



DPPH FREE RADICAL SCAVENGING ACTIVITY OF SOME FABACEAE LINDL. SPECIES

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Searching for new plant raw material with antioxidant activity is still actual. This study was aimed to evaluate the antioxidant potential of selected plants from Fabaceae: *Coronilla varia* L., *Desmodium canadense* (L.) DC., *Glycyrrhiza glabra* L., *Lathyrus grandiflorus* Sibth. & Smith, *Lespedeza bicolor* Turcz., *Onobrychis arenaria* (Kit.) D.C., *O. grandis* Lip., *Trifolium ambiguum* Bieb., *T. pannonicum* Jacq., *T. rubens* L. cv. Skif-1 at the flowering stage. Raw material was collected from the experimental collections of M.M. Gryshko National Botanical Garden of the NAS of Ukraine (NBG) in the stage of flowering. Determination of the antioxidant activity of investigated plants conducted by DPPH free radical scavenging activity according to Brand-Williams et al. (1995). The total antioxidant activity of plant whole above-ground part of methanol extracts was from 62.78 (*Coronilla varia*) to 89.99 (*Glycyrrhiza glabra*) % and water extracts from 51.28 (*Onobrychis grandis*) to 90.35 (*Lespedeza bicolor*) %. Leaf extracts exhibited DPPH scavenging activity in methanol extracts from 63.17 (*Trifolium ambiguum*) to 93.08 (*Onobrychis arenaria*) % and water extracts from 57.62 (*Coronilla varia*) to 95.36 (*Lathyrus grandiflorus*) %. Stem extracts of investigated plants showed inhibition in methanol extracts from 35.04 (*Coronilla varia*) to 90.51 (*Glycyrrhiza glabra*) % and water extracts from 20.35 (*Coronilla varia*) to 77.52 (*Lathyrus grandiflorus*) %. DPPH scavenging activity of these plants depended on species and part of plants. Thus, plants from Fabaceae family are a potential source of antioxidant activity that can be used for deep pharmacological investigations for food science.

Keywords: Fabaceae, 2,2-diphenyl-1-picrylhydrazyl, free radical scavenging activity

Introduction

The study of the antioxidant capacity of plants and finding new sources is an important direction in biological science. Natural antioxidants are widely distributed in the plant world and are represented by different classes of compounds with numerous biological activities (Xu et al., 2017). Screening of new natural sources provides an opportunity to expand the range of new products with antioxidant activity. In this case, the Fabaceae Lindl. family has numerous representatives with a spectrum of biological activities that can be used.

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Many species from Fabaceae plants (Legumes) are known as high-quality feed for livestock such as *Medicago sativa* L. and *Trifolium pratense* L. also, a rich source of phytoestrogens, nonsteroidal compounds with beneficial effect (Tucak et al., 2018). Legumes are a good source of nutrients such as proteins, carbohydrates, dietary fibers, micronutrients, vitamins, fatty acids (Gutiérrez-Urbe et al., 2016). Some representatives from Fabaceae well-known forage plants with a high content of polyphenol compounds and antioxidant activity (Shymanska et al., 2018; Vergun et al., 2020). The screening of some legumes plants determined the antimicrobial activity against gram-positive bacteria, *Candida albicans*, *Aspergillus niger* and *Pseudomonas aeruginosa* (Rosado-Vallado et al., 2000). Most of the Fabaceae plants are used as medicinal plants against numerous diseases such as asthma, gonorrhoea, ulcer, diabetes, kidney disease, rheumatism, stomach trouble, colic, etc. (Mahbubur and Parvin, 2014). There are numerous pharmacological properties of these plants studied among that contraceptive (Sethi et al., 2017), antiallergic, antimalarial, anticancer, analgesic, aphrodisiac, anti-rheumatic, anthelmintic, diuretic, antiseptic, insecticidal, toxic, etc. (Jahan et al., 2019).

Important chemical components from the plants of this family are flavonoids, coumarins, alkaloids, tannins (Ferreira Macêdo et al., 2018). Some plants, such as *Lathyrus* species, can be considered as ornamental plants (Güneş, 2019). The antioxidant activity of some Fabaceae species such as *Lathyrus binatus*, *Trifolium pannonicum* (Gođevac et al., 2008), *Glycyrrhiza glabra* (Hussein and Iqbal, 2019) was studied.

