Phytochemical screening and biological activity of extracts of plant species *Halacsya sendtneri* (Boiss.) Dörfl.

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Abstract

This study is aimed at examining total polyphenol, flavonoid, gallotannin and condensed tannins contents in acetone, chloroform, ethyl acetate and petroleum ether extracts of *Halacsya sendtneri* (Boiss.) Dörfl., their antimicrobial and antioxidant activities, as well as identifying and quantifying the phenolic components. The antioxidant activity is consistent with the results of total quantity of phenolic compound. The results showed that the acetone extract of plant species *Halascya sendtneri* (Boiss.) Dörfl. possessed the highest antioxidant activity. The IC_{50} values determined were: 9.45±1.55 μg/mL for DPPH free radical scavenging activity, 13.46±1.68 μg/mL for inhibitory activity against lipid peroxidation, 59.11±0.83 μg/mL for hydroxyl radical scavenging activity and 27.91±0.88 μg/mL for ferrous ion chelating ability. The antimicrobial activity was tested using broth dilution procedure for determination of the minimum inhibitory concentration (*MIC*). The *MIC*s were determined for 8 selected indicator strains. All of the extracts showed strong to moderate strong antimicrobial activity. The phenolic composition of *Halacsya sendtneri* extracts was determined by the HPLC method. The dominant phenolic compound in acetone, chloroform and ethyl acetate extract is rosmarinic acid. Ethyl acetate extract was also abundant in *p*-hydroxybenzoic acid and ferulic acid. The main compounds in petrol ether extract were chlorogenic acid and quercetin.

Keywords: antimicrobial activity; antioxidant activity; HPLC analysis; *Halacsya sendtneri* (Boiss.) Dörfl.

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Recently, interest for plants that is recognized in traditional medicine for prophylactic and therapeutic application increased worldwide. Interactions between plants and their environment lead to production of different biologically active substances (secondary metabolites). These substances are common for certain plants and plant families. Many of them and his extracts show clearly antimicrobial effects (against bacteria, fungi and viruses) [1–3].

Effective prevention of a number of diseases may be achieved with different parts of plants (root, leaf, flower, fruit, stem and bark). Potential toxicity of these bioactive substances has not been well established in humans [4]. Their antimicrobial and antioxidant effects affect a range of physiological processes in the human body, but may provide protection on free radicals and growth of undesirable microorganisms.

Greater consumer awareness and concern regarding synthetic chemical additives has led researchers to look for natural food additives with a large spectrum of antimicrobial effects. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have the ability to terminate lipid peroxidation, which causes liver damage and carcinogenic changes [5]. The capacity of plants to synthesize biologically active compounds and their resistance to great number of antibiotics are reasons for their use in bacterial control. In the last decade, the food industry has been trying to replace synthetic preservatives, antioxidants or other food additives by use of various plants [6]. Essential substances for antioxidant activity of medicinal herbs are phenolic compounds [7]. The abovementioned and many other compounds from plants are useful for substitutive therapy, or as models for new synthetically derived substances [8]. A great number of active substances form herbs are important for normal growth and development, or defense against infection

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and injury. Ubiquitous compounds of plants are phenolic; biologically active substances are flavonoids and other phenolics (phenolic acids, stilbenes, tannins, lignans, and lignins), too. These compounds are commonly found in both edible and nonedible plants, and they have been proven to be multiple biologically active, including antioxidant effects [9]. Various investigations have shown that total phenolic contents are closely related to antioxidant activity [10], and with flavonoids and tannins represent major herb compounds with antioxidant activity [11]. Similar studies show the importance of popular knowledge for selection of plant species with potential use in diseases treatment. Combined multidisciplinary studies (botany, pharmacology, pharmacognosy, toxicology and anthropology) show that the use of systematically tested plants over time can be applied worldwide [12]. Plant origin drugs and their use are not under permanent monitoring by health professionals, which would ensure efficacy and safety procedures. This could result in loss of biological effects or even toxic effects [13].

