

Chemical composition and antimicrobial activity of essential oils of *Juniperus excelsa* Bieb. (Cupressaceae) grown in R. Macedonia

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ABSTRACT

Background: There are no information of the yield, chemical composition and antimicrobial activity of essential oils of berries (EOB) or leaves (EOL) of *Juniperus excelsa* Bieb. (Cupressaceae) growing wild in R. Macedonia. **Materials and Methods:** Plant material was collected from two localities during two seasons. Essential oil composition was analyzed by gas chromatography/flame ionization detector/mass spectrometry (GC/FID/MS) and antimicrobial screening was made by disc diffusion and broth dilution method. **Results and Discussion:** EOB yield ranged from 1.6-9.4 ml/kg and from 8.9-13.9 ml/kg for EOL. Two chemotypes of essential oil were differentiated, α -pinene-type (with 70.81% α -pinene in EOB and 33.83% in EOL), also containing limonene, β -pinene and β -myrcene while the sabinene-type (with 58.85-62.58% sabinene in EOB and 28.52-29.49% in EOL), was rich in α -pinene, β -myrcene, limonene, *cis*-thujone, terpinolene and α -thujene. The most sensitive bacteria to the antimicrobial activity of EOB was *Haemophilus influenzae* (MIC = 31 μ l/ml). EOL have showed high activity towards: *Staphylococcus aureus*, *Streptococcus pyogenes* and *Haemophilus influenzae* (MIC = 125 μ l/ml). The pinene-type of essential oil showed moderate activity against *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Corynebacterium* spp. and *Campylobacter jejuni* (MIC > 50%). The sabinene-type of the oil showed moderate activity to *Streptococcus pyogenes*, *Haemophilus influenzae*, *Campylobacter jejuni* and *Escherichia coli* (MIC > 50%). No activity was observed toward *Candida albicans*. **Conclusion:** The analysis of EOB and EOL revealed two chemotypes (α -pinene and sabinene type) clearly depended on the geographical origin of the Macedonian *Juniperus excelsa* which also affected the antimicrobial activity of these oils.

Key words: Antimicrobial activity, essential oil, gas chromatography/flame ionization detector/mass spectrometry analysis, juniper berries and leaves, *Juniperus excelsa*

INTRODUCTION

Juniperus is one of the major genera of Cupressaceae family consisting of approximately 70 species. *Juniperus excelsa* Bieb. is large shrub or tree, spread mainly throughout the eastern Mediterranean starting from north-eastern Greece and southern Bulgaria across Turkey to the Middle-East countries (Syria and Lebanon) and the Caucasus Mountains. It occurs in Iran, Pakistan and Oman as well.^[1] The plant is known as Greek juniper, which appears as male and female plants, even some individuals could produce both sexes. Two

main subspecies of *J. excelsa* are known, subsp. *excelsa* (Greek juniper) and subsp. *polycarpus* (Persian juniper). Only subsp. *excelsa* is present in Macedonian flora and is spread throughout the whole territory of Republic of Macedonia (RM), but mostly in southern part of the country.^[2]

The *Juniperus* species are characterized by large amount of essential oil in berries and needles as well as in wood and seed. In the last decade the composition of the berries essential oils of both *J. excelsa* subspecies, *excelsa* and *polycarpus*, was investigated and some data were published recently. The berries essential oil of subsp. *excelsa* is characterized by presence of very high amounts of α -pinene,^[3,4] followed by cedrol, L-verbenol and D-verbenol as predominant components,^[5] 1,4-cineole

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and limonene,^[6] myrcene, limonene, caryophyllene, δ -elemene and cedrol,^[7] or cedrol, myrcene and limonene.^[8] The berries essential oil of subsp. *polycarpus* also contained α -pinene as predominant component,^[6-8] followed by lower amounts of 1,4-cineol, β -ocymene, δ -2-carene, 6-camphenol and sabinene^[7] or δ -3-carene and limonene.^[8] *J. excelsa* leaves essential oil was rich in cedrol (28.1%), α -pinene (22.5%) and limonene (22.7%),^[9] or α -pinene (29.7%^[5] and 32.34%^[7]) and cedrol (25.3%^[5] and 13.06%^[7]).

