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ANTIOXIDANT CAPACITY OF PLANT RAW MATERIAL OF SCUTELLARIA BAICALENSIS GEORGI

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The aim of this study was to evaluate antioxidant capacity of Scutellaria baicalensis Georgi from two regions of Ukraine: Kyiv city (M. M. Gryshko National Botanical Garden of NAS of Ukraine (NBG)) and Kherson region (Experimental Facility "Novokakhovska" of Rice Research Institute of Ukrainian Academy of Agrarian Sciences (EFN of RRI)). Observation of plants and biochemical analyses conducted with plants collected in the stage of flowering. In study investigated and compared above-ground part of plants and separated organs: inflorescences, stems, leaves. Measured morphometric parameters (height of plants, length, and width of leaves, length, and diameter of inflorescence, the diameter of the stem) showed that the most variable was the length of inflorescence (12.79%) for NBG sample and diameter of the stem (33.33%) for EFN of RRI sample. Ethanolic extracts were screened for the antioxidant capacity. As standards were used gallic acid for polyphenol content (GAE), quercetin for flavonoids (QE), caffeic acid for phenolic acids (CAE), Trolox for antioxidant capacity (TE). The total content of polyphenol compounds was 42.43 - 86.13 mg GAE.g⁻¹ DW (dry weight) (NBG sample) and 28.06 -96.76 mg GAE.g⁻¹ DW (EFN of RRI sample). The content of flavonoids was 9.39 – 62.97 mg QE.g⁻¹ DW (NBG sample) and 10.64 – 66.07 mg QE.g⁻¹ DW (EFN of RRI sample). The concentration of phenolic acids was 2.60 – 16.13 mg CA.g⁻¹ DW (NBG sample) and 12.02 – 30.12 CA.g⁻¹ DW (EFN of RRI sample). Antioxidant activity of plant extracts was measured by DPPH assay and reducing power method. The first method indicated an antioxidant ability 8.24 - 8.56 mg TE.g⁻¹ DW (NBG sample) and 7.63 – 8.83 mg TE.g⁻¹ DW (EFN of RRI sample). Reducing power of extracts was 51.48 - 306.09 mg TE.g⁻¹ DW (NBG sample) and 63.33 - 260.24 mg TE.g⁻¹ DW (EFN of RRI sample). Very strong positive correlation identified between total polyphenol content, total flavonoid content and reducing power. Scutellaria baicalensis is a rich source of antioxidants and potential raw of further pharmacological study in Ukraine as well as in other regions for improving and enrichment of relevant production.

Keywords: Scutellaria baicalensis; antioxidant activity; polyphenols; flavonoids; phenolic acids

INTRODUCTION

Species of genus Scutellaria L. belong to Lamiaceae Martinov., plants of which are known as aromatic, medicinal and food (Bazzaz et al., 2011). Among species of this genus, the most known is Scutellaria baikalensis Georgi (Huangqin) that is an important medicinal species with a rich biochemical composition that contains, also, a metabolite previously thought to be unique for Hypericum perforatum L. (Murch et al., 2004). Raw (dry roots) material of this plant widely used in Chinese herbal medicine as treatments of some diseases such as inflammation, hypertension, cardiovascular diseases, and tumor. Root extracts exhibited high antioxidant activity that appeared also due to the content of baicalin and baicalein, and other biologically active compounds (Chen et al., 2000; Liu et al., 2017; Wang et al., 2017; Cheng et al., 2018). It is described that flavonoid wogonin from Scutellaria baicalensis roots appears anti-tumor and antimetastatic action (Kimura and Sumiyoshi, 2013). According to Cheng et al. (2018), extracts and main flavonoids of *Scutellaria baicalensis* have an anticancer effect and this species is promising for anticancer therapy. As reported by **Cole et al. (2008)**, plant extracts can be effective also in the inhibition of liver fibrosis, insomnia, neuralgia etc. Besides of main flavonoids, in the plant raw material of this plant indicated flavones, diterpenes, phenylethanoid glycosides, amino acids, essential oils.

