benzenes were the most characteristic constituents in the oils from flowers and leaves (Georgieva et al., 2005). Georgieva et al. (2005) described, tridecane (5.6%), tetradecane (4.9%), pentadecane (3.8%) and tricosane (5.8%) as the main constituents of the essential oil from flowers of *G. asclepiadea,* whereas tetramethylheptane (5.2%), toluene (6.9%) and ethylbenzene (4.1%) were the main compounds of the oil from leaves. The comparison of the oils obtained from *G. asclepiadea* aerial parts from Bulgaria and Serbia showed some differences. In the study reported here, more constituents were identified in the oil from aerial parts than in the previous work. In addition, the difference in identified components and percentage amounts could be attributed mainly to drying conditions and geographic and climatic factors (Figueiredo et al., 2008).

Antimicrobial activity of essential oils

The results obtained in the evaluation of the antimicrobial activity of the essential oils against some bacterial and *C. albicans* is shown in Table 2. The oil obtained from the root of *G. asclepiadea* exhibited a significant antimicrobial activity, displaying strong activity for *K. pneumoniae*, *S. aureus*, *B. subtilis*, *E. coli* and *C. albicans* (MIC: 0.62, 2.5, 2.5, 2.5, and 5 µl/ml, respectively), while *M. lysodeikticus* seemed to be more resistant to the investigated oil. The oil from aerial part exhibited antimicrobial activity against all the tested microorganisms. The results of the microdilution method (Table 2) indicated that the most sensitive microorganisms were *M. lysodeikticus* and *C. albicans* with MIC values of 2.5 µl/ml for oil from aerial parts. The oil from the aerial part displayed less values of MIC than the oil from the underground part.

Based on the oils composition analysis, the major constituents were caryophyllene oxide, linalool and long chain alcohols and aldehydes. The antimicrobial property of linalool (Krist et al., 2008; Pattnaik et al., 1997) and caryophyllene oxide (Shunying et al., 2005; Costa et al., 2008) has been confirmed previously. The oil obtained from aerial part of *G. asclepiadea* contained elevated levels of long chain (C_6 to C_{10}) alcohols and aldehydes (Table 1) which have antimicrobial properties (Huhtanen, 1980). In fact, long chain (C_6 to C_{10}) alcohols were particularly active against Gram-positive bacteria (Delaquis et al., 2002), the antimicrobial properties of

Table 3. Antifungal activities of *G. asclepiadea* extracts.

Test fungi	MIC[a] (µg/ml)		
	MeOH extract	*n*-BuOH extract	Nystatin
P. chrysogenum	50	>2500	6.25
P. cyclopium	50	>2500	12.5
P. verrucosum	50	2500	6.25
P. canescens	50	312.5	12.5
P. fastigiata	200	>2500	12.5
A. glaucus	100	2500	6.25
A. pullulans	400	>2500	12.5
A. alternate	400	2500	12.5
T. harzianum	400	1250	7.8
T. longibrachiatum	400	1250	7.8
T. viride	100	>2500	7.8

[a] MIC, minimum inhibitory concentration (as µg/ml).

alcohols were known to increase with molecular weight (Morton, 1983).

The potent activity of the oil from underground part might be attributed to its high sesquiterpene content and their derivatives (Table 1). Sesquiterpenoids and their derivatives are credited with various biological actions, including antibacterial, antifungal, antiasthmatic, anti-inflammatory, and antineoplastic activities (Founier et al., 1997) and then, the activity could be attributed to the presence of minor components such as α-pinene (Kelen and Tepe, 2008; Cosentino et al., 1999), spathulenol (Rota et al., 2008), geraniol and eugenol (Kim et al., 1995) known already to exhibit an antibacterial activity or at least to a synergistic effect between all components (Cimanga et al., 2002).

