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ANTIOXIDANT POTENTIAL OF LAMIACEAE HERBS FROM BOTANICAL GARDEN OF SLOVAK UNIVERSITY OF AGRICULTURE IN NITRA

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Polyphenol compounds are the secondary metabolites of plant origin that are widely distributed. They exhibit a wide spectrum of biological activities, among which is antioxidant activity. Investigation of polyphenol content and their antioxidant activity of aromatic herbs was the main goal of this study. Plant material of this study was *Hyssopus officinalis* L., *Lavandula angustifolia* Mill., *Nepeta kokanica* (Regel) Kuntze, *Rosmarinus officinalis* L., *Salvia officinalis* L., *Thymus serpyllum* L., and *Th. vulgaris* L. (Lamiaceae Lindl.) harvested from the collection of the Botanical Garden of Slovak University in Nitra at the period of flowering. It was determined the total content of polyphenols (gallic acid equivalent (GAE)), flavonoids (quercetin equivalent (QE)), phenolic acids (caffeic acid equivalent (CAE)), antioxidant activity (Trolox equivalent (TE)) and reducing power (TE) of extracts. All measurements carried out on the spectrophotometer Jenway (6405 UV/Vis, England). In our study total content of polyphenols among investigated plant extracts was from 39.43 to 62.87 mg GAE/g of DW (dry weight); the content of flavonoids was from 20.80 to 36.80 mg QE/g of DW; content of phenolic acids was from 4.91 to 24.30 mg CAE/g of DW; antioxidant activity by DPPH-method was from 7.78 to 8.50 mg TE/g of DW; antioxidant activity by phosphomolybdenum method was from 122.92 to 225.36 mg TE/g of DW. Thus, we found that among investigated plants *S. officinalis* extracts had the highest amount of polyphenol compounds, phenolic acids, and flavonoids. Antioxidant activity and reducing power was maximal for plants of *H. officinalis*. All investigated plants are a potential source of natural antioxidants and have potential as therapeutic agents.

Keywords: Lamiaceae, polyphenols, flavonoids, phenolic acids, antioxidant activity.

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Introduction

Lamiaceae Lindl. is a well-known family, which includes approximately 250 genera that widely distributed in the world (Cigremis et al., 2010) and has been the subject of numerous studies for identifying the antioxidant potential (Tzima et al., 2018). Herbs from Lamiaceae such as rosemary, oregano, hyssop, lavender, thyme are known as raw with high antioxidant activity (Soleimani et al., 2011; Dauqan et al., 2017; Hawrył et al., 2019). Herbs from Lamiaceae have a long history of use as medicinal plants. It is a group of aromatics, medicinal, spicy plants also (Carović-Stanko et al., 2016; Kovtun-Vodyanytska, 2017).

Plants containing biologically active compounds such as vitamins, flavonoids, polyphenols possess high antioxidant activity (Gupta and Sharma, 2006). Plants from Lamiaceae such as *Rosmarinus officinalis* (Nieto et al., 2018), *Salvia officinalis* (Abdelkader et al., 2014; Gantner et al., 2018), *Hyssopus officinalis* (Vlase et al., 2014), *Lavandula angustifolia* (Turgut et al., 2017; Alexa et al., 2018) possess the high antimicrobial activity (Assis et al., 2018). Also, it is found that *Hyssopus officinalis* have been used as a carminative, tonic, antiseptic, expectorant, cough reliever, etc. (Khazaie et al., 2008). This species known as a culinary herb and medicinal plant may use as natural food ingredients comparing with synthetic antioxidants (López-Fernandez et al., 2003; Armatu et al., 2010). The essential oil from *Hyssopus officinalis* also a rich source of biologically active compounds (Fathiazad et al., 2011).

Investigations with *Rosmarinus officinalis* showed the antiproliferative effect of plant extracts on human prostate cancer cell lines (Bourhia et al., 2019), antifungal (Hendel et al., 2016), antimutagenic potential, reducing the DNA damage and lipid peroxidation resulting from treatment with arsenic exposure (Farias et al., 2018). The study of *Salvia officinalis* showed that raw of it demonstrated numerous activities: the anticancer, anti-inflammatory, antinociceptive, antioxidant, antimicrobial, hypoglycemic, hypolipidemic, etc. (Ghorbani and Esmaeilizadeh, 2017). Aerial parts of *Thymus serpyllum* has used as anthelmintic, antiseptic, antispasmodic, carminative, deodorant, diaphoretic, disinfectant, expectorant, sedative and tonic raw (Jarić et al., 2015).

