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Antimicrobial activity of berries and leaves essential oils of Macedonian *Juniperus foetidissima* Willd. (Cupressaceae)

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Abstract

Chemical composition and antimicrobial activity of leaves and berries essential oils from *Juniperus foetidissima* Willd. (Cupressaceae) grown in R. Macedonia (RM) was investigated. GC/FID/MS analysis was carried out and 93 components were identified, representing 89.7-96.5% of the oils. The major components of the berries essential oil were α-pinene (19.2%), limonene (24.9%) and cedrol (23.1%), followed by smaller amounts of β-funebrene, *trans*-caryophyllene, germacrene D and δ-cadinene. The composition of the leaves essential oil was variable depending on the region of collection. Accordingly, samples originated from southeastern RM contained essential oil with α-pinene (67.6%) and limonene (10.0%), from central part of RM with limonene (17.9-27.1%) and cedrol (28.8-33.9%), while samples from southwestern RM contained oil with terpinen-4-ol (19.1%), *cis*-thujone (8.3%), germacrene D (11.0%) and δ-cadinene (6.3%) as predominant components in the oil. Antimicrobial screening of the essential oils was made by disc diffusion and broth dilution method against 16 bacterial strains of Gram-positive and Gram-negative bacteria and one strain of *Candida albicans*. The leaves essential oil showed stronger antimicrobial activity against *Staphylococcus aureus, Streptococcus pyogenes* and *Haemophilus influenzae* (MIC = 125 μl/ml) and moderate activity against *Campylobacter jejuni* (MIC > 500 μl/ml). Other investigated bacterial strains and *Candida albicans* were completely resistant to the antimicrobial activity of *J. foetidissima* essential oils.

Keywords: *Juniperus foetidissima*, leaves, berries, essential oil, GC/FID/MS composition, antimicrobial activity

Introduction

Juniperus is one of the major genera of Cupressaceae family consisting of approximately 70 species variable in size and shape, from tall trees to columnar or low spreading shrubs. The plants are evergreen with needle-like or scale-like leaves. *Juniperus foetidissima* Willd. is a medium-size tree, spread mainly throughout the southeastern Europe and southwestern Asia, starting from southeastern Albania and northern Greece, across Turkey, Syria and Lebanon to the northern Iran and southwestern Turkmenistan. It often occurs together with *J. excelsa* Bieb., but it could be distinguished by its thicker shoots and green leaves (Marcysiak et al., 2007). The name *foetidissima* comes from the stinking (fetid) smell of crushed leaves.

Traditionally *Juniperus* species are used for curing different disorders and pathological conditions. There are few investigations regarding the biological activities of *J. foetidissima*, with reports on antifungal (Balaban et al., 2003), antimicrobial (Asili et al., 2010), cytotoxic (Sadaeghi-Aliabdi et al., 2009), anticholinesterase (Ozturk et al., 2010), fumigant (Tayoub et al., 2012), anti-inflamatory (Orhan et al., 2012; Lesjak et al., 2013) and antioxidant effects (Lesjak et al., 2013; Emmami et al., 2007, Emami et al., 2011). These activities have been shown due to the complex che-

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mical pattern of terpene components.

J. foetidissima contains essential oil in almost all parts of the plant, with variable composition. Thus, sabinene, α-thujone, terpinen-4-ol and γ-terpinene were reported as major components of the leaf essential oil of *J. foetidissima* from Greece (Adams, 1987; Adams, 1990). The monoterpenes sabinene, α-pinene and limonene were predominant components of the essential oils of fruits and leaves of male and female plant of *J. foetidissima* from Iran (Asili et al., 2010). Turkish *J. foetidissima* contained β-thujone and cedrol as major component in the leaf essential oil and sabinene as predominant in berry oil. The major components of seed and seedless cone berry oils were sabinene, β-thujone and abietal while in the oil from branches the most abundant was α-pinene (Tunalier et al., 2002). On the other hand, *J. foetidissima* heartwood of the root and stem was identified as the new potential source of Cedarwood oil (Tunalier et al., 2004), while in the essential oil obtained from branches of *J. foetidissima* grown in Syria, citronellol, bornyl acetate and cadalene were found as major constituents (Tayoub et al., 2012). These oils are considered as potential sources of various terpene components as well as biologically active agent.

