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Chemical characterization and radical scavenging activity of leaves of *Juniperus foetidisima*, *J. excelsa* and *J. communis* from Macedonian flora

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Abstract

Chemical characterization of three *Juniperus* species: *J. foetidisima* (JF), *J. excelsa* (JE) and *J. communis* (JC) from Macedonian flora enclosed determination of yield and essential oil composition of the oils obtained by hydro-distillation of dried leaves and determination of the content of total phenols and total flavonoids in dried plant material. GC/FID/MS analysis showed mainly monoterpene profile of the JC oil and combined monoterpene/sesquiterpene profile of JF and JE oils. Sesquiterpene cedrol was found as an important constituent of the JF and JE, thus the JF oil was characterized by three main components (α–pinene, limonene and cedrol, in amount up to 67.63%, 27.11% and 33.91%, respectively) and JE oil by four components (α–pinene, sabinene, *cis*-thujone and cedrol, in amount up to 33.83%, 29.49%, 26.20% and 24.44%, respectively). The JC oil was free of cedrol, but contained relatively large sesquiterpene fraction (sesquiterpene hydrocarbons and oxygen containing sesquiterpenes in amounts up to 28.64% and 13.57%, respectively). The JC oil was characterized by three monoterpene components (α–pinene, sabinene and terpinen-4-ol, presented up to 28.68%, 16.27% and 12.16%, respectively). The content of total phenols determined by Folin-Ciocalteu method ranged from 96.18-122.91 mg GAE/g dw (water extraction) while the content of total flavonoids ranged from 2.05-11.91 mg CE/g dw (ethanol extraction). Both water and ethanol extracts possessed radical scavenging activity against DPPH radical. Water extracts were more powerful with % of inhibition of DPPH ranging up to 64.52%, 67.40% and 78.23% for water extract (10 mg/ml) of JF, JE and JC, respectively. Obtained results showed correlation with the content of total phenols.

Keywords: *Juniperus communis, Juniperus excelsa, Juniperus foetidisima,* essential oil composition, GC/MS, total phenols, total flavonoids, DPPH.

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 leave-like or scale-like leaves. Many of *Juniperus* species are known and used as medicinal or commercially valuable plants. The common juniper, *Juniperus communis* L. (Cupressaceae), is an evergreen shrub or small coniferous tree, wide spread through the cool temperate Northern Hemisphere. *Juniperus excelsa* Bieb. is a large shrub or tree, spread mainly

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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 Mtn. It occurs in Iran, Pakistan and Oman as well (Khan et al., 2012). *Juniperus foetidissima* Willd. is a medium-size tree, spread mainly throughout the southeastern Europe and southwestern Asia, starting from south-eastern 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 above-ground parts, especially leaves and berries of *Juniperus* species are rich in essential oil that has characteristic aromatic flavour and bitter taste. Due to its diuretic and gastrointestinal properties, common juniper (*J. communis*) is used as medicinal plant for centuries. Besides berry essential oil, other juniper essential oil can be obtained from leaves, wood and seeds of the plant, usually by hydrodistillation (Orav et al., 2010a; Chatzopoulou and Katsiotis, 1993; Kumar et al., 2007). The oils are used in the pharmaceutical and cosmetic industries, for food and beverages, as well as for the production of perfumes. *J. excelsa* is a medicinal plant that has been used in folk medicine to treat dysmenorrhe, cough, bronchitis and colds, jaundice and tuberculosis and to induce menses and expel fetus (Emami et al., 2011). It is known as a remedy for diarrhea, abdominal spasm, asthma, fever, gonorrhea, headache and leucorrhoea (Khan et al., 2012). Among limited biological and pharmacological properties studied *in vitro* such as cytotoxic (Topcu et al., 2005) and antispasmodic activity (Atas et al., 2012), the most investigated were the antioxidant (Atas et al., 2012; Emami et al., 2007; Moein et al., 2010; Emami et al., 2011a) and antimicrobial activity (Ehsani et al., 2012; Atas et al., 2012; Asili et al., 2008; Unlu et al., 2008). However, there are few investigations regarding the biological activities of *J. foetidissima* also, with reports on antifungal (Balaban et al., 2007), 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; Emami et al., 2007; Emami et al., 2011b). These activities are probably result to the complex chemical pattern of terpene and other components.

There is a lot of literature data concerning the chemical composition of different *Juniperus* species, predominantly reporting the essential oil composition of berries and leaves. Thus, for *J. communis* considerable variations in the leaf oil composition were observed depending on the plant origin, however the essential oils is characterized with- α-pinene, sabinene and myrcene, followed by *trans*-(E)-caryophyllene, muurolene, germacrene D and B and humulene (Orav et al., 2010a; Chatzopoulou and Katsiotis, 1993; Kumar et al., 2003, Ottavioli et al., 2009; Filipowicz et al., 2009; Shahmir et al., 2003; Orav et al., 2010b). The major oxygen containing terpenoids were terpinen-4-ol (Chatzopoulou and Katsiotis, 1993), rarely citronellol (Koukos and Papadopoulou, 1997) and terpenyl acetate (Angioni et al., 2003). The *J. excelsa* leaf essential oil is reach in cedrol, α-pinene and limonene (Adams, 1990a), rarely α-pinene (Topcu et al., 2005; Emami et al., 2010b) or cedrol (Topcu et al., 2005; Emami et al., 2010b). *J. foetidissima* contain essential oil in almost all parts of the plant, with variable composition. Sabinene, α-thujone, terpinen-4-ol and γ-terpinene have been reported as major components of the leaf essential oil of *J. foetidissima* from Greece (Adams, 1987; Adams, 1990b). 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). Turhish *J. foetidissima* contained β-thujone and cedrol as major components of the leaf essential oil, while sabinene was predominant component in the berry oil (Tunalier et al., 2002).

