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Full Length Research Paper

Antioxidant and antimicrobial activity of different extracts from leaves and roots of *Jovibarba heuffelii* (Schott.) A. Löve and D. Löve

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The present study investigated antimicrobial and antioxidant activity, total phenolic content and flavonoid concentration of methanolic, acetone and ethyl acetate extracts from fresh leaves and roots of *Jovibarba heuffelii* (Schott.) A. Löve and D. Löve. The extracts were prepared and used to evaluate the antimicrobial activity against a panel of gram-positive and gram-negative bacteria as well as against fungi. Antimicrobial activity was evaluated using broth microdilution method. *J. heuffelii* extract showed moderate antibacterial activity, while its antifungal activity was significantly higher. The concentration of total phenolic compounds of the extracts from roots ranged from 47.98 to 31.33 mg of GAE/g. The concentrations of flavonoids in the tested plant extracts ranged from 5.26 to 16.44 mg of RuE/g. The high contents of total phenolic compounds and flavonoids indicated that these compounds contribute to the antimicrobial activity. Antioxidant activity of plant extracts was examined *in vitro* using 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent and compared with the reference substances values (3-tert-butyl-4-hydroxyanisole (BHA), rutin, chlorogenic acid). The results pointed to high antioxidant activity of phenolic compounds in *J. heuffelii* extracts.

Key words: *Jovibarba heuffelii*, antioxidant, phenolic compounds, flavonoids, antimicrobial activity.

INTRODUCTION

Jovibarba heuffelii (Schott.) A. Löve and D. Löve (syn. *Sempervivum heuffelii,* Schott, 1852) belongs to the family Crassulaceae (Gajić, 1972; Parnell and Favarger, 1993). It is a Balkan-Carpathian endemic plant with distribution in the middle part of the Balkan peninsula, and the Central and Southern parts of Karpats (Jalas et al., 1999). *J. heuffelii* is a succulent plant with a rosetted habit. It is very resistent to arid conditions and can survive without water for several months. The leaves are numerous, contiguous and are distributed in a helicoid way on the short stem. Flowering occurs after several years (at least 2 years, 3 to 4 years on average; counting starts from the germination or after the appearance of an offset). The main stem elongates and produces an erect leafy stem crowned by a several-branched inflorescence. The whole flowered rosette dies after fructification (monocarpy), but the plant survives owing to the side offsets, produced during the years before the flowering. *Jovibarba* resembles *Sempervivum* in habit, but differs by its 6-merous flowers with yellowish, fringed, erect petals. Morphology of *J. heuffelii* is very variable. The most recent studies of its morphological features showed high correlation of this variability with altitude and geological substrate (Dimitrijević et al., 2011). In Serbia, the areal of this plant is limited to high mountain's peaks, canions and gorges. It grows on rocky habitats with northern exposition. Considering geological substrate, this plant possess

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wide ecological valence and grows on limestone, silicates and serpentinites.

There are several literature data about using of the genus Sempervivum in ethnopharmacology (Bremness, 1994; Blaschek et al., 1998; Abram and Donko, 1999). Althouth they are used in folk medicine for the treatment of various inflamations and disorders, the plants from this genus are still not introduced into modern medical practice. However, since morphology and anatomy of these two plant genera (Sempervivum and Jovibarba) are very similar and familiar to profesionals only, we can assume that etnopharmacological usage utilizes both plants for the same purposes. Unlike genus *Sempervivum*, which is a well known medicinal plant, already investigated in several papers (Abram and Donko, 1999; Alberti et al., 2008; Stojičević et al., 2008), there is a complete lack of literature data about entire *Jovibarba* genus and especially for *J. heuffelii* species, considering antimicrobial, antioxidative activity and also therapeutic potentials.

