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Chemical composition and antimicrobial activity of leaves essential oil of *Juniperus communis* (Cupressaceae) grown in Republic of Macedonia

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Abstract

Chemical composition and antimicrobial activity of essential oils isolated from leaves of three different samples of wild growing *Juniperus communis* L. (Cupressaceae) from R. Macedonia was investigated. Essential oil yield ranged from 7.3 to 9.0 ml/kg. Performing GC/FID/MS analysis, ninety components were identified, representing 86.07-93.31% of the oil. The major components of the leaves essential oil (LEO) were α-pinene (21.37-28.68%) and sabinene (2.29-16.27%), followed by limonene, terpinen-4-ol, β-elemene, *trans*-(E)-caryophyllene, germacrene D and δ-cadinene. Antimicrobial screening of the LEO was made by disc diffusion and broth dilution method against 16 bacterial isolates of Gram positive and Gram negative bacteria and one strain of *Candida albicans*. Two bacteria, *Staphylococcus aureus* and *Streptococcus pyogenes* were sensitive to antimicrobial activity of LEO (MIC = 125 µl/ml). Additionally, LEO showed moderate antimicrobial activity against *Streptococcus agalactiae, Haemophilus influnzae, Corynebacterium* spp. and *Campylobacter jejuni* (MIC > 500 µl/ml). *Candida albicans, Staphylococcus epidermidis, Acinetobacter* spp*., Salmonella enteritidis, Shigella flexneri, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Proteus mirabilis* were completely resistant to the antimicrobial effects of this.

Keywords: *Juniperus communis*, leaves essential oil, oil composition, GC/FID/MS analysis, antimicrobial activity

Introduction

The common juniper, *Juniperus communis* L. (Cupressaceae), is an evergreen shrub or small coniferous tree, wide spread through the cool temperate Northern Hemisphere. The above-ground parts, especially leaves and berries of juniper are rich in essential oil that has characteristic aromatic flavour and bitter taste. Due to its diuretic and gastrointestinal properties, common juniper is used as medicinal plant for centuries. Juniper oil is used in the pharmaceutical, food and cosmetic industries, as well as for the production of perfumes. Certain beverages (gin) are made with distillation from fermented juniper berries.

According to the literature data, juniper essential oil can be obtained from berries, leaves, wood and seeds by hydrodistillation (Orav et al., 2010a; Chatzopoulou and Katsiotis, 1993; Kumar et al., 2007). The average oil yield varies from 0.5 to 2.5% (for berries) and from 0.2 to 1.0% (for needles) (Orav et al., 2010a). Dissimilarities in the oil's yield and the chemical composition can vary from the geo-

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Table 1. Plant samples of *Juniperus communis* from R. Macedonia

Species	Locality	Altitude	Herbarium voucher specimen	Sample abbreviation
Juniperus communis L.	Jelak (Shara Mtn.)	1800 m	N°JC-16/10	JcS/10
Juniperus communis L.	Velestovo (Galichica Mtn.)	1000 m	N° JC-5.1./11	JcG/11
Juniperus communis L.	Kicevo (Bistra Mtn)	600 m	N° JC-1/11	JcB/11

graphical location, age and degree of plant ripeness, harvesting methods, distillation techniques and other factors.

There are many publications reporting the essential oil composition of juniper berries and leaves (Orav et al., 2010a; Chatzopoulou and Katsiotis, 1993; Kumar et al., 2007, Ottavioli et al., 2009; Filipowicz et al., 2009; Shahmir et al., 2003; Orav et al., 2010b). Considerable variations in the oil composition were observed depending on the plant origin but often the essential oils were rich in α-pinene, sabinene and myrcene, followed by *trans*-(E)-caryophyllene, muurolene, germacrene D and B and humulene (Orav et al., 2010a). As major oxygen containing terpene were terpinen-4-ol (Chatzopoulou and Katsiotis, 1993), rarely citronellol (Koukos and Papadopoulou, 1997) and terpenyl acetate (Angioni et al., 2003).