On the other hand, there is lack of information for the chemical composition and content of other secondary metabolites derived from the leaves of *Juniperus* species. Modnicki and Labedzka (2009) reported 2.40-3.43% of total phenol contents (TPC) estimated as pyrogallol in leaves of *J. communis*. Aditionally, Moein at al. (2014) found a relationship between antioxidant potentials and phenolic compounds in fruits of *J. excelsa*, pointing out that most active fraction posses the highest reducing power (IC_{50} 61.4 μg/ml) and highest phenolic content (82.9 mg/g) (Moein et al., 2014). Further, the leave extracts of some other *Juniperus* species such as *J. oxycedrus* exhibited very high contents of polyphenols (133.08 mgGAE/g dw) and flavonoids (61.52 mg CE/g dw) (Chaouche et al., 2014).

Juniperus species occur in Macedonian flora as an important floristic elements. *J. communis* is widely spread shrub throughout the whole territory of Republic of Macedonia (RM) (Micevski, 1998). The berries of this plant are extensively utilized in production of blended teas and other herbal medicinal products, in food industry and as a spice in production of alcoholic beverages. For years, the juniper berries and the juniper essential oil are exported from RM. Additionally, the juniper leaves are used in folk medicine for various purposes. *J. excelsa* medicinal properties are also known by people of RM as pain reliever, for curing cold, asthma, edema or skin diseases. *J. foetidissima* 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). Shoots decoction of the plant is known in folk medicine for curing coughs and common cold. Up to now the chemistry of the leaves of these three *Juniperus* species from Macedonian flora is scientifically unknown and only one report on chemical composition, antioxidant and anti-inflammatory effects of Macedonian *J. foetidissima* was published (Lesjak et al., 2013). Therefore the aim of the present study was chemical characterization of the

leaves as well as assessment of radical scavenging activity of leaves extracts from *J. communis, J. excesla* and *J. foetidissima* from RM.

Material and methods

Plant material

Plant samples were collected in late autumn in 2010 and 2011. The terminal twigs of *J. communis* (JC) were collected from tree different localities in RM (Shara Mtn., Galicica Mtn. and Bistra Mtn.) (6 samples), terminal twigs with leaves of *J. excelsa* (JE) from two different localities (Velestovo and Dojran) (4 samples) and terminal plant twigs of *J. foetidissima* (JF) from four different localities in RM (Valandovo, Udovo, Veles and Velestovo) (8 samples). After air-drying in shadow the leaves were separated from stems, packed in paper bags and kept at dark and cool place until analysis.

Chemicals

All chemicals were purchased from Sigma-Aldrich (Missouri, USA), Merck (Darmstadt, Germany) and Alkaloid (Skopje, R. Macedonia).

Essential oil isolation

Essential oil isolation from plant leaves was made by steam distillation in special all-glass Clevenger type apparatus. For that purpose, 50 g of minced and dried leaves was distilled for 4 hours. After isolation, anhydrous sodium sulfate was added to remove residual water from the oil. Essential oil yield was expressed as %, calculated on dried weight (dw).

Extraction procedure

In order to determine the content of total flavonoids and total phenolic compounds, samples containing 1.0 g of powder dried plant material were processed. Two types of extracts were prepared, with 96% ethanol and water. The extraction procedure for sample preparation was performed with 10 ml of extractive solvent for 30 min in ultrasonic bath (50/60 Hz, 720 W). The extracts were filtered and the volume was made up to 10 ml. The obtained ethanol and water extracts were used for evaluation of radical scavenging activity as well.

GC/FID/MS analysis of essential oil

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. For GC/FID/MS analysis, the essential oil was dissolved in xylene to obtain 1 μl/ml oil solution.

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.

Determination of total phenolic content

The total phenolic content (TPC) of the leaves was determined with the Folin-Ciocalteu reagent according to a procedure described by Singleton et al. (1999) with slight modifications. To 1.0 ml of test sample (leaves extract), 0.5 ml (1:10 v/v diluted with distilled water) Folin-Ciocalteau reagent was added and stirred for 5 min at room temperature. After 5 min, 0.4 ml of 7.5% of sodium carbonate was added and made up to 10 ml with distilled water. These mixtures were incubated at room temperature in the dark for 2 hours. After incubation, absorbance of blue color was measured at 765 nm using a UV-Vis spectrophotometer (Agilent 8453 UV-Vis spectrophotometer, Agilent Technologies, USA). The total phenolic content was determined as mg of gallic acid equivalents per gram of dried weight of plant material (mg GAE/g dw) using an equation obtained from standard gallic acid calibration graph.

Determination of total flavonoid content

The total flavonoid content (TFC) was determined using the aluminum chloride assay described by Talari et al. (2012) with slight modification. To an aliquot of the test sample (1.0 ml of extract), 4.0 ml of distilled water and 0.3 ml of 5.0 % sodium nitrite were added and allowed to stand for 5 min. Later, 0.3 ml of 10.0 % aluminum chloride was added and the mixture was incubated for 6 min. 2.0 ml of 1 M sodium hydroxide was added and the volume was made up to 10.0 ml with distilled water. After incubation of 15 min, the mixture turned to pink and the absorbance was

measured at 510 nm using a UV-Vis spectrophotometer (Agilent 8453 UV-Vis spectrophotometer, Agilent Technologies, USA). The TFC was expressed in mg of catechin equivalents per gram of dried weight of plant material (mg CE/g dw) using an equation obtained from standard (+)-catechin calibration graph.