Phenolic compounds have been reported for anti-bacterial, antifungal, antiviral, antioxidative, antidiabetic, anticarcinogenic, antiallergic, antimutagenic and anti-inflammatory activities (Abram and Donko, 1999, Alberti et al., 2008). Flavonoids are major group of phenolic compounds reported for their antiviral, antimicrobial and antioxidant properties (Friedman, 2007). Chlorogenic, gallic, protocatechuic, caffeic, *p*-coumaric, *p*-hydroxybenzoic, *p*-hydroxyphenylacetic, vanillic, syringic, ferulic, synapic and α-rezorcylic acids were identified phenolic acids in leaves and roots of *Jovibarba sobolifera* (Sims.) Opiz. (Szewczyk and Krzaczek, 2004).

Increased concentration of free radicals causes many pathological conditions in organism such as inflammatory, neurological, psychiatric diseases and carcinogenesis (Chan, 1994, Salim, 1996, Valko et al., 2006, Ognjanović et al., 2008), and their negative effects can be largely prevented by intake of antioxidant substances (Ben-Shaul et al., 2000).

Antioxidants can be of synthetic or natural origin and a great number of secondary metabolites isolated from plants (such as various phenolic compounds) are antioxidants. The most widely used synthetic antioxidants have been suspected to cause or promote negative health influences and genotoxic effects. Natural antioxidants in most cases do not show a negative effect and allow unrestricted use (Branen, 1975, Chen et al., 1992). Secondary metabolites are involved in an important biological and pharmacological activity such as anti-oxidative, anti-allergic, antibiotic, hypoglycemic and anti-carcinogen (Mulabagal and Tsay, 2004, Katalinić et al., 2004, Borneo et al., 2008, Moein and Moein, 2010).

Till now, there was only one study dealing with *J. heuffeli,* whose results showed that diethyl-ether extract, isolated from leaves, possess moderate antibacterial activity and high antifungal activity. Also, in the same study, total phenol and flavonoid concentrations in diethyl-ether, methanolic, acetone and ethyl-acetate extracts from leaves of *J. heuffelii* were high (Dimitrijević et al., 2010).

The present investigation is prompted by the fact that there is no data about the antioxidant activity of *J. heuffelii*. Also, there is a complete lack of the literature concerning the comparative analysis of total phenolics and flavonoid concentrations of the extracts isolated from different parts of *J. heuffelii.* Considering only one study dealing with antimicrobial activity of the diethyl ether extract, antimicrobial potential of this plant is not completely investigated.

The aim of the present research was to determine antimicrobial and antioxidant activities of various extracts isolated from leaves and roots of this plant. As a logical addition to the previous study of this plant species, the content of total phenolics and flavonoid concentration in the different extracts, isolated from the roots of *J. heuffelii*, was determined.

MATERIALS AND METHODS

Plant material

Plant material was collected from its natural habitats in Serbia, locality Besna Kobila Mt. (1900 m altitude) at the full flowering stage. The collection was performed in August, 2010. The voucher specimens of *J. heuffelii* are deposited at the Herbarium BEOU (NO 16463), Belgrade, Serbia.

Chemicals

Acetone, methanol, ethyl acetate and sodium hydrogen carbonate were purchased from “Zorka pharma” Šabac, Serbia. Gallic acid, rutin hydrate, chlorogenic acid, triphenyl tetrazolium chloride (TTC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Chemicals Co., St Louis, MO, USA. Folin-Ciocalteu phenol reagent, 3-tert-butyl-4-hydroxyanisole (BHA) and aluminium chloride hexahydrate were purchased from Fluka Chemie AG, Buchs, Switzerland. Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar were obtained from Himedia, Mumbai, India. All other solvents and chemicals were of analytical grade.

Preparation of plant extracts

Three leaf and root extracts using different organic solvents (methanol, aceton and ethyl acetate) were prepared to examine the antimicrobial activity, total phenolic content, flavonoid concentration and free radical scavenging activity.

The fresh clean leaves and roots of *J. heuffelii* were cut up and pounded. Cold extraction was carried out by pouring different solvents over 10 g of the sample and allowed to stand for 7 days in darkness. After the filtration and drying over anhydrous magnesium sulfate, the extract was concentrated under rotary evaporator and stored in sample bottle in refrigerator prior the assay.