Antibacterial and antifungal activities of extracts

The methanol extract of aerial part and *n*-butanolic fraction of methanolic extract of the root from the *G. asclepiadea* were tested against a set of 7 bacteria and 12 fungi, in order to estimate their antimicrobial potentials. The results of antibacterial and antifungal activity of extracts are summarized in Tables 2 and 3. Generally, both tested extracts were found to be more or less active against one or other microorganisms. The methanol extract showed antimicrobial activities on all microorganisms tested at concentrations ranging from 50 to 1600 µg/ml while the *n*-butanol extract was found to be less effective. *G. asclepiadea* methanol extract was active against all bacteria tested, showing the lowest MIC values of 200 µg/ml against *S. aureus* (ATCC 25923), *E. faecalis* and *M. lysodeikticus*, while *B. subtilis* was inhibited using the largest concentration of the extract. According to the results, *n*-butanolic fraction of the root extract demonstrated moderate activities against tested bacteria (MIC values: 312.5 to 2500 µg/ml). The best activity was seen against *S. aureus* (ATCC 25923) with MIC of 312.5 µg/ml, while *E. coli* resisted all the extract concentrations.

The effects of extracts on the growth of twelve different pathogenic fungal strains are presented in Tables 2 and 3. The results of antifungal activity assays showed that the methanol extract has significant inhibitory effects on the growth of all *Penicillium* sp. with a MIC value of 50 µg/ml, while *C. albicans*, *A. glaucus* and *T. viride* showed moderate sensitivity to extract MIC 100 µg/ml. The MIC values of antifungal activity assays indicated that, the methanol extract of aerial part was more efficient than that of *n*-butanolic fraction of the root extract. The MIC of the *n*-butanolic fraction was seen at concentrations ranging from 312.5 to 2500 µg/ml. *P. canescens* showed best susceptibility towards the *n*-butanol extract with a MIC value of 312.5 µg/ml, while *P. chrysogenum*, *P. cyclopium*, *P. fastigiata*, *A. pullulans* and *T. viride* resisted all the extract concentrations.

Previous phytochemical analyses of *G. asclepiadea* have described the presence of secoiridoid glycosides gentiopicroside, swertiamarin and sweroside (Szücs et al., 2002). Those chemical compounds showed important antibacterial activity against pathogenic bacteria (Kumarasamy et al., 2003a, b). Pure secoiridoid glycosides isolated from *Centaurium pulchellum* (Gentianaceae) extracts demonstrated very strong antibacterial (0.01 to 0.04 mg/ml) and especially antifungal (0.001 to 0.1 mg/ml) activity (Siler et al., 2010). In addition, there are several reports about the antifungal constituents from *Gentiana* species (Tan et al., 1996a, b). The antimicrobial activity exhibited by *G. asclepiadea* extracts, observed in our experiments, may be due to the presence of xanthones. The presence of these

metabolites in *G. asclepiadea* extracts was previously confirmed (Kitanov and Spassov, 1992; Szücs et al., 2002). The mentioned metabolites were previously reported as antibacterial active substances against a range of Gram negative and Gram positive bacteria and methicillin-resistant *S. aureus* (MRSA) (Azebaze et al., 2008; Xin et al., 2010).

Conclusion

In summary, this is the first report describing the antimicrobial activities of *G. asclepiadea* L. The results of the present study revealed that the essential oils, methanol and *n*-butanol extracts of *G. asclepiadea,* inhibited microbial growth but their effectiveness varied. While the extracts showed moderate inhibitory activity against the tested microorganisms, the essential oils exhibited considerable antibiotic capabilities in these tests. The oil from the root exhibited high antimicrobial activity against important human pathogen *K. pneumoniae*. The essential oil of aerial parts displayed antimicrobial activity to varying degrees against all the tested strains.

According to the results of antimicrobial activity, *S. aureus* (ATCC 25923) was the most susceptible bacteria in test with extracts. *S. aureus* is a food-borne pathogen and a threat to global public health. It causes gastro-enteritis and poisoning and can be found in a wide variety of foodstuff (Ahmadi et al., 2010). Among the fungal strains tested, *Penicillium* sp. showed high sensitivity to methanol extract.

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