Macedonian pharmaceutical bulletin, **59** (1, 2) 41 - 48 (2013)
ISSN 1409 - 8695
UDC: 615.281.032.671:615.454.1
Short communication

Chemical composition and antimicrobial activity of berry essential oil of *Juniperus oxycedrus* L. (Cupressaceae) grown wild in Republic of Macedonia

Floresha Sela[1], Marija Karapandzova[1*], Gjose Stefkov[1], Ivana Cvetkovikj[1], Elena Trajkovska-Dokikj[2], Ana Kaftandzieva[2], Svetlana Kulevanova[1]

[1]*Institute of Pharmacognosy, Faculty of Pharmacy, University"Ss. Cyril and Methodius", Skopje, R. Macedonia*
[2]*Institute of Microbiology and Parasitology, Faculty of Medicine, University"Ss. Cyril and Methodius", Skopje, R. Macedonia*

Received: April 2013; Accepted: June 2013

Abstract

Chemical composition and antimicrobial activity of essential oil isolated from berries from 2 different samples of *Juniperus oxycedrus* L. (Cupressaceae), growing wild in Republic of Macedonia was investigated. Performing GC/FID/MS analysis, one hundred components were identified, representing 96.0-98.95% of the oil. The major components were α-pinene (22.54- 27.12%), myrcene (11.26- 15.13%) and limonene (2.78-18.06%). Antimicrobial screening of the *J. oxycedrus* 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 was *Haemophilus influenzae* (MIC = 125 μl/ml). The essential oils showed moderate antimicrobial activity against *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Streptococcus agalactiae, Streptococcus pyogenes*, *Corynebacterium* spp., *Escherichia coli* and *Campilobacter jejuni* (MIC > 500 μl/ml) and no activity against *Candida albicans, Staphylococcus epidermidis, Acinetobacter* spp*., Salmonella enteritidis, Shigella flexnery, Klebsiella pneumonia, Pseudomonas aeruginosa, Enterococcus* and *Proteus mirabilis.*

Keywords: *Juniperus oxycedrus*, berry essential oil, oil composition, GC/FID/MS, antimicrobial activity.

Introduction

Juniperus oxycedrus L. (Cupressaceae) is a hardy spreading plant, that vary in size from a low shrub to a tree of about 6 m. The leaves are tiny, narrow and prickly and are reason for people' name of the plant:"Prickly Juniper". The cones are yellow and rounded, and the fruits are berry-like, growing in clusters, and black when ripe. The plant is known as Cade juniper as well, mainly because of the oil that is obtained through destructive distillation of the wood. Cade oil is dark, aromatic oil with a strong smoky smell which is used in some cosmetics and traditional skin treatment drugs, as well as an incense (Lamnauer, 2005).

J. oxycedrus is known as Mediterranean equivalent of the common juniper, native across the Mediterranean region from Morocco and Portugal, southern France and east to Iran, Lebanon and Israel. It grows on rocky sites from sea level up to 1600 m altitude. Among *J. oxycedrus* interesting subspecies are *J. oxycedrus* var. *badia* H.Gay (syn. *J. oxycedrus* subsp. *badia* (H.Gay) Debeaux), distinguished on the basis of larger cones and described from northern Algeria, but also reported for Portugal and Spain (Salido et al., 2002; Velasco-Negueruela et al., 2003) and *J. oxycedrus* subsp. *macrocarpa* (S. et Sm.) Ball., confined to Mediterranean coastal sands, which differs in the broader

* Marija_Karapandzova@ff.ukim.edu.mk; marijakarapandzova@yahoo.com

leaves 2-3 mm wide and larger cones (Stassi et al., 1995).

