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Assessment of antioxidant activity of ethanol extracts of *Vigna* spp.

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Plants of *Vigna* Savi genus are widely used in the world as food and medicinal plants. Most investigations propagated the biochemical composition and biological activity of seeds of these plants due to the high content of proteins but fewer studies about the above-ground plant part. The aim of this study focused on the investigation of the antioxidant activity of *Vigna* spp. The plant raw of *Vigna angularis* (Willd.) Ohwi & H. Ohashi, *V. mungo* (L.) Hepper, *V. radiata* (L.) Wilczek, *V. unguiculata* (L.) Walp. was collected from the M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine experimental collection at the start of the vegetation and flowering stage. At the start of vegetation was determined 45.27–77.21 mg GAE.g^{-1} (gallic acid equivalent) of total polyphenol compounds (TPC), 8.67–20.48 mg CAE.g^{-1} (caffeic acid equivalent) of total phenolic acids (TPAC), 31.84–47.97 mg QE.g^{-1} (quercetin equivalent) of total flavonoids content (TFC), 6.97–8.14 mg TE.g^{-1} (Trolox equivalent) of antioxidant activity by DPPH method, and 110.52 to 142.61 mg TE.g^{-1} of AA by phosphomolybdenum method. At the flowering stage found the following results: 27.16–78.11 mg GAE.g^{-1} of polyphenol compounds, 4.97–17.16 mg CAE.g^{-1} of phenolic acids, 18.27–54.26 mg QE.g^{-1} of flavonoids, 4.6–6.69 mg TE.g^{-1} of AA by DPPH method, and 45.16–110.27 mg TE.g^{-1} of antioxidant activity by phosphomolybdenum method. A very strong correlation found between antioxidant activity by phosphomolybdenum method and TPAC (r = 0.883), TPC (r = 0.858), and TFC (r = 0.843) at the flowering stage. These results can be used for further biochemical and pharmacological investigations of these plants.

Keywords: *Vigna*, polyphenols, phenolic acids, flavonoids, correlation

Introduction

Representatives of *Vigna* L. genus relate to Fabaceae Lindle. plant family and consisting of more than 200 species (Harouna et al., 2020). These plants are widely distributed in tropical and subtropical regions and represented more than 80 species (Popoola et al., 2015). *Vigna* spp. is one of the most economically important plants in the world due to the wide use of seeds that is a rich source of protein (Musah et al., 2020). According to Dakora and Belane (2019), the content of leaf protein was 23–40% and seed protein up to 40%. Also, leaves and seeds are a rich source of macro- and microelements. The productivity of seeds in the conditions of the Right-Bank Forest Steppe of

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Ukraine is 486–586 g per square meter (Bondarchuk et al., 2022).

Plant raw material of *Vigna* is a rich source of protein (Sha'a, 2021), vitamins, sugars, and lipids (Vergun et al., 2022). According to Zi-Ul-Haq et al. (2014), seeds of *V. mungo* contain crude protein (25.07–28.60%), total lipids (5.13–6.22%), carbohydrates (54.81–58.13%), crude fibre (4.25–6.84%), ash (4.97–6.72%), amino acids, among which prevailed glutamic acid, aspartic acid, leucine, arginine, etc. Leaves of *V. unguiculata* contain saponins (1.34%), tannins (2.60%), terpenoids (0.47%), flavonoids (4.11%), alkaloids (3.55%), moisture (1.38%), ash (3.72%), etc. (Sha'a, 2021). As reported Wang et al. (2021), five principal fatty acids were found in seed raw such as palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid.

In addition, seed extracts of these plants exhibit various biological activities among which are antioxidant, antimicrobial, antidiabetic, hypocholesterolemic, antiviral, antifungal, thrombotic, etc. (Ibrahim et al., 2017). According to Lenny and Rizky (2020), leaves of *V. unguiculata* demonstrated antioxidant activity and were effective against *S. aureus* and *E. coli*.

Seeds of *Vigna* spp. widely used in Asian countries in food due to its rich nutritional composition and antioxidant effect (Siddhuraju and Becker, 2007; Wang et al., 2021) but exist very less information about the biochemical composition and biological activities of the above-ground part of these plants.

