Comparison of antioxidant and antimicrobial activities of extracts obtained from *Salvia glutinosa* L. and *Salvia officinalis* L.

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Abstract

Antioxidant and antimicrobial activities as well as total phenols and flavonoids contents of *Salvia glutinosa* L. (glutinous sage) and *Salvia officinalis* L. (sage) extracts were studied. Methanol and aqueous ethanol (70% v/v) were used for extraction of bioactive compounds, both in the presence and the absence of ultrasound, from herb and the spent plant material remaining after the essential oil hydrodistillation. The ratio of plant material to extracting solvent was 1:10 g/mL. Antioxidant and antimicrobial activities of the extracts were found to depend on the type of plant material and the extraction conditions. The plant materials from which essential oil had been recovered were proven to be valuable raw materials for making various herbal preparations.

Keywords: *Salvia glutinosa* L.; *Salvia officinalis* L.; antioxidant activity; flavonoids; phenols; antimicrobial activity.

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Plants from genus *Salvia* L. have a wide use as phytopreparations and dietetic products all over the world. The most popular of them, sage (*Salvia officinalis* L.), has extensive application in the production of tea mixtures [1], prevention of UV radiation [2], and treatment of Alzheimer's [3] and HIV [4] diseases. Sage is also used for food preservation (especially meat and cheese) and as a spice for flavoring [5]. Glutinous sage (*S. glutinosa* L.) is another one, which can be worthy plant in industry and traditional medicine. This plant is rich in flavonoids and other phenolics [6] as well as in sesquiterpenes [7]. Ethyl acetate, acetone, methanol and aqueous extracts of *S. officinalis* L.*, S. glutinosa* L. and *S. pratensis* L. almost completely inhibit 2,2-diphenyl-1-picrylhydrazil (DPPH) absorption [8,9]. The extracts of *S. glutinosa* and *S. officinalis* added to dough (0.1% w/w) enhance its stability and improve its rheological properties [10].

Organic solvent extraction and hydrodistillation are major extraction processes with commercial use for recovery of valuable extractable substances (ES) from plant materials. The use of ultrasound in the extraction process improves the yields of some compounds or total ES for shorter time at lower temperature, compared to classical extraction [11–14].

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Hydrodistillation of essential oils bears two by-products, the aromatic water ("hydrosol") and the spent plant material (SPM). The former is considered as a ready-to-use product in aromatherapy and cosmetic industry, and the latter is usually treated as a waste material despite its possible biological activities. There are a few reports confirming antioxidant [15] and antimicrobial [16] activities of the *Salvia* species SPM. These SPM are shown to possess flavonoids, terpenes [13] and polysaccharides [17].

Antioxidant and antimicrobial activities of extracts obtained from herb and SPM of *S. glutinosa* by ultrasound-assisted extraction (UE) and classical solvent extraction (CE) using methanol and 70% v/v aqueous ethanol as extracting solvents were studied. *S. officinalis* was included as a reference plant due to its well-known antioxidant [18,20] and antimicrobial [16,21–23] activities. The main goal was to compare antioxidant and antimicrobial activities of extracts obtained by various extraction techniques. Also, total phenolic compounds and flavonoids in the extracts obtained by different methods were compared.

EXPERIMENTAL

Plant materials

The plant materials were gathered in two locations in Serbia (*S. officinalis*: Gradište village, Sićevačka klisura gorge; the second half of May, 2010) and (*S. glutinosa*: Vučedelce village, Strešer Mt.; the first half of August, 2010). Herbaria samples are kept in General herbarium of the Balkan Peninsula (BEO) Natural His-

tory Museum in Belgrade (Serbia), under the following numbers: BEO 32149 (*S. glutinosa*) and BEO 32147 (*S. officinalis*).

The additional information on harvesting, drying, packaging and storing of the herb materials can be found elsewhere [12]. Before being used, the plant material was comminuted by a hammer mill and sieved through a 6 mm screen. After essential oil hydrodistillation using a Clevenger-type apparatus, the hydrosol was separated by vacuum filtration and the remaining SPM was dried in a well-aired place in thin layer for 5 days [13]. The moisture content, determined by drying at 105 °C to constant mass, was about 12% for all plant materials.

Chemicals

Methanol and ethanol were from Zorka-Pharma (Šabac, Serbia). Folin–Ciocalteu reagent, DPPH, gallic acid and rutin were obtained from Sigma (St. Louis, MO, USA). Sodium carbonate, potasium acetate and aluminium chloride were purchased from Merck-Alkaloid (Skopje, FYR Macedonia).

