Comparative Antioxidant Properties of *Juniperus communis* L. and *Juniperus virginiana* L. Extracts Obtained by Refluxing and Sonication Methods

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The aim of this study was to evaluate the antioxidant activity of ethyl acetate and tetrahydrofuran extracts of Juniperus berries, branches and needles, by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Two Juniperus species, Juniperus communis and Juniperus virginiana, from different regions were used. The extracts obtained by sonication and refluxing extraction were studied by gas chromatography-mass spectrometry, which revealed that the Romanian juniper extracts are largely comprised of monoterpene hydrocarbons, such as α-pinene, β-pinene, β-hellandrene, sabinene, β-cadinene, τ-cadinene, β-caryophyllene, β-cubebene, β-elemene and germacrene D. The antioxidant activity was highest in ethyl acetate Juniperus communis needles extract from Lipova (Romania) and in ethyl acetate Juniperus virginiana branches extract from Macea Botanical Garden (Romania). The mean rates (v_m) of DPPH consumption were higher for the tetrahydrofuran Juniperus communis branches extract from Lipova and ethyl acetate Juniperus virginiana branches extract than in tetrahydrofuran Juniperus communis berries extract from Lipova.

Keywords: Juniperus communis, Juniperus virginiana, sonication extraction, refluxing extraction, antioxidant activity

It is commonly accepted that free radicals play an important role in the pathogenesis of many diseases. Antioxidants, on the other hand, can control the degradation of biomolecules caused by the free radicals. Thus, considerable efforts have been made towards locating naturally occurring antioxidants for use in food or medicines, in order to replace the synthetic antioxidants [1]. The side effects of artificial antioxidants are a strong reason for considering their replacement with natural equivalents. Essential oils are one source of natural antioxidants, with a great potential for application in pharmaceutical products [2]. *Juniperus* species, for example, contain such essential oils and are used for treatment of hyperglycemia, tuberculosis, bronchitis, pneumonia, ulcers, intestinal worms and other [2].

For centuries, juniper berries have been used in folk medicine for the treatment of opportunistic infections, as a spice for meat, and as flavor in the preparation of gin and raki [3]. *Juniperus communis* oil is of interest to perfumery, cosmetic and pharmaceutical industries because of its aromatic and diuretic (based on its terpinen-4-ol content) properties [4,5].

Juniperus L. (Cupressaceae family) is a genus of evergreen shrubs or trees and the second most diverse conifer, with 67 species in the world [6]. *Juniperus communis* L. (section *Juniperus*) grows in scrubs, pastures and cliffs, from sea level to high mountain regions, throughout Europe, Asia and North America [7,8]. *Juniperus communis* is an evergreen dioecious shrub or tree, with fleshy female cones, in which the cone scales are fused resembling *berries* of dark blue – black color [8,9].

The main *Juniperus* compounds of this study are β-phellandrene, α-pinene, sabinene and germacrene D. The hydrodistillation method was previously applied to needles [4,8-10] and berries [5-7,9,11,12] of *Juniperus communis*; supercritical CO_2 extraction was also used for berries [13]. A number of studies have shown that monoterpenes, contained in juniper essential oil, enhance, through their antioxidant activity, the oxidative stress resistance of living organisms. Their antiradical activity affects the levels of the most important enzymes responsible for the neutralization of ROS: SOD, CATs, peroxidases, and glutathione transferase [3].

The purpose of this work was to evaluate the antioxidant activity of different extracts of *Juniperus communis* and *Juniperus virginiana*.

Experimental part

Materials and methods

The plant material was collected from wild growing *Juniperus communis* shrubby trees from Lipova (Arad County, Romania) and from Albac (Alba County, Romania). *Juniperus virginiana* was collected from Macea Botanical Garden (Arad County, Romania). Three kind of samples were selected, i.e. black mature berries, needles and branches, which were all dried at room temperature.

Ethyl acetate and tetrahydrofuran, both from Chimopar Bucharest, were used as solvents for refluxing extractions. The former was also used as solvent for sonication extractions. All solvents used were of *puriss* or *p.a.* grade.

All filtered extracts were dried on anhydrous sodium sulfate (Fluka Chemie AG).

Extraction methods

Refluxing extraction

The plant material was dried and stored at room temperature. Dried berries, needles, and branches (2 g in each case) were grounded, treated with 15 mL solvent (tetrahydrofuran or ethyl acetate) and refluxed for 30 min. After cooling, the extract was filtered, dried over anhydrous Na_2SO_4 and stored at -4 °C in glass containers, until the gas chromatography – mass spectrometry (GC-MS) analysis was performed.

