

Research Article



Polyphenol compounds and antioxidant activity of *Salvia officinalis* L. and *Salvia sclarea* L.

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The plants from Salvia L. (sage) genus are well-known as culinary, ornamental, aromatic, and medicine plants widely distributed in the world. The plant raw of these plants contains numerous biologically active compounds that determine the different biological activities. This study aimed to evaluate the antioxidant activity and polyphenol content of ethanol extracts of Salvia officinalis L. and S. sclarea L. during vegetation. Plant raw material was collected from an experimental collection of aromatic and medicinal plants of the Institute of Climate Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine (Kherson region, v. Plodove) in 2020-2021. It investigated polyphenol, phenolic acid, flavonoid content, molybdenum-reducing power of extracts, and radical scavenging activity by the DPPH method. The total polyphenol compound content was 24.52–95.62 mg GAE.g⁻¹ (mg gallic acid equivalent per gram) for S. officinalis and 29.39–91.02 mg GAE.g⁻¹ for S. sclarea. The total phenolic acid content of S. officinalis and S. sclarea extracts was 10.18-40.23 and 10.13-36.01 mg CAE.g⁻¹ (mg caffeic acid equivalent per gram), respectively. The total flavonoid content was from 13.73 to 55.38 mg QE.g⁻¹ (quercetin equivalen per gram) for S. officinalis and from 25.91 to 53.82 mg QE.g⁻¹for S. sclarea. The free radical scavenging activity of ethanol extracts of S. officinalis and S. sclarea was 6.05–8.59 mg TE.g⁻¹ (mg Trolox equivalent per gram) and 6.56–8.03 mg TE.g⁻¹, respectively. The extracts of S. officinalis showed molybdenum-reducing power from 56.25 to 218.67 mg TE.g⁻¹ and S. sclarea extracts from 35.42 to 162.65 mg TE.g⁻¹. A strong correlation was found between free radical scavenging activity and polyphenol compound groups (r = 0.723–0.868), and between molybdenum-reducing power and polyphenol compound groups (r = 0.759–0.927) for *S. officinalis*. Strong relations were found in extracts of *S. sclarea* between molybdenum-reducing power and investigated polyphenol compounds (r = 0.802-0.909), whereas, free radical scavenging activity weak correlated with investigated compounds. Thus, this study showed that S. officinalis and S. sclarea extracts are a source of antioxidant compounds that can be used in the pharmaceutical and food industries. Most content of antioxidant compounds is found in the leaf and inflorescence extracts.

Keywords: sage, polyphenols, flavonoids, phenolic acids, correlation

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Introduction

The species from Salvia L. (sage) genus are well-known ornamental, aromatic, and medicine plants widely distributed in European countries, and their number achieved 500 (Korablova et al., 2019). It's one of the most species-rich genera and the exact number of species is still being determined (González-Gallegos et al., 2020). These species were *domestica*ted more than 3,500 years BC, used as food plants in some countries, and are perspective nutraceutical crops (Sosa et al., 2016). These are perennial and annual plants, woody shrubs, and habitats related to seed dispersal and can be from desert to dry shrubland, etc. (Zona, 2017). Many species from this genus are used as culinary herbs in different countries (Hamidpour et al., 2014) and used in pharmaceutical and cosmetic industries (Grdiša et al., 2015).

As aromatic cultures, plants *S. officinalis* and *S. sclarea* are characterized by the rich content of essential oil with numerous biological activity compounds (Vergine et al., 2019; Ovidi et al., 2021). The essential oil composition of these species is α -pinene, camphene, β -pinene, *p*-cymene, 1,8-cineole, γ -terpinene, α -thujone, chrysanthenone, camphore, etc. (Ovidi et al., 2021).