Halacsya sendtneri is a member of the monotypic genus *Halacsya* of the family Boraginaceae, its range being limited to parts of the habitat in the central Balkans. The species inhabits open serpentine rocky landscapes at altitudes ranging from 190 to 1500 m [14]. In Serbia, it is found along the serpentines of certain mountains, as well as in some gorges. *H.sendtneri* is considered a Tertiary relict [15]. It is qualified as a vulnerable species (V) in the European Red List (marked as +) [16]. Apart from being highly important in terms of world plant gene pool preservation, endemic plants can also contribute substantially to studies on antimicrobial activity [17]. Various investigations implied that total phenolic compounds are closely related to antioxidant activity, with flavonoids and tannins being major plant compounds with antioxidant activity [18]. No previous studies on the biological activity or chemical constituents of *Halacsya sendtneri* have been reported in the literature.

EXPERIMENTAL

Plant material

The test plant was collected at Ilijak Hill (Central Serbia) in May/June 2008. The species was identified and the voucher specimen was deposited at the Department of Botany, Faculty of Biology, University of Belgrade (16336 BEOU, Lakušić Dmitar).

Chemicals

Methanol (HPLC, gradient grade), quercetin and formic acid (HPLC) were supplied by Merck KGaA (Darmstadt, Germany). Folin-Ciocalteau's reagent and standard substances including gallic acid, protocatechuic acid, caffeic acid, vanillic acid, chlorogenic acid, syringic acid, ferulic acid, rutin, myricetin, rosmarinic acid, *trans*-cinnamic acid, naringenin, luteolin, kaempferol, apigenin and aloe-emodin were purchased from Sigma-Aldrich GmbH (Sternheim, Germany). Water used throughout the experiments was purified using a Millipore, Elix UV and Simplicity Water Purification System (Milford, MA, USA). 1,1-Diphenyl-2-picrylhydrazyl hydrate (DPPH), Folin–Ciocalteu, Muller–Hinton broth, ascorbic acid, butylated hydroxytoluene (BHT), nystatin and pyrocatechol were purchased from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Hydrochloric acid, formaldehyde, anhydrous sodium carbonate, potato glucose agar, methanol, ethanol, acetone and petroleum ether were purchased from Centrohem (Centrohem, Stara Pazova, Serbia). All other chemicals and reagents were of analytical reagent grade.

Spectrophotometric measurements

Spectrophotometric measurements were performed using a UV–Vis spectrophotometer MA9523-Spekol 211 (ISKRA, Horjul, Slovenia).

Preparation of the extracts

The plant material was air-dried at room temperature (26 °C) for one week, after which it was ground to a uniform powder. Mean particle size d = 0.388 mm was determined using sieve sets (Erweka, Germany). All extracts (acetone, chloroform, ethyl acetate and petroleum ether) were prepared by soaking 500 g dry powdered plant material in 2000 mL of solvent at room temperature for 3 days. The extracts were filtered through a Whatman No. 42 (125 mm) filter paper, concentrated using a rotary evaporator and were dried at 60 °C to the constant mass.

Test microorganisms

The antimicrobial activity of the plant extracts were tested *in vitro* against the following bacteria: *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 13315, *Proteus mirabilis* ATCC 14153, *Bacillus subtilis* ATCC 6633, and fungi; *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404. The fungi were cultured on potato-glucose agar for 7 days at room temperature of 20 °C under alternating light and dark conditions. They were recultured on a new potato-glucose substrate for another 7 days. The culturing procedure was performed four times until pure culture was obtained. The identification of the test microorganisms was confirmed by the Laboratory of Mycology, Department of Microbiology, Torlak Institute, Belgrade, Serbia.

Determination of total phenolic, flavonoid, condensed tannins and gallotannins content

Total phenols were estimated according to the Folin-Ciocalteu method [20]. The extract was diluted to

the concentration of 1 mg/mL, and aliquots of 0.5 mL were mixed with 2.5 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and 2 mL of $NaHCO_3$ (7.5%). After 15 min at 45 °C, the absorbance was measured at 765 nm using a spectrophotometer against a blank sample. Total phenols were determined as gallic acid equivalents (mg GAE/g extract), and the values are presented as means of triplicate analyses.