Aromatical oils from different parts of *J. oxycedrus* have been used since antiquity for fragrance, flavoring, medicinal, antibacterial, insecticidal, and cosmetic purposes. In folk medicine, they have been recommended as a mouth analgesic and for stomach disorders. Berries of this plant are used as a spice, particularly in European cuisine, and essential oil is used for production of beverages such as gin, to which the oil gave distinguishing flavor. The composition of leaf essential oil of *J. oxycedrus* from Morocco (Derwich and Chabir, 2011), Tunisia (Medini et al., 2010), Greece (Adams et al., 1999), Corsica (Boti et al., 2006), and Allgeria (Dob et al., 2006) has been reported in varying details. The oil is characterized by large amount of α-pinene followed by smaller amounts of sabinene, limonene, β-pinene, caryophyllene oxide and myrcene (Derwich et al., 2010; Derwich and Chabir, 2011; Adams et al., 1999). Berry essential oil of *J. oxycedrus* was examined in Italy and similar composition to that of the leaf essential oil was found with predominant amounts of α-pinene followed by smaller amounts of β-pinene, δ-3-carene, sabinene, myrcene, α-phellandrene, limonene and germacrene D (Angioni et al., 2003). Additionally, authors found that ripeness of the berries does not have large influence on the oil composition. Boti et al. (2006) found α-pinene, β-phellandrene and δ-3-carene as the most abundant constituents in the berry essential oil from Corsica. The berry essential oil of *J. oxycedrus* ssp. *badia* from Spain contained α-pinene and myrcene as the predominant components (Velasco-Negueruela et al., 2003), while the oil of *J. oxycedrus* ssp. *macrocarpa* contained α-pinene, germacrene D, myrcene, abietadiene and *cis*-calamenene as major constituents (Medini et al., 2012).

J. oxycedrus essential oil possess broad spectrum of biological activities: antioxidant (Loizzo et al. 2007), antimicrobial (Angioni et al., 2003; Medini et al., 2012), antifungal (Cavaleiro et al., 2006), wound healing (Tumen et al., 2012), insecticidal (Athanassiou et al., 2013), herbicidal (Ismail et al., 2011), etc. The oil with predominated content of α-pinene and β-myrcene possesses antiviral activity against HIV-1 (Loizzo, 2008). Antibacterial and antifungal activity against different pathological strains that grow on food makes possible use of *J. oxycedrus* essential oil as food preservative (Stassi at al. 1996).

In Macedonian flora, only *J. oxycedrus* L. subsp. *oxycedrus* is present (Micevski, 1989). This species is spread throughout the whole territory of Republic of Macedonia, except on very high altitudes on mountains in the continental part. Up to now, *J. oxycedrus* was not chemically investigated and no testing of possible biological and pharmacological activities was performed. Therefore the aim of the present study was analysis of chemical composition of berry essential oil of this plant and evaluation of its antimicrobial activity.

Material and methods

Plant materials

The terminal twigs with berries were collected from two different localities in R. Macedonia: Velestovo village near Ohrid Lake and Vodno Mtn., near Skopje, in late autumn 2010 and 2012. Plant identity was verified as *Juniperus oxycedrus* L., and herbarium voucher specimen N° JO-1/10, N° JO-1/12, were deposited at the Department of Pharmaceutical Botany, Institute of Pharmacognosy, Faculty of Pharmacy, Skopje, R. Macedonia.

The plant material was dried at room temperature. Just before essential oil isolation, the berries were separated from leaves and branches 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

Essential oil isolation from plant material was made by steam distillation in special 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, Haemophylus influenzae, Proteus mirabilis, Salmonella enteritidis, 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 from Merck (Darmstadt, Germany), blood agar (Oxoid, Basingstoke, UK) and Sabouraund agar (bioMerieux, Durham, NC) were used for growing of the microbial strains.

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 microorganisms - S).