This study aimed to evaluate the antioxidant potential of selected Fabaceae plants and to compare this parameter in different parts of plants. The results of this assessment are essential for further pharmacological study.

Materials and methodology

Biological material

It was investigated some representatives from the Fabaceae Lindl.: *Coronilla varia* L., *Desmodium canadense* (L.) D.C., *Glycyrrhiza glabra* L., *Lathyrus grandiflorus* Sibth. & Smith, *Lespedeza bicolor* Turcz., *Onobrychis arenaria* (Kit.) D.C., *O. grandis* Lip., *Trifolium ambiguum* Bieb., *T. pannonicum* Jacq., *T. rubens* L. cv. Skif-1. An experiment carried out during 2018–2019 at the laboratory of Cultural Flora Department of M.M. Gryshko National Botanical Garden of the N.A.S. of Ukraine. Plants samples took at the flowering stages and dried at 45 °C for 72 hours. All investigated plants are perennial.

DPPH scavenging activity determination

1 g of dried and milled plant raw extracted in the 25 ml of solvent (methanol and water) for 24 hours. After the filtration procedure obtained extracts used to determined antiradical activity on a spectrophotometer Unico UV 2800 (Russia). A working solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) prepared the following way: 25 mg of radical dissolved in 100 ml of methanol. Obtained radical's solution dissolved in 10 times till optical density was in the range of 0.700–0.800. The procedure of measuring was conducted by Brand-Williams et al. (1995). 3.9 ml of radical solution mixed with 100 µl of plant extract and put for 10 min in the

dark. During the procedure of measuring on a spectrophotometer at a wavelength of 515 nm used value of radical solution and value of the radical solution with the sample. Obtained results expressed in percentages.

Statistical analysis

The mean values of three replicates and the standard deviation are given. Data submitted with ANOVA and differences between means compared using the Tukey-Kramer test ($\alpha = 0.05$).

Results and discussion

There are numerous methods to determine the antioxidant activity, among which the DPPH scavenging activity has been found as simple, inexpensive, and allows to evaluate of the antioxidant potential at the screening process. However, it is necessary to consider that one method can't be universal for all plants (Kedare and Singh, 2011). This method is based on the reaction discoloration of radical (2,2-diphenyl-1-picrylhydrazyl) solvent by plant extract (Alam et al., 2013). In this study, different plant parts of selected plants were used to extract two solvents.

Coronilla varia is a perennial legume used for cardio-tonic, diuretic, prostate diseases. Coumarins and glycosides were isolated from this species and raw demonstrated antimicrobial and insecticidal effects (Al-Snafi, 2016).

The free radical scavenging activity of *Coronilla varia* methanol extracts was from 35.04 (stems) to 87.05 (leaves) % (Figure 1). In the water extracts, this parameter was from 20.35 (stems) to 65.54 (inflorescences). Renda et al. (2019) determined in methanol extracts (25–100 $\mu\text{g/ml}$) of *C. varia* the DPPH scavenging activity from 9.89 to 39.40% and in water extracts from 8.64 to 27.31% depending on initial concentration. Also, in this study detected for methanol extracts of *C. orientalis* the DPPH antioxidant activity from 12.59 to 41.91% and for water extracts from 8.14 to 20.45%.