The biochemical composition of plant raw of *S. officinalis* L. is terpenoids, flavonoids (Topçu, 2006; Topcu and Kusman, 2014), polyphenols, flavones, proteins, reducing sugars (Neagu et al., 2014), polyunsaturated acids (Sosa et al., 2016), phenolic acids (Paje et al., 2022). Seeds of some species id a good source of α -linolenic acid (Nitrayová et al., 2014). Different extracts of *Salvia* species exhibited various biological activities such as antioxidant, anti-cancer,

anti-diabetic, anti-diarrheal, decreasing of cholesterol levels, improving memory (Hamidpour et al., 2014), antimicrobial, and anti-inflammatory (Sharopov et al., 2018).

The pharmacological activities of phenolic acids of *Salvia* species are anti-oxygenation, antithrombotic, anti-liver injury activity, anti-tumour, anti-hypertensive effect, antiviral, etc. (Wang et al., 2019). The comparable analysis of *S. officinalis* and *S. sclarea* antioxidant activity showed that oil and seeds of the first species exhibited the highest values (Živković et al., 2017). Extracts of *S. officinalis* and *S. sclarea* were effective against *E. coli*, *P. fluorescens*, *A. bohemicus*, *K. marina*, and *B. cereus* by disc diffusion method (Ovidi et al., 2021).

This study aimed to estimate the polyphenol content and antioxidant activity of two *Salvia* species in the South region of Ukraine as a potential source of antioxidants that can be used in further pharmacological research.

Material and methodology

Biological material

The plants of *Salvia officinalis* L. and *S. sclarea* L. (Figure 1) were investigated in this study from the experimental collection of the Institute of Climate Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine (Kherson region, v. Plodove) in 2020–2021. The plant raw took at the budding (buds, leaves, and all above-ground part), flowering (leaves, inflorescences, and all above-ground part), and fruitage (leaves, fruits, and all above-ground part).

All biochemical analyses were conducted at the Slovak University of Agriculture in Nitra (Slovak Republic).



Figure 1 Plants of Salvia officinalis L. (1) and S. sclarea L. (2) at the flowering stage

Chemicals

All chemicals used were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA) and CentralChem (Slovakia).

Preparations of extracts

An amount of 0.25 g of each sample was extracted with 20 mL of 80% ethanol for 2 h in a laboratory shaker GFL 3005 (GFL, Burgwedel, Germany). Then, the samples were centrifuged at 4605 RCF (Rotofix 32 A, Hettich, Germany) for 10 min and the supernatant was used for measurement of FRSA (antiradical activity) using DPPH, MRAP (antioxidant activity) using phosphomolybdenum method and measurement of other antioxidant properties (detection of total polyphenol, total flavonoid, and phenolic acid content).

Total polyphenol content of extracts

The total polyphenol content (TPC) was measured by the method of Singleton and Rossi (1965) using the Folin-Ciocalteu reagent. A quantity of 0.1 mL of each sample was mixed with 0.1 mL of the Folin-Ciocalteu reagent, 1 mL of 20% (w/v) sodium carbonate, and 8.8 mL of distilled water. After 30 min in darkness, the absorbance at 700 nm was measured with the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25–300 mg.L⁻¹; R² = 0.998) was used as the standard. The results were expressed in mg.g⁻¹ DW gallic acid equivalent.

Total phenolic acid content

The content of phenolic acids was determined using Farmakopea Polska (1999). 0.5 ml of sample extract was mixed with 0.5 ml of 0.5 M hydrochloric acid, 0.5 ml Arnova reagent, 0.5 ml of 1 M sodium hydroxide (w/v), and 0.5 ml of distilled water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid 1–200 mg.l⁻¹ ($R^2 = 0.999$) was used as a standard. The results were expressed in mg.g⁻¹ caffeic acid equivalents (CAE).

Total flavonoid content of extracts

The total flavonoid content (TFC) was determined by the modified method described by Shafii et al. (2017). An aliquot of 0.5 mL of the sample was mixed with 0.1 mL of 10% (w/v) ethanolic solution of aluminium chloride, 0.1 mL of 1 M potassium acetate, and 4.3 mL of distilled water. After 30 min in darkness, the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (1–400 mg.L⁻¹; $R^2 = 0.9977$) was used as the standard. The results were expressed in $mg.g^{-1}$ DW quercetin equivalent.