Sonication extraction

In order to obtain the volatile compounds from the three anatomical parts (berries, needles, and branches) of the two *Juniperus* species, 1 g of dried and grounded plant material was placed in a vial with 6 mL solvent (ethyl

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acetate). The vial was covered and then placed in the sonication water bath (HK2200, 100 W, 50 kHz) for 10 min [14]. After sonication, the extract was filtered, dried over anhydrous Na_2SO_4 and stored at -4°C until the GC-MS analysis was performed.

GC-MS analysis

The extracts obtained by refluxing and sonication were analyzed by GC-MS in order to identify the main components. A Hewlett Packard HP 6890 Series gas chromatograph coupled with a Hewlett Packard 5973 mass selective detector (GC-MS) system was used. A HP-5 MS capillary column was used for the GC system. The temperature program was set from 50 to 250°C, with a rate of 6°C/min, using helium as carrier gas. The relative percentage concentrations of the volatile compounds for the two species of juniper were computed from the GC peak areas. The identification of the main compounds was performed by matching the experimental mass spectra with those from the NIST/EPA/NIH Mass Spectral Library 2.0.

2,2-Diphenyl-1-picrylhydrazyl radical-scavenging

A DPPH solution of approximately 1 mM was prepared in ethanol (96 %) and diluted to obtain a standard curve for DPPH. The absorbance was recorded at 517 nm (Lambda 25 UV-Vis Spectrophotometer) using ethanol as blank. The mean rate (v_m) of DPPH consumption was calculated for all extracts as the ratio between the decrease in concentration (Δc, μM) and the time interval (Δt, s), according to equation 1 [15].

$$v_m = -\frac{\Delta c}{\Delta t} \quad (1)$$

The antioxidant activity was calculated according to equation 2.

$$A\% = \frac{A_{(t=0)} - A_{(t=15)}}{A_{(t=0)}} \times 100 \quad (2)$$

where $A_{(t=0)}$ is the absorbance for the positive control (DPPH solution, without the sample) and $A_{(t=15)}$ is the absorbance after 15 min.

Results and discussions

Composition of Juniperus extracts

The amount of the extracted compounds is expressed as a percentage of the obtained peak area, compared with the total area of all peaks (table 1). Of the large number of compounds found in all extracts (over 100), only the most important (26 compounds) were discussed.

The GC-MS analyses showed that α-pinene is present in all extracts. α-Pinene is present in higher amount (26.70%) in *J. communis* branches from Lipova ethyl acetate extract obtained by sonication, by comparison with the supercritical CO_2 extraction (α-pinene 7.11 %) [13]. Also, germacrene D is present in a higher amount (14.42 %) in *J. communis* branches from Lipova ethyl acetate extract prepared by sonication, along with sabinene (10.22%) from the *Juniperus virginiana* needles extract in tetrahydrofuran from Macea Botanical Garden, when compared with commercial essential oil from Bulgaria (sabinene 5.8%) [3].

Table 1

THE MAIN COMPONENTS OBTAINED BY REFLUXING AND SONICATION EXTRACTION FROM *JUNIPERUS COMMUNIS* AND *JUNIPERUS VIRGINIANA* NEEDLES, BERRIES AND BRANCHES (% OF TOTAL AREA)

No.	Compounds	% of total area								
		Refluxing extraction								Sonication
		E1 [16]	E3 [16]	E4	E5	E6	E7 [16]	E8	E9	E2
1.	α-Pinene	19.73	13.81	9.36	0.66	2.57	14.36	1.51	1.74	**26.70**
2.	β-Pinene	**10.10**	0.47	0.85	0.28	4.27	0.85	0.33	2.59	2.47
3.	Bisabolene epoxide	0.16	0.10	0.13	0.03	0.11	**0.19**	-	-	-
4.	3-Carene	**0.61**	-	0.15	-	0.05	-	0.08	-	-
5.	β-Cadinene	0.65	0.47	-	0.51	-	0.37	0.43	0.11	**2.72**
6.	τ-Cadinene	0.20	0.18	-	**0.32**	-	0.16	0.26	0.12	1.13
7.	τ-Cadinol	0.08	-	-	-	-	-		-	**2.50**
8.	Camfene	**0.17**	0.08	0.08	-	-	-	0.03	0.03	-
9.	Cariophylene	1.83	0.44	0.10	1.23	1.92	**3.10**	1.18	1.20	1.88
10.	Carveol	0.06	0.01	**0.36**	0.09	0.04	0.09	-	0.03	0.03
11.	Cedrene	-	0.12	-	0.02	-	-	0.03	-	**0.14**
12.	Copaene	**0.55**	0.04	-	-	-	-	-	-	0.11
13.	β-Cubebene	-	**2.14**	0.42	-	1.75	-	0.07	1.12	-
14.	β-Elemene	2.04	0.88	-	0.13	0.15	0.10	0.22	0.18	**3.85**
15.	τ-Elemene	**4.43**	1.89	0.20	0.14	0.25	-	0.20	0.09	4.25
16.	β-Phellandrene	10.51	8.12	0.26	7.98	**46.64**	10.51	0.11	26.95	4.71
17.	Germacrene D	3.42	3.49	-	3.05	6.07	2.09	2.76	3.26	**14.42**
18.	α-Humulene	**1.59**	0.27	0.03	0.13	0.18	0.35	0.12	0.10	1.49
19.	Limonene	4.75	2.08	1.37	0.54	1.21	0.66	0.60	0.83	**5.05**
20.	Linalool	0.07	-	-	0.06	0.06	**0.73**	0.06	0.03	-
21.	Ocimene	-	0.02	-	0.06	0.09	0.33	-	-	**0.72**
22.	Sabinene	-	**12,07**	7,70	-	-	-	10,22	-	0.46
23.	Spatulenol	**0.56**	-	0.07	0.09	-	-	-	-	0.48
24.	cis-β-Terpineol	**0.76**	0.05	0.69	0.17	0.42	0.24	0.45	0.62	0.11
25.	τ-Terpinene	0.28	-	0.23	0.42	0.09	-	0.19	**1.02**	0.36
26.	Terpinolene	**0.76**	0.32	0.39	0.24	0.45	0.25	0.13	0.32	0.33