Extracts of *Scutellaria baicalensis* possess high potency to reduce lipid peroxidation (**Gabrielska et al., 1997**). Study of **Gaire Prasad et al. (2014**) resulted that raw of *Scutellaria baicalensis* and its individual components showed a neuroprotective effect that combined from some pharmacological effects. The physiological effect of baicalein is regulation of apoptosis in damaged roots (**Hirunuma et al., 2011**). Significant antioxidant activity was found in the extract of this plant (**Li et al., 2015**).

The aim of this study was a comparing study of the antioxidant activity, total polyphenols, flavonoids, phenolic acid content of plant raw material of selected species of the genus of *Scutellaria baicalensis* Georgi from two areas of Ukraine.

Scientific hypothesis

Comparative assessment of morphometric and antioxidant parameters of *Scutellaria baicalensis* plants from two regions of Ukraine.

MATERIAL AND METHODOLOGY

Plant materials

The plants material of *Scutellaria baicalensis* Georgi was collected from experimental collection of Cultural Flora Department of M. M. Gryshko National Botanical Garden of the NAS of Ukraine (NBS) (Kyiv; 50°24'55"N, 30°33'45"E) and collection of aromatic and medicinal plants of Experimental Facility "Novokakhovska" of Rice Research Institute of Ukrainian Academy of Agrarian Sciences (EFN of RRI) (v. Plodove; 46°45'16.2"N 33°20'55.1"E). The following morphometric parameters of investigated plants were conducted at the conditions of NBG (Figure 1) and EFN of RRI (Figure 2) in the period of flowering: height of plants in cm, length of leaf in cm, the width of leaf in cm, length of inflorescence in cm, the diameter of the stem in cm.



Figure 1 *Scutellaria baicalensis* Georgi in the period of flowering in the M. M. Gryshko National Botanical Garden of NAS, Kyiv.



Figure 2 *Scutellaria baicalensis* Georgi in the period of flowering-frutage in the Experimental Facility "Novokakhovska" of Rice Research Institute of Ukrainian Academy of Agrarian Sciences.

In this study investigated parameters of antioxidant activity of ethanol extracts of *S. baicalensis* from two regions of Ukraine in the stage of flowering. Plant samples were dried at 35 °C for four days. All biochemical analyses were conducted at the Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Resources, Department of Plant Storage and Processing.

Chemicals

All chemicals were analytical grade and were purchased from Reachem (Slovakia) and Sigma Aldrich (USA).

Sample preparation

0.2 g of dried plant raw material was extracted with 20 mL of 80% ethanol for 2 hours. After centrifugation at 4000 g (Rotofix 32 A, Hettich, Germany) for 10 min, the supernatant was used for the next measurements: antioxidant activity, polyphenols, and flavonoids. All data expressed in mg of standard compound per gram of dry weight (DW).

Total polyphenol content (TPC)

Total polyphenol content extracts were measured by the method of **Singleton and Rossi** (1965) using Folin-Chiocalteu reagent. 0.1 mL of each sample extract was mixed with 0.1 mL of the Folin-Chiocalteu 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 using the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25 – 250 mg.L⁻¹; $R^2 = 0.996$) was used as the standard and the results were expressed in mg.g⁻¹ gallic acid equivalents.

Total flavonoid content (TFC)

Determination of total flavonoids content was conducted using the procedure described by **Shafii et al. (2017)**. 0.5 mL of sample extract was mixed with 0.1 mL of 10% (w/v) ethanolic solution of aluminum chloride, 0.1 mL of 1 M sodium 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 (0.01 – 0.5 mg.L⁻¹; $R^2 = 0.997$) was used as the standard and the results were expressed in µg.g⁻¹ quercetin equivalents.

Total phenolic acid content (TPAC)

Determination total phenolic acids content of extracts was carried out using the method of **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 and the results were expressed in mg.g⁻¹ caffeic acid equivalents.