Free radical scavenging activity - DPPH assay

The scavenging activity of DPPH free radical of leaves extracts was done according to the method reported by Gyamfi et al. (1999) with minor modifications. 200 µl of different concentrations of tested samples (100, 50, 20 and 10 mg/ml for ethanol extracts and 10, 5, 2 and 1 mg/ml for water extracts) were placed in a cuvette and 4 ml of 100 µm methanolic solution of DPPH was added. Mixtures were shaken vigorously for 1 min and left to stand 10 min in the dark at ambient temperature. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm. Methanol was used as control.

The percentage of inhibition was calculated from the absorbance of the control (A_c) and the sample (A_s) using the following equation: Inhibition (%) = $[(A_c - A_s) / A_c]$ x 100

Results and discussion

Essential oil yield and chemical composition

The essential oil yield of *Juniperus* leaves was found in variable amounts in all investigated species, ranging from 0.50-1.38 %, 0.16-3.30 % and 0.75-0.90 % dw for JF, JE and JC, respectively (Table 1). GC/FID/MS analysis showed presence of four main classes of components in all investigated samples of oils: monoterpene hydrocarbons (MH), oxygen-containing monoterpenes (OM), sesquiterpene hydrocarbons (SH) and oxygen-containing sesquiterpenes (OS). In some samples diterpenes (D) were also determined in very small amounts as well as some non-terpene components (NT) (Table 1). The monoterpene hydrocarbons were the most abundant fraction in all investigated oils ranging from 39.97% to 53.39%, 40.96% to 42.80% and 38.22% to 83.21%, in the essential oils of JC, JE and JF, respectively. The second dominated fraction was variable for different oils, thus for JC it was sesquiterpene hydrocarbons (12.27-28.64%), while for other two species it was oxygen containing sesquiterpenes, presenting a broad range from 10.10% to 39.71% and from 0.67% to 25.28%, for essential oil of JF and JE, respectively (Table 1).

The two main components in JC essential oil were α-pinene (21.27-28.68%) and sabinene (2.29-16.27%). Additionally, limonene was presented up to 6.95% and terpinene-4-ol up to12.16%. The monoterpene α-pinene was extremely high in some samples of JE and JF (up to 33.83% and up to 67.61%, for JE and JF, respectively). Additionally, the percentage of limonene was high, ranging up to 6.14% and 27.11% in samples of JE and JF, respectively. Some samples of JE contained high percentage of *trans*-sabinyl acetate (up to 10.38%). In the same time, these oils were the only one containing thujones (*cis*-thujone up to 26.20% and *trans*-thujone up to 12.86%).

Concerning the presence of sesquiterpene components, obtained results could not be classified and generalized as deferent components were present in deferent oils in a very different amount, from traces to very high percentage. Interesting component was sesquiterpene cedrol, found in JF and JE from 9.60-33.90% and from 0.0-24.44%, respectively, while in JC it was not detected (Table 1). Other often identified sesqiterpene components were: *trans*-(E)-caryophyllene (0.22-3.305 and 0.81-3.45%, for JF and JC, respectively, not detected in JF), germacrene D (1.21-4.16% and 1.43-3.23% in JE and JC, respectively and only 0.2-0.5% in JF), δ-cadinene (0.2-1.5%, 0.31-3.95% and 2.05-7.98% for JF, JE and JC, respectively), α-cadinol (up to 2.1%, 1.31% and 6.05% for JF, JE and JC, respectively). For JC oils, γ-cadinene and *epi*-α-muurolol were characteristic, presented up to 2.26% and 4.13%, respectively.

In general, considering the maximum determined percentages of the constituents of the oils, JF oil was characterized by three main components (α–pinene, limonene and cedrol, presented up to 67.63%, 27.11% and 33.91%, respectively), JE oil by four components (α–pinene, sabinene, *cis*-thujone and cedrol, presented up to 33.83%, 29.49%, 26.20% and 24.44%, respectively) and JC oil by three components (α–pinene, sabinene and terpinen-4-ol, presented up to 28.68%, 16.27% and 12.16%, respectively).

Compared to literature data, similarity in the composition of the leave essential oil was found with the Greek *J. communis* where α-pinene (41.25%) and sabinene (17.4%) have been found as 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 and Katsiotis, 1993). Estonian *J. communis* leave oil was 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 presented in smaller amounts (Orav et al., 2010a, 2010b). 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 reported that juniper leaves essential oil was rich in sabinene (40.7%), followed by 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 leave essential oil 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).

Considering leaves essential oils of *J. excelsa*, many authors reported higher amounts of α-pinene and cedrol. In

Table 1. Essential oil yields (%) and chemical composition of leaves essential oils of *Juniperus foetidissima* (JF), *Juniperus excelsa* (JE) and *Juniperus communis* (JC) from R. Macedonia (%)