Testing of antimicrobial activity

The extracts were tested against a panel of microorganisms including Gram-positive *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 6538, *S. aureus* (clinical isolate), *S. aureus* (isolate

Table 1. Minimal inhibitory (MIC) and minimal bactericidal/fungicidal (MBC/MFC) concentrations of *Jovibarba heuffelii* extracts.

Bacterial strain		Methanolic extract (leaves)		Methanolic extract (root)		Ethyl acetate extract (leaves)		Acetone extract (leaves)		Tetracycline (µg/ml)	Nystatin (µg/ml)
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MIC
E. coli	ATCC 8739	30.00	30.00	>32.00	>32.00	>12.00	>12.00	>32.00	>32.00	1.56	NT*
E. coli	Isolate from food	3.250	>30.00	32.00	>32.00	6.00	>12.00	8.00	>32.00	0.19	NT
E. coli	Clinical isolate	3.250	>30.00	16.00	>32.00	12.00	>12.00	8.00	16.00	0.19	NT
K. pneumoniae	ATCC 10031	15.00	30.00	>32.00	>32.00	>12.00	>12.00	16.00	>32.00	0.39	NT
K. pneumoniae	Isolate	1.62	>30.00	4.00	>32.00	6.00	>12.00	2.00	>32.00	0.39	NT
S. enteritidis	ATCC 13076	15.00	30.00	>32.00	>32.00	>12.00	>12.00	>32.00	>32.00	0.19	NT
S. lutea	ATCC 9431	0.81	7.50	32.00	>32.00	1.50	6.00	8.00	>32.00	0.39	NT
S. aureus	ATCC 6538	1.62	7.50	2.00	4.00	3.00	6.00	2.00	16.00	0.09	NT
S. aureus	ATCC 27853	7.50	30.00	32.00	>32.00	12.00	>12.00	16.00	>32.00	0.09	NT
S. aureus	clinical isolate	7.50	30.00	32.00	>32.00	12.00	>12.00	16.00	>32.00	0.09	NT
S. aureus	Isolate from food	15.00	>30.00	>32.00	>32.00	>12.00	>12.00	16.00	>32.00	0.19	NT
B. cereus	Isolate	1.62	15.00	16.00	>32.00	3.00	12.00	4.00	>32.00	0.19	NT
B. subtilis	ATCC 6633	3.50	15.00	4.00	4.00	0.18	6.00	2.00	16.00	0.09	NT
M. flavus	ATCC 10240	15.00	30.00	>32.00	>32.00	>12.00	>12.00	8.00	16.00	0.39	NT
C. albicans	ATCC 10231	0.20	3.25	4.00	16.00	1.50	1.50	1.00	4.00	NT	6.25

NT-not tested.

from food), *Sarcina lutea* ATCC 9431, *Micrococcus flavus* ATCC 10240, *Bacillus subtilis* ATCC 6633, *B. cereus* (isolate from food), Gram-negative *Escherichia coli* ATCC 8739, *E. coli* (clinical isolate), *E. coli* (isolate from food), *Klebsiella pneumoniae* ATCC 10031, *K. pneumoniae* (clinical isolate), *Salmonella enteritidis* ATCC 13076 and yeast *Candida albicans* ATCC 10231.

Bacterial isolates were clinical isolates from the Institute of Public Health, Kragujevac and Institute of Public Health, Niš, stored in microbiological collection at the Microbiology Laboratory (Faculty of Sciences and Mathematics, Department of Biology and Ecology, University of Niš).