Ahmad et al. (2015) described ethnobotanical use of some Lamiaceae species: flowers, leaves and essential oil of *Hyssopus officinalis* exhibited expectorant and antiviral activities; *Nepeta kokanica* leaves and flowers characterized by anthelmintic, emetic, digestive activity. Also, some studies demonstrated the allelopathic activity that is characterized by a low level of toxicity (Yunosheva and Ellanska, 2018).

Taking into account the useful properties of these plants the aim of the research is the determination of the total antioxidant activity and the content of phenolic compounds of the leaves of selected Lamiaceae herbs in the period of flowering.

Materials and methodology

Biological material

Plant material of this study was above-ground parts plants of *Hyssopus officinalis* L., *Lavandula angustifolia* Mill., *Nepeta kokanica* (Regel) Kuntze, *Rosmarinus officinalis* L., *Salvia officinalis* L.,

Thymus serpyllum L., and *Th. vulgaris* L. harvested from the collection of the Botanical Garden of Slovak University in Nitra at the period of flowering. Plant raw dried in the oven (Binder 115, Germany) at 38 °C temperature, 48 hours. Biochemical analyses conducted at the Slovak Agricultural University in Nitra.

Chemicals

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

Preparation of sample extracts

The dry above-ground parts of plants were used for the detection of total phenolic content and total flavonoid content. An amount of 0.2 g of each sample was extracted with 20 mL of 80% ethanol for 24 h. Then, the sample in 80% ethanol was centrifuged at 4605 RCF (Rotofix 32 A, Hettich, Germany) for 10 min and the supernatant was used for measurement with the DPPH and molybdenum reducing antioxidant power methods.

Determination of antioxidant activity

Total polyphenol content

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; R^2 = 0.998) was used as the standard. The results were expressed in mg/g DM gallic acid equivalent (GAE).

Total flavonoid content

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 0.1mL of 10% (w/v) ethanolic solution of aluminum 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; R^2 = 0.9977) was used as the standard. The results were expressed in mg/g DM quercetin equivalent (QE).

Total phenolic acid content

Total phenolic acid (TPA) content was determined using the method of Farmakopea Polska (1999). A 0.5 mL of sample extract was mixed with 0.5 mL of 0.5 M hydrochloric acid, 0.5 mL Arnova reagent (10% $NaNO_2$ + 10% Na_2MoO_4), 0.5 mL of 1 M sodium hydroxide (w/v) and 0.5 mL of water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid (1–200 mg/L, R^2 = 0.999) was used as a standard and the results were expressed in mg/g DM caffeic acid equivalents (CAE).

Free radical scavenging activity

Free radical scavenging activity of samples was measured using the 2.2-diphenyl-1-picrylhydrazyl (DPPH) (Sanches-Moreno et al., 1998). An amount of 0.4mL of the sample was mixed with 3.6mL of DPPH solution (0.025 g DPPH in 100 mL methanol). 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; R^2 = 0.989) was used as the standard and the results were expressed in mg/g DM Trolox equivalents (TE).

Molybdenum reducing antioxidant power

Molybdenum reducing (MRP) antioxidant power 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 rapidly cooled. The absorbance at 700 nm was detected with the spectrophotometer Jenway (6405 UV/Vis, England). Trolox (10–1,000 mg/L; R^2 = 0.998) was used as the standard and the results were expressed in mg/g DM Trolox equivalent (TE).

Statistical analysis

Data were analyzed with ANOVA test and differences between means compared through the Tukey-Kramer test (α = 0.05).

Results and discussion

Polyphenols are the group of biologically active compounds, the main classes of which are phenolic acids and flavonoids. Epidemiological studies showed that diet dense in polyphenols has a higher chance of cardiac safety. Polyphenols exhibit anticancer and anti-diabetic activity (Abbas, 2017). Plant phenolic compounds play a protective role against degenerative diseases due to their antioxidative action (Beer et al., 2002).

The number of different groups of polyphenol compounds varied with the investigated plant species (Figure 1). Total phenolics were ranged from 39.43 (*Lavandula angustifolia*) to 62.87 (*Salvia officinalis*) mg CAE/g DW.

According to Fathiazad et al. (2011), the content of polyphenol compounds of n-butanol and ethyl acetate extracts of *Hyssopus officinalis* was 246 mg GAE/g and 51 mg GAE/g, respectively. In our study ethanol extracts had value 45.47 mg GAE/g DW.

As reported by Al'udatt et al. (2015), the total phenolic content of different extracts of *Thymus vulgaris* was from 0.92 to 2.49 mg GAE/g. In other studies, polyphenol content for *Th. vulgaris* was 0.58 mg GAE per 100 g DW (Wojdyło et al., 2007), 158 mg GAE/mg DW (Köksal et al., 2017), 32 mg GAE/mg DW (Armatu et al., 2010). In our study, this parameter had value 52.1 mg GAE/mg DW.