Microorganisms and nutrition media

Escherichia coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10231, *Saccharomyces cerevisiae* ATCC 9763 and *Aspergillus niger* ATCC 16404 (Oxoid, England) were used as test microroganisms.

Trypton soya agar (for bacteria cultures) and Sabouraud dextrose agar (for yeast and mold cultures) were used as nutrition media. Plate count agar was used for determining the total number of microorganisms (CFU/ /mL). All nutrition media were made by Merck (Germany).

Extraction of plant materials

The plant material (10 g) and the extracting solvent (methanol and 70% v/v aqueous ethanol, 100 mL) were placed in an Erlenmeyer flask (250 mL). The sonication was performed using an ultrasonic cleaning bath (Sonic, Niš, Serbia; total nominal power: 3×50 W; internal dimensions: 30 cm×15 cm×20 cm; frequency: 40 kHz; temperature: 40±1 °C) for 20 min [12]. CE was performed under the same conditions, except the ultrasound generator was switched off.

At the end of an extraction cycle, the liquid extracts were separated from the plant material by vacuum filtration. The solvent was evaporated using a rotary vacuum evaporator at 50 °C. The extract was then dried under vacuum at 50 °C to constant weight.

Determination of antioxidant activity

The free radical-scavenging activity of plant extracts was evaluated using the stable radical DPPH [24]. A series of extracts with seven different concentrations (0.01–1.00 mg/mL) were prepared in methanol. The extract (2.5 mL) and the 3×10^{-4} M DPPH solution in methanol (1 mL) were mixed and placed in the dark at room temperature for 30 min. Then, the absorbance of each plant extract containing DPPH was measured at 517 nm using a spectrophotometer (Varian Cary-100). The plant extract solution (2.5 mL) plus methanol (1.0 mL) and the DPPH solution (2.5 mL) plus methanol (1.0 mL) were used as a blank and a control, respectively. All determinations were performed in triplicate. The percentage of DPPH radical scavenging capacity of each plant extract was calculated using the Eq. (1):

$$\text{DPPH radical scavenging capacity (\%)} = 100\left(1-\frac{(A_s - A_b)}{A_c}\right) \quad (1)$$

where A_s is the absorbance in the presence of the plant extract in DPPH solution, A_b is the absorbance of the sample extract solution without DPPH and A_c is the absorbance of the control solution (containing only DPPH).

Determination of total phenolic content

Total phenols were determined by Folin–Ciocalteu reagent using gallic acid as a standard [25]. The total phenols were expressed as mg gallic acid equivalents per g dry extract. Standard curve equation (R^2 = 0.9994) was as follows:

$$\text{Absorbance at 765 nm} = 12.722c_{ga} + 0.0034 \quad (2)$$

where c_{ga} is the gallic acid concentration μg/mL). Each of plant extracts (0.2 mL, 1 mg/mL) or gallic acid was mixed with Folin–Ciocalteu reagent (1 mL) and aqueous sodium carbonate (0.8 mL, 7.5%). The mixtures were allowed to stand at room temperature for 30 min, and the absorbance of the reaction mixture was measured at 765 nm.

Determination of total flavonoid content

The total flavonoid content was determined according to the aluminium chloride colorimetric method [26]. Each plant extracts (2 mL, 0.5 μg/mL) in methanol were mixed with 10% aluminium chloride solution (0.1 mL), 1 M potassium acetate (0.1 mL) and distilled water (2.8 mL). After incubation at room temperature for 30 min, absorbance of the reaction mixture was measured at 415 nm against distilled water. Rutin was chosen as a standard and the total flavonoid content was expressed as *mg* rutin equivalents per *g* of dry extracts. Results were expressed as a mean of three replicate measurements. Standard curve equation (R^2 = 0.9919) was as follows:

$$\text{Absorbance at 415 nm} = 7.2328c_r - 0.2286 \quad (3)$$

where c_r is the rutin concentration (μg/mL).