Antioxidant activity

Radical scavenging assay (DPPH)

The radical scavenging activity of samples was measured using 2.2-diphenyl-1-picrylhydrazyl (DPPH) (Sánchéz-Moreno, Larrauri and Saura-Calixto, 1998). The extracts (0.5 mL) were mixed with 3.6 mL of radical solution (0.025 g of DPPH in 100 mL ethanol). The absorbance of the sample extract was determined using the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2carboxylic acid) (10 – 100 mg.L⁻¹; $R^2 = 0.988$) was used as the standard and the results were expressed in mg.g⁻¹ Trolox equivalents.

Reducing power (RP)

Reducing the power of extracts was determined by the phosphomolybdenum method of **Prieto, Pineda and Aguilar (1999)** with slight modifications. The mixture of 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) incubated at 90 °C for 120 min, then rapidly cooled and detected by monitoring absorbance at 700 nm using the spectrophotometer Jenway (6405 UV/Vis, England). Trolox (10 – 1000 mg.L⁻¹; $R^2 = 0.998$) was used as the standard and the results were expressed in mg.g⁻¹ Trolox equivalents.

Statistical analysis

The statistically treated data are given in tables as the arithmetical mean values and their standard errors. Data were submitted ANOVA and differences between means compared through the Tukey-Kramer test ($\alpha = 0.05$). We used the Dixon's Q test (DQn) at the significance level of p < 0.05. Correlation analysis was performed using Pearson's criterion. Variability of all these parameters was evaluated using descriptive statistics. Level of variability determined by **Stehlíková (1998)**.

RESULTS AND DISCUSSION

The morphometric parameters of plants of Scutellaria baicalensis in conditions of M. M. Gryshko National Botanical Garden represented in Table 1. Variability of measured parameters was from 2.45 to 12.79%, the most variable parameter was the length of inflorescence and the least variable the width of the leaf. The morphometric parameters of plants of Scutellaria baicalensis in conditions of Experimental Facility "Novokakhovska" of Rice Research Institute of Ukrainian Academy of Agrarian Sciences represented in Table 2. The most variable parameter was the diameter of the stem and the least height of plants. Based on obtained results, it's should be noted that significant differences found when comparing the diameter of inflorescences. Investigation of biological activities of plant raw material one of the most discussed areas of plant raw material study (Cocan et al., 2018; Ohigashi et al., 1991). One of the most known biological activity of plant raw material is antioxidant capacity (Kavalcová et al., 2014). In addition, plant raw material of Lamiaceae herb is a rich source of antioxidant compounds (Matkowski, Tasarz and Szypuła, 2008). Antioxidant properties of herb from Lamiaceae studied in the context of the condition of raw. The higher values obtained by studying the dried material than fresh and frozen (Adámková, Kouřimská and Kadlecová, 2015; Kouřimská, Ešlerová and Khatri, 2016).

According to **Liu et al. (2011)**, 80% ethanol extract of *Scutellaria baicalensis* demonstrated higher antioxidant capacity than other concentration of alcohol solution. Also, flavonoid, phenolic acids content and antimicrobial activity were higher at the 80% ethanol extraction.

The antioxidant capacity of plant extracts has been attributed to their phenolic contents that were determined by Folin-Ciocalteu reagent. The amount of total polyphenol content of Scutellaria baicalensis extracts is shown in Table 3 and Table 4. The content of polyphenols for plants of NBG was in the range from 42.43 to 86.13 mg GAE per g of DW (dried weight) depending on part of the plant. Plants collected from EFN of RRI accumulated polyphenols from 28.06 to 96.76 mg GAE per g DW. It should be noted the most content of polyphenol compounds indicated in the leaves.

Study of **Seo et al. (2013)** showed that content of different polyphenol compounds using liquid chromatography (HPLC-UV) depends on the organ of *Scutellaria baicalensis* and was 1715.7 mg.kg⁻¹ fresh weight of fruits (FW) for roots, 885.0 mg.kg⁻¹ FW for leaves, 622.4 mg.kg⁻¹ ¹ FW for flowers and 307.4 mg.kg⁻¹ FW for stems.