Free radical scavenging activity

Free radical scavenging activity (FRSA) of samples (antiradical activity) was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sánchéz-Moreno et al., 1998). An amount of 0.4 mL of sample was mixed with 3.6 mL of DPPH solution (0.025 g DPPH in 100 mL ethanol). The absorbance of the reaction mixture was determined with the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10–100 mg.L⁻¹; $R^2 = 0.989$) was used as the standard and the results were expressed in mg.g⁻¹ DM Trolox equivalents.

Molybdenum-reducing power of extracts

The molybdenum-reducing power (MRP) of samples was determined by the method of Prieto et al. (1999) with slight modifications. The mixture of the sample (1 mL), monopotassium phosphate (2.8 mL, 0.1 M), sulfuric acid (6 mL, 1 M), ammonium heptamolybdate (0.4 mL, 0.1 M), and distilled water (0.8 mL) was incubated at 90 °C for 120 min, then cooled to room temperature. The absorbance at 700 nm was detected with the spectrophotometer Jenway (6405 UV/Vis, England). Trolox (10–1000 mg.L⁻¹; R² = 0.998) was used as the standard and the results were expressed in mg.g⁻¹ DM Trolox equivalent.

Statistical analysis

The results are expressed as mean values of three replications \pm standard deviation (SD); hierarchical cluster analyses of similarity between samples were computed based on the Euclidean similarity index. Data were analyzed with the ANOVA test and differences between means were compared through the Tukey-Kramer test (p <0.05).

Results and discussions

The study of antioxidant activity is one of the distributed topics of the last decades and is related to phenolic substances (Nićiforović et al., 2010). Polyphenol compounds are a group of antioxidants that includes flavonoids, phenolic acids, and their subclasses that are found in teas, fruits, juices, wines, olive oil, and chocolates. They have synthetic, medicinal, and industrial value (Handique and Baruah, 2002; Perron and Brumaghim, 2009). Polyphenol compounds are interesting as anti-inflammatory, anti-cancer, and anti-ageing agents, widely used for cosmetic and nutraceutical purposes (Munin and Edwards-Lévy, 2011). Polyphenol compounds of *Salvia* species include rosmarinic acid, vanillin, chlorogenic acid, catechin, quercetin, and p-coumaric acid (Rowsan and Najafian, 2020).

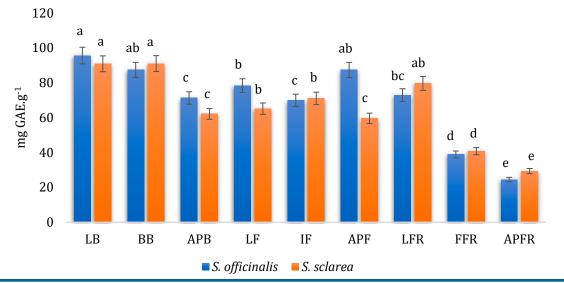
The content of polyphenol compounds of ethanol extracts of S. officinalis and S. sclarea was from 24.52 (S. officinalis, leaves at the budding) to 95.62 (S. officinalis, above-ground parts at the fruitage) mg GAE.g⁻¹ (Figure 2). The polyphenol compound content of S. officinalis raw was 24.25-87.36 mg GAE.g⁻¹ during vegetation. The different parts of S. sclarea demonstrated the polyphenol content from 29.39 to 91.02 mg GAE.g⁻¹. The content of these compounds in the herb (all above-ground parts) was minimal at the budding stage and fruitage for both species, whereas minimal polyphenol content was only in S. sclarea raw of herb at the flowering stage. The above-ground part of S. officinalis at the flowering stage demonstrated the highest content of polyphenols than other parts at this period.