Total flavonoids were determined according to [21]. A total of 0.5 mL of 2% aluminium chloride ($AlCl_3$) in methanol was mixed with the same volume of methanol solution of plant extract. After 1 hour of staying at room temperature, the absorbance was measured at 415 nm in a spectrophotometer against the blank sample. Total flavonoids were determined as rutin equivalents (mg RUE/g dry extract), and the values are presented as means of triplicate analyses.

The method for determination of condensed tannins relies on the precipitation of proanthocyanidins with formaldehyde [22]. First, total phenolics were measured using the Folin-Ciocalteu reagent as described above. A 0.5 mole equivalent of phloroglucinol was added for every gallic acid equivalent in the extract. An aliquot of 2 mL of the extract dissolved in methanol was mixed with the calculated amount of phloroglucinol, followed by 1 mL of 2:5 HCl /H_2O solution and 1 mL of formaldehyde solution (13 mL of 37% formaldehyde diluted to 100 mL in water). After overnight incubation at room temperature, unprecipitated phenols were estimated in the supernatant by the Folin-Ciocalteu method. The precipitate contains proanthocyanidins and the known amount of phloroglucinol, which is always quantitatively precipitated. The concentration of condensed tannins was calculated as residuum of the total phenolic and unprecipitated phenol concentrations, and expressed as gallic acid equivalents. The results are given in Table 1, and presented as means of triplicate analyses.

Gallotannins are hydrosoluble tannins containing a gallic acid residue esterified to a polyol. Gallotannins can be detected quantitatively by the potassium iodate assay. This assay is based on the reaction of potassium iodate (KIO_3) with galloyl esters [18], which will form a red intermediate and ultimately a yellow compound. The concentration of the red intermediate can be measured spectrophotometrically at 550 nm. The reaction was performed by adding 1.5 mL of a saturated potassium iodate solution to 3.5 mL of extract at a temperature of over 40 °C until maximum absorbance was reached (regardless of time). Gallotannin content was determined using gallic acid as standard. The results are given in Table 1, and presented as means of triplicate analyses.

HPLC Analysis

Quantification of individual phenolic compounds was performed by reversed phase HPLC analysis, using a modified method of Mišan *et al*. [28]. HPLC analysis was performed by using a liquid chromatography (Agilent 1200 series), equipped with a diode array detector (DAD), Chemstation Software (Agilent Technologies), a binary pump, an online vacuum degasser, an autosampler and a thermostated column compartment, on an Agilent Zorbax Eclipse Plus-C18, 1.8 µm, 600 bar, 2.1×50 mm column, at a flow rate of 0.8 mL/min. Gradient elution was performed by varying the proportion of solvent A (methanol) to solvent B (1% formic acid in water (v/v)) as follows: initial 0–2 min, 100% B; 2–4 min, 100-98% B; 4–6 min, 98–95% B; 6–7 min, 95–73% B; 7–10 min, 75–48% B; 10–12 min 48% B; 12–20 min, 48–40% B. The total running time and post-running time were 21 and 5 min, respectively. The column temperature was 30 °C. The injected volume of samples and standards was 5 µL and it was done automatically using an autosampler. The spectra were acquired in the range 210–400 nm and chromatograms plotted at 280, 330 and 350 nm with a bandwidth of 4 nm, and with reference wavelength/bandwidth of 500/100 nm.

Determination of total antioxidant activity of the *H. sendtneri* extracts and antioxidants

Determination of total antioxidant activity

The total antioxidant activity of the *Halacsya sendtneri* extract was evaluated by the phosphomolybdenum method [23]. The assay is based on the reduction of Mo (VI)–Mo (V) by antioxidant compounds and subsequent formation of a green phosphate/Mo (V) complex at acid pH. A total of 0.3 mL of sample extract was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95 °C for 90 min. Then, the absor-

Table 1. Total phenolics, flavonoids, condensed tannins, gallotannins and total antioxidant capacity of the H. sendtneri extracts (results are mean values ± SD from three experiments; abbreviations: AA – ascorbic acid, GAE – gallic acid, RUE – rutin)