Taking this into account, the main goal of this study was to evaluate the antioxidant activity of the extracts of herbs of *Vigna* spp. as a potential source of polyphenol compounds.

Material and methodology

Biological material

The above-ground part of four species of the *Vigna* L. genus was used in this study. Plant raw of *Vigna angularis* (Willd.) Ohwi & H. Ohashi, *V. mungo* (L.) Hepper, *V. radiata* (L.) Wilczek, *V. unguiculata* (L.) Walp. were collected from an experimental collection of the M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (Kyiv) at the start of the vegetation and flowering stage (Figure 1) in 2020–2021.

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

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

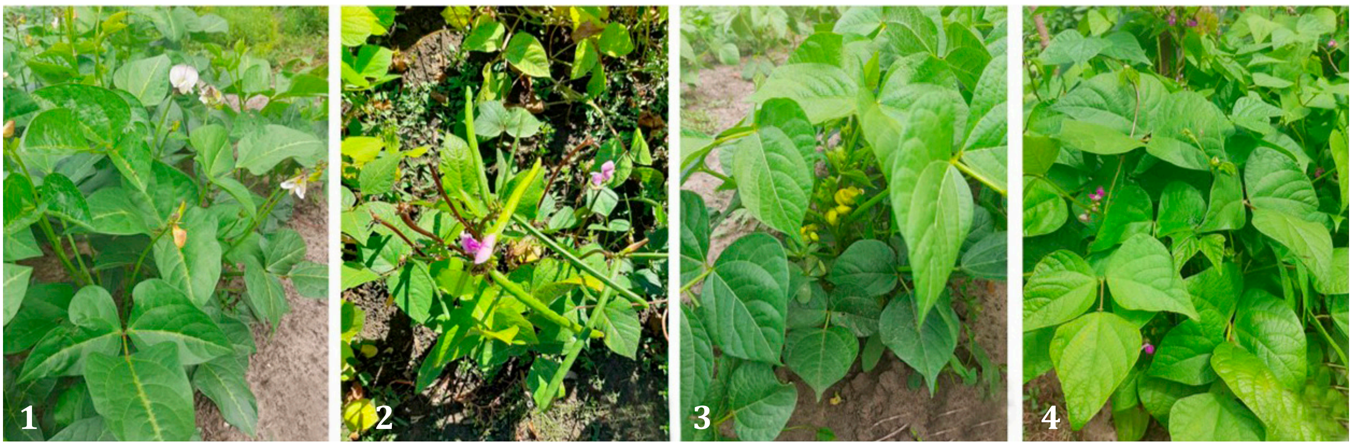

Figure 1 Plants of *Vigna* spp. at the flowering stage
1 – *Vigna angularis* (Willd.) Ohwi & H. Ohashi; 2 – *V. mungo* (L.) Hepper; 3 – *V. radiata* (L.) Wilczek; 4 – *V. unguiculata* (L.) Walp.

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 $mg.L^{-1}$; $R^2 = 0.998$) 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

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 $mg.L^{-1}$; $R^2 = 0.998$) 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 ± 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

Plants belonging to the Fabaceae family include a large group of plants with antioxidant and antimicrobial properties (Obistioiu et al., 2021). The plant raw materials of Fabaceae plants are a rich source of polyphenol compounds (Doblado et al., 2005). Seeds of *Vigna* spp. plants along with other raw characterized by antioxidant activity due to the content of polyphenol compounds (Zi-Ul-Haq et al., 2013; Dalaram, 2015; Mahmoudi et al., 2020), and antioxidant properties depend on the processing of seeds (Yadav et al., 2018). According to Tungmunnithum et al. (2021), the total content of polyphenols in the seeds of *V. angularis* was 25.47–69.77 mg $GAE.g^{-1}$, *V. mungo* 26.35–54.72 mg $GAE.g^{-1}$, *V. radiata* 10.76–16.04 mg $GAE.g^{-1}$, and *V. unguiculata* 33.76–71.73 mg $GAE.g^{-1}$.