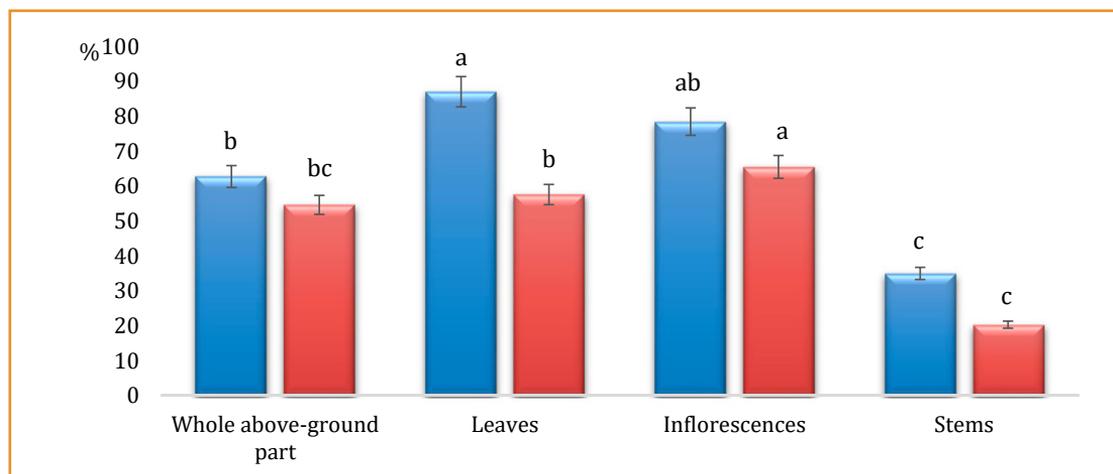


Figure 1 DPPH scavenging activity of *Coronilla varia* L. at the stage of the flowering (means in columns followed by different letters are different at $p < 0.05$. Each value represents the mean of three independent experiments (\pm SD))

The methanol extracts of *Desmodium canadense* demonstrated inhibition of radical solution from 60.01 (stems) to 85.66 (whole above-ground part) % (Figure 2). Tsai et al. (2011) investigated ten species of *Desmodium* genus and found a high content of phenolic compounds in some species (*D. sequax*) that correlated with antioxidant activity. Venkatachalam and Muthukrishnan (2012) determined the DPPH scavenging activity of different concentration extracts of *D. gangeticum* from 25.12 to 54.14%. Ayoola et al. (2018) identified in leaf extracts radical scavenging activity of 3.17–91.3%, and in stem extracts of 2.87–61.65%. *D. canadense* leaf extracts are a source of polyphenol compounds (flavonoids and phenolic acids) with high antioxidant activity by Trolox equivalent methods (Vergun et al., 2019).

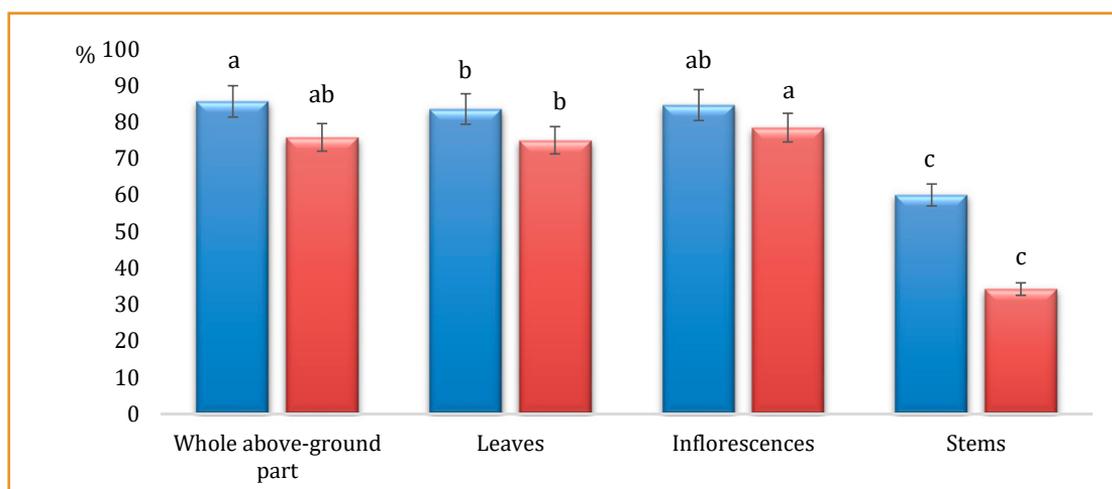


Figure 2 DPPH scavenging activity of *Desmodium canadense* (L.) D.C. at the stage of the flowering (means in columns followed by different letters are different at $p < 0.05$. Each value represents the mean of three independent experiments (\pm SD))