Broth dilution method

This method was used in order to determine minimal inhibitory concentration (MIC) of the particular essential oil (50% solution in DMSO) that had revealed antimicrobial activity by disc diffusion method. 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 (Mueller-Hinton agar, blood agar, Sabouraund 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 obtained essential oils of *J. oxycedrus* were transparent, agile, light yellowish liquids with specific and very strong turpentine odor. Using GC/FID/MS a total of 100 components were identified, representing 96.01-98.95% of the oils. Among different classes of components, the most abundant fraction for Velestovo and Vodno oils were the monoterpene hydrocarbons (MH) (59.23% and 60.43%, respectively), followed by the sesquiterpene hydrocarbons (SH) (21.58% and 28.72%, respectively) (Table 1). Both samples of essential oils, contained smaller amounts of oxygen-containing monoterpenes (OM) (5.63% and 3.67%, respectively) as well as oxygen-containing sesquiterpenes (OS) (9.13% and 5.75%, respectively) (Table 1).

Chemical analysis of the berry essential oil of *J. oxycedrus* showed presence of tree main components: α-pinene (22.54% and 27.12%), β-myrcene (11.26% and 15.13%) and limonene (18.06% and 2.78%). Additionally, β-pinene (2.60% and 3.14%), *trans*-caryophyllene (6.18% and 2.67%), α-humulene (4.65% and 2.68%), and δ-cadinene (4.16% and 2.23% for Velestovo and Vodno, respectively) were determined in smaller amounts. Larger amounts of germacrene D (11.50%) were found only in the sample from Vodno.

Table 1. Chemical composition (%) of *Juniperus oxycedrus* berry essential oils

No.	Components	KIL	KIE	Velestovo 2010	Vodno 2012
1	Tricyclene	921	938.9	0.02	0.03
2	α-Thujene	931	940.8	0.26	0.75
3	α-Pinene	932	949.4	22.54	27.12
4	Camphene	946	955.6	0.45	0.26
5	Verbenene	961	966.9	-	0.04
6	Sabinene	969	975.1	1.15	9.17
7	β-Pinene	974	976.2	2.60	3.14
8	β-Myrcene	988	990.6	11.26	15.13
9	α-Phellandrene	1002	997.1	-	0.05
10	δ^3-Carene	1008	1001.5	-	0.13
11	α-Terpinene	1014	1007.2	0.61	0.27
12	*p*-Cymene	1020	1014.3	0.13	0.09
13	Limonene	1024	1018.6	18.06	2.78
14	β-Phellandrene	1025	1019.9	-	-
15	*Z*-β-Ocimene	1032	1037.1	-	0.02
16	*E*-β-Ocimene	1044	1037.3	-	0.04
17	γ-Terpinene	1054	1046.4	1.14	0.54
18	*cis*-Sabinene hydrate	1065	1054.2	-	0.13
19	Terpinolene	1086	1076.3	1.03	0.94
20	Linalool	1095	1091.2	0.69	0.77
21	2-Methyl butyl isovalerate	1103	1095.6	0.22	-
22	*cis*-*p*-Menth-2-en-1-ol	1118	1114.2	-	0.05
23	α-Campholenal	1122	1121.0	-	0.03
24	*trans*-Pinocarveol	1135	1135.4	-	0.03
25	*trans*-*p*-Menth-2-en-1-ol	1136	1138.2	-	0.05
26	*trans*-Verbenol	1140	1143.9	-	0.06
27	Citronelall	1148	1155.0	-	0.02
28	β-Pinene oxide	1154	1159.4	-	0.03
29	Borneol	1165	1163.2	0.14	0.04
30	*p*- Menth-1,5-dien-8-ol	1166	1166.2	-	0.02
31	Terpinen-4-ol	1174	1162.3	1.60	1.13
32	*p*-Cymene-8-ol	1179	1184.4	-	0.04
33	α-Terpineol	1186	1184.6	0.60	0.18
34	Myrtenol	1194	1188.5	-	0.05
35	Methyl chavicol	1195	1190.4	-	0.03
36	Verbenone	1204	1197.4	-	0.04
37	*endo*-Fenchyl acetate	1218	1207.5	-	0.02
38	β-Citronellol	1223	1220.1	0.30	0.15
39	*trans* -Chrysanthenyl acetate	1235	1224.6	-	0.05
40	Thymol methyl ether	1232	1225.3	-	-
41	Neral	1235	1232.2	-	0.08
42	Hexyl isovalerate	1241	1232.6	0.05	-
43	Isoamyl hexanoate	1246	1240.9	0.08	-
44	Geraniol	1249	1247.3	-	0.06
45	*cis*-Myrtanol	1250	1249.2	-	tr
46	Methyl citronellate	1257	1252.7	-	0.05
47	Geranial	1264	1261.8	-	0.07
48	Bornyl acetate	1284	1261.6	0.09	0.25
49	*trans*-Sabinyl acetate	1289	1279.6	1.29	-
50	2-Undecanone	1293	1282.3	-	0.07
51	Terpinen-4-ol-acetate	1299	1286.9	-	0.02
52	Myrtenil acetate	1324	1310.6	-	0.12
53	δ-Elemene	1335	1322.4	0.04	0.22
54	α-Cubebene	1345	1335.3	0.09	0.59
55	Citronellyl acetate	1350	1339.2	-	0.08
56	Dihydro carveol acetate	1356	1350.0	-	0.03