Previous data about the content of flavonoids concerning mainly of their concentration in the roots of *S. baicalensis* (Kimura and Sumiyoshi, 2013; Kosakowska, 2017). According to Kosakowska (2017), the mean value of the content of flavonoids in the roots was 0.33%. *Scutellaria baicalensis* flavonoids are active compounds of anti-inflammatory herbal medicine in China (Gao et al., 1999). The most known active ingredients of genus *Scutellaria* are flavonoids such as baicalin, baicalein, and wogonin, which play important role in biological activities of these plants: antimicrobial, antifungal, antiviral (Bazzaz et al., 2011).

According to **Cheng et al.** (2018), more than 40 flavonoids identified from the raw of this plant. **Cole et al.** (2008) reported that baicalin and baicalein the most common flavonoids from the *Scutellaria baicalensis*. As resulted in **Grzegorczyk-Karolak**, **Wysokińska and Olas** (2015), the content of some flavonoids was much lower than in roots and shoots. In the stems and leaves 21 flavonoids were detected (Liu et al., 2011). The main flavonoids of the aboveground part of this plant are scutellarin, dihydroscitellarin, and glucuronides of apigenin and luteolin (Olennikov, Chirikova and Tankhaeva, 2010).

In our study content of flavonoids compared with quercetin content. As noticed **Gao et al. (1999)**, flavonoids such as quercetin, luteolin, and catechin are better antioxidants than for example ascorbic acid or vitamin E. Total flavonoid content in ethanol extracts of *Scutellaria baicalensis* from NBG was from 9.39 to 62.97 mg QE per g DW and for it plants from EFN of RRI from 10.64 to 66.07 mg QE per g (DW) (Table 3 and Table 4). The least content of flavonoids was found in the stems of both samples. The high concentration of flavonoids indicated in all plants of the NBG sample and in the leaves of EFN of RRI sample.

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Parameter	min	max	mean	SD	V(%)
Height of plant, cm	60.02	70.00	64.30	1.17	5.68
Length of leaf, cm	4.00	4.40	4.24	0.04	2.98
Width of leaf, cm	0.22	0.51	0.35	0.03	2.45
Length of inflorescence, cm	12.00	17.00	14.30	0.58	12.79
Diameter of inflorescence, cm	7.11	9.00	8.21	0.26	10.21
Diameter of stem, cm	0.21	0.52	0.31	0.03	8.01

Table 1 Morphometric parameters of Scutellaria baicalensis Georgi plants in conditions of M. M. Gryshko National Botanical Garden.

Note: min, max – minimal and maximal measured values; mean – arithmetic mean; SD – standard error of mean; V – coefficient of variation (%).

Table 2 Morphometric parameters of *Scutellaria baicalensis* Georgi plants in conditions of Experimental Facility "Novokakhovska" of Rice Research Institute of Ukrainian Academy of Agrarian Sciences.

Parameter	min	max	mean	SD	V (%)
Height of plant, cm	60.11	70.02	66.30	2.14	4.39
Length of leaf, cm	3.50	4.00	3.77	0.19	5.80
Width of leaf, cm	0.53	0.90	0.66	0.09	18.18
Length of inflorescence, cm	11.00	16.00	13.80	1.64	13.62
Diameter of inflorescence, cm	4.90	7.10	5.80	0.74	15.13
Diameter of stem, cm	0.20	0.45	0.33	0.09	33.33

Note: min, max – minimal and maximal measured values; mean – arithmetic mean; SD – standard error of mean; V – coefficient of variation (%).

Table 3 Total polyphenol, flavonoid and phenolic acid content of plants of *Scutellaria baicalensis* Georgi (NBS).

Part of plant	Total polyphenols (mg GAE.g ⁻¹)	Total flavonoids (mg QE.g ⁻¹)	Total phenolic acid (mg CAE.g ⁻¹)
Inflorescences	42.43 ± 1.20	26.43 ±2.36	10.68 ±0.93
Leaves	86.13 ±2.06	61.53 ± 1.91	13.98 ± 0.25
Stems	45.36 ± 0.56	9.39 ±0.52	2.60 ± 0.26
All plant	81.24 ±3.91	62.97 ±0.63	16.13 ±0.53

Note: GAE (gallic acid equivalent); QE (quercetin equivalent); ± (standard deviation of the mean).