Table 2

THE ABBREVIATION FOR ANTIOXIDANT ACTIVITY EXTRACTS

Abbreviation	Extracts for DPPH assay
E_1	*Juniperus communis* berries extract in ethyl acetate from Albac
E_2	*Juniperus communis* branches extract in ethyl acetate from Albac
E_3	*Juniperus communis* needles extract in ethyl acetate from Lipova
E_4	*Juniperus communis* branches extract in tetrahydrofuran from Lipova
E_5	*Juniperus virginiana* needles extract in ethyl acetate from Macea Botanical Garden
E_6	*Juniperus virginiana* berries extract in ethyl acetate from Macea Botanical Garden
E_7	*Juniperus virginiana* branches extract in ethyl acetate from Macea Botanical Garden
E_8	*Juniperus virginiana* needles extract in tetrahydrofuran from Macea Botanical Garden
E_9	*Juniperus virginiana* berries extract in tetrahydrofuran from Macea Botanical Garden

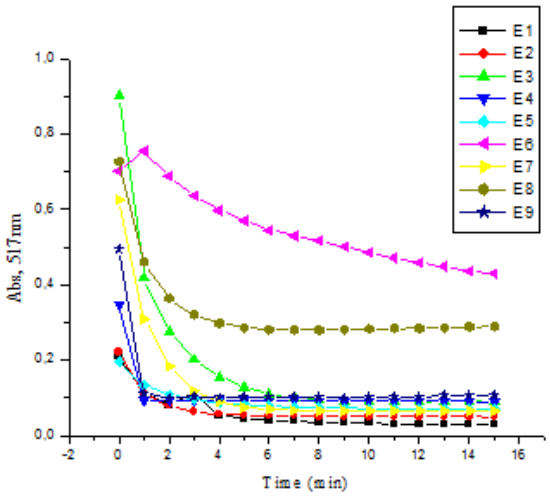

Fig. 1. Reduction of DPPH by juniper extracts

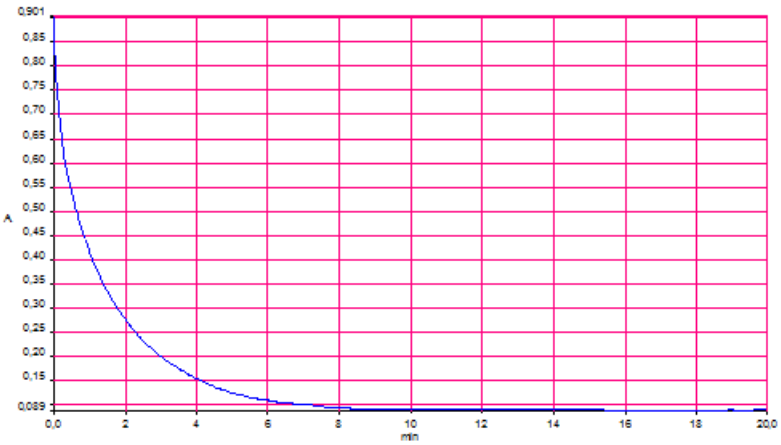

Fig. 2. Antioxidant activity of *Juniperus communis* needles extract in ethyl acetate from Lipova

To conclude, the extracts are mainly comprised of monoterpenes (limonene, τ-terpinene, terpinolene, cis-β-terpineol, β-phellandrene, α-pinene, β-pinene) and sesquiterpenes (β-cadinene, τ-cadinene, β-caryophyllene, germacrene D, α-humulene).