In vitro antimicrobial (antibacterial and antifungal) activity of the berries essential oil was studied and the results showed strong to moderate antimicrobial activity (Filipowitz et al. 2003; Stassi et al. 1995). Other results from the antimicrobial assessment of the leaves essential oil (LEO) demonstrate no or weak antimicrobial activity against various tested microbial strains (Asili et al., 2008; Angioni et al., 2003).

Common juniper, *Juniperus communis,* is widely spread shrub throughout the territory of Republic of Macedonia (Micevski, 1998). The berries of this plant are extensively utilized in production of blended teas and other herbal medicinal products, in food industry, as a spice, in production of alcoholic beverages, *etc*. For years, the juniper berries and the juniper essential oil are exported from R. Macedonia. On the other hand the juniper leaves are used in folk medicine for various purposes. Up to date there is no information of the composition and antimicrobial activity of the leaves' essential oil from Macedonian juniper. Therefore the aim of the present study was to investigate the chemical composition and the antimicrobial activity of the leaves essential oil of *Juniperus communis* grown wild in R. Macedonia.

Material and methods

Plant materials

The terminal twigs of *Juniperus communis* were collected from tree different localities in R. Macedonia in late autumn 2010 and 2011. Plant identity was verified as *Juniperus communis* L. and herbarium voucher specimen were deposited at the Department of Pharmaceutical Botany, Institute of Pharmacognosy, Faculty of Pharmacy, Skopje, R. Macedonia (Table 1).

The plant material was dried at room temperature. Just before essential oil isolation, the juniper leaves were separated and minced properly.

Chemicals

Dimethylsulfoxide was purchased from Sigma-Aldrich (Steinheim, Germany), sodium chloride and anhydrous sodium sulfate from Merck (Darmstadt, Germany) and from Kemica (Zagreb, Croatia), respectively, while xylene was purchased from Alkaloid (Skopje, R. Macedonia).

Essential oil isolation

The essential oils were obtained from dried plant material through steam distillation using all glass Clevenger-type apparatus. For that purpose, 20 g of minced plant material was distilled for 4 hours. After isolation, anhydrous sodium sulfate was added to remove residual water from the oil. The essential oil yield was calculated on dried plant material and was expressed in ml/kg. For GC/FID/MS analysis, the essential oil was dissolved in xylene to obtain 1 μl/ml oil solution.

Gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS)

Essential oil samples were analyzed on Agilent 7890A Gas Chromatography system equipped with FID detector and Agilent 5975C Mass Quadrupole detector as well as capillary flow technology which enables simultaneous analysis of the samples on both detectors. For that purpose, HP-5ms capillary column (30 m x 0.25 mm, film thickness 0.25 μm) was used. Operating conditions were as follows: oven temperature at 60 °C (5 min), 1 °C/min to 80 °C (2 min) and 5 °C/min to 280 °C (5 min); helium as carrier gas at a flow rate of 1ml/min; injector temperature 260 °C and that of the FID 270 °C. 1μl of each sample was injected at split ratio 1:1. The mass spectrometry conditions were: ionization voltage 70 eV, ion source temperature 230

°C, transfer line temperature 280 °C and mass range from 50 - 500 Da. The MS was operated in scan mode.

Identification of the components

Identification of the components present in essential oils was made by comparing mass spectra of components in essential oils with those from Nist, Wiley and Adams mass spectra libraries, by AMDIS (Automated Mass Spectral Deconvolution and Identification System) and by comparing literature and estimated Kovat's (retention) indices that were determined using mixture of homologous series of normal alkanes from C_9 to C_{25} in hexane, under the same above mentioned conditions.

The percentage ratio of essential oils components was computed by the normalization method of the GC/FID peak areas without any correction factors.

Antimicrobial activity: microbial strains and cultures

16 bacterial isolates representing both Gram positive and Gram negative bacteria and one strain of *Candida albicans* were used for antimicrobial screening. Five isolates were standard strains (*Staphylococcus aureus* ATCC 29213, *Escherichia coli* 25927, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231). The remaining 12 bacterial strains (*Staphylococcus epidermidis, Enterococcus, Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus pneumoniae, Haemophilus influenzae, Proteus mirabilis, Salmonella enteritidis, Corynebacterium* spp., *Salmonella enteritidis, Shigella flexneri, Campylobacter jejuni and Acinetobacter spp*.) were clinical isolates provided from the Institute of Microbiology and Parasitology, Faculty of Medicine, Skopje, R. Macedonia.