Antimicrobial activity was evaluated using a broth microdilution method according to the NCCLS method (NCCLS, 2003). Minimum inhibitory concentrations (MIC) determination was performed by a serial dilution method in 96 well microtitre plates. The test species were cultured at 37℃ in Mueller Hinton agar for bacteria and Sabouraud dextrose agar for yeast (30℃). After 18 h of cultivation, bacterial suspensions were made in Mueller Hinton broth and their turbidity was standardized to 0.5 McFarland turbidity standard (corresponding to ~1.5 × 10^8 colony forming units per milliliter (CFU/ml) for bacteria and 1.5 × 10^7 CFU/ml for yeasts). Optical density of every suspension was confirmed on spectrophotometer (UV-VIS 1650, Shimadzu). The final density of bacterial and yeast's inoculum was 5 × 10^5 CFU/ml.

Stock solutions of the extracts were prepared in 70% ethanol and than serial doubling dilutions of the extracts were made in a concentration range from 0.005 to 32 mg/ml. Concentration of ethanol in each well never exceeded 5%. The inoculum was added to all wells and the plates were cultivated at 37℃ during 24 h (bacteria) or at 30℃ for 48 h (fungal strains). Tetracycline and Nystatin served as a positive control, while the solvent was used as a negative control. One inoculated well was included, to allow control of the adequacy of the broth for organism growth. One non inoculated well, free of antimicrobial agent, was also included, to ensure medium sterility.

The bacterial growth was determined by adding 20 µl of 0.5% TTC (triphenyl tetrazolium chloride) aqueous solution. MIC was defined as the lowest concentration of the extract that inhibited visible growth (red coloured pellet on the bottom of the wells after the adding of TTC). To determine MBC/MFC (minima bactericidal/fungicidal concentration), broth was taken from each well without visible growth and inoculated in Mueller Hinton agar (MHA) for 24 h at 37℃ for bacteria or in Sabouraud dextrose agar (SDA) at 30℃ (yeasts). The MBC/MFC is defined as the lowest concentration of the extracts at which inoculated microorganisms were 99.9% killed. The experiments were done in triplicate and the mean values are presented in the Table 1.

Determination of total phenolic content

The amount of total phenolic compound in the *J. heuffelii* root extract was determined spectrophotometrically using Folin-Ciocalteu (FC) reagent according to the method by

Singleton et al. (1999). The methanolic solution of the extract in concentration of 1 mg of dry extract /ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of the extract, 2.5 ml of 10% FC reagent and 2 ml of 7.5% $NaHCO_3$. The blank was concomitantly prepared containing 0.5 ml of methanol, 2.5 ml of 10% FC reagent dissolved in water and 2 ml of 7.5% of $NaHCO_3$. After 15 min of incubation at 45°C, absorbance was determined using spectrophotometer (Iskra, MA9523-SPEKOL 211) at λ_{max} = 765 nm. Based on the measured absorbance, the concentration of total phenolic compounds was read from the calibration line; then the concentration of total phenolic compounds in the extract was expressed in terms of gallic acid equivalent (mg of GAE /g).

Determination of total flavonoid content

The content of flavonoids in the *J. heuffelii* root extract was determined using spectrophotometric method (Quettier et al., 2000). The sample contained methanol solution of the extract in concentration of 1 mg of dry extract/ml and 2% $AlCl_3$ solution dissolved in methanol in equal volumes. The sample was incubated for an hour at room temperature. The absorbance was determined using spectrophotometer (Iskra, MA9523-SPEKOL 211) at λ_{max} = 415 nm. The sample was prepared in triplicate for analysis and the mean value of the absorbance was obtained. The same procedure was repeated for the solution of rutin (0 to 50 mg/l) as reference substance and the calibration line was constructed. Based on the measured absorbance, the concentration of flavonoids was read on the calibration line; then, the content of flavonoids in the extract was expressed **in** terms of rutin equivalent, (mg of RuE/g).