No.	Components	KIL	KIE	Velestovo 2010	Vodno 2012
57	α-Ylangene	1373	1356.7	-	0.02
58	α-Copaene	1374	1361.5	0.20	0.58
59	Geranyl acetate	1379	1368.9	-	0.23
60	β-Bourbonene	1387	1369.9	0.03	-
61	β-Elemene	1389	1378.2	0.23	2.09
62	Longipinene	1400	1384.6	-	-
63	Sibirene	1400	1386.8	-	0.31
64	β–Longifolene	1407	1390.2	-	tr
65	α-*cis*-Bergamotene	1411	1397.9	-	0.01
66	*trans*-(E)-Caryophyllene	1417	1404.0	6.18	2.67
67	β-Copaene	1430	1413.0	0.11	0.35
68	γ-Elemene	1434	1418.3	0.45	2.08
69	α-Guaiene	1437	1419.2	0.07	-
70	6,9-Guaidiene	1442	1424.6	0.08	
71	Unknown	/	1435.6	-	0.19
72	α-Humulene	1454	1439.3	4.65	2.68
73	*cis*-Muurola-4,(14),5-diene	1465	1448.0	tr	0.14
74	Germacrene D	1484	1471.5	1.89	11.50
75	β-Selinene	1485	1473.8	0.11	tr
76	Bicyclogermacrene	1494	1501.7	0.20	-
77	α-Muurolene	1500	1483.7	1.07	1.71
78	γ-Cadinene	1513	1496.9	1.16	0.69
79	δ-Cadinene	1522	1506.8	4.16	2.23
80	*trans*-Cadina-1,4-diene	1533	1514.7	0.15	0.12
81	α-Cadinene	1537	1519.9	-	1.71
82	Elemol	1549	1532.1	0.10	0.19
83	Unknown	/	1537.4	0.42	0.09
84	Germacrene B	1559	1543.3	0.41	1.73
85	Germacrene D-4-ol	1574	1560.7	-	1.37
86	Spathulenol	1577	1563.0	0.06	tr
87	Caryophyllene oxide	1581	1593.2	1.63	0.17
88	Humulene epoxide II	1608	1593.6	1.80	0.17
89	1,10-di-*epi*- Cubenol	1618	1596.5	tr	tr
90	Junenol	1618	1602.4	0.17	tr
91	1-*epi*-Cubenol	1627	1610.7	0.26	0.25
92	γ-Eudesmol	1630	1613.9	0.17	-
93	τ-Murolol	1640	1624.7	1.55	0.88
94	α-Muurolol	1645	1613.4	0.38	tr
95	α-Cadinol	1653	1638.8	2.60	1.16
96	Cadalene	1675	1657.1	tr	-
97	Abieta-8,12-diene	2022	2004.0	tr	tr
98	Abietatriene	2054	2024.8	tr	tr
99	Abietadiene	2080	2172.8	tr	tr
100	Sandarocopimarinal	2184	2172.5	tr	tr
	Total			96.01	98.95
	Monoterpene hydrocarbons (MH)			59.23	60.43
	Oxygen containing monoterpenes (OM)			5.63	3.67
	Sesquiterpene hydrocarbons (SH)			21.58	28.72
	Oxygen containing sesquiterpenes (OS)			9.13	5.75