Components	RI	RIE	JF min	JF max	JE min	JE max	JC min	JC max
Tricyclene	921	930.4	tr	0.12	-	-	-	0.04
α-Thujene	931	933.8	-	-	0.84	1.42	-	2.43
α-Pinene	932	937.2	9.62	67.63	1.76	33.83	21.27	28.68
Camphene	946	945.5	0.21	1.01	-	0.47	-	0.91
Sabinene	969	962.8	-	-	0.34	29.49	2.29	16.27
β-Pinene	974	964.1	0.21	1.42	-	-	-	2.28
β-Myrcene	988	976.4	0.62	1.94	0.46	2.78	0.55	2.12
Δ^3-Carene	1008	989.8	0.41	3.42	-	-	-	3.15
α-Terpinene	1018	998.3	-	-	-	1.87	-	0.12
p-Cymene	1020	1001.8	0.21	0.32	0.19	0.88	tr	5.78
Limonene	1024	1006.7	10.0	27.11	1.75	6.14	-	6.95
γ-Terpinene	1054	1033.4	0.12	0.53	tr	3.53	-	0.23
α-Terpinolene	1086	1062.5	0.33	0.73	-	1.02	-	0.21
cis-Thujone	1101	1079.2	-	-	-	26.20	-	-
trans-Thujone	1112	1090.9	-	-	-	12.86	-	-
trans-Pinocarveol	1135	1117.1	-	-	-	-	-	0.47
trans-Sabinol	1140	1120.3	-	-	-	1.74	-	1.16
Camphor	1141	1123.7	0.12	0.22	-	-	-	-
Borneol	1165	1150.0	tr	0.12	-	-	-	0.28
Terpinen-4-ol	1174	1161.8	-	0.11	-	5.87	0.55	12.16
p-Cymene-8-ol	1179	1171.1	-	0.31	-	-	-	0.24
α-Terpineol	1186	1175.1	-	tr	-	-	-	0.37
Bornyl acetete	1284	1263.4	-	0.11	-	-	-	0.78
trans-Sabinyl acetate	1289	1268.5	-	-	-	10.38	-	0.13
δ-Elemene	1335	1306.0	-	0.32	-	0.12	-	0.14
α-Cubebene	1345	1318.6	-	0.21	tr	0.17	-	0.12
α-Copaene	1374	1345.4	tr	0.21	0.05	0.28	-	0.67
β-Bourbonene	1387	1354.5	-	0.11	0.06	0.25	-	0.32
β-Elemene	1389	1362.2	-	-	-	-	1.37	4.17
Sibirene	1400	1380.1	-	-	-	-	0.31	2.31
β-Funebrene	1413	1381.3	-	4.32	-	-	-	-
trans-Caryophyllene	1418	1386.9	tr	tr	0.22	3.30	0.81	3.45
β-Cedrene	1419	1387.7	0.54	2.72	-	-	-	-
β-Copaene	1430	1396.1	-	-	0.05	1.19	-	0.20
γ-Elemene	1434	1399.7	-	-	-	2.17	0.58	2.95
α-Humulene	1452	1421.8	tr	0.24	tr	0.45	0.07	2.89
γ-Muurolene	1478	1444.6	-	0.24	0.06	0.92	-	-
Germacrene D	1484	1449.9	0.27	0.54	1.21	4.16	1.43	3.23
β-Selinene	1489	1455.1	-	-	-	-	0.11	0.64
α-Selinene	1498	1463.2	-	-	-	-	0.71	1.07

Components	RI	RIE	JF min	JF max	JE min	JE max	JC min	JC max
α-Muurolene	1500	1468.3	-	-	0.21	1.83	0.45	1.32
γ-Cadinene	1513	1482.1	-	-	0.11	1.92	0.60	2.26
δ-Cadinene	1522	1490.3	0.21	1.51	0.31	3.95	2.05	7.98
trans-Cadina-1,4-diene	1533	1538.8	-	0.52	-	0.51	-	0.31
α-Cadinene	1537	1504.1	-	-	-	0.17	-	0.50
Germacrene B	1559	1525.6	-	-	-	1.91	-	0.49
Spatulenol	1577	1546.0	-	-	-	-	-	1.67
Caryophyllene oxide	1581	1552.6	0.11	0.42	-	-	-	1.73
Cedrol	1600	1576.2	9.62	33.91	-	24.44	-	-
Humulene epoxide II	1608	1578.2	-	-	-	-	0.71	1.17
1,10-di-*epi*-Cubenol	1618	1582.8	-	-	-	0.08	-	tr
1-*epi*-Cubenol	1627	1595.2	0.12	0.82	0.05	0.93	0.39	2.31
epi-α-Muurolol	1640	1609.7	-	0.21	-	-	1.07	4.13
α-Muurolol	1645	1613.4	-	0.41	-	0.02	-	0.44
α-Cadinol	1653	1621.9	-	2.11	-	1.31	1.67	6.05
Manool oxide	1987	1962.4	-	0.12	-	-	-	0.27
Abietatriene	2054	2024.7	-	tr	tr	0.08	-	0.62
Abietadiene	2080	2051.9	tr	0.12	tr	0.47	-	0.12
4-*epi*-Abietal	2298	2264.5	-	0.13	tr	0.21	-	0.04
Total (%)			93.23	96.55	82.69	95.61	86.07	96.31
Monoterpene hydrocarbons (MH)			38.27	83.25	40.96	42.80	39.97	53.39
Oxygen containing monoterpenes (OM)			0.55	1.75	0.34	44.97	3.89	12.16
Sesquiterpene hydrocarbons (SH)			2.32	15.45	6.91	16.11	12.27	28.64
Oxygen containing sesquiterpenes (OS)			10.15	37.94	0.67	25.28	5.98	13.57
Diterpenes (D)			tr	0.37	tr	0.76	-	1.05
Non-terpene components (NT)			0.3	0.12	-	-	-	0.04
Essential oil yield (%, dw)			0.50	1.38	0.16	3.30	0.75	0.90

RI - Retention index - literature data (Adams, 2007); RIE - Retention index experimentally determined with reference to a homologous series of *n*-alkanes on HP-5ms column (AMDIS); (-) - not found, tr - traces < 0.02

some earlier papers 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 (Adams, 1990). Also, Turkish authors found α-pinene (29.7%) and cedrol (25.3%) (Topcu et al., 2005) as dominant for this species. Moreover, Iranian researchers confirmed these two components as predominant in the leaf essential oil from wild growing *J. excelsa* from Iran (α-pinene 32.34% and cedrol 13.06%) (Emami et al., 2011a). Recently, 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 (Adams et al., 2013) where cedrol was found to be the most abundant constituent of the oils, ranging from 11.3 to 35.8%. These data are in correlation with our findings for some samples of *J. excelsa,* but some other samples from Republic of Macedonia possess essential oil with deferent oil composition characterized with larger percentages of sabinene and thujone (*cis* + *trans*) (29.49% and 39.06%, respectively).