J. excelsa subsp. *excelsa* is a medicinal plant that has been used in folk medicine to treat dysmenorrhea, cough, bronchitis and colds, jaundice and tuberculosis and to induce menses and expel fetus.^[7] It is known as a remedy for diarrhea, abdominal spasm, asthma, fever, gonorrhoea, headache and leucorrhoea.^[1] Among limited biological and pharmacological properties studied *in vitro* (cytotoxic,^[5] antioxidant^[4,7,8,10] and antispasmodic activity)^[4] the most investigated was the antimicrobial activity (antibacterial^[3,4,6,11] and antifungal).^[8,12]

J. excelsa medicinal properties are also known by people of RM and it is used as pain reliever and for curing cold, asthma, edema or skin diseases. Up to now no chemical investigations were done on this plant and no testing of possible biological and pharmacological activities were performed. Therefore the aim of the present study is analysis of chemical composition of leaves and berries essential oils and evaluation of their antimicrobial activity.

MATERIALS AND METHODS

Plant materials

The terminal twigs with leaves and berries were collected from two different localities in R. Macedonia: Lake Dojran (altitude - 140 m) and Lake Ohrid (village Velestovo, National Park Galicica, altitude - 1060 m), in late autumn 2010 and 2011. Plant identity was verified as *Juniperus excelsa* M. Bieb. and herbarium voucher specimen N° JE-1/10, N° JE-3/10 and N° JE-1/11 were deposited at the Department of Pharmaceutical Botany, Institute of Pharmacognosy, Faculty of Pharmacy, Skopje, RM.

The plant material was dried at room temperature, the berries and the leaves were separated and minced properly.

Chemicals

Dimethylsulfoxide (DMSO) 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, RM).

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 gas chromatography/flame ionization detector/mass spectrometry (GC/FID/MS) analysis, the essential oil was dissolved in xylene to obtain 1 μ l/ml oil solution.

Gas chromatography and gas chromatography/mass spectrometry

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-5 ms capillary column (30 m \times 0.25 mm, film thickness 0.25 μ m) 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 1 ml/min; injector temperature 260°C and that of the FID 270°C. One microliter 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 comparison of their mass spectra with those from Nist, Wiley and Adams mass spectra libraries, by AMDIS (Automated Mass Spectral Deconvolution and Identification System) and by comparing literature^[13] and estimated Kovat's (retention) indices that were determined using mixture of homologous series of normal alkanes from C₉ to C₂₅ 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*, *Acinetobacter* spp., *Corynebacterium* spp., *Shigella flexneri* and *Campylobacter jejuni*) 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 McFarland (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 μ l of 50% solutions of essential oils in (DMSO (Sigma-Aldrich, Germany) and one disc was filled only with DMSO as a control. The plates were incubated at 37°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 mm 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 μ l of those essential oils were diluted in equal quantities of 0.9% sodium chloride solution, to make them with concentration of 25%. This concentration was decreased five times, subsequently, by adding 25 μ l of each bacterial or fungal suspension, thus the final concentrations were: 12.5%, 6.2%, 3.1%, 1.5% and 0.7% or 125 μ l/ml, 62 μ l/ml, 31 μ l/ml, 15 μ l/ml and 7 μ l/ml, respectively. 15 μ l 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 MIC.

RESULTS AND DISCUSSION

Both berries and leaves essential oils were transparent, agile, light yellowish liquids with specific and very strong turpentine odor. The berries essential oil (EOB) yield ranged from 1.6-9.4 ml/kg and from 8.9-13.9 ml/kg for leaves essential oil (EOL). Table 1 shows the GC/FID/MS analysis of the investigated oils isolated from *Juniperus excelsa* berries and leaves samples collected from two different localities in R. Macedonia: Lake Dojran (from south-eastern part of RM) and Lake Ohrid (from south-western part of RM).