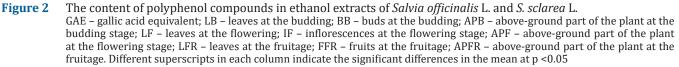
The study of eight *Salvia* species showed that total polyphenol content was in the range of 50.3–167.1 mg GAE.g⁻¹ depending on the species (Tosun et al., 2009). The comparative study of Iranian *Salvia* species showed that the total polyphenol content was from 38 to 326 mg GAE.g⁻¹ (Asadi et al., 2010). According to Jasicka-Misiak et al. (2018), the total content of polyphenols of *S. officinalis* and *S. sclarea* extracts was 63.9–93.8 mg GAE.g⁻¹ and 96.1–134.4 mg GAE.g⁻¹,

respectively. According to Afonso et al. (2019), the total polyphenol content in water extracts of S. mexicana, S. officinalis, and S. africana was 158, 229, and 350.6 μg GAE.mg $^{\text{-1}}$ extracts. The study of three Salvia species showed that methanol extracts had a polyphenol content of 658.3–1805.9 mg caffeic acid equivalent per 100 g FW (fresh weight) (Sharopov et al., 2018). According to Mňahončaková et al. (2019), the polyphenol content in an extract of Slovakian S. officinalis was 62.87 mg GAE.g⁻¹ at the stage of flowering was less compared with our study (87.36 mg GAE.g⁻¹). The polyphenol content of another Lamiaceae species Scutellaria baicalensis Georgi from the same region of Ukraine (Kherson area) was 96.54 mg GAE.g⁻¹ (Vergun et al., 2019). Also, the study of *Thymus* herb demonstrated that total polyphenol content varied from 56.12 to 98.36 mg GAE.g⁻¹ depending on species at the flowering stage (Vergun et al., 2022).

A phenolic acid is a group of phenolic compounds that play an important role as an antiaging agent, and demonstrate antitumor, antimicrobial, and anti-inflammatory properties. These biologically active molecules are found in edible and nonedible plants (Jitan et al., 2018). As reported Wang et al. (2019), phenolic acids are the main active polyphenol compounds of *Salvia* species with high therapeutic functions, among which caffeic acid and danshensu are structural units.

The total content of phenolic acids in the ethanol extracts was from 10.13 to 40.23 mg CAE.g⁻¹ during vegetation depending on species (Figure 3). The minimal content





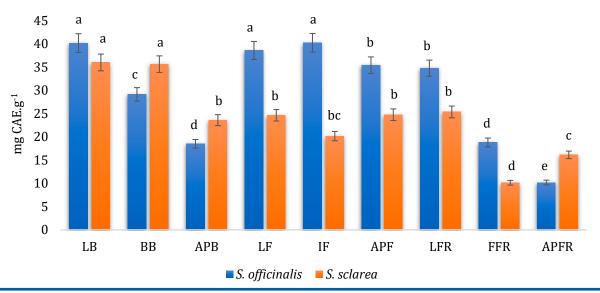


Figure 3 The content of phenolic acids in ethanol extracts of *Salvia officinalis* L. and *S. sclarea* L. CAE – caffeic acid equivalent; LB – leaves at the budding; BB – buds at the budding; APB – above-ground part of the plant at the budding stage; LF – leaves at the flowering; IF – inflorescences at the flowering stage; APF – above-ground part of the plant at the flowering stage; LFR – leaves at the fruitage; FFR – fruits at the fruitage; APFR – above-ground part of the plant at the fruitage. Different superscripts in each column indicate the significant differences in the mean at p <0.05

of phenolic acids for both species was determined at the budding stage in the herb. The maximal values of phenolic acids at the flowering stage were determined for *S. officinalis* raw. The highest values of these compounds of *S. officinalis* extracts were found at the budding and fruitage. The phenolic acids in *S. sclarea* extracts accumulated unevenly.