According to literature data leaf essential oil of *J. foetidissima* was characterized by monterpene hydrocarbons as predominated constituents of the oil. For the leaves essential oil of *J. foetidissima* from Greece, Adams reported sabinene (19.6%), α-thujone (18.6%), terpinen-4-ol (17.6%) and γ-terpinene (6.5%) as major components (Adams, 1990b). 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%). Iranian researchers found that 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). Considering essential oil composition of *J. foetidissima* from Balkans, only one article was published by Lesjak et al. (2013) who reported 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. Our findings showed higher percentage of α–pinene (67.63%), followed by larger amounts of cedrol (up to 33.91%) and limonene (27.11%).

The contents of total phenols and total flavonoids and DPPH radical scavenging activity

The obtained values for total phenols content determined with the Folin-Ciocalteu reagent (TPC-FC) as well as total flavonoid content determined spectrophotometrycally using $AlCl_3$ as chelating agent (TFC-$AlCl_3$) are presented in Table 2. In all investigated samples of *Juniperus* leaves the content of TPC-FC was almost twice higher in water extracts (96.18-122.91 mg GAE/g dw) compared to ethanol extracts (47.72-64.42 mg GAE/g dw). On the contrary, the contents of TFC-$AlCl_3$ were slightly higher in ethanol extracts (2.05-11.91 mg CE/g dw) compared to water extracts (1.95-10.25 mg CE/g dw). Leaves extract of JF and JE, both water and ethanol contained much higher content of TFC-$AlCl_3$ than JC extracts.

Leaves extracts of investigated *Juniperus* species (JF, JE and JC), both water and ethanol extracts, showed radical scavenging capacity expressed as % of inhibition of the DPPH radical that had broad range depending on the extract concentration. In both cases more concentrated extracts have shown better results and water extracts have demonstrated stronger scavenging activity (Table 3). Thus, the highest radical scavenging activity against DPPH radi-

Table 2. The content of total phenols (TPC - FC, mg GAE/g dw) and total flavonoids (TFC - $AlCl_3$, mg CE/g dw) in leaves of *Juniperus foetidissima* (JF), *Juniperus excelsa* (JE) and *Juniperus communis* (JC)

Specimen	TPC-FC water extract	TPC-FC ethanol extract	TFC-FC water extract	TFC-$AlCl_3$ ethanol extract
JF	118.87 ± 0.68	60.45 ± 1.62	10.25 ± 1.16	11.91 ± 1.24
JE	122.91 ± 0.35	64.42 ± 1.34	8.85 ± 0.94	8.92 ± 1.84
JC	96.18 ± 1.22	47.72 ± 0.86	1.95 ± 0.04	2.05 ± 0.52

(n=3)

Table 3. Radical scavenging activity against DPPH radical expressed as % of inhibition for leaves extracts of *Juniperus foetidissima* (JF), *Juniperus excelsa* (JE) and *Juniperus communis* (JC)

Sample	% of inhibition of DPPH radical		
	JF	JE	JC
Ethanol extracts (mg/ml)			
100	18.32 - 20.12	48.73 - 48.82	74.4 - 76.7
50	17.25 - 18.36	39.32 - 42.12	55.7 - 70.7
20	5.61- 6.64	33.03 - 34.32	27.3 - 42.8
10	0.0	14.42 - 16.24	11.2 - 23.1
Water extracts (mg/ml)			
10	60.56 - 64.52	59.74 - 67.4	60.56 - 78.23
5	45.40 - 48.25	40.72 - 72.6	41.95 - 45.40
2	18.23 - 20.19	15.04 - 43.4	19.86 - 20.19
1	10.42 - 12.32	8.54 - 25.71	11.19 - 12.31

cal showed water extract of JF, JE and JC leaves in concentration 10 mg/ml, with % of inhibition of DPPH ranging up to 64.52%, 67.40% and 78.23%, respectively.

Tavares et al. (2009) evaluated the possibility of application of *Juniperus* leaves from species naturally occurring in Portugal (*J. phoenicea* subsp. *phoenicea*, *J. turbinata*, *J. oxycedrus* subsp. *oxycedrus*, *J. oxycedrus* subsp. *badia* and *J. navicularis*) against some diseases in which oxidative reactions play a crucial role. They found that all species exhibited minimum polyphenol and flavonoid contents in March/April and July and therefore a reduced antioxidant activity while the maximum concentrations of these compounds were detected in November/December, when they demonstrated higher antioxidant capacity (Taveras et al., 2009).

The antioxidant activity of leaves and fruits of 11 different conifer taxons growing wild in Iran were evaluated by Emami et al. (2007). Methanol extract of leaves and fruits were prepared and antioxidant activity of each extracts was measured using two different tests (the ferric thiocyanate method and thiobarbituric acid test, TBA). Results indicated that the methanol extracts of leaves, of male and female, and fruits of all these species possessed antioxidant activity when tested with both methods. The antioxidant capacity was then compared with those of α-tocopherol (a natural antioxidant) and butylated hydroxytoluene (BHT, a synthetic antioxidant). Methanol extract of *Juniperus* species, especially *J. excelsa, J. excelsa* ssp. *polycarpos, J. oblonga* and *J. foetidisima,* demonstrated antioxidant activity comparable to the BHT and even higher than α-tocopherol (Emami et al., 2007).