KIL - Kovat's (retention) index - literature data (Adams, 2007); KIE – Kovat's (retention) index experimentally determined (AMDIS); (-) - not found, tr – traces < 0.02.

Table 2. Antimicrobial activity of the berry essential oil of *Juniperus oxycedrus*.

Microorganism		Velestovo 2010	Vodno 2012
Streptococcus pneumoniae	DD	M	R
	MIC	> 500	n.m.
Staphylococcus aureus	DD	M	R
	MIC	> 500	n.m.
Staphylococcus epidermidis	DD	R	R
	MIC	n.m.	n.m.
Streptococcus agalactiae	DD	M	R
	MIC	> 500	n.m.
Streptococcus pyogenes	DD	M	M
	MIC	> 500	> 500
Enterococcus	DD	R	R
	MIC	n.m.	n.m.
Corynebacterium spp.	DD	M	R
	MIC	> 500	n.m.
Haemophilus influenzae	DD	S	S
	MIC	125	125
Acinetobacter spp.	DD	R	R
	MIC	n.m.	n.m.
Escherichia coli	DD	R	M
	MIC	n.m.	> 500
Salmonella enteritidis	DD	R	R
	MIC	n.m.	n.m.
Shigella flexneri	DD	R	R
	MIC	n.m.	n.m.
Campylobacter jejuni	DD	M	M
	MIC	> 500	> 500
Klebsiella pneumoniae	DD	R	R
	MIC	n.m.	n.m.
Pseudomonas aeruginosa	DD	R	R
	MIC	n.m.	n.m.
Proteus mirabilis	DD	R	R
	MIC	n.m.	n.m.
Candida albicans	DD	R	R
	MIC	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.

Similar composition of the berry essential oil was found for the Spanish *J. oxycedrus* ssp. *badia* with α-pinene (59.8-61.5%) and myrcene (18.5-18.6%) as major constituents of the oil (Velasco-Negueruela et al., 2003). Furthermore, in unripe berries, Salido et al. (2002) found essential oil rich with α-pinene (65%) and smaller amounts of myrcene, limonene, germacrene D and γ-muurolene. Medini et al. (2011) reported that the amounts of α-pinene, germacrene D, myrcene, abietadiene and *cis*-calamenene vary according to the phonological stage of *J. oxycedrus* ssp. *macrocarpa*. Berry essential oil of *J. oxycedrus* ssp. *macrocarpa* from Greece contained 63.03% of α-pinene (Stassi et al., 1995). Comparing to the leaf essential oil composition of *J. oxycedrus*, similarities could be found as the leaf essential oil contain α-pinene as the most abundant constituent (Derwich et al., 2010; Derwich et al., 2011; Boti et al., 2006; Salido et al., 2002; Adams et al., 1999; Stassi et al., 1995; Medini et al., 2010; Angioni et al., 2003).

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 antimicrobial effects of *Juniperus oxycedrus* berry essential oils was *Haemophilus influnzae* (MIC = 125 μl/ml) (Table 2).

The berry essential oil showed moderate antimicrobial activity against *Streptococcus pneumoniae*, Staphylococcus aureus, Streptococcus agalactiae, Streptococcus pyogenes, Corynebacterium spp., Escherichia coli and Campilobacter jejuni with MIC > 500 μl/ml. No activity against Candida albicans, Staphylococcus epidermidis, Acinebacter spp., Salmonella enteritidis, Shigella flexnery, Klebsiella pneumonia, Pseudomonas aeruginosa, Enterococcus and Proteus mirabilis was found.