Determination of antimicrobial activity

The agar well-diffusion method was employed for the determination of antimicrobial activities of extracts [27]. Microorganism suspension (0.1 mL), formed of 24 h culture on obliquely agar with 10 mL of sterile 0.9% NaCl, was suspended into 10 mL of the nutrition medium (ca. 10^6 CFU/mL). A Petri dish (86 mm internal diameter) was filled with this system. The wells (10 mm in diameter) were cut from the agar and the extract solution (30 µl, 20 mg/mL in methanol) was delivered into them. As controls, methanol (30 µl) was delivered into a well per each Petri dish. Erythromycin (997 µg/mg; [114--07-8]; Sigma) and tylosin tartarat (950 µg/mg; [74610-55-2]; Sigma) were used as a positive control (concentration in methanol 0.05 mg/mL). All dilutions were filtrated using a 0.45 µm membrane filter (Sartorius, Germany) and performed in three replicates. After incubation at 37 °C for 24 h, agar plates were examined for any zones of inhibition. Diameters of inhibition zones (mm) were measured by a Fisher Lilly Antibiotic Zone Reader (USA). Data were analyzed by Duncan's test at 5% significance level [28].

RESULTS AND DISCUSSION

Yield of ES

The ES yield depended on the plant species, the plant material, the extracting solvent and the extraction technique as can be seen in Table 1. The ES yield was higher from *S. glutinosa* than from *S. officinalis*, except from herbal material when aqueous ethanol (70% v/v) was used as extracting solvent. In earlier work [12,13], it was reported that the ES yield from *S. officinalis* using aqueous ethanol was higher than that from *S. glutinosa*. As expected, the ES yield was higher from the herbal material than that from SPM, independently of the other extraction conditions. The ES yields from the *S. glutinosa* and *S. officinalis* SPM were from 28 to 34% and from 66 to 85%, respectively, of those from the corresponding herbal material. Aqueous ethanol (70% v/v) ensured higher ES yields than methanol at the same other extraction conditions. Finally, the efficiency of the UE exceeded that of the CE.

Antioxidant capacity

It has been recently shown [18] that the radical scavenging capacity (RSC) determined by the DPPH method, compared to the linoleic acid peroxidation inhibition, was greater in the case of polar methanolic extracts of *S. officinalis*, which contained both diterpenoids (carnosic acid and carnosol) and rosmarinic acid, and lower in the case of the less polar acetone extracts. Therefore, the DPPH method was used for the RSC determination in the present study. The percentage of DPPH reduction is plotted against the plant extract concentration in Figure 1. For comparing the antioxidant activities of the extracts, the ES concentration producing 50% reduction of the radical absorbance (EC_{50}) was used as an index. The EC_{50} values, calculated by sigmoid non-linear regression model using SigmaPlot 2000 Demo, are given in Table 2. Independently of the type of solvent and plant material, the contents of phenols and flavonoids as well as the antioxidant activity were greater in the extracts obtained by CE than in the other extracts. In addition, independently of the type of solvent, plant material and extraction technique, *S. glutinosa* was shown to have a firm antioxidant activity, while *S. officinalis* was richer in phenolic compounds. The extracts of *S. glutinosa* herb contained more flavonoids than those of *S. officinalis* independently of the type of extraction technique and solvent. On the other hand, the SPM extracts of *S. officinalis* had more flavonoids than those of *S. glutinosa*.

All EtOH extracts, independently of the extraction techniques and the plant material, had a greater content of phenolic compounds and flavonoids, and showed a higher antioxidant activity than MeOH extracts. In addition, all extracts from herbal materials showed a higher antioxidant activity and had higher contents of phenolic compounds and flavonoids than SPM extracts. Table 2 shows also that *S. glutinosa* extracts have better antioxidant capacity than *S. officinalis* ones, which differs from the results of Miliauskas *et al.* [8].

Ultrasound showed a positive effect on the yield of ES from both herb and SPM, but the negative effect on the content of total phenolic compounds and flavonoids. This was explained by degradation of a part of these compounds by interaction with highly reactive hydroxyl radicals formed during sonication [29].

Table 1. Yield of extractable substances obtained by different extraction techniques (g ES/100 g of plant material); EtOH – 70 % v/v aqueous ethanolic extracts; MeOH – methanolic extracts; CE – classical extraction; UE – ultrasound-assisted extraction; H – herb; SPM – spent plant material

Plant material	EtOH 70 % v/v				MeOH			
	UE		CE		UE		CE	
	H	SPM	H	SPM	H	SPM	H	SPM
S. glutinosa	13.20	4.51	13.03	4.34	7.01	2.00	6.51	1.97
S. officinalis	11.53	7.62	8.41	6.82	9.33	6.24	7.12	6.05