Type of extract	Total phenolics mg GAE/g	Flavonoids mg RUE/g	Condensed tannins mg GAE/g	Gallotanins mg GAE/g	Total antioxidant capacity µg AA/g
Acetone	97.26±0.69	31.24±0.55	78.65±0.75	31.74±1.05	98.45±0.98
Chloroform	91.34±0.56	29.45±0.78	73.56±0.48	29.45±0.78	89.75±0.25
Ethyl acetate	92.45±0.55	30.12±0.65	75.87±0.35	29.47±0.54	94.34±0.43
Petroleum ether	85.45±0.95	29.25±0.98	75.87±0.35	26.78±0.87	79.94±0.54

bance of the solution was measured at 695 nm using spectrophotometer against the blank after cooling to room temperature. Methanol (0.3 mL) instead of extract was used as the blank. Ascorbic acid (AA) was used as the standard and total antioxidant capacity was expressed as milligrams of ascorbic acid per gram of dry extract.

Determination of DPPH free radical scavenging activity

The method used by Takao [24] was adopted with suitable modifications from Kumarasamy [25]. DPPH (2,2-dephenyl-1-picrylhydrazyl) (8 mg) was dissolved in 99% methanol (100 mL) to obtain a concentration of 80 μg/mL. Serial dilutions were carried out with the stock solution (1 mg/mL) of the extract. Solutions (2 mL each) were then mixed with DPPH (2 mL) and allowed to stand for 30 min for any reaction to occur, and the absorbance was measured at 517 nm. Ascorbic acid (AA), gallic acid (GA) and butylated hydroxytoluene (BHT) were used as reference standards and dissolved in methanol to make the stock solution with the same concentration (1 mg/mL). The control sample was prepared containing the same volume without test compounds or reference antioxidants. Methanol (95%) was used as a blank. The DPPH free radical scavenging activity (%) was calculated using the following equation:

$$\text{Inhibition} = 100\frac{A_c - A_s}{A_c} \quad (1)$$

where A_s is absorbance of sample solution and A_c is absorbance of control.

The IC_{50} value, defined as the concentration of the test material that leads to 50% reduction in the free radical concentration, was calculated as μg/mL through a sigmoidal dose-response curve.

Determination of inhibitory activity against lipid peroxidation

Antioxidant activity was determined by the thiocyanate method [26]. Serial dilutions were carried out with the stock solution (1 mg/mL) of the extracts, and 0.5 mL of each solution was added to linoleic acid emulsion (2.5 mL, 40 mM, pH 7.0). The linoleic acid emulsion was prepared by mixing 0.2804 g linoleic acid, 0.2804 g Tween-20 as emulsifier in 50 mL 40 mM phosphate buffer and the mixture was then homogenized. The final volume was adjusted to 5 mL with 40 mM phosphate buffer, pH 7.0. After incubation at 37 °C in the dark for 72 h, a 0.1 mL aliquot of the reaction solution was mixed with 4.7 mL of ethanol (75%), 0.1 mL $FeCl_2$ (20 mM) and 0.1 mL ammonium thiocyanate (30%). The absorbance of the mixture was measured at 500 nm and the mixture was stirred for 3 min. Ascorbic acid, gallic acid, α-tocopherol and BHT were used as reference compounds. To eliminate the solvent effect, the control sample, which contained the same amount of solvent added to the linoleic acid emulsion in the test sample and reference compound, was used. Inhibition percent of linoleic acid peroxidation was calculated using the same equation [1].

Determination of hydroxyl radical scavenging activity

The ability of *Halacsya sendtneri* to inhibit non site-specific hydroxyl radical-mediated peroxidation was carried out according to the method described in literature [27]. The reaction mixture contained 100 μL of extract dissolved in water, 500 μL of 5.6 mM 2-deoxy-D-ribose in KH_2PO_4–NaOH buffer (50 mM, pH 7.4), 200 μL of premixed 100 μM $FeCl_3$ and 104 mM EDTA (1:1 v/v) solution, 100 μL of 1.0 mM H_2O_2 and 100 μL of 1.0 mM aqueous ascorbic acid. Tubes were vortexed and incubated at 50 °C for 30 min. Thereafter, 1 mL of 2.8% TCA and 1 mL of 1.0% TBA were added to each tube. The samples were vortexed and heated in a water bath at 50 °C for 30 min. The extent of oxidation of 2-deoxyribose was estimated from the absorbance of the solution at 532 nm. The percentage inhibition values were calculated from the absorbance of the control A_c and of the sample A_s, where the controls contained all the reaction reagents except the extract or positive control substance. The values are presented as the means of triplicate analyses.