Total of 74 components were identified in the analyzed samples of *J. excelsa* berries and leaves essential oils, representing 82.69-98.13% of the oil. Data analysis of the chemical composition revealed six different classes of components: Monoterpene hydrocarbons (MH), oxygen containing monoterpenes (OM), sesquiterpene hydrocarbons (SH), oxygen containing sesquiterpenes (OS), diterpenes (D) and other, non-terpene components (NT). Among different classes of terpenes present in the berries essential oil from Dojran, the MH fraction was the most abundant with total participation of 80.05%. On the other hand mass part of SH was only 3.54% [Table 1]. The samples of essential oil obtained from the plant material from Ohrid (Velestovo village), for two years of collection, 2010 and 2011, also contained MH as predominant fraction (from 79.92-80.33%) followed by larger amount of OM (from 7.85-10.51%). Fractions of sesquiterpenes, SH and OS, were much lower, ranging from 3.45-6.82% and from 0.12-1.31%, respectively [Table 1].

Component analysis of the *J. excelsa* EOB showed existence of two chemotypes of the oils (pinene- and sabinene-type). The sample from south-east RM (around Lake Dojran) was rich in α -pinene (70.81%), while the most abundant component in the samples from south-west RM (around Lake Ohrid) was sabinene (from 54.85-62.58%). Both “chemotypes” of berries oils had more than 80% of MH + OM fractions. Pinene-type of the essential oil was rich in: Limonene (4.00%), β -pinene (2.54%) and β -myrcene (1.58%), while sabinene-type contained following monoterpenes: α -pinene (4.51-7.09%), β -myrcene (5.06-5.65%), limonene (2.20-2.23%), *cis*-thujone (1.72-3.01%), terpinolene (1.52-1.75%) and α -thujene (1.03-1.17%). On the other hand, the sesquiterpene fraction was very low in both types of essential oils. Only germacrene D was presented in larger amounts in sabinene-type of the oil (1.21-3.12%).

Table 1: Chemical composition of berries and leaves essential oils from wild growing *Juniperus excelsa* in R. Macedonia