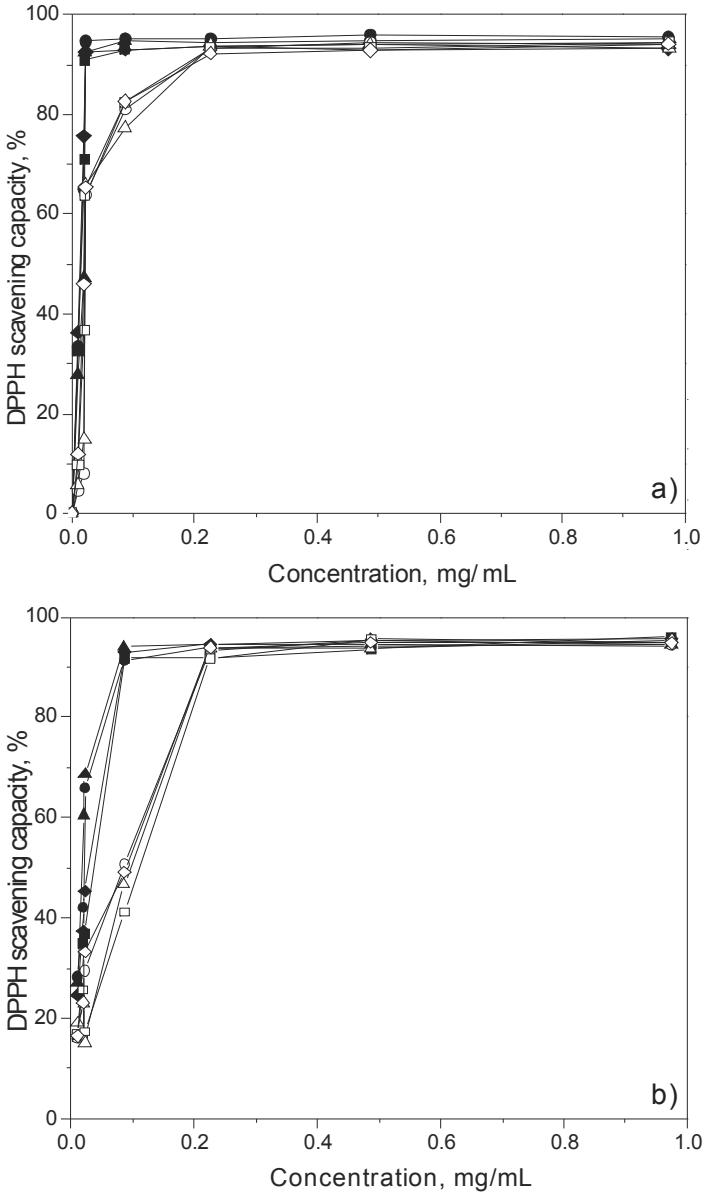

Figure 1. DPPH radical scavenging capacity for the S. glutinosa (a) and S. officinalis (b) extracts obtained by different extraction techniques (herbal material – black symbols; SPM – white symbols; aqueous ethanol, 70% v/v: UE – circles and CE – triangles; methanol: UE – squares and CE – rhombs).

Antimicrobial activity

Table 3 shows that the *S. glutinosa* extracts from **1** to **5** were firmly active against the Gram-negative bacteria, while the extracts from **6** to **8** showed a firm activity against the Gram-positive bacteria. The greatest activity was against the yeasts (*S. cerevisiae*, *C. albicans*), while there was no activity against the mould *A. niger*. The control treatment using methanol had no inhibitory effect on any of the test microorganisms. The extracts **5** and **6** were active against the all microorganisms tested, but the former one showed the antimicrobial activity against the Gram-negative bacteria and the latter one was firmly active against the Gram-positive bacteria. Generally, the SPM extracts obtained by the same solvent and extraction technique showed a higher antimicrobial activity against the all microorganisms than the herbal extracts. The CE extracts showed firm antimicrobial activity on the Gram-negative bacteria. Generally, the EtOH extracts had a greater antimicrobial activity than the MeOH ones.