The study of *Glycyrrhiza glabra* showed the presence of alkaloids, glycosides, carbohydrates, phenolic compounds, proteins, etc. Plant raw exhibited antidepressant, antimicrobial, anticancer, antioxidant, anti-inflammatory, antidiabetic, hypolipidemic, etc. (Al-Snafi, 2018). Traditionally, this plant is recommended as prophylaxis of gastric and duodenal ulcers, dyspepsia (Harwansh et al., 2011), effective against vitiligo (Herath et al., 2018). Pharmacological activity appeared against various microorganisms, parasites, viruses (Batiha et al., 2020). Biological activities of this species have studied basically with roots as useful raw (Chopra et al., 2013). The essential oil of this plant also characterized by numerous biological activities such as antioxidant, anticancer, antifungal, etc. (Ali, 2013).

The free radical scavenging activity of methanol extracts of *Glycyrrhiza glabra* was from 89.99 (whole above-ground part) to 91.58 (leaves) % (Figure 3). In the water extracts, this activity was from 75.79 (stems) to 91.61 (inflorescences) %. Al-Snafi (2018) determined in methanol extracts of roots the inhibition of DPPH radical 67.22%.

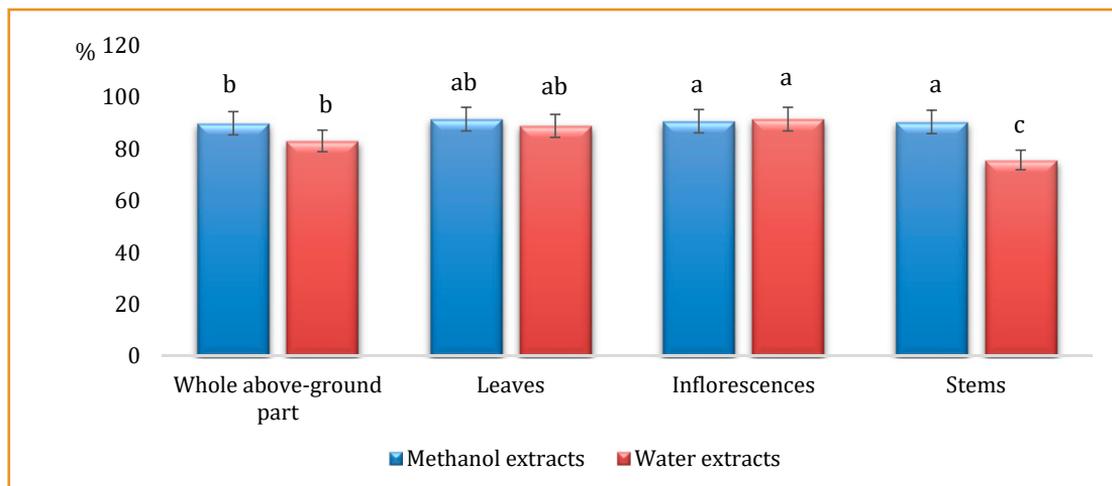


Figure 3 DPPH scavenging activity of *Glycyrrhiza glabra* L. at the stage of the flowering (means in columns followed by different letters are different at $p < 0.05$. Each value represents the mean of three independent experiments (\pm SD))

Lathyrus species are known as a source of protein, but few of them are cultivated as food plants. Raw of them also contains flavonoids, fatty acids, proanthocyanidins, triterpene saponins, etc. (Heydari et al., 2015).

The free radical scavenging activity of methanol extracts of *Lathyrus grandiflorus* was from 40.07 (stems) to 84.81 (leaves) % (Figure 4). In the water extracts, this activity was from 77.52 (stems) to 95.36 (leaves) %. Heydari et al. (2015) investigated five species of *Lathyrus* and in some cases, DPPH scavenging activity was up to 90%.