Components	KIL	KIE	Dojran		Ohrid			
			Berries	Leaves	Berries	Leaves	Berries	Leaves
			2010		2010		2011	
Tricyclene	921	930.2	0.10	-	tr	-	-	-
α -Thujene	931	933.7	-	-	1.03	1.42	1.17	0.84
α -Pinene	939	937.5	70.81	33.83	4.51	1.76	7.09	2.59
Camphene	953	945.8	0.51	-	0.12	-	0.41	0.47
Sabinene	969	966.4	-	0.34	62.58	28.52	58.85	29.49
β -Pinene	974	973.8	2.54	-	-	-	-	-
β -Myrcene	991	976.3	1.58	0.46	5.06	2.09	5.65	2.78
α -Terpinene	1018	995.8	-	-	1.10	1.87	0.99	0.99
<i>p</i> -Cymene	1025	1002.6	0.22	0.19	0.16	0.83	0.41	0.88
Limonene	1031	1005.9	4.00	6.14	2.20	1.76	2.23	1.75
γ -Terpinene	1062	1033.7	0.16	tr	1.82	3.53	1.60	1.75
<i>cis</i> -Sabinene hydrate	1068	1041.5	-	-	0.58	0.60	0.51	0.12
Terpinolene	1088	1052.6	0.23	-	1.75	1.02	1.52	0.68
<i>cis</i> -Thujone	1102	1079.8	-	-	1.72	5.49	3.01	26.20
<i>trans</i> -Thujone	1114	1079.9	-	-	0.68	1.44	0.57	12.86
Menth-2-en-1-ol	1118	1120.1	-	-	0.16	-	-	-
<i>trans</i> -Sabinol	1140	1121.6	-	-	0.35	1.74	-	0.67
Thujan-3-ol	1164	1162.8	-	-	-	0.12	-	0.23
Terpinen-4-ol	1177	1162.2	-	-	2.47	5.87	2.70	4.52
α -Terpineol	1189	1174.6	-	-	0.07	0.21	0.06	0.18
<i>cis</i> -Piperitol	1193	1195.7	-	-	-	0.19	-	tr
Thymol methyl ether	1232	1221.1	-	-	0.08	-	0.04	-
<i>trans</i> -Sabinene hydrate acetate	1261	1229.8	-	-	0.06	-	0.04	-
Bornyl acetate	1285	1260.9	0.06	0.34	0.04	0.13	0.06	-
<i>trans</i> -Sabinyl acetate	1291	1268.0	-	-	4.30	10.38	0.83	0.14
Terpinen-4-ol acetate	1340	1274.2	-	-	tr	0.11	0.03	0.05
δ -Elemene	1339	1306.2	-	0.12	tr	0.08	0.03	-
α -Cubebene	1351	1319.2	0.07	0.17	0.06	tr	0.06	-
α -Copaene	1376	1345.9	0.07	0.28	0.02	0.17	0.14	0.05
β -Bourbonene	1384	1354.9	-	0.25	-	0.11	0.02	0.06
β -Elemene	1391	1361.9	-	0.29	0.06	0.31	0.25	0.20
Sibirene	1409	1371.4	-	-	-	-	0.02	-
<i>trans</i> -(E)-Caryophyllene	1418	1387.3	0.54	3.30	0.58	0.59	0.70	0.22
β -Copaene	1420	1396.4	-	1.19	tr	0.17	0.17	0.05
γ -Elemene	1433	1400.1	-	-	0.24	0.77	0.29	2.17
Aromadendrene	1439	1412.1	-	-	0.13	-	0.10	-
<i>cis</i> -Muurola-3,5-diene	1448	1418.2	0.03	-	tr	-	0.02	-
<i>trans</i> -Muurola-3,5-dien	1451	1431.8	-	0.40	-	0.08	-	-
α -Humulene	1454	1422.4	0.10	tr	0.42	0.45	0.40	0.14
<i>cis</i> -Muurola-4 (14),5-diene	1465	1432.0	-	-	0.05	0.19	0.11	0.04
<i>trans</i> -Cadinane-1 (6),4-diene	1467	1442.1	-	-	-	0.14	0.02	-
γ -Muurolene	1477	1445.0	0.74	0.92	0.12	0.46	0.08	0.06
Germacrene D	1480	1450.1	0.37	2.26	1.21	4.16	3.12	1.21
α -Muurolene	1495	1468.0	0.16	1.83	0.12	1.05	0.46	0.21
γ -Cadinene	1513	1481.7	0.80	1.92	0.20	1.50	0.16	0.11
δ -Cadinene	1524	1490.1	0.55	2.67	0.15	3.95	0.43	0.31
<i>trans</i> -Cadinane-1,4-diene	1532	1538.8	0.09	0.51	tr	0.15	0.03	-
α -Cadinene	1538	1504.1	0.02	-	tr	0.06	0.07	0.17
Elemol	1549	1529.4	-	-	-	0.11	-	0.05
Germacrene B	1556	1525.3	-	-	0.09	0.64	0.14	1.91
Germacrene D-4-ol	1574	1544.1	-	-	-	0.10	0.34	0.03
Caryophyllene oxide	1581	1552.6	-	-	0.06	-	0.04	-
Viridiflorol	1590	1563.4	-	-	-	0.16	0.02	0.05
Humulene epoxyde II	1606	1578.6	-	-	-	-	0.06	-
Cedrol	1607	1590.0	-	24.44	-	0.62	-	-
1,10-di- <i>epi</i> Cubenol	1614	1594.9	-	-	-	0.08	-	-
1- <i>epi</i> -Cubenol	1627	1595.4	-	0.65	-	0.20	0.05	0.05
τ -Muurolol	1641	1609.2	-	0.19	0.02	0.93	0.27	0.19
α -Muurolol (Torreyol)	1645	1613.4	-	-	tr	0.20	0.07	tr
α -Cadinol	1653	1621.8	0.04	-	0.04	1.31	0.44	0.30
Shyobunol	1685	1660.4	-	-	-	-	0.02	-

Contd..