Table 4 Total polyphenol, flavonoid and phenolic acid content of plants of Scutellaria baicalensis Georgi (EFN of RRI).

Part of plant	Total polyphenols (mg GAE.g ⁻¹)	Total flavonoids (mg QE.g ⁻¹)	Total phenolic acid (mg CAE.g ⁻¹)
Inflorescences	67.50 ±0.89	45.89 ±0.98	15.40 ±0.32
Leaves	96.76 ±2.18	72.66 ± 2.69	23.40 ± 1.13
Stems	28.06 ± 1.91	10.64 ±0.29	12.02 ± 0.17
All plant	96.54 ±0.68	66.07 ± 0.67	30.12 ±2.66

Note: GAE (gallic acid equivalent); QE (quercetin equivalent); ± (standard deviation of the mean).

Table 5 The correlation coefficients of a linear relationship between the different parameters of antioxidant activity of investigated plants of *Scutellaria baicalensis* Georgi from Kyiv region of Ukraine.

Parameter	TPC	TFC	TPAC	DPPH
TFC	0.943*			
TPAC	0.772*	0.939*		
DPPH	-0.065*	-0.358	-0.625	
RP	0.942	0.969*	0.878*	-0.177

Note: *Significant according to the *t*-test (p < 0.05).

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Parameter	TPC	TFC	TPAC	DPPH
TFC	0.995*			
TPAC	0.887	0.843*		
DPPH	0.519*	0.481*	0.447*	
RP	0.933*	0.906	0.980	0.356*

Table 6 The correlation coefficients of a linear relationship between the different parameters of antioxidant activity of investigated plants of *Scutellaria baicalensis* Georgi from Kherson region of Ukraine.

Note: *Significant according to the *t*-test (p < 0.05).

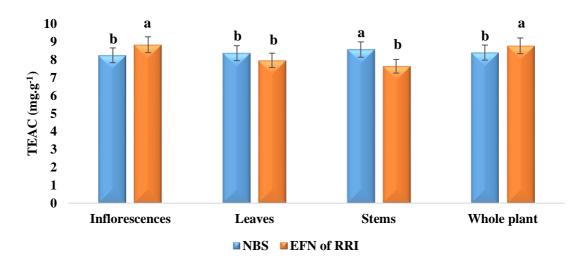


Figure 3 Antioxidant activity of extracts of plants of *Scutellaria baicalensis* Georgi depending on the region of growing (means in columns followed by different letters are different at p = 0.05. Each value represents the mean of three independent experiments ($\pm SD$)).

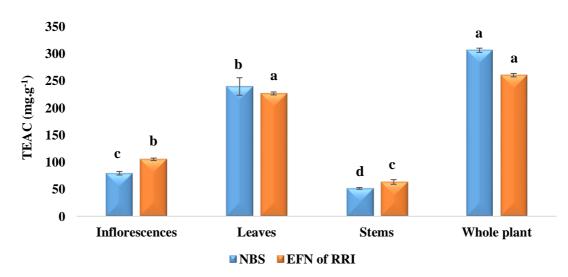


Figure 4 Reducing power of extracts of plants of *Scutellaria baicalensis* Georgi depending on the region of growing (means in columns followed by different letters are different at p = 0.05. Each value represents the mean of three independent experiments ($\pm SD$)).

Phenolic acids are derivates of benzoic and cinnamic acid that can exist in free and bound forms (Leváková and Lacko-Bartošová, 2017). As flavonoids, phenolic acids play an important role as antioxidant protectors against diseases such as cardiovascular, cancer, inflammatory bowel syndrome etc. (Saxena, Saxena and Pradhan, 2012). Also, this group of phenolic compounds connected with diverse functions in plant organism such as nutrient

uptake, protein synthesis, enzyme activity, photosynthesis etc. The concentration of phenolic acids depends on conditions of growth, among which is temperature **(Robbins, 2003)**.

In this study total phenolic acid content was found in the range from 2.60 to 16.13 mg CAE per g (DW) for NBG sample and from 12.02 to 30.12 mg CAE per g DW for EFN of RRI sample. In addition, stems of both samples

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accumulated the least content of phenolic acids and all plant raw had the higher content of it.