As reported Orhan et al. (2012), the extracts of Turkish species of *Salvia* weren't identified as gallic acid. According to Mňahončaková et al. (2019), the phenolic acid content in *S. officinalis* extracts was 24.30 mg CAE.g⁻¹. The phenolic acid content of *Scutellaria baicalensis* from the same region of Ukraine was 30.12 mg CAE.g⁻¹ (Vergun et al., 2019). The most abundant phenolic acid of *Salvia* species is rosmarinic acid (Paje et al., 2022). Extracts of *Thymus* species demonstrated from 26.19 to 40.46 mg CAE.g⁻¹ depending on species at the flowering stage which is close to the results in this study (Vergun et al., 2022).

Flavonoids are a versatile class of natural compounds that demonstrated different biological activities such as antimicrobial and antifungal (Saleem et al., 2018). These polyphenol compounds are abundant in fruits, vegetables, and grains, and have antioxidant, antiinflammatory activity and reduce the risk of diseases (Shen et al., 2022). Flavonoids from some *Salvia* species had α -amylase and α -glucosidase inhibitory effect that has an important antidiabetic effect (Asghari et al., 2015). The content of flavonoids in ethanol extracts of investigated *Salvia* species was from 13.73 to 55.38 mg QE.g⁻¹ during the flowering period depending on the species (Figure 4). The content of flavonoids in raw of *S. officinalis* and *S. sclarea* was 13.73–55.38 and 25.91–53.82 mg QE.g⁻¹, respectively. The highest content of flavonoids of *S. officinalis* raw accumulated in leaf extracts during vegetation, whereas *S. sclarea* accumulated these compounds in the leaves at the budding and fruitage. At the flowering stage, flavonoid content was maximal in the inflorescences extracts of *S. sclarea*.

As reported Asadi et al. (2010), the total flavonoid content in extracts of Iranian species was from 91 to 253 mg of catechin per gram extracts. According to Sharopov et al. (2018), the flavonoid content of methanol extracts of these species varied from 13 to 184.9 mg QE.100 g⁻¹ FW. According to Mňahončaková et al. (2019), the flavonoid content in extracts of *S. officinalis* growing in Slovakia was 36.80 mg QE.g⁻¹. The flavonoid content of *Scutellaria baicalensis* from the same region of Ukraine was 66.07 mg QE.g⁻¹ (Vergun et al., 2019) which was higher than in extracts of investigated plants of *Salvia*. A similar content of flavonoids was found for *Thymus* species at the flowering stage and was 24.59–49.29 mg QE.g⁻¹ (Vergun et al., 2022).

Antioxidants play an essential role in living organisms on a cellular level and this is important to search for new natural sources of these compounds (Lourenço et al., 2019). The value of the antioxidant activity of

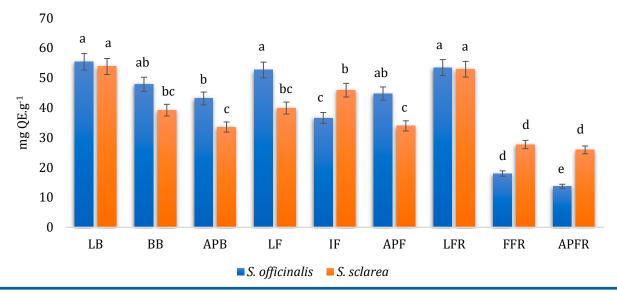
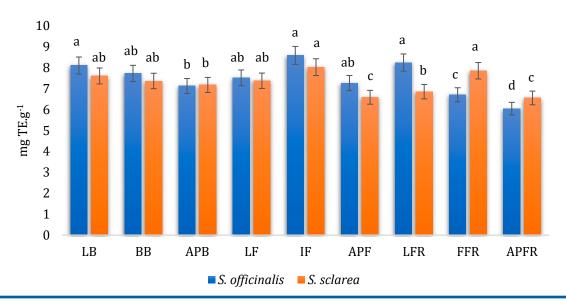
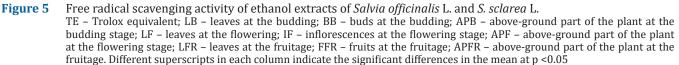