J. foetidissima occurs in the flora of Republic of Macedonia (RM). Mainly, it grows in southern parts of the country, but could be found in the valleys of the River Crn Drim and the River Treska in western and Karadzica Mtn. in central RM (Micevski, 1998). Up to now only one report on chemical composition, an antioxidant and anti-inflammatory effects of one sample of Macedonian *J. foetidissima* was published (Lesjak et al., 2013). The present study was aimed to determine the chemical composition and to evaluate the antimicrobial activity of the essential oils obtained from berries and leaves from *J. foetidissima* collected on several locations in RM.

Material and methods

Plant materials

The terminal plant twigs were collected from four different localities in RM (Table 1). Plant identity was verified as *Juniperus foetidissima* Willd. and herbarium voucher specimens were deposited at the Department of Pharmaceutical Botany, Institute of Pharmacognosy, Faculty of Pharmacy, Skopje, RM. The plant material was dried at room temperature. Just before hydroisolation, leaves and berries were separated from the branches and minced properly.

The plant material was collected from 4 localities from central (Veles), southern (Udovo), southeastern (Valandovo) and southwestern (Ohrid) RM. According to this the samples of leaves oils were marked as Ve-LEO, U-LEO, Val-LEO and Oh-LEO, respectively. The sample of berries essential oil was marked as Val-BEO (Table 1).

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, RM).

Essential oil isolation

The essential oils were obtained from dried plant material through distilled stem 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

Table 1. Plant material and samples of essential oil (LEO - leaves essential oil, BEO -berries essential oil)

Locality	m.a.s.l.	Voucher specimen N°	Abbrev. in the text
Region of Valandovo, southeastern RM	100 m	N° JF-1/11	Val-LEO Val-BEO
v. Udovo, River Vardar (Axios), south RM	100 m	N° JF-2/11	U-LEO
Taorska Gorge, River Vardar (Axios), region of Veles, central RM	250 m	N° JF-3/11	Ve-LEO
v. Velestovo, National Park Galichica, Ohrid Lake, southwestern RM.	1000 m	N° JF-4/11	Oh-LEO

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 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 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 of 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 11 bacterial strains (*Staphylococcus epidermidis, Enterococcus, Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus pneumoniae, Haemophilus influenzae, Proteus mirabilis, Corynebacterium* spp*., Shigella flexneri, Campylobacter jejuni, Salmonella enteritidis* 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. Only, *Campylobacter* was incubated in microaerophilic atmosphere, at 42°C for 48 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 microorganisms (S)).

Broth dilution method

This method was used in order to determine minimal inhibitory concentration (MIC) of the particular essential oil that had revealed antimicrobial activity by disc diffusion method. For that purposes, 25 μl of those essential oils (50% solution of essential oil in DMSO = 500 μl/ml) were diluted in equal quantities of 0.9% sodium chloride solution, to make them with concentration of 25% (250 μl/ml). 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, 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 discusion

The chemical composition of essential oils

The chemical composition of the essential oils was analyzed by GC/FID/MS and 93 components in total were identified, representing 89.7-96.5% of the oils (Table 2). Among different classes of terpenes present in the berries oil isolated from samples from Valandovo, the monoterpene hydrocarbons (MH) and the oxygen-containing sesquiterpenes (OS) were the major fractions with total participation of 47.2% and 26.8%, respectively. The mass part of sesquiterpene hydrocarbons (SH) and oxygen containing monoterpenes (OM) were 19.7% and 0.7%, respectively (Table 2). The major components of the essential oil were limonene (24.9%), α-pinene (19.2%) and cedrol (23.1%).

Table 2. Chemical composition (%) of leaves (LEO) and berries (BEO) essential oils of *Juniperus foetidissima* from R. Macedonia