A nutrient (Mueller Hinton) agar (Merck, Darmstadt, Germany), blood agar (Oxoid, Basingstoke, UK) and Sabouraud agar (bioMerieux, Durham, NC) were used for growing of the microbes.

Disc diffusion method

Disc diffusion method was used for screening the antimicrobial activity of all essential oils in order to determine the growth inhibition zones of studied microorganisms that occur around certain essential oil. In this regard, microorganisms were suspended in sterile broth with turbidity corresponding to 0.5 and 1 Mc Farland (approximate by 10^7-10^8 CFU/ml) for all bacteria and for *Candida albicans*, respectively. The microbial suspensions were streaked over the surface of the agar media using a sterile cotton swabs to ensure uniform inoculation. After inoculation of microorganisms, discs of 6 mm in diameter were made at well-spaced intervals. They were filled with 85 μl of 50% solutions of essential oils in dimethylsulfoxide (DMSO, Sigma-Aldrich, Germany) and one disc was filled only with DMSO as a control. The plates were incubated at 37 ^{0}C, aerobically for 24 hours. The growth inhibition zones were measured after incubation of the isolates under their optimal growth conditions and were ranged between 6 mm and 30 mm in diameter. The antimicrobial activity was determined according to the diameters of the inhibition zones (0-14 mm resistant - R, 14-19 mm moderate susceptible - M and 19-30 susceptible - S microorganisms).

Broth dilution method

This method was used in order to determine minimal inhibitory concentration (MIC) of the particular essential oil prepared as 50% solution in DMSO). For that purposes, 25 μl of those essential oils were diluted in equal quantities of 0.9 % sodium chloride solution, to make them with the concentration of 25%. This concentration was decreased five times, subsequently, by adding 25 μl of each bacterial or fungal suspension, thus the final concentrations were: 12.5%, 6.2%, 3.1%, 1.5% and 0.7% or 125 μl/ml, 62 μl/ml, 31 μl/ml, 15 μl/ml and 7μl/ml, respectively.15 μl of each bacterial or fungal suspensions with these particular concentrations were inoculated on solid media (Miller-Hinton agar, blood agar, Sabouraud agar), depending on the type of microorganism. The growth of any microorganism was evaluated after its incubation under the optimal growth conditions. The lowest concentration of essential oil which was able to inhibit the growth of the particular microorganism was considered as its minimal inhibitory concentration (MIC).

Results and discussion

The yields of the leaves essential oil were: 7.3, 7.3 and 9.0 ml/kg for JcS/10, JcB/11 and JcG/11 respectively. Percentage presence with Kovat's retention indices of ninety identified components representing 86.07-93.31% of the oil are presented in Table 2. Data analysis of the chemical composition revealed four main classes of components: monoterpene hydrocarbons (MH), oxygen-containing monoterpenes (OM), sesquiterpene hydrocarbons (SH) and oxygen-containing sesquiterpenes (OS). Diterpenes (D) were present in small amounts as well as some non-terpene components (NT). Monoterpene hydrocarbons were the most abundant fraction in all investigated oils (39.97%, 52.32% and 53.39%, for the samples from Shara Mtn., Galicica Mtn. and Bistra Mtn., respectively), followed by SH (28.64%, 20.66% and 12.27%, respectively) (Table 2). Oxygen-containing monoterpenes were present in much smaller amounts (3.89% in samples from Shara Mtn., 6.69% in samples from Galicica Mtn. and 12.16% in samples from Bistra Mtn.) as well as the oxygen-containing sesquiterpenes (13.57%, 11.53% and 5.98%, respectively). The ratios between monoterpenes (M) and sesquiterpenes (S) were 1:1, 2:1 and 3:1 for LEO from Shara Mtn., Galicica Mtn. and Bistra Mtn., respectively (Fig. 1).