Radical scavenging activity of the ethanol extracts of investigated samples of Scutellaria baicalensis was screened against DPPH radical which is the most frequently used to determine the antiradical activity of several natural compounds (Marinova and Batchvarov, 2011; Alam, Bristi and Rafiguzzaman, 2013). Also, in a review of Alam, Bristi and Rafiguzzaman (2013) described that ethanol the most frequently used for extraction of plant samples to detect the antioxidant capacity. In Figure 3 represented comparable results of radical scavenging assay of ethanolic extracts of two samples of Scutellaria baicalensis. The difference in this parameter wasn't significant and for sample growing in the condition of NBG, it was of 8.24 – 8.56 mg Trolox per g (DW). In another sample (EFN of RRI) this parameter was 7.63 – 8.83 mg Trolox per g.

According to **Seo et al.** (2013), antioxidant activity by DPPH method was maximal in flowers and stems of investigated plants. In our study, the maximal value of this parameter was in the stem for plant sample from NBG and in inflorescences for EFN of RRI plant samples. Measuring of reducing the power of plant extracts one of the methods to determine the antioxidant capacity of plants. Determination of this parameter by phosphomolybdenum method based on reduction Mo (VI) to Mo (V) (Alam, Bristi and Rafiguzzaman, 2013).

Results of reducing power measuring of ethanolic extracts investigated species of *Scutellaria baicalensis* showed in Figure 4. In total, the value of this parameter for NBG sample was of 51.48 - 306.09 mg Trolox per g, for EFN of RRI sample of 63.33 - 260.24 mg Trolox per g. The fewer results were obtained for stems of investigated plants but stems of EFN of RRI demonstrated higher reducing power than other parts of the plant. The highest reducing power exhibited extracts of all plant. As studied by **Seo et al.** (2013), reducing power value was maximal for flowers of *Scutellaria baicalensis*, however, measured by another method than us.

Pearson's correlation analyses conducted for investigated plants (Table 5, Table 6). According to Li, Wu and Huang (2009), between antioxidant activity and phenolic compounds exhibited significant correlation. This fact depends on investigated extract and structure of phenolic compounds (Tatiya et al., 2011). In the study of Vamanu et al. (2011) wasn't found a correlation between phenolic compounds and antioxidant activity by DPPH method. Moreover, this research showed that existed a direct relationship between reducing power and antioxidant activity of investigated extracts, while in our study we didn't find a significant correlation between these parameters (very weak) for both samples.

We found a moderate positive correlation between total phenolic acids content, flavonoid content and total phenolic content and antioxidant activity by DPPH method for EFN of RRI sample, although NBS sample didn't show this relationship. Strong positive correlation identified between all investigated phenolic compounds and reducing power of extracts for both samples. Thus, in our case, we identified a significant relationship between the content of investigated polyphenol compounds and reducing power of extracts of two tested samples.

CONCLUSION

Investigated plant extracts of different parts of Scutellaria baicalensis Georgi from two Ukrainian regions exhibited high antioxidant activity in the stage of flowering. The high content of polyphenol compounds was identified in the leaves of both investigated samples. The present study demonstrates that the highest concentration of phenolic acids detected in all parts of plants. Some difference noticed in the accumulation of flavonoids: the highest values found in all parts of plants of NBG sample, whereas, for EFN of RRI sample maximum found in the leaves. Radical scavenging activity hasn't significant values that can say that this parameter does not depend on the area of growth for this species. Extracts of all plant demonstrated higher antioxidant activity by DPPH method and by phosphomolybdenum method than extracts of separated organs. The lowest values of these parameters found for stems. Correlation analyses found significant values in the relationship between investigated phenolic compounds and reducing the power of extracts. Moderate positive correlation noticed between phenolic compounds content and antioxidant activity by DPPH method for a sample from the Kherson region. Thus, two samples of Scutellaria baicalensis from two Ukrainian regions appears high potency of antioxidant activity that can be used for further study especially pharmacological.

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