Minimum inhibitory concentration (*MIC*)

Minimum inhibitory concentrations (*MIC*) of the extract and cirsimarin against the test bacteria were determined by microdilution method in 96 multi-well microtiter plates [19]. All tests were performed in Muller–Hinton broth (MHB) with the exception of the yeast, in which case Sabouraud dextrose broth was used. A volume of 100 μL stock solutions of oil (in methanol, 200 μL/mL) and cirsimarin (in 10% DMSO, 2 mg/mL) was pipetted into the first row of the plate. 50 μL of Mueller–Hinton or Sabouraud dextrose broth (supplemented with Tween 80 at a final concentration of 0.5% (v/v) for analysis of oil) was added to the other wells. A volume of 50 μL from the first test wells was pipetted into the second well of each microtiter line, and then 50 μL of scalar dilution was transferred from the second to the twelfth well. 10 μL of resazurin indicator solution (prepared by dissolution of a 270 mg tablet in 40 mL of sterile distilled water) and 30 μL of nutrient broth were added to each well. Finally, 10 μL of bacterial suspension (10^6 CFU/mL) and yeast spore suspension (3×10^4 CFU/mL) was added to each well. For each strain, the growth conditions and the sterility of the medium were checked. Standard antibiotic amracin was used to control the sensitivity of the tested bacteria, whereas ketoconazole was used as control against the tested yeast. Plates were wrapped loosely with cling film to ensure that bacteria did not become dehydrated and prepared in triplicate, and then they were placed in an incubator at 37 °C for 24 h for the

bacteria and at 28 °C for 48 h for the yeast. Subsequently, color change was assessed visually. Any color change from purple to pink or colorless was recorded as positive. The lowest concentration at which color change occurred was taken as the *MIC* value. The average of 3 values was calculated, and the obtained value was taken as the *MIC* for the tested compounds and standard drug.

Statistical analysis

The results are presented as mean ± standard deviations of three determinations. Statistical analyses were performed using Student's *t*-test and one-way analysis of variance. Multiple comparisons of means were done by *LSD* (least significant difference) test. A probability value of 0.05 was considered significant. All computations were made by employing the statistical software (SPSS, version 11.0). IC_{50} values were calculated by nonlinear regression analysis from the sigmoidal dose-response inhibition curve.

RESULTS AND DISCUSSION

Phenolic compounds and flavonoids have been reported to be associated with antioxidant action in biological systems, mainly due to their red-ox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [29]. Antioxidant activity of flavonoids makes them valuable for therapeutic and prophylactic applications, *e.g*., after infection, inflammation, burns, or radiation injury [30]. The antioxidant activity of crude methanol extracts is due to the presence of flavonoid monomers and polymers (condensed tannins), hydrolyzable tannins, and phenolics. Recently, polyphenolic compounds from plants such as condensed and hydrolyzable tannins have been shown to be powerful antioxidants [31]. Furthermore, tannins are reported to be 15–30 times more effective in quenching peroxyl radicals than simple phenolics. Therefore, tannins should be considered as important biological antioxidants [32]. The results on total phenolic, flavonoids, condensed tannins, gallotannins and total antioxidant capacity are given in Table 1. Acetone extract of plant species *Halascya sendtneri* shows the highest activity, and chloroform and ethyl acetate extracts shows lower activity, while petroleum ether extract possesses the lowest activity.