Table 1: Chemical composition of berries and leaves essential oils from wild growing *Juniperus excelsa* in R. Macedonia

Components	KIL	KIE	Dojran		Ohrid			
			Berries	Leaves	Berries	Leaves	Berries	Leaves
			2010		2010		2011	
Sclarene	1974	1963.7	-	-	-	-	-	-
Manool oxide	1987	1976.2	-	tr	-	tr	-	-
Abieta-8,12-diene	2022	1989.6	-	-	tr	tr	0.16	0.02
Abietatriene	2055	2024.8	tr	tr	tr	tr	0.16	0.08
Abietadiene	2087	2052.6	tr	tr	tr	tr	0.91	0.47
Abieta-8 (14),13 (15)-diene	2153	2118.9	-	-	-	tr	0.14	0.03
Sandaracopimarinal	2184	2157.5	-	-	tr	tr	0.27	0.03
4- <i>epi</i> -Abietal	2298	2264.2	tr	tr	-	tr	0.35	0.21
Abieta-7,13-diene-3-one	2312	2278.1	-	-	-	-	0.04	-
4- <i>epi</i> -Abetol	2343	2311.6	-	-	-	-	0.03	-
Abietol	2401	2360.9	-	-	-	-	0.06	-
Octacosane	2800	2770.2	-	-	-	-	0.07	-
Other non-terpene compounds (NT)			0.10	-	tr	-	0.07	-
Monoterpene hydrocarbons (MH)			80.05	40.96	80.33	42.80	79.92	42.22
Oxygen containing monoterpenes (OM)			0.06	0.34	10.51	26.28	7.85	44.97
Sesquiterpene hydrocarbons (SH)			3.54	16.11	3.45	15.03	6.82	6.91
Oxygen containing sesquiterpenes (OS)			0.04	25.28	0.12	3.71	1.31	0.67
Diterpenes (D)			tr	tr	tr	tr	2.16	0.84
Total			83.79	82.69	94.41	87.82	98.13	95.61

KIL=Kovats's (retention) index - literature data^[6]; KIE=Kovats's (retention) index experimentally determined (AMDIS); (-) - not found; tr=traces<0.02

Furthermore, in the essential oil from leaves can be distinguish the same two chemotypes (pinene and sabinene) as with EOB. The differences between berries and leaves essential oil exist in the sesquiterpenes fractions which is higher in the oils distilled from leaves. This can be seen in the samples originated from south-east RM where the SH and OS fractions are accounted for 16.11% and 25.28%, respectively. However, these fractions were lower in the EOL from south-west RM, ranging from 6.91%-15.03% (for SH) and from 0.67%-3.71% (for OS). Despite the large amount of α -pinene (33.83%), leaves essential oil from Dojran was the only investigated oil that contains large amount of cedrol (24.44%). Additionally, this oil contains the following components: limonene 6.14%, caryophyllene 3.3%, germacrene D 2.26% and γ -cadinene 2.67%. There is difference in the composition of the leaves essential oil among samples from south-western part of RM and those from south-east part, which can be noticed in the EOB samples as well. The EOL was rich in: Sabinene (28.52-59.25%), *cis*-thujone (2.91-26.20%) and *trans*-thujone (0.47-12.86%), followed by large amounts of α -pinene (1.76-6.48%), β -myrcene (2.09-4.81%), γ -terpinene (1.51-3.53%), terpinen-4-ol (2.58-5.87%) and germacrene D (1.21-4.16%). No cedrol was identified in this oil.

Further, comparing to literature data a lot of similarities and differences could be found. Many authors found α -pinene as the most abundant component of *J. excelsa* berries essential oil,^[3-7] presented in amounts of 46.1%,^[4] 34%^[5] or 47.64%.^[7] Turkish authors found that despite