Table 3 shows the results of antimicrobial activity tests for the *S. officinalis* extracts, too. The extracts from **1'** to **5'** were firmly active against the Gram-negative bacteria. The most pronounced acting was against the yeast *C. albicans* and the mould *A. niger*, while there was no activity against the yeast *S. cerevisiae*. The extracts **3'** and **4'** had the highest activity against the all microorganisms, the extract **3'** being more active

Table 2. DPPH radical scavenging capacity, total amount of plant phenolic compound and flavonoids of S. glutinosa and S. officinalis extracts; H – herb; SPM – spent plant material; EtOH – 70 % v/v aqueous ethanolic extracts; MeOH – methanolic extracts; CE – classical extraction; UE – ultrasound-assisted extraction

Plant species	Plant material	Extraction technique	Solvent	Total phenolics (mg gallic acid/g of dry extract)	EC_{50} / μg mL^{-1}	Total flavonoids (mg rutin/g of dry extract)
S. glutinosa	H	UE	MeOH	84.0±0.76	19.65±0.24	94.6±0.55
			EtOH	137.3±0.42	13.43±0.20	101.3±0.40
		CE	MeOH	85.8±0.91	14.29±0.31	99.2±0.24
			EtOH	121.0±0.49	12.61±0.25	108.5±0.57
	SPM	UE	MeOH	67.4±0.42	45.28±1.78	60.3±0.78
			EtOH	133.2±0.52	24.41±0.18	69.8±0.98
		CE	MeOH	66.9±0.50	37.52±0.42	63.0±0.78
			EtOH	132.6±0.21	20.88±0.41	75.4±0.73
S. officinalis	H	UE	MeOH	119.0±0.49	25.97±0.49	91.4±0.55
			EtOH	138.4±1.48	23.86±0.51	94.4±0.40
		CE	MeOH	123.6±0.80	24.61±1.03	92.6±0.09
			EtOH	143.6±0.16	21.60±0.62	106.5±0.40
	SPM	UE	MeOH	61.3±0.41	71.03±2.36	59.6±0.51
			EtOH	75.6±0.28	67.51±2.46	63.2±0.31
		CE	MeOH	64.6±0.16	62.65±2.42	60.7±0.16
			EtOH	79.6±0.73	64.31±1.89	66.0±0.69

Table 3. Antimicrobial activity (zone size in mm) of S. glutinosa and S. officinalis extracts (EtOH – 70 % v/v aqueous ethanolic extracts; MeOH – methanolic extracts; CE – classical extraction; UE – ultrasound-assisted extraction; E – erythromycin; T – tylosin tartarat; values for a microorganism designated with the same letter do not significantly differ at 5% error (Duncan's test) where erythromycin is used as a control)

Mocroorganism	Antibiotics		Herb				Spent plant material			
			EtOH		MeOH		EtOH		MeOH	
			CE	UE	CE	UE	CE	UE	CE	UE
			S. glutinosa							
	E	T	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
E. coli	21.2	18.4	14.0 d	13.4 g	13.7 e,f	13.1	20.3	19.7	13.5 f,g	14.0 d,e
P. aeruginosa	25.2	17.6	13.8 f,g	13.6 g,h	13.5 h,i	13.3 i	21.1	20.6 a	14.0 f	12.4
B. subtilis	19.1	17.3	13.3 f	13.3 e,f	13.0	12.7	20.0	20.8	18.4	18.7
S. aureus	23.6	18.5	12.6 e,f	12.6 g,f	13.3 b,d	12.7 e,g	19.7	20.7	17.8 k	18.6
C. albicans	23.0	16.2	24.7	22.6	23.8	26.1 a	32.5	30.8 b	25.4	26.3 a
S. cerevisiae	0	0	23.2	30.5	29.8 a	29.7 a	33.5	34.2	28.7	29.0
A. niger	20.5	18.1	0	0	0	0	0	0	0	0
			S. officinalis							
			1'	**2'**	**3'**	**4'**	**5'**	**6'**	**7'**	**8'**
E. coli			17.0 b,c	17.0 c	21.8	20.9	17.9	17.3 a	17.6	17.1 a,b
P. aeruginosa			14.8 c,e	16.1 d,e	20.5 a	19,8	17.2	16.9 b	16.7 b,c,d	17.5
B. subtilis			15.7 e	16.0	20.3	19.6	17.1 b,c,d	17.1 a,d	17.0 a,b	16.7 c
S. aureus			14.2 j	13.3 c,d	17.7 k	17.0	14.5 h,a	14.7 h,i	13.5 b,c	14.6 i,j,a
C. albicans			27.9	31.0 b	34.8	34.5	31.9	29.4 c	29.4 c	30.4
S. cerevisiae			0	0	0	0	0	0	0	0
A. niger			28.5	28.8 b	35.5	33.9	31.1 a	31.1 a	29.3	28.9 b

than the extract **4'**. Generally, the **5'** and **6'** SPM extracts showed a firmer antimicrobial activity compared to the **1'** and **2'** herbal extracts, while the **3'** and **4'** herbal extracts had a greater antimicrobial activity than the **7'** and **8'** SPM extracts. In addition, the CE extracts showed a firm antimicrobial activity on microorganisms.