The results of our experiments with extracts of *Halascya sendtneri* sequence indicate the content of polyphenol components and antioxidant activiti is: acetone extract > cholorophorm extract > ethyl acetate extract > petroleum ether extract, which is in total agreement with the sciential works which confirm interdependence of the antioxidant activity and the amount of phenolic compounds presented in plant. Antioxidant activities of the extracts were evaluated using the DPPH and hydroxyl radical scavenging, lipid peroxidation and metal chelating assays. The results on antioxidant activity were compared with control antioxidants, ascorbic acid, gallic acid, α-tocopherol and BHT. Results showed that the acetone extract of plant species *Halascya sendtneri* possesses the highest antioxidant activity. IC_{50} values were determinated formal measurements: 9.45±1.55 μg/mL for DPPH free radical scavenging activity, 13.46±1.68 μg/mL for inhibitory activity against lipid peroxidation, 59,11±0.83 μg/mL for hydroxyl radical scavengind activity and 27.91±0.88 μg/mL for ferrous ion chelating ability (Table 2). Cholophorm, ethyl acetate and petroleum ether extracts shows various activity among acetone *H. sendtneri* extract. Also, it can be confirmed that the DPPH free radical scavenging activity of *H. sendtneri* extract possesses higher activity than BHT, and lower activity than ascorbic and gallic acid. All *Halacsya sendtneri* extracts possess high and intermediate antioxidant activity.

HPLC method was used for identification and quantification of dominant metabolites in this plant. Tables 3–6 show the dominant components of the acetone, chloroform, ethyl acetate and petroleum ether extacts. The dominant component in acetone, chloroform and ethyl acetate extract is rusmaric acid, with the excep-

Table 2. Antioxidant activity (IC_{50}, μg/mL, determined by nonlinear regression analysis)of the H. sendtneri extracts and antioxidants; results are mean values ± SD from three experiments; abbreviations: DPPH – 2,2-dephenyl-1-picrylhydrazyl, BHT – Butylated hydroxytoluene

Type of extract	DPPH scavenging activity	Inhibitory activity against lipid peroxidation	Metal chelating activity	Hydroxyl radical scavenging activity
Acetone	9.45±1.55	13.46±1.68	27.91±0.88	59,11±0.83
Chloroform	18.29±0.64	31.23±1.45	37.89±0.76	88.47±0.58
Ethyl acetate	11.18±1.05	29.34±0.78	31.15±0.95	67.25±1.15
Petroleum ether	34.89±1.75	94.89±1.55	56.89±1.35	94.89±1.24
Gallic acid	3.79±0.69	255.43±11.68	–	59.14±1.10
Ascorbic acid	6.05±0.34	> 1000	–	160.55±2.31
BHT	15.61±1.26	1.00±0.23	–	33.92±0.79
α-Tocopherol	–	0.48±0.05	–	–

Table 3. Quantitative and qualitative contents of phenolic components in H. sendtneri acetone extract

Component Number	Component	Retention time, min	Concentration, mg/g extract
1	Gallic acid	0.452	0.154
2	Protocatehuic acid	0.893	0.102
3	*p*-Hydroxybenzoic acid	1.500	0.255
4	Caffeic acid	3.468	0.085
5	Vanillic acid	3.669	0.104
6	Chlorogenic acid	5.821	0.083
7	*p*-Coumaric acid	6.216	0.062
9	Ferulic acid	8.512	0.094
10	Synapic acid	8.868	0.210
11	Rutin	9.468	3.039
13	Rosmarinic acid	9.876	14.019
14	Cinnamic acid	10.033	0.083
16	Naringenin	10.558	1.123
17	Luteolin	10.815	0.391
18	Kaempferol	11.167	0.301
19	Apigenin	11.379	0.360

Table 4. Quantitative and qualitative contents of phenolic components in chloroform extract of plant species H. sendtneri

Component Number	Component	Retention time, min	Concentration, mg/g extract
3	*p*-Hydroxybenzoic acid	1.520	0.051
6	Chlorogenic acid	5.683	0.109
7	*p*-Coumaric acid	6.279	0.019
11	Rutin	9.689	0.098
13	Rosmarinic acid	9.884	0.167
14	Cinnamic acid	10.056	0.115
16	Naringenin	10.449	0.019
17	Luteolin	10.834	0.028
19	Apigenin	11.366	0.021

Table 5. Quantitative and qualitative contents of phenolic components in ethyl acetate extract of plant species H. sendtneri