α -pinene, cedrol (12.3%) and verbenol (L-verbenol 5.4% and D-verbenol 4.4%) were important constituents of the oil.^[5] In the samples of oil that originated from Iran, except α -pinene, the dominant constituents were: β -myrcene (5.91%), limonene (4.5%), caryophyllene (3.6%), γ -elemene (5.5%) and cedrol (12.01%).^[7] Opposite to this, data for sabinene-type of *J. excelsa* berries essential oil is rarely reported. Serbian authors found very high amounts of sabinene (75.40%) in berries essential oil of *J. excelsa* from Serbia, followed by: α -pinene (5.2%), β -myrcene (4.96%), limonene (2.25%), terpinene (1.39%), terpinen-4-ol (1.15%) and germacrene D (2.15%).^[12] Iranian authors reported completely different berries oil composition characterized by large amounts of cedrol (28%), α -pinene (22.5%) and limonene (22.7%).^[14]

Considering *J. excelsa* leaves essential oil, Adams reported that the oil from wild growing *J. excelsa* from Greece contained cedrol (28.1%), α -pinene (22.5%) and limonene (22.7%) as predominant constituents.^[9] Some former data point out on *J. excelsa* leaves essential oil as a natural source of cedrol, aroma component valuable for perfume production.^[15] Turkish authors found that *J. excelsa* leaves essential oil consists mainly of α -pinene (29.7%) and cedrol (25.3%).^[5] Furthermore, Iranian researchers confirmed that these two components are predominant in the leaves essential oil from wild growing *J. excelsa* from Iran (α -pinene-32.34% and cedrol-13.06%).^[7] These data are similar with our findings for leaves essential oil composition of *J. excelsa* growing in south-east of RM. On the contrary, the results that we present for *J. excelsa* samples from south-western

part of RM have sabinene and thujone as predominant compounds of the leaves oil [Table 1], a combination of constituents which is reported for the first time. This characteristic essential oil composition is most likely due to the specific geographic origin where the samples were collected. Some authors pointed out on seasonal variations of *J. excelsa* berries and leaves essential oils, putting stress on harvest season as very important factor that influence the oil composition.^[16] Adams *et al.*, revealed presence of moderate geographical variations in the volatile leaves oil of *J. excelsa*, comparing the samples from Greece, Bulgaria, Turkey and Cyprus.^[17] Cedrol was found as the most abundant constituent of the oils, ranged continuously from 11.3-35.8%.

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*. The most sensitive bacteria to berries essential oil was *Haemophilus influenzae* (MIC = 31 µl/ml) [Table 2]. The pinene-type of essential oil showed moderate antimicrobial activity against *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Corynebacterium* spp. and *Campylobacter jejuni* with MIC >50%. On the other hand, the sabinene type of the oil showed moderate activity toward four bacteria: *Streptococcus pyogenes*, *Haemophilus influenzae*, *Campylobacter jejuni* and *Escherichia coli* (MIC >50%). Both type of berries essential oil, pinene and sabinene types, showed no activity against *Candida albicans*. *J. excelsa* leaves essential oil has activity against: *Staphylococcus aureus*, *Streptococcus pyogenes* and *Haemophilus influenzae* with MIC values of 125 µl/ml [Table 2]. *Candida albicans* was resistant to the antimicrobial activity of the tested oils.

Antimicrobial activity of berries and leaves essential oil of *J. excelsa* was previously investigated and literature data showed wide range of antimicrobial activity against various tested microbial strains.^[6,8,11] *J. excelsa* essential oil have shown strong antimicrobial effects against anaerobic bacterium *Clostridium perfringens* and moderate activity toward *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Mycobacterium smegmatis*, *Candida albicans* and *Candida krusei*.^[11] α-Pinene has been considered as antimicrobial active component responsible for the activity of *J. excelsa* essential oils.^[11] Besides moderate to no activity against *Candida*, berries essential oil of *J. excelsa* have shown strong antifungal activity against 10 strains of pathogenic fungi such as *Aspergillus niger*, *A. flavus*, *Fusarium tricinctum*, *Penicillium ochrochloron*, *P. funiculosum*, *Phomopsis helianthi*, *Trichoderma viride*, *Trichophyton mentagrophytes*, *Microsporum canis* and *Epidermophyton floccosum*. The MIC and MFC (minimal fungicidal concentration) values were very low, ranging between 6-35 µl/ml and 8-45 µl/ml, respectively.^[12] In

Table 2: Antimicrobial activity of berries and leaves essential oils of *J. excelsa*