No.	Components	RI	RIE	Val-BEO	Val-LEO	U-LEO	Ve-LEO	Oh-LEO
1	Tricyclene	921	930.4	tr	0.1	tr	tr	-
2	α-Thujene	931	933.8	-	-	-	-	tr
3	α–Pinene	932	937.2	19.2	67.6	9.6	14.7	-
4	Camphene	946	945.5	0.6	1.0	0.5	0.2	1.3
5	Sabinene	969	962.8	tr	-	-	-	1.5
6	β-Pinene	974	964.1	0.4	1.4	02	0.1	-
7	β-Myrcene	988	976.4	1.1	1.9	0.7	0.6	0.2
8	Δ^3-Carene	1008	989.8	-	0.4	-	3.4	-
9	*p*-Cymene	1020	1001.8	0.2	0.3	0.3	0.2	-
10	Limonene	1024	1006.7	24.9	10.0	27.1	17.9	0.8
11	γ-Terpinene	1054	1033.4	0.3	0.5	0.2	0.1	1.3
12	α-Terpinolene	1086	1062.5	0.4	0.7	0.3	0.6	0.8
13	Linalool	1095	1077.0	-	-	-	-	0.4
14	*cis*-Thujone	1101	1079.2	-	-	-	-	8.3
15	*trans*-Thujone	1112	1090.9	-	-	-	-	2.6
16	*trans*-Pinocarveol	1135	1117.1	-	-	-	-	-
17	Camphor	1141	1123.7	0.1	0.2	0.1	0.2	-
18	Borneol	1165	1150.0	tr	0.1	tr	-	-
19	*cis*-Pinocamphone	1172	1157.4	-	-	tr	-	-
20	Terpinen-4-ol	1174	1161.8	0.1	-	0.1	0.1	19.1
21	*p*-Cymene-8-ol	1179	1171.1	-	-	0.3	-	-
22	α-Terpineol	1186	1175.1	-	-	tr	-	0.5
23	Myrtenal	1195	1176.8	-	-	-	-	-
24	*cis*-Piperitol	1195	1177.7	-	-	-	-	0.6
25	Verbenone	1204	1187.4	-	0.1	-	0.1	-
26	*trans*-Piperitol	1207	1187.4	-	-	tr	-	0.4
27	Fenchyl acetate	1218	1194.6	0.2	0.1	0.4	0.2	-
28	*cis*-Mentha-1(7),8-dien-2-ol	1227	1200.9	-	-	0.4	-	-
29	Carvone	1239	1218.8	-	-	0.4	-	-
30	Carvacrol methyl ether	1241	1219.6	-	-	-	-	-
31	Linalool oxide acetate	1287	1263.4	0.1	-	0.1	0.1	0.4
32	*trans*-Sabinyl acetate	1289	1268.5	-	-	-	-	2.1
33	2E,4Z-Decadienal	1292	1269.2	-	-	0.1	0.1	-
34	2E, 4E-Decadienol	1319	1287.8	-	-	2.3	2.9	-
35	Dihydrocarveol acetate	1326	1297.3	-	-	0.4	-	-
36	δ-Elemene	1335	1306.0	-	-	0.1	0.3	0.1
37	α-Cubebene	1345	1318.6	0.2	-	0.1	0.2	0.1
38	α-Copaene	1374	1345.4	0.3	tr	0.1	0.2	3.7
39	β-Bourbonene	1387	1354.5	0.1	-	0.1	0.1	0.1
40	7-*epi*-Sesquithujene	1390	1359.7	-	-	0.2	0.2	-
41	β-Elemene	1389	1362.2	-	-	-	-	0.7
42	Sesquithujene	1405	1374.1	0.1	tr	0.1	0.1	-
43	β-Funebrene	1413	1381.3	3.3	-	4.3	3.9	-
44	*trans*-Caryophyllene	1418	1386.9	1.8	tr	tr	-	1.8
45	β-Cedrene	1419	1387.7	tr	0.5	2.7	2.0	-
46	β-Copaene	1430	1396.1	-	-	-	-	0.6
47	*cis*-Thujopsene	1430	1397.4	0.7	0.2	0.8	0.7	-
48	γ-Elemene	1434	1399.7	-	-	-	-	1.6
49	α-Guaiene	1437	1405.3	0.1	-	-	0.1	0.1
50	Aromadendrene	1439	1412.1	tr	-	-	-	0.5