Evaluation of antioxidant activity

The ability of the plant extract to scavenge DPPH free radicals was assessed by the standard method (Tekao et al., 1994), adopted with suitable modifications (Kumarasamy et al., 2007) DPPH (20 mg) was dissolved in methanol (250 ml) to obtain the concentration of 80 µg/ml. The stock solution of the plant extract was prepared in methanol to achieve the concentration of 1 mg of dry extract/ml. Dilutions were made to obtain concentrations of 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.99 and 0.97 µg/ml. Diluted solutions were mixed with DPPH. After 30 min in darkness at room temperature, the absorbance (A) was recorded using spectrophotometer (Iskra, MA9523-SPEKOL 211) at λ_{max} = 517 nm. The percentage inhibition was calculated using Equation 1, while IC_{50} values were estimated from the % inhibition versus concentration sigmoidal curve, using a non-linear regression analysis. A parallel analysis of the reference synthetic antioxidants (BHA, rutin and chlorogenic acid) was carried out and compared with the values related to *J. heuffelli*. Results are displayed as DPPH scavenging effect (% inhibition) of the methanolic solution of extracts in 1 mg/ml of concentration and IC_{50} values in µg/ml:

$$\%\ inhibition = \left(\frac{A \text{ of control} - A \text{ of sample}}{A \text{ of control}} \right) x\,100 \quad (1)$$

Statistical analysis

All experimental measurements were carried out in triplicate and are expressed as average of three analyses ± standard deviation. The magnitude of correlation between variables was done using a SPSS (Chicago, IL) statistical software package (SPSS for Windows, ver. 17, 2008).

RESULTS

Antimicrobial activity

Antimicrobial activity of *J. heuffelii* extracts is presented in Table 1. The results of minimum inhibitory (MIC) and minimum bactericidal/fungicidal concentrations (MBC/MFC) showed moderate activity of *J. heuffelii* extracts. The highest activity was exhibited by leaf methanolic extracts, with determined inhibitory activity against all tested strains. On the other hand, ethyl acetate leaf extract showed the weakest antimicrobial activity with inhibitory effect against 10 out of the 15 tested strains. The activity of the extracts can be presented in the following order: methanolic leaf extract > aceton leaf extract > ethyl acetate leaf extract > methanolic root extract. The most sensitive strains against all tested extracts were *C. albicans* (ATCC 10231), *S. aureus* (ATCC 6538), *B. subtilis* (ATCC 6633) followed by isolates of *K. pneumoniae* and *B. cereus*. The most resistants strains were *E. coli* (ATCC 8739), *S. enteritidis* ATCC 13076 and isolate of *S. aureus* (Table 1).

Total phenolic content

The concentration of total phenolic compounds in the examined plant extracts using the FC reagent was expressed in terms of gallic acid equivalent, (the standard curve equation: y = 7.026x – 0.0191, r^2 = 0.999) as mg of GAE/g (Table 2). The obtained values of total phenolic content in root extracts ranged from 31.33 to 41.98 mg GAE/g. Methanolic extract (41.98 mg GAE/g) contained the highest concentration of phenolic compounds.

Flavonoid concentration

The concentration of flavonoids in plant extract isolated from roots of *J. heuffelii* was determined using spectrophotometric method with $AlCl_3$. The concentration of flavonoids was expressed in terms of rutin equivalent, Ru (the standard curve equation: y = 17.231x – 0.0591, r^2 = 0.999), mg of RuE/g. The quantity of flavonoids identified in the tested extracts is presented in Table 2. Values obtained for the concentration of flavonoids in the root extracts ranged from 5.26 to 16.44 mg of RuE/g. High concentrations of flavonoids were determined in the ethyl acetate extracts.

Antioxidant activity

Antioxidant activity of the *J. heuffelii* different plant extracts was determined using methanol solution of DPPH reagent. In recent years, DPPH method has been used to quantify antioxidants in complex biological systems. This method is based on the reduction of

Table 2. Free radical scavenging activity, total phenol and flavonoid concentration of *Jovibarba heufelii* extracts.