CONCLUSION

Antioxidant and antimicrobial activities as well as total phenols and flavonoids contents of the extracts obtained from herb and SPM (remaining after the essential oil hydrodistillation) of glutinous sage (*Salvia glutinosa* L.) by extraction using methanol and aqueous ethanol (70% v/v) were studied in the presence and the absence of ultrasound. Therefore, all extracts obtained by the UE showed weaker antioxidant, compared to the extracts obtained by CE. In addition, the SPM extracts were shown to have a higher antimicrobial activity than the herbal extracts. Although SPM extracts of *S. glutinosa* showed approximately twice smaller antioxidant activities than its herbal extracts, independently of the extraction technique and solvent applied, this SPM can be used in the extract production as being a valuable source of natural products with potential application in the protection and preservation of certain foods and nutraceuticals. Thus, the use of this secondary priceless raw material from essential oil production is expected to have technological, economical and ecological justification, as safer alternative food preservation additives.

Abbreviations

CE – Classical extraction;
CFU – Colony forming units;
DPPH – 2,2-Diphenyl-1-picrylhydrazil;
EC_{50} – The concentration of extract necessary to decrease DPPH radical concentration by 50%;
ES – Extractable substances;
RSC – Radical scavenging capacity;
SPM – Spent plant material;
UE – Ultrasound-assisted extraction.

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IZVOD

POREĐENJE ANTIOKSIDANTNE I ANTIMIKROBNE AKTIVNOST EKSTRAKTA *Salvia glutinosa* L. I *Salvia officinalis* L.

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(Naučni rad)

Ispitivana je antioksidantna i antimikrobna aktivnost, kao i sadržaj ukupnih fenola i flavonoida, ekstrakta lepljive žalfije (*Salvia glutinosa* L.) i žalfije (kadulje) (*Salvia officinalis* L.). Ekstrakcija bioaktivnih komponenata je vršena ultrazvukom i klasičnom ekstrakcijom (maceracijom) iz suve herbe, kao i biljnog materijala koji je ostao nakon izolacije etarskih ulja postupkom hidrodestilacije. Za ekstrakciju su korišćeni rastvarači metanol i vodeni rastvor etanola (70% v/v) pri hidromodulu 1:10 (g/mL), pri čemu je upotrebom drugog rastvarača dobijen veći prinos ekstraktivnih supstanci. Antioksidantna aktivnost dobijenih ekstrakata ispitana je DPPH testom, dok je njihova antimikrobna aktivnost određena disk difuzionom metodom. Pokazalo se da navedene aktivnosti ekstrakata zavise od tipa biljnog materijala i uslova ekstrakcije. Nezavisno od tipa rastvarača, biljnog materijala i tehnike ekstrakcije, *S. glutinosa* je pokazala snažniju antioksidantnu aktivnost, dok je *S. officinalis* bila bogatija fenolnim komponentama. Takođe, ekstrakti herbe *S. glutinosa* sadržali su više flavonoida od ekstrakata *S. officinalis*. Svi etanolni ekstrakti pokazali su veću antioksidantnu aktivnost od metanolnih ekstrakata. Posebno se ističu ekstrakti herbe, koji su bogatiji fenolnim komponentama i flavonoidima od ekstrakata rezidualnog biljnog materijala. Za razliku od ekstrakata *S. officinalis*, ekstrakti *S. glutinosa* su pokazali bolji antioksidantni kapacitet. Generalno, ekstrakti *S. glutinosa* dobijeni iz rezidualnog biljnog materijala, pokazali su jaču antimikrobnu aktivnost protiv svih mikroorganizama u poređenju sa ekstraktima herbe. I u ovom slučaju, etanolni ekstrakti su pokazali jaču antimikrobnu aktivnost od metanolnih ekstrakata. Biljni materijal (SPM) iz kojeg je prethodno izolovano etarsko ulje sadrži i dalje komplekse aktivnih materija koje pokazuju značajne biološke aktivnosti, što ga svrstava u vrednu sirovinu za izradu raznovrsnih fitopreparata.

Ključne reči: *Salvia glutinosa* L. • *Salvia officinalis* L. • Antioksidantna aktivnost • Flavonoidi • Fenoli • Antimikrobna aktivnost