No.	Components	RI	RIE	Val-BEO	Val-LEO	U-LEO	Ve-LEO	Oh-LEO
51	*trans*-Muurola-3,5-dien	1451	1418.2	0.5	tr	0.2	0.2	0.2
52	α-Humulene	1452	1421.8	0.9	0.2	tr	tr	1.4
53	β-Farnesene	1454	1422.5	tr	-	0.8	0.7	-
54	α-Acoradiene	1464	1434.4	0.3	0.1	0.4	0.3	-
55	10-*epi*-β-Acoradiene	1474	1434.4	0.3	0.1	0.4	0.4	-
56	*trans*-Cadina-1(6),4-diene	1475	1441.3	1.0	-	0.6	0.6	0.2
57	γ-Muurolene	1478	1444.6	0.3	0.2	-	-	0.62
58	Germacrene D	1484	1449.9	1.5	0.2	0.5	0.4	11.0
59	α-Muurolene	1500	1468.3	1.8	-	-	-	2.2
60	β-Himachalene	1500	1467.8	-	0.1	0.3	0.3	-
61	Cuprenene	1505	1474.2	0.3	0.1	0.5	0.4	0.2
62	α-Alaskene	1512	1480.0	0.8	0.3	1.0	1.1	-
63	γ-Cadinene	1513	1482.1	0.8	-	-	-	1.4
64	δ-Cadinene	1522	1490.3	2.9	0.2	1.5	1.2	6.3
65	*trans*-Cadina-1,4-diene	1533	1538.8	0.6	-	0.4	0.5	0.2
66	α-Cadinene	1537	1504.1	0.1	-	-	-	0.6
67	Hedycariol	1546	1556.0	-	-	-	-	0.1
68	Germacrene B	1559	1525.6	-	-	-	-	1.1
69	β-Calacorene	1564	1530.5	0.1	-	0.2	-	-
70	Germacrene D-4-ol	1574	1542.9	-	-	tr	-	1.1
71	Caryophyllene oxide	1581	1552.6	0.2	0.4	0.2	0.1	0.2
72	*allo*-Cedrol	1589	1560.1	1.7	-	1.9	2.2	-
73	Cedrol	1600	1576.2	23.1	9.6	28.8	33.9	-
74	β-Oplopenone	1607	1577.8	-	-	-	-	0.9
75	1,10-di-*epi*-Cubenol	1618	1582.8	-	-	-	-	0.1
76	1-*epi*-Cubenol	1627	1595.2	1.3	0.1	0.7	0.8	0.2
77	α-Acorenol	1632	1601.7	0.2	-	0.2	0.3	-
78	*epi*-α-Muurolol	1640	1609.7	0.4	-	0.2	-	2.1
79	α-Muurolol	1645	1613.4	0.1	-	0.1	0.4	0.5
80	α-Cadinol	1653	1621.9	0.2	-	2.1	0.2	3.4
81	β-Atlantone	1668	1629.4	-	-	0.2	-	-
82	*iso*-Cedranol	1672	1648.9	-	-	0.2	-	-
83	*epi*-α-Bisabolol	1683	1650.2	-	-	0.1	-	-
84	*cis*-14-nor-Muurol-5-en-4-one	1688	1655.2	0.1	-	0.1	-	0.2
85	Cedryl acetate	1767	1732.8	0.1	-	0.1	0.1	-
86	Sandarocopimara-8(14),15-diene	1968	1933.5	-	-	-	-	0.3
87	Manool oxide	1987	1962.4	0.1	-	tr	0.1	2.0
88	Abieta-8,12-diene	2022	1989.0	-	-	-	-	tr
89	Abietatriene	2054	2024.7	tr	-	tr	-	0.2
90	Abietadiene	2080	2051.9	0.1	0.1	tr	0.1	1.2
91	Abieta-8(14),13(15)-diene	2135	2118.9	0.1	-	-	-	0.1
92	Sandaracopimarinal	2184	2157.3	-	-	-	-	0.2
93	4-*epi*-Abietal	2298	2264.5	0.1	-	0.1	0.1	1.6
	Total (%)			95.3	96.5	93.2	93.2	89.7
	Monoterpene hydrocarbons (MH)			47.2	83.2	38.2	38.9	6.0
	Oxygen containing monoterpenes (OM)			0.7	0.5	1.7	0.6	34.8
	Sesquiterpene hydrocarbons (SH)			19.7	2.3	15.4	13.7	34.6
	Oxygen containing sesquiterpenes (OS)			26.8	10.1	34.7	37.9	8.8
	Diterpenes (D)			0.5	0.1	0.1	0.1	5.5
	Non-terpene components (NT)			0.4	0.3	3.1	3.1	0.1

RI - Retention index - literature data (Adams, 2007); RIE – Retention index experimentally determined with reference to a homologous series of *n*-alkanes on an HP-5ms column (AMDIS); (-) - not found, tr - traces < 0.05;