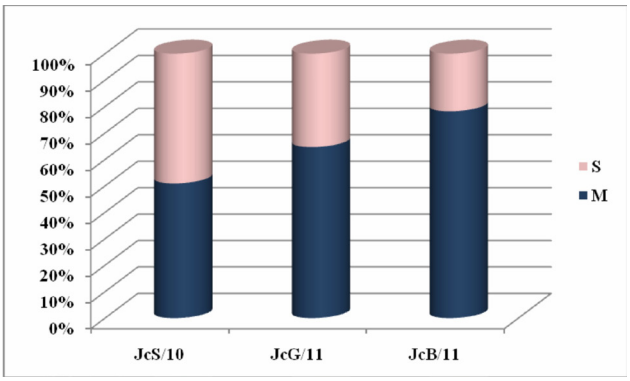

Fig. 1. The monoterpenes (M) / sesquiterpenes (S) ratio of leaves essential oil from Macedonian juniper

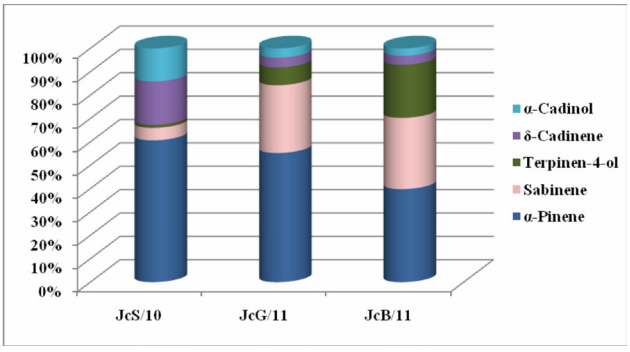

Fig. 2. Predominant components in the leaves essential oil from wild growing *J. communis* in R. Macedonia

GC/FID/MS analysis of the juniper leaves essential oil showed presence of two main components in the samples from Galicica Mtn. and Bistra Mtn.: α-pinene (28.68% and 21.27%, respectively) and sabinene (15.5% and 16.27%, respectively). Additionally, limonene was identified with 2.82% and 6.95%, respectively, while terpinene-4-ol was the most abundant oxygen-containing monoterpenes (3.98% and 12.16%, respectively). Concerning the sesquiterpene fraction, β-elemene, *trans*-(E)-caryophyllene, α-humulene, germacrene D, δ-cadinene and α-cadinol were present in amounts between 1.37 and 3.45%. Likewise LEO from Shara Mtn. contained α-pinene (26.05%) as predominant component, followed by smaller amounts of δ-3-carene (3.15%), β-phellandrene (4.37%) and sabinene (2.29%). Terpinene-4-ol was present with 0.55%. The sesquiterpene components (β-elemene, δ-cadinene and α-cadinol) were present in amounts up to 7.98%. Evaluation of the occurrence of the main components that characterized the leaves essential oil of Macedonian juniper (Fig.2), showed that sabinene was found in Bistra Mtn. and Galicica Mtn., terpinen-4-ol only in Bistra Mtn., while the sesquiterpenes, δ-cadinene and α-cadinol were characteristic for the LEO from Shara Mtn.