Polyphenols are bioactive and multi-functional compounds with antioxidant, anti-inflammatory, antitumoral, etc., activities (Cutrum and Cortez Sloboda, 2018). This group of compounds is the most abundant among antioxidants and plays an important role in human nutrition (Scalbert et al., 2005) and health benefits (Ignat et al., 2011). There are plant metabolites that are widely distributed in plants and plant products (Petti and Scully, 2009).

Previous studies of different raw Fabaceae representatives showed the high antioxidant potential of methanol, ethanol, and water extracts (Vergun et al., 2020a).

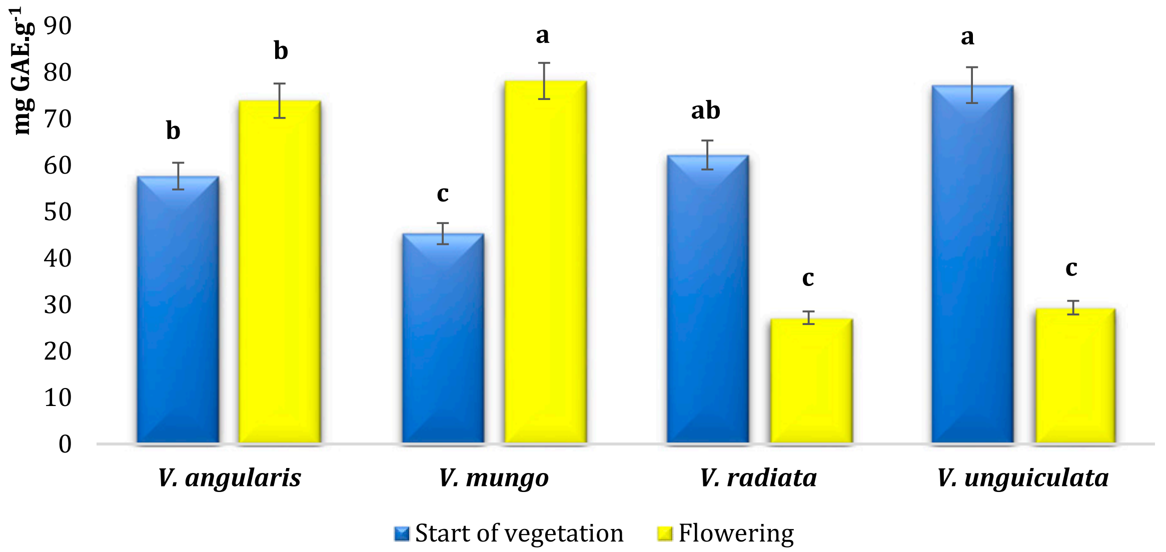

Figure 2 The content of polyphenol compounds in ethanol extracts of *Vigna* spp.
GAE – gallic acid equivalent; different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

The total content of polyphenol compounds at the start of vegetation of investigated plants was from 45.27 to 77.21 mg GAE.g^{-1} depending on the species (Figure 2). During the flowering period, polyphenol content in ethanol extracts of investigated species was from 27.16 to 78.11 mg GAE.g^{-1}.

According to Geđevac et al. (2008), the content of polyphenols in raw of nine Fabaceae species from natural flora was from 38 (*Coronilla emerus* L.) to 180.88 (*Lathyrus binatus* Pancic) mg GAE.g^{-1} depending on species. Total phenolic content of *Melilotus officinalis* (L.) Pall. was 21.37 mg GAE.g^{-1} in ethanol extracts (Mladenović et al., 2016). As reported Lee et al. (2018), polyphenol content in leaf extracts of *V. angularis* was in the range of 2.9–14.7 mg GAE.g^{-1}. The study of leaf extracts of other species *Desmodium canadensis* DC. showed that the total polyphenol content was 71.43 mg GAE.g^{-1} (Vergun et al., 2019).