The composition of leaves essential oil was variable depending on the geographical origin. In Val-LEO the MH was the major fraction (83.7%), followed by smaller fraction of OS (10.1%). The predominant components in this oil were: α-pinene (67.6%), limonene (10.0%) and cedrol (9.6%). Ve-LEO and U-LEO were characterized by limonene (17.9% and 27.1%), α-pinene (14.7% and 9.6%) and cedrol (33.9% and 28.8%, respectively). These oils were characterized by almost equal amounts of MH (38.9 and 38.2%) and OS (34.7 and 37.9%, respectively) (Table 1). On the other hand, Oh-LEO contained mainly SH (34.6%) and OM (34.8%). The major components of this oil were completely different from previous oils. Terpinen-4-ol (19.1%), *cis*-thujone (8.3%), germacrene D (11.0%), δ-cadinene (6.3%) and α-cadinol (3.4%) were predominant constituents (Table 2). On the other hand, α-pinene, limonene and cedrol were not identified or were present in amounts < 0.1%. This oil contain larger amounts of diterpenes (5.5%), mainly with abietane skeleton such as abietadiene, abietal, abieta-1,12-dine, abieta-8(14),13(15)-dine, abietatriene and sandaracopimarinal. Val-LEO and Ve-LEO contained some of the mentioned diterpenes in amounts < 0.1% or in traces.

Comparing to literature data, similarities and differences could be noticed. Adams found sabinene (19.6%), α-thujone (18.6%), terpinen-4-ol (17.6%) and γ-terpinene (6.5%) being the major components of the leaf essential oil of *J. foetidissima* collected in Greece (Adams, 1990). At the same time, the minor compounds of this essential oil were α-terpinene (4.3%), β-thujone (3.5%), cedrol (3.2%), myrcene (2.7%) and α-pinene (2.6%). According to the Iranian researchers, the major components of the essential oils of fruits, leaves of male and leaves of female plant of *J. foetidissima* were sabinene (37.1, 19.9 and 16.8%), α-pinene (29.9, 22.2 and 18.6%) and limonene (11.8, 20.9 and 13.6%), respectively (Asili et al., 2010). Tunalier et al. found β-thujone and cedrol as predominant components of the essential oil of leaf and sabinene as major component in the berry essential oil of *J. foetidissima* from Turkey (Tunalier et al., 2002). Considering essential oil composition of *J. foetidissima* from Balkans, only one article was published. Lesjak et al. examined one sample from region of Prespa Lake from RM and found sabinene (39.9%), γ-terpinene (10.1%) and terpinen-4-ol (17.0%) as major monoterpenes and germacrene D (0.7%) and γ-cadinene (2.9%) as major sesquiterpene (Lesjak et al., 2013). Although the mentioned region of collection was geographically close to Ohrid Lake, where our samples were collected, the leaves oil composition differed mainly in the percentage amounts of *trans*-thujone (2.6%) and *cis*-thujone (8.3%) that were present in our oil samples, but were not identified by Lesjak et al.

Antimicrobial activity of essential oils

Antimicrobial screening of the essential oils of Macedonian *J. foetidissima* 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 results obtained showed weak antimicrobial activity of both leaves and berries essential oils. The sensitive bacteria to antimicrobial effects of leaves essential oils were *Staphylococcus aureus, Streptococcus pyogenes* and *Haemophilus influenzae* (MIC = 125 μl/ml). The berry essential oil showed moderate activity against *Streptococcus pyogenes* and *Haemophilus influenzae* (MIC > 500 μl/ml). Both leaves and berries essential oils showed moderate antimicrobial activity against *Campylobacter jejuni* (MIC > 500 μl/ml). Other investigated bacterial strains and *Candida albicans* were completely resistant to the antimicrobial effects of *J. foetidissima* essential oils (Table 3).