Similarity in the composition of the leaves essential oil was found with the Greek *J. communis* where α-pinene (41.25%) and sabinene (17.4%) have been predominant constituents followed by smaller amounts of limonene (4.2%), terpinen-4-ol (2.7%), β-myrcene (2.6%) and β-pinene (2.0%) (Chatzopoulou et al., 1993). Further, the Estonian *J. communis* LEO has been also rich in α-pinene (33.3-45.6%) and sabinene (0.2-15.4%) while limonene, *trans*-(E)-caryophyllene, α-humulene and germacrene D were present in smaller amounts (Orav et al., 2010a, 2010b). Raal et al. (2010) reported similar composition for the essential oil obtained from branches of *J. communis* from Estonia comparing to the leaves essential oil where α-pinene (40.4-62%) and limonene (4.2-10%) were dominant components, followed by α-cadinol, δ-cadinene, γ-muurolene and germacrene. LEO from *J. communis* from Lithuania was rich in α-pinene (38.5-59.9%), accompanied by β-phellandrene (4.1-11.4%) or α-cadinol (one sample of tested essential oil contained 8.7%) (Butkine et al., 2005). Butkine et al. (2005) have identified 143 components in the juniper LEO which were divided in two groups according to the monoterpenes/sesquiterpenes ratio: M:S = 5:1 and M:S = 2:1. Filipowicz et al. (2009) have reported that populations of *J. communis* from Northern Poland have essential oils with different α-pinene/sabinene ratio. Iranian authors found that juniper leaves essential oil was rich in sabinene (40.7%), than α-pinene (12.5%) and terpinen-4-ol (12.3%) (Shahmir et al., 2003). Asili et al. (2008), confirmed α-pinene as predominant component in the Iranian *J. communis* subsp. *hemisphaerica* LEO, while Ottavioli et al. (2009) for French *J. communis* subsp. *alpina* reported limonene (9.2-53.9%), β-phellandrene (3.7-25.2%), α-pinene (1.4-33.7%) and sabinene (0.1-33.6%) as major constituents. The LEO from Indian *J. communis* contained predominantly sabinene (22.8%), β-pinene (10.7%), *trans*-sabinene hydrate (6.0%) and γ-cadinene (10.6%) (Kumar et al., 2007).

Antimicrobial activity

Antimicrobial screening of the essential oils was made by disc diffusion and broth dilution method against 16 bacterial isolates of Gram positive and Gram negative bacteria and one strain of *Candida albicans* (Table 3). The highest MIC (125 µl/ml) of LEOs were towards *Staphylococcus aureus* and *Streptococcus pyogenes*, and moderate antimicrobial activity against *Streptococcus agalactiae, Haemophillus influnzae, Corynebacterium* spp. and *Campylobacter jejuni* (MIC > 500 µl/ml). *Candida albicans, Staphylococcus epidermidis, Acinetobacter* spp., *Salmonella enteritidis, Shigella flexneri, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Proteus mirabilis* were completely resistant to the antimicrobial activity of juniper oil.

Antimicrobial activity of juniper essential oils was previously investigated so the available literature pointed out no activity to some antimicrobial effects against vari-

Table 2. The chemical composition of the leaves essential oil (LEO) of *Juniperus communis* from R. Macedonia

No.	Components	KIL	KIE	JcS/10	JcG/11	JcB/11
1	Tricyclene	921	930.5	-	0.04	-
2	α-Thujene	924	933.3	-	0.79	2.43
3	α-Pinene	932	937.5	26.05	28.68	21.27
4	Camphene	946	946.3	0.18	0.91	-
5	n-Heptanol	959	946.5	-	-	5.69
6	Sabinene	969	962.6	2.29	15.05	16.27
7	β-Pinene	974	964.2	2.28	tr	-
8	β-Myrcene	988	975.7	0.55	1.69	2.12
9	δ-2-Carene	1001	982.1	0.16	0.17	-
10	α-Phellandrene	1002	995.2	0.38	-	-
11	δ-3-Carene	1008	989.7	3.15	0.22	-
12	α-Terpinene	1014	995.3	-	0.12	-
13	*p*-Cymene	1020	1002	0.56	1.43	tr
14	*o*-Cymene	1022	1003	-	-	4.35
15	Limonene	1024	1005	-	2.82	6.95
16	β-Phellandrene	1025	1016	4.37	-	-
17	γ-Terpinene	1054	1033	-	0.23	-
18	Terpinolene	1086	1062	-	0.21	-
19	Isopentyl 2-methylbutanoate	1100	1083	-	0.1	-
20	α-Campholenal	1122	1102	-	0.16	-
21	*trans*-Pinocarveol	1135	1118	0.47	0.18	-
22	*trans*-Verbenol	1140	1128	1.16	0.37	-
23	3-methyl-2-butenyl 3-methyl-Butanoate	1147	1138	-	0.02	-
24	Borneol	1165	1150	0.28	0.09	-
25	Terpinen-4-ol	1174	1161	0.55	3.96	12.16
26	*p*-Cymene-8-ol	1179	1170	-	0.24	-
27	α-Terpineol	1186	1174	0.37	0.33	-
28	Myrtenol	1194	1178	-	0.29	-
29	Verbenone	1204	1187	tr	0.27	-
30	*trans*-Carveol	1215	1195	-	0.08	-
31	β-Citronellol	1223	1205	-	0.1	-
32	Thymol methyl ether	1232	1210	-	0.05	-
33	*cis*-Myrtanol	1249	1248	0.15	-	-
34	Bornyl acetate	1284	1260	0.78	0.42	-
35	*trans*-Sabinyl acetate	1289	1280	0.13	-	-
36	Terpinen-7-al	1290	1268	-	0.03	-
37	δ-Elemene	1335	1305	0.14	0.07	-
38	α-Cubebene	1345	1319	0.12	0.09	-
39	α-Ylangene	1373	1341	-	0.04	-
40	α-Copaene	1374	1345	-	0.62	0.67
41	β-Bourbonene	1387	1354	-	0.32	-
42	β-Elemene	1389	1362	4.17	2.62	1.37
43	Sibirene	1400	1384	2.31	0.39	0.31
44	2-*epi*-Funebrene	1411	1394	0.08	-	-