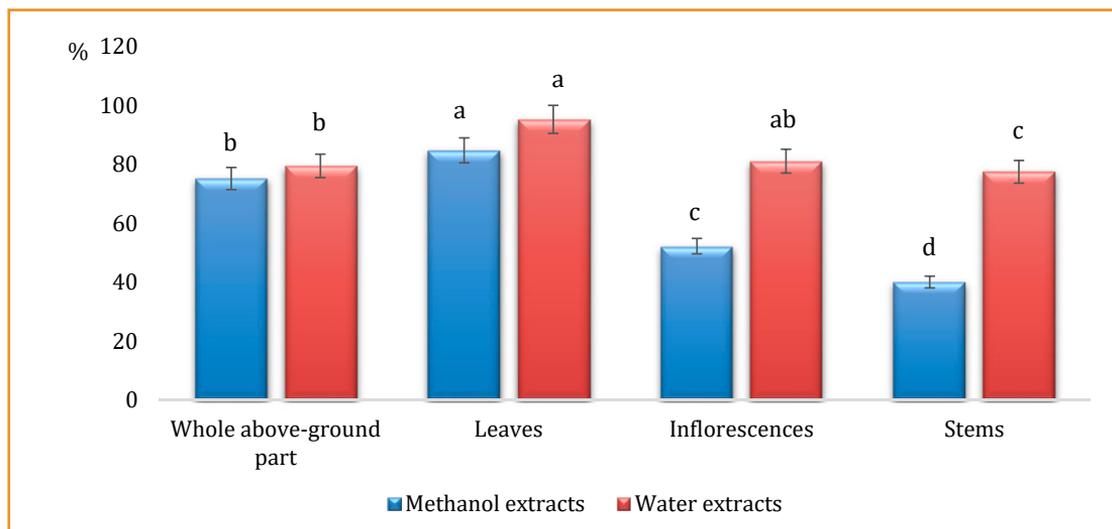


Figure 4 DPPH scavenging activity of *Lathyrus grandiflorus* Sibth. & Smith at the stage of flowering (means in columns followed by different letters are different at $p < 0.05$. Each value represents the mean of three independent experiments (\pm SD))

The free radical scavenging activity of methanol extracts of *Lespedeza bicolor* was from 53.54 (stems) to 92.07 (inflorescences) % and water extracts from 70.06 (stems) to 92.55 (leaves) % (Figure 5). Lee et al. (2016) investigated extracts of this plant and established the anti-inflammatory, depigmentation, and antioxidant activity effect. Besides the antioxidant effect, *L. bicolor* extracts demonstrated antifungal and antimicrobial activities (Sami, 2017).

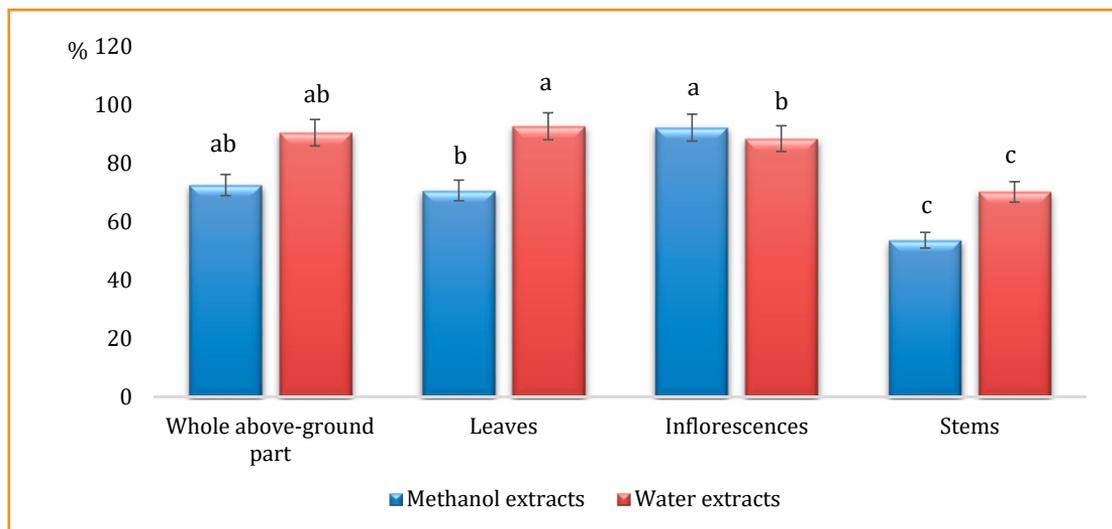


Figure 5 DPPH scavenging activity of *Lespedeza bicolor* Turcz. at the stage of flowering (means in columns followed by different letters are different at $p < 0.05$. Each value represents the mean of three independent experiments (\pm SD))