Along with the polyphenol compounds study we used it to determine total phenolic acid content. Phenolic acids are a group of phenolic compounds that play an important role as antiaging agents and demonstrate antitumor, antimicrobial, and anti-inflammatory properties. These biologically active molecules are found in edible and nonedible plants (Jitan et al., 2018; Kumar and Goel, 2019). Phenolic acids released from emerging roots in Fabaceae plants during seed germination and in root nodules of *V. mungo* stimulate

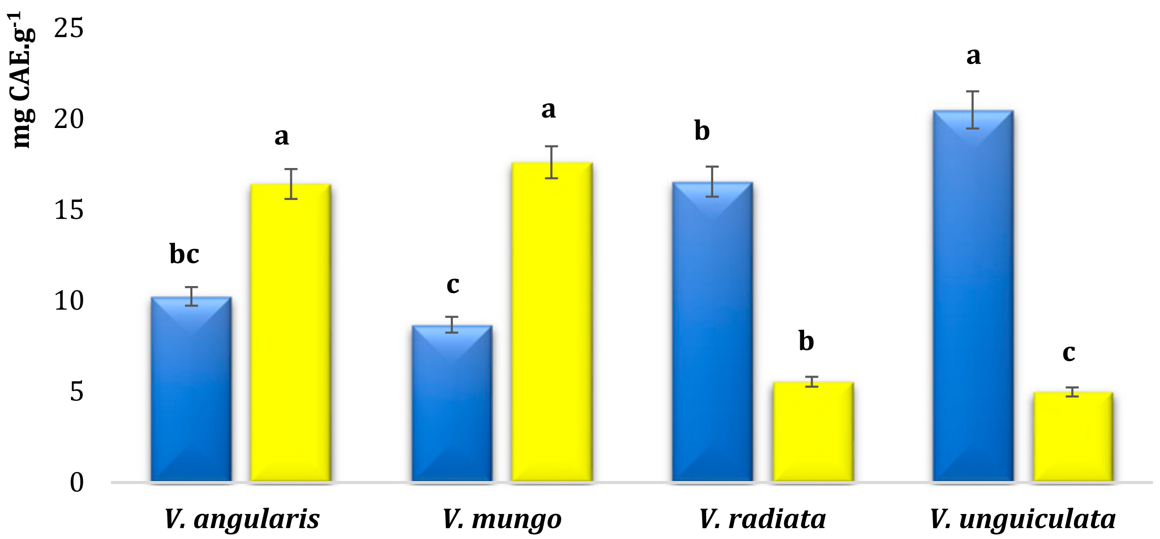

Figure 3 The content of phenolic acids in ethanol extracts of *Vigna* spp.
CAE – caffeic acid equivalent; different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

IAA production and nodules morphogenesis (Mandal et al., 2010).

The total content of phenolic acids in the ethanol extracts of investigated species was from 8.67 to 20.48 mg CAE.g^{-1} at the start of vegetation (Figure 3). This parameter was from 4.97 to 17.16 mg CAE.g^{-1} at the flowering stage depending on species. It should be noted that plants of *V. angularis* and *V. mungo* accumulated phenolic acids from the start of vegetation to the flowering stage and plants of *V. radiata* and *V. unguiculata* opposite.

The total phenolic acid content of ethanol extracts of *Galega* spp. was 14.13–16.73 mg CAE.g^{-1} at the start of vegetation and 11.62–16.22 mg CAE.g^{-1} at the flowering stage (leaves) (Vergun et al., 2020b). The study of leaf extracts of other species *Desmodium canadensis* showed that the total phenolic acid content was 8.70 mg CAE.g^{-1} (Vergun et al., 2019).

Also, we were used to detect the total flavonoid content in ethanol extracts of *Vigna* species. Flavonoids are a versatile class of natural compounds that demonstrated different biological activities such as antimicrobial and antifungal (Saleem et al., 2017). These polyphenol compounds are abundant in fruits, vegetables, and grains, have antioxidant, and anti-inflammatory activity and reduce the risk of diseases (Shen et al., 2022).

The total content of flavonoids in the ethanol extracts of investigated species of *Vigna* was from 31.84 to 47.97 mg QE.g^{-1} at the start of vegetation (Figure 4). The content of flavonoids was from 18.27 to 54.26 mg QE.g^{-1} during the flowering period depending on the species.