No.	Components	KIL	KIE	JcS/10	JcG/11	JcB/11
45	*trans*-(E)-Caryophyllene	1417	1387	0.81	3.45	1.52
46	β-Copaene	1430	1396	0.13	0.2	-
47	γ-Elemene	1434	1400	2.95	0.58	0.67
48	Sesquterpene[*1]	/	1405	-	0.04	-
49	Sesquterpene[*2]	/	1411	-	0.11	-
50	*trans*-Muurola-3,5-diene	1451	1418	0.1	tr	-
51	α-Humulene	1452	1421	1.07	2.89	1.37
52	*cis*-Muurola-4(14),5-diene	1465	1431	0.26	0.21	-
53	Germacrene D	1484	1450	2.45	3.23	1.43
54	β-Selinene	1489	1455	0.1	0.53	0.64
55	α-Selinene	1498	1463	1.07	0.88	0.7
56	α-Muurolene	1500	1468	1.32	0.61	0.45
57	δ-Amorphene	1511	1487	0.21	-	-
58	γ-Cadinene	1513	1481	2.26	0.9	0.6
59	δ-Cadinene	1522	1490	7.98	2.15	2.05
60	*trans*-Cadina-1.4-diene	1533	1498	0.31	0.1	-
61	α-Cadinene	1537	1517	0.5	-	-
62	α-Calacorene	1544	1510	0.2	0.22	-
63	Elemol	1548	1516	0.31	0.16	-
64	Germacrene B	1559	1525	-	0.41	0.49
65	Nerolidol E	1561	1528	-	0.5	-
66	Germacrene D-4-ol	1574	1544	-	tr	-
67	Spathulenol	1577	1546	-	1.67	1.06
68	Caryophyllene oxide	1582	1552	-	1.73	0.78
69	Viridiflorol	1592	1563	-	0.29	-
70	Humulene epoxide II	1608	1578	0.77	1.17	0.71
71	1,10-di-*epi* 1-*epi*-Cubenol	1618	1582	tr	tr	-
72	1-*epi*-Cubenol	1627	1595	2.31	0.51	0.39
73	τ-Murolol (*epi*-α-Muurolol)	1640	1609	4.13	1.32	1.07
74	α-Muurolol	1644	1613	-	0.44	0.3
75	α-Cadinol	1652	1622	6.05	2.04	1.67
76	Cadalene	1675	1624	-	tr	-
77	Sesquterpene[*3]	/	1654	-	0.34	-
78	Shyobunol	1685	1659	-	0.31	-
79	8-Cedren-13- ol	1688	1676	-	0.26	-
80	Sesquterpene[*4]	/	1690	-	0.21	-
81	Oplopanone	1739	1705	-	0.23	-
82	Sesquterpene[*5]	/	1778	-	0.23	-
83	(Z)-Lanceol	1760	1790	-	0.12	-
84	Pimaradiene	1948	1934	-	0.06	-
85	Manool oxide	1987	1962	-	0.27	-
86	Abietatriene	2055	2025	-	0.62	0.32
87	Abietadiene	2087	2052	-	0.12	-
88	dehydro-Abietal	2274	2238	-	0.03	-
89	Abietal	2313	2278	-	0.04	-