Type of extract	% of inhibition[1]	IC_{50} values (µg/ml)	Flavonoid contents (mg of RuE/g of extract)	Phenol concentration (mg of GAE/g of extract)
Leaves				
Methanolic	76.51 ± 0.9	422.69 ± 1.5	ND[2]	ND
Acetone	74.12 ± 0.7	396.81 ± 1.7	ND	ND
Ethyl acetate	55.04 ± 1.2	708.96 ± 2.1	ND	ND
Roots				
Methanolic	78.94 ± 0.9	304.19 ± 1.9	5.26 ± 0.4	41.98 ± 0.7
Acetone	65.88 ± 0.81	480.55 ± 1.36	15.12 ± 0.77	37.54 ± 0.69
Ethyl acetate	58.45 ± 1.12	550.14 ± 1.1	16.44 ± 0.83	31.33 ± 0.24
Standard substances				
Rutin	93.71 ± 1.1	9.28 ± 0.27	-	-
Chlorogenic acid	96.60 ± 1.8	11.65 ± 0.52	-	-
BHA	93.37 ± 2.5	5.39 ± 0.31	-	-

All values are average of three analysis ± SD. [1] Presented as percentage of inhibition in the 1 mg/1 ml solution of extracts; [2] ND- not determined (already published in Dimitrijević et al., 2010).

methanolic solution of colored free radical DPPH by free radical scavenger. The scavenging activity was measured as the decrease in absorbance of the samples versus DPPH standard solution.

The antioxidant activity of the six different extracts from leaves and roots of *J. heuffelii* is expressed as DPPH scavenging effect (% inhibition) of the methanolic solution of extract in concentration of 1 mg of dry extract/ml and IC_{50} values. (Table 2). Parallel to the examination of the antioxidant activity of the plant extracts, the antioxidant activities of rutin, chlorogenic acid and BHA were determined and used as a reference values (Table 2).

Percentage values for antioxidant activity of leaves ranged from 55.04 to 76.51%. The largest capacity to neutralize DPPH radicals was measured in methanolic and acetone extracts (Table 2). The antioxidant activity of the same extracts, but expressed as IC_{50} ranged from 396.81 to 708.96. The highest capacity to neutralize DPPH radicals was measured in the acetone extract, which neutralized 50% of free radicals at the concentration of 396.81 µg/ml.

Percentage values for antioxidant activity of roots from *J. heuffelii* ranged from 58.45 to 78.94%. Once again, the methanolic extract showed the largest antioxidant potential. Values for antioxidant activity expressed as IC_{50} ranged from 304.19 to 550.14. The largest capacity to neutralize DPPH radicals was measured in the methanolic extract, which neutralized 50% of free radicals at the concentration of only 304.19 µg/ml.

DISCUSSION

The antimicrobial activity of the tested *J. heuffelii* extracts was in the range from 0.18 to 32.00 mg/ml, depending on the extraction solvent, concentration of the extract and the tested organism. The range of the determined active concentrations was wide and can be characterized as high or, in more cases, moderate antimicrobial activity. *J. heuffelii* extracts were bactericidal at very high concentrations, or in most cases, there was no bactericidal activity at the tested concentrations.

According to this, one can conclude that *J. heuffelii* extracts exhibit mostly inhibitory but not cidal effect to microorganisms. The extracts from leaves showed higher antimicrobial effect than the one obtained from the roots, indicating higher concentration of antimicrobial compounds in the leaves. Also, when observing antimicrobial activity against bacterial species, it can be noticed that Gram-positive bacteria were more susceptible. This can be attributed to the differences in the cell envelope composition between Gram-positive and Gram-negative bacteria, which affect permeability and susceptibility of these organisms to different compounds (Sikkema, 1995).

According to the results obtained, we can conclude that methanol extracted the compounds with the highest antimicrobial activity and that these compounds are located in the leaves of the plant. This was expected, since ethnopharmacological usage of this plant describes the leaves as the active plant parts. Also, it can be concluded that phenolic compounds, well known by their antimicrobial activity (Burt, 2007) are mostly responsible for the obtained results. Due to the slight disagreement of the antimicrobial activity and phenol composition results (significant concentration in the roots but low antimicrobial activity), we can assume that some other compounds, such as flavonoids (determined in low amount

in the root extract), possess significant role in the antimicrobial effect of the tested extracts.