A correlation was found between the primary antioxidant activity and the total phenolic contents in different *Juniperus* species (*J. communis* var. *communis* (Jcc), *J. communis* var. *saxatilis* Pall. (Jcs), *J. drupacea* Labill. (Jd), *J. oxycedrus* subsp. *oxycedrus* (Joo) and *J. oxycedrus* subsp. *macrocarpa* (Sibth. & Sm.) Ball. (Jom)) from Turkey by Taviano et al. (2011). Both in DPPH and TBA test, Jom resulted the most active (IC_{50} = 0.034 ± 0.002 mg/ml and 0.287 ± 0.166 μg/ml, respectively). Different extracts of leaves, ripe fruits, and unripe fruits of *Juniperus* species, including *J. communis*, were studied for antioxidant activity by the ferrous ion-chelating, superoxide radical scavenging and ferric-reducing antioxidant power (FRAP) assays. It was found that all investigated *Juniperus* samples possessed antioxidant activity, but the leaves extracts usually had higher antioxidant activity (Orhan et al., 2011).

Conclusion

Chemical characterization of three *Juniperus* species, *J. foetidisima* (JF), *J. excelsa* (JE) and *J. communis* (JC) from Macedonian flora enclosed determination of yield and essential oil composition of the oils obtained by hydrodistillation of dried leaves and determination of the content of total phenols and total flavonoids in dried plant material. With GC/FID/MS analysis the essential oil profile of all investigated species was characterized dominantly with monoterpene hydrocarbon fraction. Sesquiterpene cedrol was found as an important constituent of the JF and JE oils, however the essential oil from JF was additionally characterized by two other main components (α–pinene and limonene), while the JE oil by three components (α–pinene, sabinene and *cis*-thujone). The essential oil of JC was free of cedrol, despite the fact that the fraction of sesquiterpene components of this oil was relatively high, but it consisted of many components presented in lower percentages and this oil was characterized mainly by three monoterpene components (α–pinene, sabinene and terpinen-4-ol).

The content of total phenols determined by Folin-Ciocalteu method ranged from 96.18-122.91 mg GAE/g dw (water extraction) while the content of total flavonoids ranged from 2.05-11.91 mg CE/g dw (ethanol extraction). Both water and ethanol extracts possessed radical scavenging activity against DPPH radical but water extract were more powerful with % of inhibition of DPPH ranging up to 64.52%, 67.40% and 78.23% for water extract (10 mg/ml) of JF, JE and JC, respectively. Obtained results showed correlation with the content of total phenols as the water extracts contained higher amounts of total phenols and exhibited better antioxidant activity.

The leaves of *Juniperus* species from Macedonian flora (*J. foetidisima, J. excelsa* and *J. communis*) can be taken in further consideration as a plant source for isolation of essential oil as well for extraction of phenolic compounds with promising antioxidant activity. Further research is needed to evaluate the chemical and biological potential of this raw plant material.

References

Adams, R. 1987. Investigation of *Juniperus* Species of the United States for New Sources of Cedarwood Oil I. Economic Botany 41, 48-54.

Adams, R.P. 1990a. The chemical composition of leaf oils of *Juniperus excelsa* M. Bieb. J. Essent. Oil Res. 2, 45-8.

Adams, R. 1990b. Variation in the chemical composition of the leaf oil of *Juniperus foetidissima* Willd. J. Essent. Oil Res. 2, 67-70.

Adams, R., 2007. Identification of essential oil components by gas chromatography/mass spectrometry. 4th Ed. Illinois: Allured Publishing Corporation IL, USA, pp 9-31.

Adams, R.P., Tashev, A.N., Baser, K.H.K., Christou, A.K. 2013. Geographic variation in volatile leaf oils of *Juniperus excelsa* M. Bieb. Phytologia 95(4), 279-285.

Angioni, A., Barra, A., Russo, M.T., Coroneo, V., Dessi, S., Cabras, P., 2003. Chemical composition of the essential oils of *Juniperus* from ripe and unripe berries and leaves and their antimicrobial activity. J. Agric. Food Chem. 51 (10), 3073-3078.

Asili, J., Emami, S.A., Rahimizadeh, M., Fazly-Bazzaz, B.S., Hassanzadeh, M.K. 2008. Chemical and Antimicrobial Studies of *Juniperus excelsa* subsp. *excelsa* and *Juniperus excelsa* subsp. *polycarpos* essential oils. J. Essent. Oil Bear.

Pl. 11(3), 292-302.

Asili, J., Emami, S.A., Rahimizadeh, M., Fazly-Bazzaz, B.S., Hassanzadeh, M.K. 2010. Chemical and antimicrobial studies of *Juniperus sabina* L. and *Juniperus foetidissima* Willd. essential oils. J. Essent. Oil Bear. Pl. 13, 25-36.

Atas, A.D., Goze, I., Alim, A., Cetinus, S.A., Durmus, N., Vural, N., Cakmak, O. 2012. Chemical Composition, Antioxidant, Antimicrobial and Antispasmodic Activities of the Essential Oil of *Juniperus excelsa* subsp. *excelsa*. J. Essent. Oil Bear. Pl. 15(3), 476-483.