According to literature data, essential oils of *J. oxycerdrus, J. phoenicea* and *J. communis* were tested against *Candida albicans, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* The results obtained led to a non-significant inhibitory effect, although essential oil of *J. phoenicea* and leaf oil of *J. oxycerdrus* exhibited rather good or weak activity against *Candida albicans* and *Staphylococcus aureus* (Angioni et al., 2003). The study of the antimicrobial activity of leaf essential oil of *J. oxycedrus* from Tunisia showed that *Escherichia coli* was found to be extremely resistant to this oil while *Staphylococcus aureus* was the most sensitive strain with MIC ranged from 600 to 650 μg/ml (Medini et al., 2012). *J. oxycerdrus* leaf essential oil provided to be an emergent alternative as antifungal agent against dermatophyte strains. δ-3-carene was shown to be a fundamental compound for this activity (Cavaliero et al., 2006).

Conclusion

The major components in the essential oil of two samples of berries of *Juniperus oxycedrus* L. (Cupressaceae), growing wild in R. Macedonia were α-pinene, myrcene and limonene. β-pinene, caryophyllene, α-humulene and δ-cadinene were determinate in smaller amounts while larger amount of germacrene D was found only in one sample of the oil. Antimicrobial screening of the essential oils showed that the most sensitive bacteria was *Haemophilus influenzae* (MIC = 125 μl/ml). The *J. oxycedrus* essential

oil showed moderate antimicrobial activity against *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Streptococcus agalactiae, Streptococcus pyogenes*, *Corynebacterium* spp., *Escherichia coli* and *Campilobacter jejuni* (MIC > 500 μl/ml) and no activity against other investigated bacteria and *Candida albicans*.

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Резиме

Хемиски состав и антимикробна активност на етерично масло од бобинки од диворастечка црвена смрека *Juniperus oxycedrus* L. (Cupressaceae) од Р. Македонија

Флореша Села[1], Марија Карапанџова[1]*, Ѓоше Стефков[1], Ивана Цветковиќ[1], Елена Трајковска-Докиќ[2], Ана Кафтанџиева[2], Светлана Кулеванова[1]

[1]*Институт за фармакогнозија, Фармацевтски факултет, Универзитет "Св. Кирил и Методиј", Скопје, Република Македонија*

[2]*Институт за микробиологија и паразитологија, Медицински факултет, Универзитет "Св. Кирил и Методиј", Скопје, Република Македонија*

Клучни зборови: *Juniperus oxycedrus*, етерично масло од бобинки, состав на етерично масло, GC/FID/MS, антимикробна активност.

Хемискиот состав и антимикробната активност се испитувани на 2 различни примероци на етерично масло од бобинки од диворастечкиот *Juniperus oxycedrus* L. (Cupressaceae) од Република Македонија. Со GC/FID/MS анализа, идентификувани се 100 компоненти и истите сочинуваат 96,00-98,95 % од маслото. Најзастапените компоненти во маслото се α-пинен (22,54-27,12 %), мирцен (11,26-15,13 %) и лимонен (2,78-18,06 %). Антимикробната активност на етеричните масла е испитувана со диск дифузиона и диск дилуциона метода на 16 бактериски изолати на Грам позитивни и Грам негативни бактерии и еден изолат на габата *Candida albicans*. Најголема осетливост на дејството на маслата покажа бактеријата *Haemophilus influenzae* (MIC=125 μl/ml). Етеричните масла покажаа умерена антимикробна активност кон *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Corynebacterium* spp., *Escherichia coli* и *Campilobacter jejuni* (MIC> 500 μl/ml). Етеричните масла не покажаа активност кон *Candida albicans*, *Staphylococcus epidermidis*, *Acinetobacter* spp., *Salmonella enteritidis*, *Shigella flexnery*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Enterococcus* и *Proteus mirabilis*.