Component Number	Component	Retention time, min	Concentration, mg/g extract
1	Gallic acid	0.354	0.072
2	Protocatehuic acid	0.881	0.136
3	*p*-Hydroxybenzoic acid	1.514	0.283
4	Caffeic acid	3.767	0.047
6	Chlorogenic acid	5.827	0.059
7	*p*-Coumaric acid	6.268	0.005
8	Syringic acid	6.859	0.060
9	Ferulic acid	8.549	0.187
11	Rutin	9.517	0.111
13	Rosmarinic acid	9.878	0.147
14	Cinnamic acid	10.054	0.127
17	Luteolin	10.800	0.037
19	Apigenin	11.371	0.070

Table 6. Quantitative and qualitative contents of phenolic components in petroleum ether extract of plant species H. sendtneri

Component Number	Component	Retention time, min	Concentration, mg/g extract
3	*p*-Hydroxybenzoic acid	1.491	0.037
4	Caffeic acid	3.611	0.031
6	Chlorogenic acid	5.795	0.083
7	*p*-Coumaric acid	6.389	0.040
8	Syringic acid	7.218	0.017
11	Rutin	9.382	0.035
14	Cinnamic acid	10.102	0.015
15	Qercetin	10.526	0.065
16	Naringenin	10.615	0.038
17	Luteolin	10.837	0.044
19	Apigenin	11.259	0.039

tion of ethyl acetate extract where it is mainly found in the same amount as *p*-hydroxybenzoic acid and ferulic acid, while in petrol ether extract, chlorogenic acid and quercetin are dominant. The results of antimicrobial activity obtained by the dilution method are given in Table 7; *MIC*s were determined for eight selected indicator strains. The results presented in Table 7 reveal antimicrobial activity of the acetonic extract, chloroform extract, ethyl acetate extract and petroleum ether extract of *Halacsya sendtneri* within the concentration range of 15.62 μg/mL to 62.50 μg/mL. Acetone extract of *Halacsya sendtneri* showed the highest antimicrobial susceptibility of *Staphylococcus aureus, Klebsiella pneumoniae and Bacillus subtilis* (*MIC* = 15.62 μg/mL) and yeasts *Candida albicans* (*MIC* = = 15.62 μg/mL). Intermediate antimicrobial susceptibility was shown by *Escherihia colli* bacteria and *Aspergillus niger* fungi (*MIC* = 31.25 μg/mL). The lowest antimicrobial susceptibility was shown by *Proteus vulgaris* and *Proteus mirabilis* (*MIC* = 62.50 μg/mL). Chloroform extract of *Halacsya sendtneri* gave the highest antimicrobial susceptibility of *Staphylococcus aureus* and *Bacillus subtilis* (*MIC* = 15.62 μg/mL) and fungi *Aspergillus niger* (*MIC* = 15.62 μg/mL). Intermediate antimicrobial susceptibility was shown by *Escherihia colli, Klebsiella pneumoniae* bacteria and yeast *Candida albicans* (*MIC* = = 31.25 μg/mL). The lowest antimicrobial susceptibility was of *Proteus vulgaris* and *Proteus mirabilis* (*MIC* = 62.50 μg/mL). The ethyl acetate extract of *Halacsya sendtneri* presented the highest antimicrobial susceptibility of *Escherihia colli, Bacillus subtilis* (MIC = 15.62 μg/mL) and fungi *Aspergillus niger* (*MIC* = 15.62 μg/mL). Intermediate antimicrobial susceptibility was presented by *Proteus mirabilis, Klebsiella pneumoniae* bacteria and yeast *Candida albicans* (*MIC* = 31.25 μg/mL). The lowest antimicrobial susceptibility was shown by *Pro-*

Table 7. Minimum inhibitory concentration (MIC, μg/mL) of the acetonic, chloroform, ethyl acetate and petroleum ether extracts of H. sendtneri