Balaban, M., Atik, C., Ucar, G. 2003. Fungal growth inhibition by wood extracts from *Juniperus foetidissima* and *J. oxycedrus*. Holz als Roh und Werkstoff 61, 231-232.

Chaouche, T.M., Haddouchi, F., Ksouri, R., Medini, F., Atik-Bekara, F. 2013. In vitro evaluation of antioxidant activity of the hydro-methanolic extracts of *Juniperus oxycedrus* subsp. *oxycedrus*, Phytothérapie 11 (4), 244-249.

Chatzopoulou, P.S., Katsiotis, S.T., 1993. Chemical investigation of the leaf oil of *Juniperus communis* L. J. Essent. Oil Res. 5 (6), 603-607.

Ehsani, E., Akbari, K., Teimouri, M., Khadem, A. 2012. Chemical composition and antibacterial activity of two *Juniperus* species essential oils. Afr J Microbiol Res. 6(38), 6704-10.

Emami, S.A., Abedindo, B.F., Hassanzadeh-Khayyat, M. 2011a. Antioxidant Activity of the Essential Oils of Different Parts of *Juniperus excelsa* M. Bieb. subsp. *excelsa* and *J. excelsa* M. Bieb. subsp. *polycarpos* (K. Koch) Takhtajan (Cupressaceae). Iran J Pharm Res. 10(4), 799-810.

Emami, S.A., Asgary, S., Naderi, G.A., Shams Ardekani, M.R., Kasher, T., Aslani, S., Airin, A., Sahebkar, A. 2011b. Antioxidant activities of *Juniperus foetidissima* essential oils against several oxidative systems. Revista Brasileira de Farmacognosia 21, 627-634.

Emami, S.A., Asili, J., Mohagheghi, Z., Hassanzadeh, M.K. 2007. Antioxidant Activity of Leaves and Fruits of Iranian Conifers. Evidence Based Complementary and Alternative Medicine 4, 313–319.

Filipowicz, N., Madanecki, P., Gołębiowski, M., Stepnowski, P., Ochocka, R.J., 2009. HS-SPME/GC analysis reveals the population variability of terpene contents in *Juniperus communis* needles. Chem. Biodiv. 6(12), 2290-2301.

Gyamfi, MA, Yonamine M, Aniya Y. 1999. Free-radical scavenging action of medicinal herbs from Ghana *Thonningia sanguinea* on experimentally-induced liver injuries. Gen Pharmacol. 32, 661-667.

Khan, M., Khan, A., Rehman, N., Gilani, A.H. 2012. Pharmacological explanation for the medicinal use of *Juniperus excelsa* in hepatoprotective gastrointestinal and respiratory disorders. *J. Nat. Med.* 66, 292-301.

Koukos, P.K., Papadopoulou, K.I., 1997. Essential oil of *Juniperus communis* L. grown in northern Greece: variations of fruit oil yield and composition. J. Essent. Oil Res. 9 (1), 35-39.

Kumar, A., Yadav, L.B.S., Ahmad, J., Dubey, N., Puri, S., 2007. Chemical composition of commercial *Juniperus communis* L. leaf oil. J. Essent. Oil Bear. Pl. 10 (4), 310-313.

Lesjak, M.M., Beara, I.N., Orcic, D.Z., Ristic, J.D., Anackov, G.T., Bozin, B.N., Mimica-Dukic, N.M. (2013) Chemical characterisation and biological effects of *Juniperus foetidissima* Willd. 1806. Food Science & Technology 53, 530-539.

Marcysiak, K., Mazur, M., Romo, A., Montserrat, J.M., Didikuh, Y., Baratynska, K., Jasinski, A., Kosinski, P., Boratynski, A. (2007) Numerical taxonomy of *Juniperus thurifera, Juniperus excelsa* and *Juniperus foetidissima* (Cupressaceae) based on morphological characters. Botanical Journal of the Linnean Society 155, 483-495.

Micevski, K., 1998. *Flora na R. Makedonija*. 1st Ed. Skopje: Makedonska Akademija na Naukite i Umetnostite (MANU), Skopje, pp. 81-84.

Modnicki, D., Łabędzka, J. 2009. Estimation of the total phenolic compounds in juniper sprouts (*Juniperus communis* L., Cupressaceae) from different places at the kujawsko-pomorskie province, Herba Polonica 55 (3), 127-132.

Moein, M.R., Ghasemi, Y., Moein, S., Nejati, M. 2010. Analysis of antimicrobial, antifungal and antioxidant activities of *Juniperus excelsa* M. B. subsp. *polycarpos* (K. Koch) Taghtajan essential oil. Pharmacog. Res. 2(3), 128–31.

Moein, M.R., Moein, S., Mousavi, F. 2014. Study on relationship between antioxidant potential and phenolic contents of *Juniperus excelsa* fruit, International Journal of Pharmacy and Pharmaceutical Science 6(7), 192-194.

Orav, A., Kailas, T., Müürisepp, M., 2010a. Chemical investigation of the essential oil from berries and needles of common juniper (*Juniperus communis* L.) growing wild in Estonia. *Nat. Prod. Res*. 24 (19), 1789-1799b.

Orav, A., Koel, M., Kailas, T., Müürisepp, M., 2010b. Comparative analysis of the composition of essential oils and supercritical carbon dioxide extracts from the berries and needles of Estonian juniper (*Juniperus communis* L.). Procedia Chem. 2, 161–167.

Orhan, N., Akkol, E., Ergun, F. 2012. Evaluation of anti-inflammatory and antinociceptive effects of some *Juniperus* species growing in Turkey. Turkish Journal of Biology 36, 719-726.