The free radical scavenging activity of methanol extracts of *Onobrychis arenaria* was from 63.08 (stems) to 93.08 (leaves) % (Figure 6). In the water extracts, this activity was from 59.85 (stems) to 89.85 (leaves) %. In methanol extract of *O. grandis* determined DPPH scavenging activity from 37.39 (stems) to 86.31 (inflorescences) %, in water extracts from 31.31 (stems) to 86.01 (leaves) %. Karamian and Asadbegy (2016) detected in methanol extracts of *O. viciifolia*, *O. melanotria* and *O. sosnovskyi* inhibition of DPPH as 92.70, 92.27 and 91.38%, respectively.

Al-Snafi (2018) determined in methanol extracts of roots the inhibition of DPPH radical 67.22%.

The methanol extracts of *Trifolium ambiguum* exhibited the DPPH scavenging activity from 28.02 (stems) to 94.07 (inflorescences) % and water extracts from 41.84 (stems) to 93.33 (inflorescences) % (Figure 7). *T. pannonicum* showed scavenging activity in methanol extracts from 75.48 (stems) to 88.24 (inflorescences) % and in water extracts from 38.76 (stems) to 88.24 (whole above-ground part). In methanol extracts of *T. rubens* determined DPPH scavenging activity from 24.85 (stems) to 84.45 (inflorescences) % and in water extracts from 47.56 (stems) to 74.0 (whole above-ground part) %.

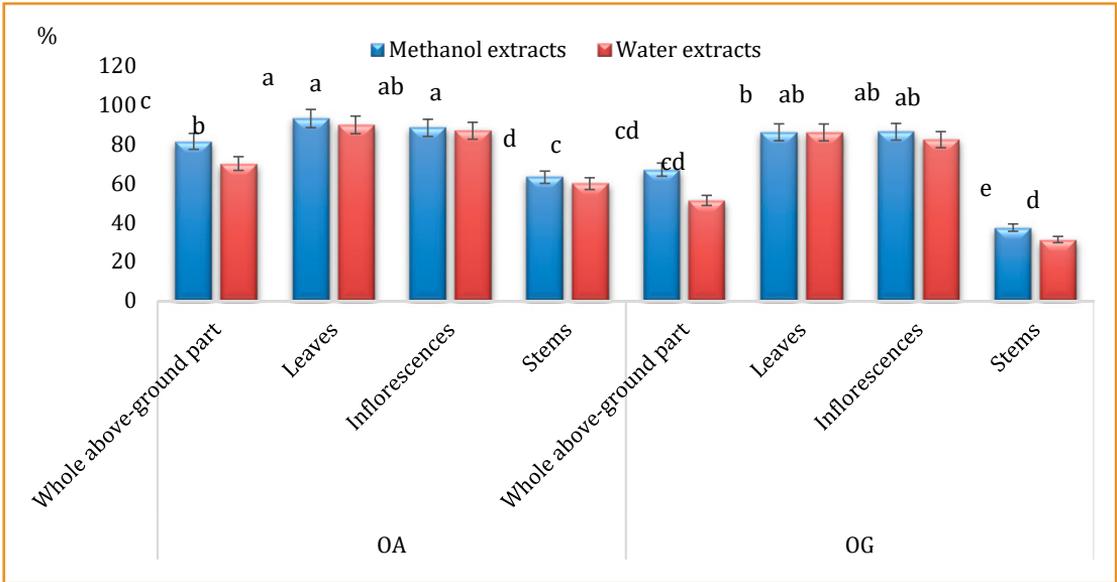


Figure 6 DPPH scavenging activity of *Onobrychis arenaria* (Kit.) D.C. (OA) and *O. grandis* Lip. (OG) at the stage of flowering (means in columns followed by different letters are different at $p < 0.05$. Each value represents the mean of three independent experiments (\pm SD))