The total phenolic content of herb extracts of *Salvia officinalis* in some reports was 8.25 mg GAE per 100 g DW (Wojdyło et al., 2007), 31.5 mg GAE/g (Abdelkader et al., 2014), 32 mg GAE/g (Armatu et al., 2010). In our study extracts of this species had the most content of polyphenols and was determined as 62.87 mg GAE/g DW.

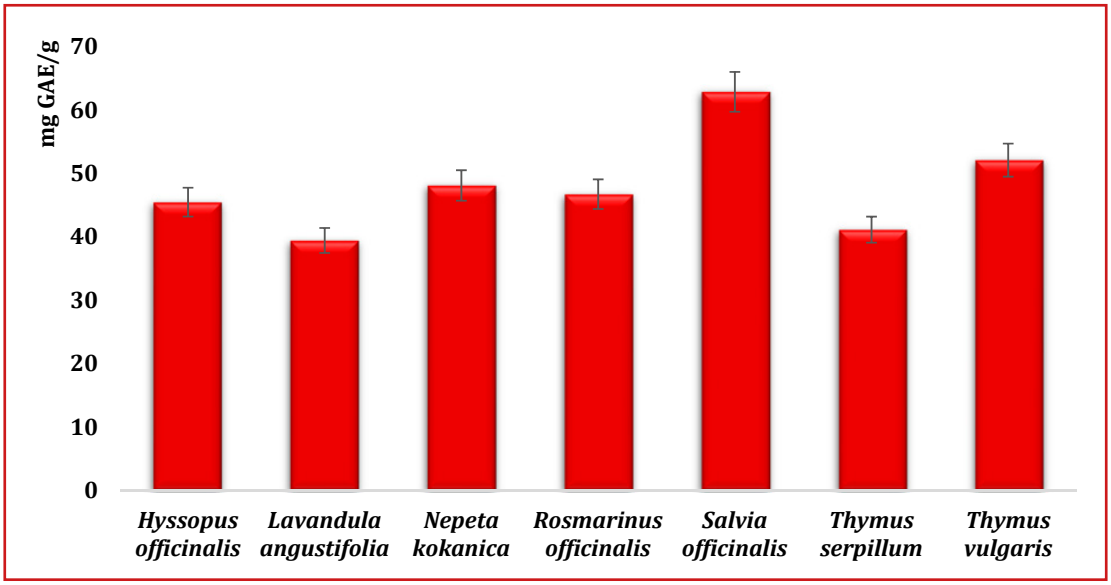

Figure 1 Total content of polyphenol compounds of ethanol extracts of Lamiaceae herbs in the period of flowering

According to Wojdyło et al. (2007), the total phenolic content of herb extracts of *Rosmarinus officinalis* 1.71 mg GAE per 100 g DW. Bourhia et al. (2019) resulted, that ethanol extracts of *R. officinalis* demonstrated polyphenol content from 74.15 to 146.63 mg GAE/mg depending on the origin. According to Hendel et al. (2016), total polyphenol content in this species was 128.98 mg GAE/g. Armatu et al. (2010) found 22 mg GAE/g polyphenol content of this species. In our research value of this parameter for *R. officinalis* was 46.73 mg GAE/g DW. Andrys et al. (2017) reported that polyphenol content in *Lavandula angustifolia* extracts was 12.3–21.7 mg GAE/g. Duda et al. (2015) found polyphenol content in *L. angustifolia* 12.44 –18.16 mg GAE/g DW. It should be noted that in our study ethanol extracts of *L. angustifolia* had the least value of polyphenol compounds content among other investigated species.

But should be taken into account that fact that the physicochemical nature of the individual phenolics in the extracts may be more important in contributing to the antioxidant activity than the total phenolic content (Santos et al., 2012).

The simplest phenolic compounds found in plants are derivates of benzoic and cinnamic acids, which related to the group of phenolic acids among them gallic, coumaric, caffeic, ferulic, etc (Beer et al., 2002). Some phenolic acids found in free (many fruits and vegetables) and bound form (Abbas,2017). The phenolic acid content of investigated extracts was from 4.91 (*Nepeta kokanica*) to 24.30 (*Salvia officinalis*) mg CAE/g DW (Figure 2).