Figure 4The content of flavonoids in ethanol extracts of Salvia officinalis L. and S. sclarea L.
QE – quercetin equivalent; LB – leaves at the budding; BB – buds at the budding; APB – above-ground part of the plant at the
budding stage; LF – leaves at the flowering; IF – inflorescences at the flowering stage; APF – above-ground part of the plant
at the flowering stage; LFR – leaves at the fruitage; FFR – fruits at the fruitage; APFR – above-ground part of the plant at the
fruitage. Different superscripts in each column indicate the significant differences in the mean at p <0.05</td>

plant raw depends on many factors among which are a method of determination and the type of solvent (Dawidowicz et al., 2012). As reported Francik et al. (2020), extracts of *S. officinalis* leaves, for example, are characterized by higher polyphenol content than infusions. One of the most popular methods is DPPH (radical scavenging activity) which is widely used to determine antioxidants with a polyphenol nature (Marinova and Batchvarov, 2011). Numerous studies demonstrated that *Salvia* extracts had significant antioxidant activity by different methods among which DPPH, FRAP, TEAC, and ABTS (Asadi et al., 2010; Kačmárová et al., 2016).

The antioxidant activity of the DPPH method was from 6.97 to 8.14 mg TE.g⁻¹ at the start of vegetation and from 4.6 to 6.69 mg TE.g⁻¹ at the flowering stage (Figure 5). The maximal values of antioxidant activity by the DPPH





method were found for leaf, inflorescences, and fruit extracts.

According to Mňahončaková et al. (2019), the free radical scavenging activity of *S. officinalis* extracts was 7.78 mg TE.g⁻¹. *Thymus* extracts of plants at the flowering stage exhibited free radical scavenging activity from 8.0 to 8.74 mg TE.g⁻¹ (Vergun et al., 2022).

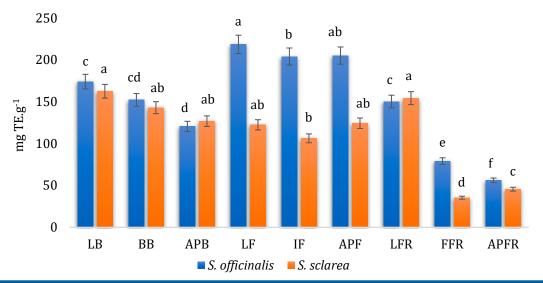
Among the numerous methods of antioxidant activity assessment also well-known molybdenumreducing power of extracts that are based on reducing molybdenum by anti-oxygen agents (Vasyliev et al., 2020). The antioxidant activity by the phosphomolybdenum method of investigated plants was from 56.25 to 218.67 mg TE.g⁻¹ for *S. officinalis* and from 35.42 to 162.65 mg TE.g⁻¹ depending on species and part of plants (Figure 6). The highest molybdenumreducing power was found for leaves of S. officinalis extracts during vegetation. The extracts of S. sclarea demonstrated maximal values of this parameter in the leaves at the budding and fruitage and herbs at the flowering stage.

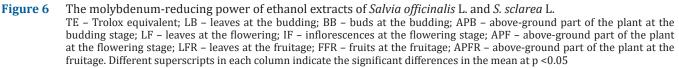
According to Mňahončaková et al. (2019), the molybdenum-reducing power of *S. officinalis* extracts was 174.50 mg TE.g⁻¹. This parameter was varied in extracts of *Scutellaria baicalensis* from the same region of Ukraine (63.33–260.24 mg TE.g⁻¹) but above-ground parts demonstrated the highest reducing power of extracts compared with separate organs at the flowering stage (Vergun et al., 2019). *Thymus* species extracts demonstrated reducing power in the range of 87.56–160.94 mg TE.g⁻¹ (Vergun et al., 2022).