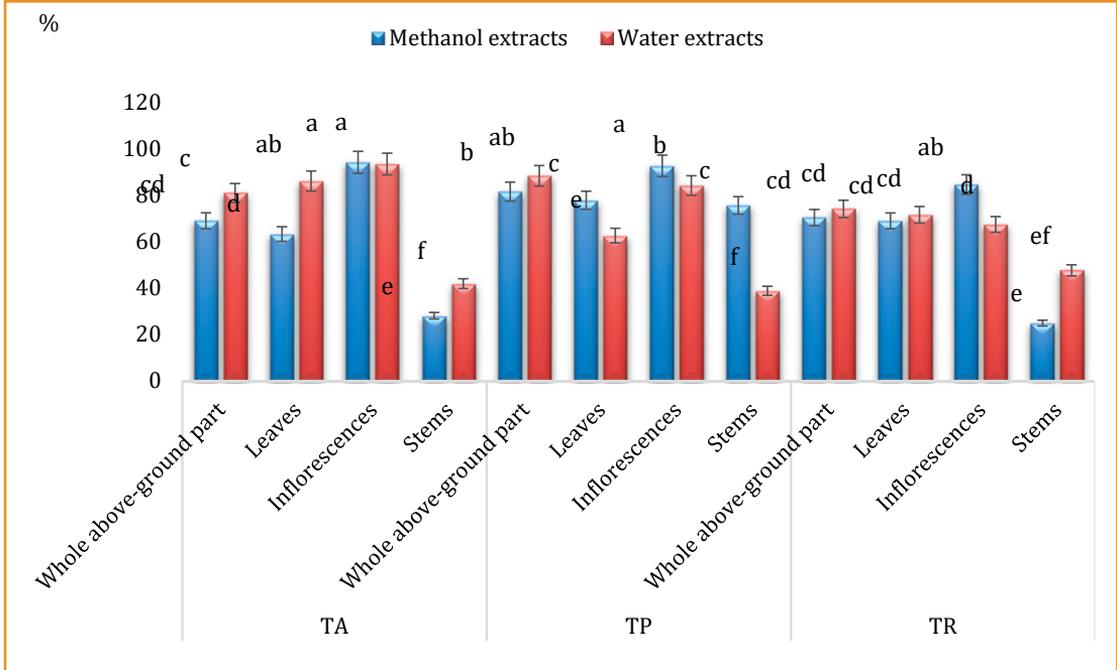


Figure 7 DPPH scavenging activity of *Trifolium ambiguum* Bieb. (TA), *T. pannonicum* Jacq. (TP) and *T. rubens* L. cv. Skif-1 (TR) at the stage of flowering (means in columns followed by different letters are different at $p < 0.05$. Each value represents the mean of three independent experiments (\pm SD))

The study of other Fabaceae species *Indigofera tinctoria* L. showed the antioxidant activity by DPPH of water extracts was 52.08% (Srinivasan et al., 2016).

Conclusions

Different extracts of crops from the Fabaceae family collected in the M.M. Gryshko National Botanical Garden exhibited an antioxidant potential. It's should be concluded that the scavenging activity of these plants depended on species and part of plants. The total antioxidant activity of plant whole above-ground part was lowest in methanol extracts of *Coronilla varia* and highest in *Glycyrrhiza glabra* and the minimal result showed water extract of *Onobrychis grandis* and maximal *Lespedeza bicolor*. Leaf extracts exhibited a minimal value of DPPH scavenging activity in methanol extracts of *Trifolium ambiguum* and maximum in *Onobrychis arenaria* and water extracts *Coronilla varia* and *Lathyrus grandiflorus*, respectively. Stem extracts of investigated plants showed the lowest inhibition in methanol extracts for *Coronilla varia* and highest for *Glycyrrhiza glabra* and in water extracts *Coronilla varia* and *Lathyrus grandiflorus*, respectively. Plants from the Fabaceae family are a potential source of antioxidant activity that can be used for deep pharmacological investigations for food science.

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