Microbial strain	Acetonic extract	Chloroform extract	Ethyl acetate extract	Petroleum ether extract	Amracin	Ketoconazole
Staphylococcus aureus ATCC 25923	15.62	15.62	15.62	31.25	0.97	–
Klebsiella pneumoniae ATCC 13883	15.62	31.25	31.25	62.50	0.49	–
Escherichia coli ATCC 25922	31.25	31.25	15.62	15.62	0.97	–
Proteus vulgaris ATCC 13315	62.50	62.50	62.50	62.50	0.49	–
Proteus mirabilis ATCC 14153	62.50	62.50	31.25	31.25	0.49	–
Bacillus subtilis ATCC 6633	15.62	15.62	15.62	15.62	0.24	–
Candida albicans ATCC 10231	15.62	31.25	31.25	31.25	–	1.95
Aspergillus niger ATCC 16404	31.25	15.62	15.62	15.62	–	0.97

teus vulgaris and *Proteus mirabilis* (*MIC* = 62.50 μg/mL). The petroleum ether extract of *Halacsya sendtneri* showed the highest antimicrobial susceptibility of *Escherichia colli, Bacillus subtilis* (*MIC* = 15.62 μg/mL) and fungi *Aspergillus niger* (*MIC* = 15.62 μg/mL). Intermediate antimicrobial susceptibility was shown by *Proteus mirabilis, Staphylococcus aureus* bacteria and yeast *Candida albicans* (MIC = 31.25 μg/mL). The lowest antimicrobial susceptibility was shown by *Proteus vulgaris* and *Klebsiella pneumoniae* (*MIC* = 62.50 μg/mL).

CONCLUSIONS

Antioxidant and antimicrobial properties of various extracts of many plants are of great interest in both fundamental science and alternative medicine, since their potential use as natural extracts has emerged from a growing tendency to replace synthetic antioxidants by natural ones. The present study confirmed the antimicrobial and antioxidant activities of acetone, chloroform, ethyl acetate and petroleum ether extracts of the Serbian plant *Halacsya sendtneri.* Determination of polyphenolic components by HPLC analysis revealed the presence of high amounts of rosmarinic acid, ferulic acid, p-hydroxybenzoic acid, chlorogenic acid and quercetin responsible for the reported antimicrobial activity of *H. sendtneri*. The obtained results suggest that the extracts of the endemic species *H. sendtneri* shows antimicrobial activity under *in vitro* conditions against the test fungi as well as antioxidant activity relative to the control antioxidants.

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IZVOD

FITOHEMIJA I BIOLOŠKA AKTIVNOST EKSTRAKATA BILJNE VRSTE *Halacsya sendtneri* (BOISS.) DÖRFL.

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(Naučni rad)

Ovaj rad je imao za cilj ispitivanje sadržaja ukupnih fenola, flavonoida, galotanina i kondenzovanih tanina u acetonskom, hloroformskom, etil-acetatnom i petroletarskom ekstraktu biljne vrste *Halacsya sendtneri* (Boiss.) Dörfl., njihovu antimikrobnu i antioksidativnu aktivnost, kao i identifikaciju i kvantifikaciju fenolnih komponenti. Antioksidativna aktivnost je u saglasnosti sa rezultatima ukupne količine fenolnih jedinjenja. Rezultati su pokazali da acetonski ekstrakt biljne vrste *Halascya sendtneri* (Boiss.) Dörfl. poseduje najveću antioksidativnu aktivnost. IC_{50} vrednost su formalno određena merenjem: 9,45±1,55 μg/ml za DPPH uklanjenje slobodnih radikala, 13,46±1,68 μg/ml za inhibicuju lipidne peroksidacije, 59,11±0,83 μg/ml za antioksidativnu aktivnost na nivou hidroksilnih radikala i 27,91±0,88 μg/ml za helatacionu aktivnost. Antimikrobna aktivnost je testirana koristeći metodu razblaženja za određivanje minimalne inhibitorne koncentracije (*MIC*). *MIC* su određivane za 8 bakterijskih sojeva. Svi ekstrakti su pokazali jaku do umereno jaku antimikrobnu aktivnost, fenolne komponente ekstrakta biljke *H. sendtneri* određivane su HPLC metodom. Dominantna komponenta acetonskog, hloroformskog i etil acetatnog ekstrakta je ruzmarinska kiselina, sa izuzetkom etil acetatnog ekstrakta, gde je pronađena, u priblizno istoj količini i *p*-hidroksi benzoeva kiselina i ferulična kiselina, dok su u petroletarskom ekstraktu, dominantna hlorogenska kiselina i kvercetin.

Ključne reči: Antimikrobna aktivnost • Antiooksidativna aktivnost • *Halacsya sendtneri* (Boiss.) Dörfl.