Polyphenol compounds demonstrated the scavenging ability of free radicals which determines the antioxidant activity of raw materials (Tosun et al., 2009). As a result, the studied parameters of both S. officinalis and S. sclarea extracts showed a very strong correlation between all investigated parameters and the molybdenum-reducing power of extracts (Table 1). So, a very strong correlation was found between phenolic acid content and reducing power of extracts (r = 0.927), total phenolic content and reducing power of extract (r = 0.810), and total flavonoid content and molybdenum reducing power of extract (r = 0.759) for S. officinalis. A strong correlation was also found between both methods of antioxidant activity determination (r = 0.732). A very strong relations determined between free radical scavenging activity and phenolic acid content (r = 0.868), flavonoid content (r = 0.732), and phenolic content (r = 0.723).

It should be noted that a weak correlation was found between free radical scavenging activity and all groups of phenolic compounds of *S. sclarea* extracts. However, molybdenum reducing power of extracts strong correlated with total polyphenol content (r = 0.909), total phenolic acids (r = 0.887), and total flavonoid content (r = 0.802).

The study of antioxidant activity and polyphenol content of different herbs showed that the correlation between investigated parameters depended on species and extracts (Kiselova et al., 2006). Comparing with other species showed that the reducing power of extracts had a very strong relationship with polyphenols,





Parameter	ТРС	TPAC	TFC	RSA	MRP
		S. offic	cinalis		
TPAC	0.800**	1	0.776**	0.868**	0.927**
TFC	0.928*	0.776**	1	0.732**	0.759**
RSA	0.723**	0.868**	0.732**	1	0.732**
MRP	0.810**	0.927**	0.759**	0.732**	1
		S. sci	larea		
ТРАС	0.882**	1	0.643	-0.036	0.887**
TFC	0.840**	0.643*	1	0.266	0.802**
RSA	0.284*	-0.036	0.266*	1	-0.042
MRP	0.909**	0.887**	0.802**	-0.042	1

Note: TPC – total phenolic compounds; TPAC – total phenolic acid content; TFC – total flavonoid content; RSA – free radical scavenging activity; MRP – molybdenum reducing the power of extracts. ** Correlation is significant at $p \le 0.01$; * correlation is significant at $p \le 0.05$

flavonoids, and phenolic acids (r = 0.906–0.980), however, between both methods of antioxidant activity determination found a weak or negative correlation in case of *Scutellaria baicalensis* extracts (Vergun et al., 2019). However, the investigation of extracts of certain Lamiaceae species showed that the ratio between polyphenol compounds and method of antioxidant activity can be varied and differ from other results. The study of *Thymus* species demonstrated that values of the coefficient of correlation depended on species, so, *Th. vulgaris* extracts characterized by the strong correlation between polyphenol compounds and two assays of detection of antioxidant activity compared with other species (Vergun et al., 2022).

Conclusions

Thus, the plant raw material of two investigated Salvia species is a source of polyphenol compounds with high antioxidant activity. The distribution of polyphenols, phenolic acids, and flavonoids was uneven and depended on species, stage of growth, and part of the plant. The highest values of investigated parameters were found in leaf and inflorescences extracts. The minimal values of polyphenol compound content, flavonoid content, and free radical scavenging activity of extracts were found for S. officinalis, whereas phenolic acid content and molybdenum-reducing power of extracts for *S. sclarea*. The maximal values of phenolic acid content and molybdenum-reducing power of extracts were determined for S. officinalis, whereas total phenolic compounds content, flavonoid content, and free radical scavenging of extracts for S. sclarea. Both S. officinalis and *S. sclarea* extracts demonstrated a strong correlation between molybdenum-reducing power and all investigated polyphenol group compounds.

Free radical scavenging activity of *S. sclarea* extracts demonstrated a weak correlation with investigated compounds. Thus, this study showed that *S. officinalis* and *S. sclarea* extracts are a source of antioxidant compounds that can be used in the pharmaceutical and food industries.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

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