No.	Components	KIL	KIE	JcS/10	JcG/11	JcB/11
90	Octacosane	2800	2822	-	0.93	-
	Non-terpene components (NT)			-	0.97	5.69
	Monoterpene hydrocarbons (MH)			39.97	52.32	53.39
	Oxygen-containing monoterpenes (OM)			3.89	6.69	12.16
	Sesquiterpene hydrocarbons (SH)			28.64	20.66	12.27
	Oxygen-containing sesquiterpenes (OS)			13.57	11.53	5.98
	Diterpenes (D)			-	1.14	0.32
	Total (%)			86.07	93.31	89.81

KIL - Kovat's (retention) index - literature data (Adams, 2007); KIE – Kovat's (retention) index experimentally determined (AMDIS); (-) - not found, tr – traces < 0.02, *1, 2, 3, 4, 5 - tentative identification.

Table 3. Antimicrobial activity of the leaves essential oil of *Juniperus communis*

No.	Microorganism		JcS/10	JcG/11	JcB/11
1	*Streptococcus pneumoniae*	DD	R	R	R
		MIC	n.m.	n.m.	n.m.
2	*Staphylococcus aureus*	DD	S	S	S
		MIC	125	125	125
3	*Staphylococcus epidermidis*	DD	R	R	R
		MIC	n.m.	n.m.	n.m.
4	*Streptococcus agalactiae*	DD	M	R	R
		MIC	>500	n.m.	n.m.
5	*Streptococcus pyogenes*	DD	S	M	M
		MIC	125	>500	>500
6	*Enterococcus*	DD	R	R	R
		MIC	n.m.	n.m.	n.m.
7	*Corynebacterium* spp.	DD	M	R	R
		MIC	>500	n.m.	n.m.
8	*Haemophilus influenzae*	DD	M	M	M
		MIC	>500	>500	>500
9	*Acinetobacter* spp.	DD	R	R	R
		MIC	n.m.	n.m.	n.m.
10	*Escherichia coli*	DD	R	R	R
		MIC	n.m.	n.m.	n.m.
11	*Salmonella enteritidis*	DD	R	R	R
		MIC	n.m.	n.m.	n.m.
12	*Shigella flexneri*	DD	R	R	R
		MIC	n.m.	n.m.	n.m.
13	*Campylobacter jejuni*	DD	M	M	M
		MIC	>500	>500	>500
14	*Klebsiella pneumoniae*	DD	R	R	R
		MIC	n.m.	n.m.	n.m.
15	*Pseudomonas aeruginosa*	DD	R	R	R
		MIC	n.m.	n.m.	n.m.
16	*Proteus mirabilis*	DD	R	R	R
		MIC	n.m.	n.m.	n.m.
17	*Candida albicans*	DD	R	R	R
		MIC	n.m.	n.m.	n.m.

DD – Disc diffusion (zone of inhibition including the diameter of disc 6 mm), R - resistant with zone of inhibition 0 - 14 mm, M - moderate susceptible with zone of inhibition 14 - 19 mm and S - susceptible microorganism with zone of inhibition 19 - 30 mm); MIC – minimum inhibitory concentration (µl/ml); n.m. – not measured.