Antimicrobial activity of *Juniperus* essential oils was previously investigated and literature data pointed out wide range of activity from no antimicrobial effects to some antimicrobial activity against various tested microbial strains. Juniper essential oil obtained from the juniper berry (*J. communis*) have shown bactericidal activities against Gram-positive and Gram-negative bacteria species, 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 (from 0.39 to 2%, V/V). GC/MS analysis of this essential oil showed that the main compounds in the oil were α-pinene (29.17%) and β-pinene (17.84%), sabinene (13.55%), limonene (5.52%), and myrcene (0.33%) (Pepelnjak et al., 2005). The essential oil of *J. communis* growing wild in Kosovo, showed moderate to high activity against *Staphylococcus aureus*, *Escherichia coli* and *Hafnia alvei*. *Pseudomonas aeruginosa* was resistant to this essential oil (Haziri et al., 2013). Furthermore, *J. excelsa* essential oil have shown strong activity against anaerobic bacteria *Clostridium perfringens* and moderate activity against *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumonia, Mycobacterium smegmatis, Candida albicans* and *Candida krusei* (Inlu et al., 2008). Besides moderate to no activity against *Candida*, berry essential oil of *J. excelsa* have shown strong antifungal activity toward 10 strains of pathogenic fungi. In this way, authors suggested that this essential oil can be used in production of food as natural preservative of fungal contamination. Additionally, α-pinene has been considered as antimicrobial active component responsible for the activity of *J. excelsa* essential oils (Sokovic et al., 2004). The antimicrobial activity of essential oil of *J. phoenicea* from Algeria was tested on nine bacterial strains. Variable degree of antimicrobial activity was achieved (Ramadani et al., 2013) and again, the activity was probably due to the high amount of α-pinene in the oil. Angioni *et al*. tested the essential oils of *J. oxycedrus, J. phoenicea* and *J. communis* against *Candida albicans, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* The results ob-

Table 3. Antimicrobial activity of essential oils of *J. foetidissima*

Microorganism		Val-BEO	Val-LEO	Oh-LEO
Streptococcus pneumoniae	DD	R	R	R
	MIC	n.m.	n.m.	n.m.
Staphylococcus aureus	DD	R	S	S
	MIC	n.m.	125	125
Staphylococcus epidermidis	DD	R	R	R
	MIC	n.m.	n.m.	n.m.
Streptococcus agalactiae	DD	R	R	R
	MIC	n.m.	n.m.	n.m.
Streptococcus pyogenes	DD	M	S	M
	MIC	> 500	125	> 500
Enterococcus	DD	R	R	M
	MIC	n.m.	n.m.	> 500
Corynebacterium spp.	DD	R	R	R
	MIC	n.m.	n.m.	n.m.
Haemophilus influenzae	DD	M	S	S
	MIC	> 500	125	125
Acinetobacter spp.	DD	R	R	R
	MIC	n.m.	n.m.	n.m.
Escherichia coli	DD	R	R	R
	MIC	n.m.	n.m.	n.m.
Salmonella enteritidis	DD	R	R	R
	MIC	n.m.	n.m.	n.m.
Shigella flexneri	DD	R	R	R
	MIC	n.m.	n.m.	n.m.
Campylobacter jejuni	DD	M	M	R
	MIC	> 500	> 500	n.m.
Klebsiella pneumoniae	DD	R	R	R
	MIC	n.m.	n.m.	n.m.
Pseudomonas aeruginosa	DD	R	R	R
	MIC	n.m.	n.m.	n.m.
Proteus mirabilis	DD	R	R	R
	MIC	n.m.	n.m.	n.m.
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.

tained led to a non-significant inhibitory effect, although essential oil of *J. phoenicea* and *J. oxycedrus* 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 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., 2010). Regarding antifungal activity, *J. oxycedrus* leaf essential oil provided to be an emergent alternative as antifungal agent against dermatophyte strains. Delta-3-carene was shown to be a fundamental compound for this activity (Cavaleiro et al., 2006). The evaluation of the biological activity of berry essential oil of Iranian *J. foetidissima* showed no antimicrobial activity against *Candida albicans*, *Escherichia coli* and *Pseudomonas aeruginosa*. The leaf essential oil of the same plant showed moderate activity against *Bacillus subtilis, Candida albicans, Escherichia coli, Pseudomonas aeruginosa* (MIC values between 3.125 and 6.25 mg/ml) and *Staphylococcus aureus* (MIC = 25 mg/ml) (Asili et al., 2010).

In general, *Juniperus* essential oils (*J. communis, J. excelsa, J. phoenicea, J. oxycedrus* and *J. foetidissima*) have

shown stronger antimicrobial activity against *Staphylococcus aureus* and *Candida albicans*, rarely against other bacterial or fungal strains. Only few authors found susceptible antimicrobial activity against *Escherichia coli, Streptococcus pyogenes* and *Streptococcus pneumonia.* Comparing to our findings, leaves essential oil of Macedonian *J. foetidissima* showed similar antimicrobial activity against *Staphylococcus aureus* and *Streptococcus pyogenes.* Promising antimicrobial activity of leaves essential oil against *Haemophilus influenzae* was found for the first time. It is worth to mention that besides good antifungal activity presented in the literature (Asili et al., 2010; Haziri et al., 2013; Unlu et al., 2008), essential oils from Macedonian *J. foetidissima* have shown no activity against *Candida albicans.*