According to Berber et al. (2014), extracts of plants *Adenocarpus complicatus* (L.) Gay demonstrated 8.89 mg RE.g^{-1} (rutin equivalent) in fruits and 36.67 mg RE.g^{-1} of total flavonoid content in mixed raw. The ethanol extracts of other species from Fabaceae such as *Galega* spp. had a flavonoid content of 38.79–44.27 mg QE.g^{-1} at the start of vegetation and 40.09–44.91 mg QE.g^{-1} at the flowering stage (leaves) (Vergun et al., 2020b). The study of leaf extracts of other species *Desmodium canadensis* showed that the total flavonoid content was 61.05 mg QE.g^{-1} (Vergun et al., 2019). The content of flavonoids in seeds of *V. unguiculata* was from 30.5 to 46.3 mg RE.g^{-1} (rutin equivalent) (Nassourou et al., 2016).

The polyphenol compounds act as antioxidant agents and the antioxidant activity of plant raw is caused by the presence of these compounds. It exists numerous methods to determine it such as 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity (DPPH), ferric reducing assay (FRAP), Trolox equivalent antioxidant capacity, etc. (Moharram and Youssef, 2014; Chaves et al., 2020). The extracts of different plant parts such as leaves, stems, inflorescences, and fruits exhibited antioxidant potential depending on the species (Krishnaiah et al., 2011).

This study used two methods to evaluate the antioxidant activity such as DPPH and the phosphomolybdenum method which are, according to Alam et al. (2013), related to *in vitro* methods and also are the most widely used. A previous study about antioxidant activity by the DPPH method of Fabaceae species showed high values in the methanol and water extracts (Vergun et al., 2020a).

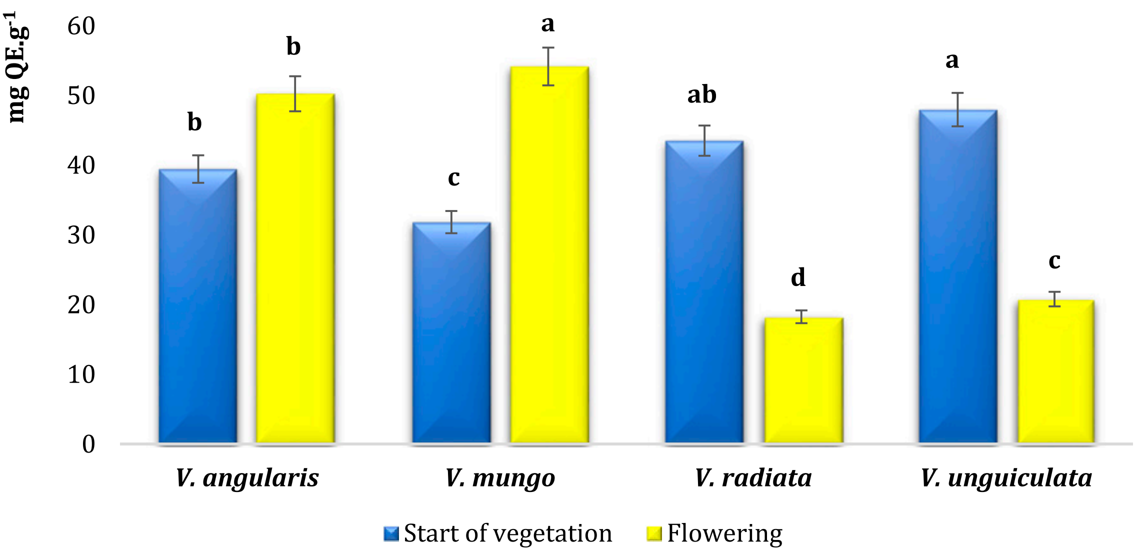

Figure 4 The content of flavonoids in ethanol extracts of *Vigna* spp.
QE – quercetin equivalent; different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