Antioxidant activity

The DPPH assay test was used in this study in order to determine the ability of juniper extract to act as hydrogen atom donors. All nine extracts reduce the DPPH radical from a deep violet color to colorless when neutralized, as can be seen in figure 1. By monitoring the reaction at 517 nm, a decrease in absorbance for extracts E_1, E_4, E_5 and E_7 in the first three minutes can be observed (table 2). For branches, this decrease was slower than for needles and berries.

The antioxidant activity was calculated by using the absorbance values for the positive control and for the sample after 15 min (eq. 2, table 3). The obtained antioxidant activity values were between 33.31% (E_5) and 89.98% (E_3). *Juniperus communis* needles extract in ethyl acetate from Lipova had the highest antioxidant activity (fig. 2); also, for *Juniperus communis* branches extract in ethyl acetate from the Macea Botanical Garden, the value was very close (89.69%, fig. 3).

Juniperus communis branches extract in tetrahydrofuran from Lipova had the highest mean rate (v_m), calculated with equation 1, (0.0212 μM/s), followed by the *Juniperus communis* branches extract in ethyl acetate from Macea Botanical Garden (0.0122 μM/s). *Juniperus virginiana* berries extract in ethyl acetate from Macea Botanical Garden had the lowest values (0.026 μM/s), (table 3).

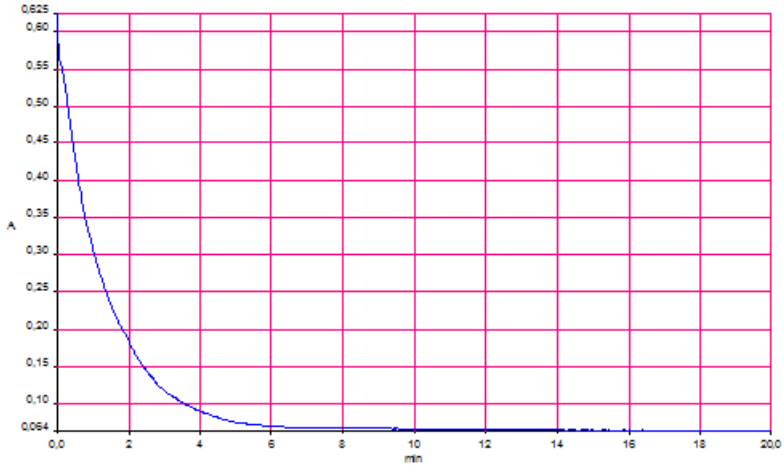

Fig. 3. Antioxidant activity of *Juniperus communis* branches extract in ethyl acetate from Macea Botanical Garden

Table 3
ANTIOXIDANT ACTIVITY AND MEAN RATE FOR JUNIPER EXTRACTS

Extracts	A% (DPPH)	Mean rate, v_m (μM/s)
E_1	85.82	0.0050
E_2	77.54	0.0043
E_3	**89.98**	0.0065
E_4	74.01	**0.0212**
E_5	33.31	0.0032
E_6	38.93	0.0026
E_7	89.69	0.0122
E_8	60.28	0.0049
E_9	78.35	0.0034

Conclusions

Nine juniper extracts, obtained using ethyl acetate and tetrahydrofuran as solvents, were used for antioxidant activity determinations. Sonication extracts showed the higher contents of α-pinene and limonene (5.05 %, comparable with the essential oil from literature [5]) in *Juniperus communis* branches extract in ethyl acetate from Albac (Romania). By refluxing extraction, the sabinene from *Juniperus communis* needles extract in ethyl acetate from Lipova (Romania) showed the highest percent. The highest antioxidant activity was attributed to the *Juniperus communis* needles extract in ethyl acetate from Lipova, which was follow by the *Juniperus communis* branches extract in ethyl acetate from Macea Botanical Garden. The mean rate of *Juniperus communis* branches extract in tetrahydrofuran from Lipova and *Juniperus virginiana* branches extract in ethyl acetate from Macea Botanical Garden was higher than in the *Juniperus virginiana* berries extract in ethyl acetate from Macea Botanical Garden.

Acknowledgements: This work was partially supported by the strategic grant POSDRU 6/1.5/S/13 (2008) from the Ministry of Labor, Family and Social Protection, Romania, co-financed by the European Social Fund – Investing in People. Vasile Goldis Western University of Arad is gratefully acknowledged for providing the Juniperus virginiana samples.

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Manuscript received: 23.12.2016