Orhan, N., Orhan, I. E., & Ergun, F. 2011. Insights into cholinesterase inhibitory and antioxidant activities of five *Juniperus* species. Food and Chemical Toxicology 49(9), 2305–2312.

Ottavioli, J., Gonny, M., Casanova, J., Bighelli, A., 2009. Chemical variability of the needle oil of *Juniperus communis* ssp. *alpina* from Corsica. Chem. Biodivers. 6 (12), 2192-2199.

Ozturk, M., Tumen, I., Ug'ur, S., Aydog, F., Topcu, G. 2010. Evaluation of fruit extractas of six Turkish *Juniperus* species for their antioxidant, anticholinesterase and antimicrobial activities. Journal of the Science of Food and Agriculture 91, 867-876.

Sadaeghi-Aliabadi, H., Emami, A., Saidi, M., Sadeghi, B., Jafarain, A. 2009. Evaluation of in vitro cytotoxic effects of *Juniperus foetidissima* and *Juniperus sabina* extracts against a panel of cancer cells. Iranian Journal of Pharmaceutical Research 8, 281-286.

Shahmir, F., Ahmadi, L., Mirza, M., Korori, S.A.A., 2003. Secretory elements of needles and berries of *Juniperus communis* L. ssp. *communis* and its volatile constituents. Flavour Frag. J. 18 (5), 425-428.

Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299,152-178.

Talari, S., Rudroju, S., Penchala, S., Nanna Rama, S. 2012. Quantification of total phenolic and total flavonoid contents in extracts of *Oroxylum indicum* L. Kurz, Asian J. Pharm. Clin. Res. 5(4), 177-179.

Tavares, L., Pimpao, R.C., Santos, C., McDougall, G.J., Stewart,

D., Ferreira, RB. 2009. Phytochemical characterization of *Juniperus* spp. leaves, Planta Med. 75 - PA73, DOI: 10.1055/s-0029-1234398.

Taviano, M.F., Marino, A., Trovato, A., Bellinghieri, V., La Barbera, T.M., Guvenc, A., Hurkul, M.M., Pasquale, R., Miceli, N. 2011. Antioxidant and antimicrobial activities of branches extracts of five *Juniperus* species from Turkey. Pharm. Biol. 49, 1014-1022.

Tayoub, G., Odeh, A., Ghanem, I. 2012. Chemical composition and efficacy of essential oil from *Juniperus foetidissima* Willd against the Kharpa Beetle. International Journal of Medicinal and Aromatic Plants 2, 501-508.

Topçu, G., Gören, A.C., Bilsel, G., Bilsel, M., Çakmak, O., Schilling, J., Kingston, D.G.I. 2005. Cytotoxic Activity and Essential Oil Composition of Leaves and Berries of *Juniperus excelsa*. Pharmaceutical Biology 43(2), 125-8.

Tunalier, Z., Kirimer, N., Baser, K.H.C. 2002. The composition of essential oils from various parts of *Juniperus foetidissima*. Chemistry of Natural Compounds 38, 43-47.

Unlu, M., Vardar-Unlu, G., Vural, N., Domnez, E., Cakmak, O. 2008. Composition and antimicrobial activity of *Juniperus excelsa* essential oil. Chemistry of Natural Compounds 44 (1), 129-131.

Резиме

Хемиска карактеризација и инхибиторна радикалска активност на листови од *Juniperus foetidisima*, *J. excelsa* и *J. communis* од Македонска флора

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Клучни зборови: *Juniperus communis, Juniperus excelsa, Juniperus foetidosima,*состав на етерично масло, GC/MS, вкупни феноли, вкупни флавоноиди, DPPH.

Хемиската карактеризација на три *Juniperus* вида, *J. foetidisima* (JF), *J. excelsa* (JE) и *J. communis* (JC) од Македонската флора, вклучува определување на содржина и состав на етерични масла добиени со дестилација со водена пареа на суви листови, како и определување на содржина на вкупни феноли и вкупни флавоноиди во сувиот материјал. Со GC/FID/MS анализата утврден е монотерпенски профил на маслото од JC и монотерпенско/сесквитерпенски профил на маслата од JF и JE. Сесквитерпенот цедрол е идентификуван како важен конституент на маслата од JF и JE, при што маслото од JF се карактеризира со три главни компоненти (α–пинен, лимонен и цедрол, во количини што се движат до 67,63%, 27,11% и 33,91%, соодветно), додека маслото од JE се карактеризира со четири главни компоненти (α–пинен, сабинен, *cis*-тујон и цедрол, присутни во количини до 33,83%, 29,49%, 26,20% и 24,44%, соодветно). Маслото од JC не содржи цедрол, иако содржи релативно висок удел на сесквитерпени (сесквитерпенски јаглеводороди и сесквитерпени со кислорд во количини што се движат до 28,64% и до 13,57%, соодветно). Ова масло се карактеризира главно со три монотерпенски компоненти (α–пинен, сабинен и терпинен-4-ол, застапени до 28,68%, до 16,27% и до 12,16%, соодветно). Содржината на вкупните феноли определена со метод по Folin-Ciocalteu се движи од 96,18 до122,91 mg GAE/g (водена екстракција), а содржината на вкупни флавоноиди од 2,05 до 11,91 mg CE/g (етанолна екстракција). Двата екстракти (водениот и етанолниот) поседуваат инхибирачка активност во однос на DPPH радикалот. Водените екстракти се помоќни бидејќи во концентрација од 10 mg/ml покажуваат % на инхибиција на DPPH радикалот кој се движи до 64.52%, до 67.40% и до 78.23%, за JF, JE и JC, соодветно. Добиените резултати покажуваат корелација со содржината на вкупните феноли.