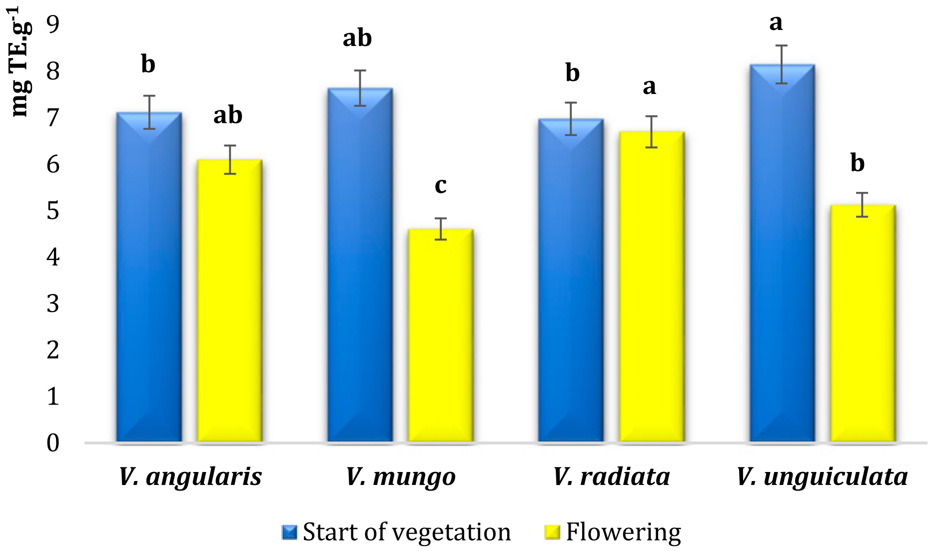

Figure 5 Antioxidant activity of ethanol extracts of *Vigna* spp. by DPPH method
TE – Trolox equivalent; different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

The antioxidant activity of investigated plant extracts by 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 phosphopmolybdenum method of antioxidant activity determination is based on a redox antioxidant reaction where phosphate-Mo (VI) is reduced to phosphate-Mo (V) (Phatak and Hendre, 2014). As described Diwan et al. (2012), the DPPH scavenging assay usually detected the polyphenols and flavonoids, and the phosphomolybdenum assay is used for some phenolics, usually its ascorbic acid, carotenoids, etc. The antioxidant activity of investigated plant extracts by the phosphomolybdenum method was from 110.52 to 142.61 mg $TE.g^{-1}$ at the start of vegetation and from 45.16 to 110.27 mg $TE.g^{-1}$ at the flowering stage (Figure 6).

As reported Berber et al. (2014), *Adenocarpus complicatus* extracts had antioxidant activity by phosphomolybdenum method 207.53 mg $TE.g^{-1}$ in fruits and 251.53 mg $TE.g^{-1}$ in the mixed raw.

The study of leaf extracts of other species *Desmodium canadensis* showed that antioxidant activity by the phosphomolybdenum method was 190.64 mg $TE.g^{-1}$ (Vergun et al., 2019).

The correlation analyses between antioxidant parameters were conducted (Table 1). A very strong