Conclusion

The berries essential oil of Macedonian *J. foetidissima* was characterized by large amount of monoterpene hydrocarbons (MH) (47.2 %) and smaller amounts of oxygen-containing sesquiterpenes (OS) (26.8 %), with: limonene (24.9%), α-pinene (19.2%) and cedrol (23.1%) as predominant constituents. The composition of leaves essential oil was variable depending on the geographical origin. In the oil obtained from samples from south-eastern part of RM, the major fraction was MH (83.7%), followed by smaller fraction of OS (10.1%). The predominant components were: α-pinene (67.6%), limonene (10.0%) and cedrol (9.6%). In the essential oils obtained from samples originated from central part of RM the predominant components were also limonene (17.9% and 27.1%), α-pinene (14.7% and 9.6%) and cedrol (33.9% and 28.8%) respectively. These oils were characterized by almost equal amounts of MH (38.9 and 38.2%) and OS (34.7 and 37.9%, respectively). On the other hand, the essential oil obtained from samples originated from south-western part of RM, contained mainly SH (34.6%) and OM (34.8%) and the major components of this oil were completely different from previous oils. Those were: terpinen-4-ol (19.1%), *cis*-thujone (8.3%), germacrene D (11.0%), δ-cadinene (6.3%) and α-cadinol (3.4%). This oil contain larger amounts of diterpenes (5.5%), mainly with abietane skeleton such as abietadiene, abietal, abieta-1,12-dine, abieta-8(14),13(15)-dine, abietatriene and sandaracopimarinal. The both types of essential oils possessed low capacity for antimicrobial activity. Bacteria sensitive to the antimicrobial effects of leaves essential oils were: *Staphylococcus aureus, Streptococcus pyogenes* and *Haemophilus influenzae* (MIC = 125 μl/ml). The berry essential oil showed moderate activity against *Streptococcus pyogenes* and *Haemophilus influenzae* (MIC > 500 μl/ml). Both leaves and berries essential oils showed moderate antimicrobial activity against *Campylobacter jejuni* (MIC > 500 μl/ml). Other investigated bacterial strains and *Candida albicans* were completely resistant to the antimicrobial effects of *J. foetidissima* essential oils.

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Резиме

Антимикробна активност на етерично масло од бобинки и од игличи од македонскиот *Juniperus foetidissima* Willd. (Cupressaceae)

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Клучни зборови: *Juniperus foetidissima*, иглички, бобинки, етерично масло, GC/FID/MS состав, антимикробна активност

Хемискиот состав и антимикробната активност се испитувани кај етеричното масло од иглички и од бобинки од *Juniperus foetidissima* Willd. (Cupressaceae) кој расте на територија на Р. Македонија. Со GC/FID/MS анализа се идентификувани 93 компоненти кои сочинуваат од 89,7 до 96,5% од маслото. Доминантни компоненти во етеричното масло од бобинки се α-пинен (19,2%), лимонен (24,9%) и цедрол (23,1%), следени со пониска застапеност на β-фунебрен, *trans*-кариофилен, гермакрен D и δ-кадинен. Составот на етеричното масло од иглички варира во зависност од локалитетот на собирање на растителниот материјал. Според тоа, примероците што потекнуваат од југоисточна Македонија содржат етерично масло со доминантни α-пинен (67,6%) и лимонен (10,0%), од централна Македонија лимонен (17,9-27,1%) и цедрол (28,8-33,9%), додека примероците од југозападна Македонија содржат етерично масло со доминантни терпинен-4-ол (19,1%), *cis*-тујон (8,3%), гермакрен D (11,0%) and δ-кадинен (6,3%). Антимикробната активност на етеричните масла е испитувана со агар дифузиона и со агар дилуциона метода кон 16 бактериски изолати на Грам позитивни и Грам негативни бактерии и еден изолат на габата *Candida albicans*. Етеричните масла од иглички покажаа подобра антимикробна активност кон *Staphylococcus aureus, Streptococcus pyogenes* и *Haemophilus influenzae* (МИК = 125 μl/ml) и умерена активност кон *Campylobacter jejuni* (МИК > 500 μl/ml). Останатите испитувани бактерии и *Candida albicans* покажаа резистентност кон антимикробната активност на етерични масла од *J. foetidissima*.