ous tested microbial strains. Essential oil (*Juniperi aetheroleum*) obtained from the juniper (*J. communis*) berries was evaluated for the antimicrobial activity against sixteen bacteria, seven yeast-like fungi, three yeasts and four dermatophyte strains. Juniper essential oil showed similar bactericidal activities against Gram-positive and Gram-negative bacterial strains, with MIC values between 8 and 70% (V/V), as well as a strong fungicidal activity against yeasts, yeast-like fungi and dermatophytes, with MIC values below 10% (V/V). The strongest fungicidal activity was recorded against *Candida* spp. (MIC from 0.78 to 2%, V/V) and dermatophytes (MIC from 0.39 to 2%, V/V). GC/MS analysis of the essential oil showed that predominant constituents in this oil were α-pinene (29.17%), β-pinene (17.84%), sabinene (13.55%), limonene (5.52%) and β-myrcene (0.33%) (Pepelnjak et al., 2005). The essential oil of *J. communis* growing wild in Kosovo, showed moderate to high activities against *Staphylococcus aureus*, *Escherichia coli* and *Hafnia alvei*, while *Pseudomonas aeruginosa* was resistant to the antimicrobial effects of the oil (Haziri et al., 2013). The leaves essential oils of *J. communis* subsp. *hemisphaerica* and *J. oblonga* from Iran did not show noticeable activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* (Asili et al., 2008). Angioni et al. reported similar results concerning the antimicrobial activity of the essential oils from ripe and unripe berries and leaves of Italian *J. communis* against the most of the above mentioned microbial.

Conclusion

The essential oil (LEO) isolated from tree different leaves samples of wild growing *Juniperus communis* L. (Cupressaceae), from R. Macedonia was characterized with presence of α-pinene (21.37-28.68%) and sabinene (2.29-16.27%), followed by limonene, terpinen-4-ol, β-elemene, *trans* (E)-caryophyllene, germacrene D and δ-cadinene. Antimicrobial screening of the LEOs against 16 bacterial isolates of Gram positive and Gram negative bacteria and one strain of *Candida albicans,* showed the strongest antimicrobial activity towards *Staphylococcus aureus* and *Streptococcus pyogenes* (MIC = 125 μl/ml) and moderate antimicrobial activity against *Streptococcus agalactiae, Haemophillus influnzae, Corynebacterium* spp. and *Campylobacter jejuni* (MIC > 500 μl/ml). On the other hand, *Candida albicans, Staphylococcus epidermidis, Acinetobacter* spp*., Salmonella enteritidis, Shigella flexneri, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Proteus mirabilis* were completely resistant to the antimicrobial effects of *J. communis* LEO.

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Резиме

Хемиски состав и антимикробната активност на етерично масло изолирано од листовите на *Juniperus communis* L. (Cupressaceae) од Република Македонија

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Клучни зборови: *Juniperus communis*, етерично масло од листови, состав на масло, GC/FID/MS анализа, антимикробно дејство

Главна цел на студијата беше испитување на хемискиот состав и антимикробната активност на етеричното масло изолирано од листовите на три различни популации на диво растечки *Juniperus communis* L. (Cupressaceae) од Република Македонија. Приносот на дестилираните етерични масла се движи од 7.3 до 9.0 ml/kg. Со GC/FID/MS анализа беа идентификувани вкупно деведесет компоненти што претставуваат 86.07-93.31 % од маслото. Најзастапени компоненти во етеричното масло од листовите беа: α-пинен (21.37-28.68 %) и сабинен (2.29-16.27 %), проследени со помали количества на лимонен, терпинен-4-ол, β-елемен, Е-кариофилен, гермакрен D и δ-кадинен. Антимикробната активност на маслото беше определена со диск дифузиона и агар дилуциона метода на 16 бактериски изолати на грам позитивни и грам негативни бактерии и еден вид на *Candida albicans*. Две бактерии, *Staphylococcus aureus* и *Streptococcus pyogenes* беа чувствителни на маслото од смрека, со MIC=125 µl/ml. Етеричното масло од листови покажа умерено антимикробно дејство против *Streptococcus agalactiae*, *Haemophilus influnzae*, *Corynebacterium* spp. и *Campylobacter jejuni* (MIC> 50 %). *Candida albicans, Staphylococcus epidermidis, Acinetobacter* spp.*, Salmonella enteritidis, Shigella flexneri, Klebsiella pneumonia, Pseudomonas aeruginosa* и *Proteus mirabilis* беа потполно резистентни на маслото изолирано од иглички на *J. communis.*