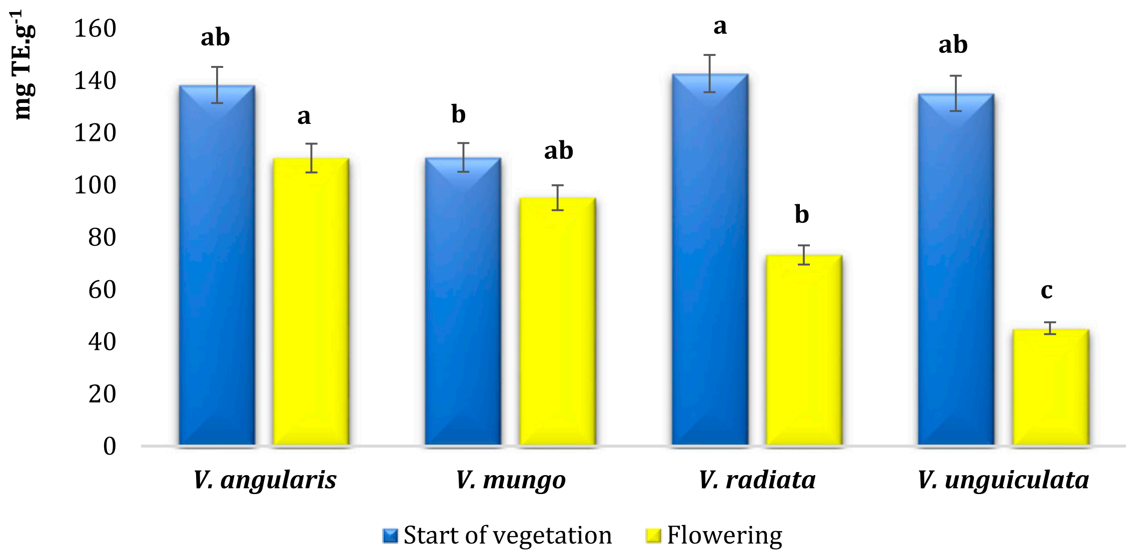

Figure 6 Antioxidant activity of ethanol extracts of *Vigna* spp. by phosphomolybdenum method
TE – Trolox equivalent; different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

Table 1 Correlation analysis between antioxidant parameters of *Vigna* spp.

Parameter	TPC	TPAC	TFC	DPPH	PHOMO
Start of vegetation					
TPAC	0.937**	1	0.938**	0.405*	0.577*
TFC	0.976**	0.938**	1	0.227*	0.793**
DPPH	0.423*	0.405*	0.227*	1	-0.389
PHOMO	0.663**	0.577*	0.793**	-0.389	1
Flowering					
TPAC	0.997**	1	0.996**	-0.36*	0.883**
TFC	0.999**	0.996**	1	-0.433	0.843**
DPPH	-0.405	-0.365	-0.433	1	0.111*
PHOMO	0.858**	0.883**	0.843**	0.111*	1

Note: TPC – total phenolic content; TPAC – total phenolic acid content; TFC – total flavonoid content; DPPH – antioxidant activity by DPPH method; PHOMO – antioxidant activity by phosphomolybdenum method. ** Correlation is significant at p ≤0.01; * correlation is significant at p ≤0.05

correlation at the start of vegetation found between TPC and TFC (r = 0.976), TPAC and TFC (r = 0.938) and TPC and TPAC (r = 0.937). A strong correlation at the start of vegetation was detected between TFC and PHOMO (r = 0.793) and TPC and PHOMO (r = 0.663). Between the content of all phenolic compounds and antioxidant activity by the DPPH method at the start of vegetation determined a moderate or weak correlation (r = 0.227–0.423). The negative correlation between antioxidant activity by DPPH and phosphomolybdenum method (r = -0.389).

A very strong correlation was found between the following investigated parameters at the flowering stage: TPC and TFC (r = 0.999), TPC and TPAC (r = 0.997), TPAC and TFC (r = 0.996). A very strong correlation was found between PHOMO and all phenolic compound groups investigated in this study (r = 0.843–0.883). Negative relations were found between antioxidant activity by the DPPH method and investigated parameters.

Due to existing fewer data about correlation analysis between antioxidant parameters of *Vigna* spp. above-ground part, it is difficult to compare obtained results. A negative correlation between antioxidant activity by two methods such as DPPH and phosphomolybdenum was found in another study (Kasangana et al., 2015). According to Lee et al. (2018), the leaves extracts study of *V. angularis* demonstrated a negative correlation between DPPH scavenging activity and total phenolic content (r = -0.722) whereas in our study this correlation was moderate.

Conclusions

Taking the obtained data into account it should be noted that investigated species of the *Vigna* genus are a good source of antioxidants. The study of ethanol extracts of above-ground parts of four species showed some patterns in the accumulation of selected polyphenol compounds. So, the accumulation of total polyphenol compounds, phenolic acids, and flavonoids in *V. angularis* and *V. mungo* extracts was higher at the flowering stage than at the start. The opposite was indicated for extracts of *V. radiata* and *V. unguiculata*, where all investigated polyphenol compounds were higher at the start of vegetation. The antioxidant activity by the phosphomolybdenum method was less at the flowering stage for all investigated species. In this study, a very strong correlation was found between polyphenol compounds and antioxidant activity by the phosphomolybdenum method at the flowering stage, whereas relations between polyphenols and the DPPH method of antioxidant activity determination were weaker. These results can be used for further biochemical and pharmacological investigations.

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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