Microorganism	Dojran		Ohrid	
	Berries	Leaves	Berries	Leaves
	2010		2011	
<i>Streptococcus pneumoniae</i>				
DD	M	R	R	R
MIC	>50%	n.m.	n.m.	n.m.
<i>Staphylococcus aureus</i>				
DD	M	S	R	S
MIC	>50%	125	n.m.	125
<i>Staphylococcus epidermidis</i>				
DD	R	R	R	R
MIC	n.m.	n.m.	n.m.	n.m.
<i>Streptococcus agalactiae</i>				
DD	M	R	R	R
MIC	>50%	n.m.	n.m.	n.m.
<i>Streptococcus pyogenes</i>				
DD	M	R	M	S
MIC	>50%	n.m.	>50%	125
<i>Enterococcus</i>				
DD	R	R	R	M
MIC	n.m.	n.m.	n.m.	>50%
<i>Corynebacterium</i> spp.				
DD	M	R	R	R
MIC	>50%	n.m.	n.m.	n.m.
<i>Haemophilus influenzae</i>				
DD	S	M	M	S
MIC	31	>50%	>50%	125
<i>Acinetobacter</i> spp.				
DD	R	R	R	R
MIC	n.m.	n.m.	n.m.	n.m.
<i>Escherichia coli</i>				
DD	R	R	M	R
MIC	n.m.	n.m.	>50%	n.m.
<i>Salmonella enteritidis</i>				
DD	R	R	R	R
MIC	n.m.	n.m.	n.m.	n.m.
<i>Shigella flexneri</i>				
DD	R	R	R	R
MIC	n.m.	n.m.	n.m.	n.m.
<i>Campylobacter jejuni</i>				
DD	M	M	M	R
MIC	>50%	>50%	>50%	n.m.
<i>Klebsiella pneumoniae</i>				
DD	R	R	R	R
MIC	n.m.	n.m.	n.m.	n.m.
<i>Pseudomonas aeruginosa</i>				
DD	R	R	R	R
MIC	n.m.	n.m.	n.m.	n.m.
<i>Proteus mirabilis</i>				
DD	R	R	R	R
MIC	n.m.	n.m.	n.m.	n.m.
<i>Candida albicans</i>				
DD	R	R	R	R
MIC	n.m.	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

this regard, authors suggested that this essential oil could be used in production of food as natural preservative of fungal contamination.

CONCLUSION

Chemical composition of the essential oil of Macedonian *J. excelsa* was variable depending on the regions of collection, therefore two different chemotypes of berries and leaves essential oils were distinguished: Pinene-type, originated from south-eastern part of RM and sabinene-type, from south-western part of the country. The most abundant components in both chemotypes were monoterpenes. The pinene chemotype contained 70.81% (in EOB) and 33.83% (in EOL) α -pinene, followed by: limonene, β -pinene and myrcene, while the sabinene-type of oils despite sabinene (EOB = 58.85-62.58%; EOL = 28.52-29.49%), were rich in α -pinene, β -myrcene, limonene, *cis*-thujone, terpinolene and α -thujene. However the chemotype did not influenced the sesquiterpenes which were present in smaller amounts in the berries essential oils compared to the leaves essential oils.

The leaves essential oil from south-eastern part of RM was rich in cedrol (24.44%), a component absent from the EOLs from south-western part and has tujones as one of the predominant constituents.

The most sensitive bacteria to the antimicrobial activity of berries essential oil was *Haemophilus influenzae* (MIC = 31 μ l/ml). The pinene-type of EOB showed moderate antimicrobial activity against *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Corynebacterium* spp. and *Campylobacter jejuni* (MIC > 50%). The sabinene type of the oil showed moderate activity to *Streptococcus pyogenes*, *Haemophilus influenzae*, *Campylobacter jejuni* and *Escherichia coli* (MIC > 50%). However, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Haemophilus influenzae* (MIC = 125 μ l/ml) were sensitive to *J. excelsa* leaves essential oil. Both chemotypes of berries and leaves essential oils showed no activity against *Candida albicans*.

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