The results of antimicrobial activity showed the highest effect against fungi *C. albicans*, which is in accordance with our previous results obtained for diethyl ether extract. This extract was active against five bacterial and six fungal strains and the results showed that it exhibited higher activity against fungi (Dimitrijević et al., 2010). Further comparison with the previous results is possible only for relative species, *Sempervivum tectorum*, since there are no any more papers dealing with the plant investigated in the present paper. The mentioned study showed significant antimicrobial activity against *S. aureus*, *B. cereus*, *E. fecalis* and *Geotrichum sp.*, while *E. coli*, *P. morgani* and *S. cerevisiae* showed higher resistance to the activity of the extract, where only inhibitory activity, but not bactericidal or fungicidal, was determined (Abram and Donko 1999). In the study of Stojičević et al. (2008), methanolic extract of the *S. marmoreum* exhibited activity only against fungal species *Aspergillus niger* and *C. albicans*. This can be used as an argument for the conclusion that plants belonging to these two closely related genera possess compounds which can interact more easily with the fungal membranes, and further investigations should be focused to the detection and identification of these active principles.

Previous results for the concentrations of total phenolic compounds in the leaf extracts of this plant showed that all tested extracts, except the ethyl acetate one, contained significant concentration of the phenolic compounds (Dimitrijević et al., 2010). Methanolic extract from the roots showed an appreciable concentration of phenolic compounds, but lower than the methanolic and acetone leaf extract. Considering the concentration of flavonoids, it can be noticed that only a small fraction of the mentioned methanolic extract (5.26 mg Ru/g extr.) belongs to the flavonoids subclass. This is very different result when compared to the previous ones, obtained for the various extracts isolated from leaves of this plant, where concentrations of flavonoids ranged from 10.00 to 30.00 mg Ru/g of extract (Dimitrijević et al., 2010).

Comparison of the results for the reference substances and the results indicated that *J. heuffelii* has a high ability of DPPH radicals inhibition. Among the tested extracts, the one obtained from the roots exhibited the highest potential for free radical inhibition, while the extract isolated using ethyl acetate showed the lowest inhibition of free radicals. In the previous phytochemical investigations of *J. heuffelii,* kaempferol and myricetin were identified in vegetative parts (Stevens et al., 1996). For these flavonol aglycons, number of studies demonstrated strong antioxidant activity (Tokuşoğlu et al., 2003). It was confirmed that kaempferol and kaempferol-3-glucoside significantly contribute to the antioxidative properties of the juice from fresh leaves of *S. tectorum* (Šentjurc et al., 2003).

Comparison of the phenolic compounds concentration and antioxidative activity points to the fact that the extracts with the highest concentrations of phenolic compounds also possess strong scavenging effect. Based on these results, each extract of *J. heuffelii* exhibited phenolic concentration-dependent scavenging effects. Investigations of the antioxidant activity of plant extracts from related species have confirmed a high linear correlation between the values of the phenolic content and antioxidant activity (Stojičević et al., 2008).

In addition, till date, the phenolic contents of the tested extracts of *J. heufelii* depended on the plant part used in the experiment and solvents used for the extraction. Also, it can be concluded that not only the concentration of phenolic contents but also properties of these compounds contributed to the activities of different extracts. The obtained results indicated that the methanolic extract from the roots of *J. heuffelii* showed a stronger antioxidant activity than those obtained by other solvents.

Results of our analysis suggest that the species *J. heuffelii* has a significant potential for use in pharmacy and phytotherapy. Based on this information, parts of this plant are natural sources of antimicrobial and antioxidant substances of high importance. Further studies of this plant species should be directed to a detailed qualitative analysis of all its parts and carry out *in vivo* studies of its medically active components in order to prepare a natural pharmaceuticals of high value.

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