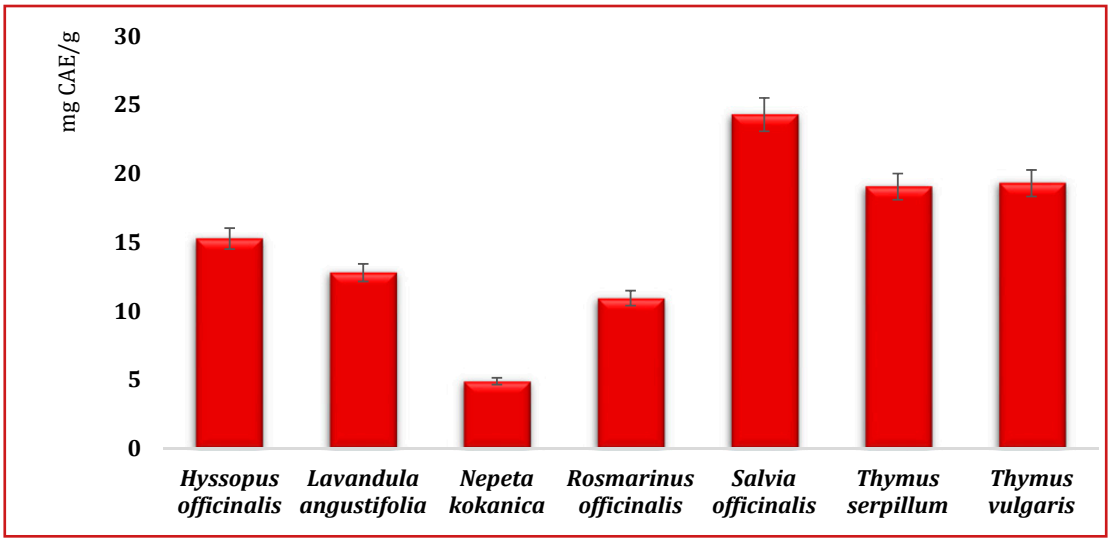

Figure 2 Total content of phenolic acids of ethanol extracts of Lamiaceae herbs in the period of flowering

Flavonoids are polyphenol compounds that are widely distributed in plants that perform many functions such as chemical messengers, physiological regulators, cell cycle inhibitors (Köksal et al., 2017). The content of flavonoids was from 20.80 to (*Thymus* serpillum) to 36.80 (*Salvia officinalis*) mg QE/g DW (Figure 3).

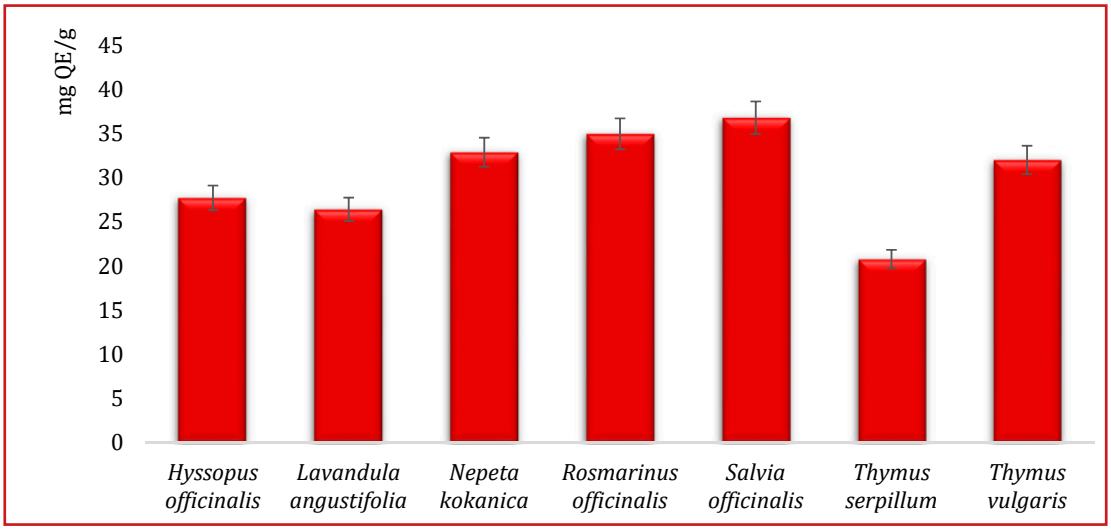

Figure 3 Total content of flavonoids of ethanol extracts of Lamiaceae herbs in the period of flowering

Purified flavonoids from *Hyssopus officinalis* demonstrated a high antioxidant activity. The most known flavonoid from the aerial part of this species is apigenin (Fathiazad et al., 2011). Duda et al. (2015) found the content of flavonoids in *Lavandula angustifolia* 3.37–4.85 mg QE/g DW. As reported Hendel et al. (2016), flavonoids content for *Rosmarinus officinalis* was

38.02 mg QE/g. Abdelkader et al. (2014) determined in extracts of *Salvia officinalis* flavonoids 18.46 mg QE/g. The study of Köksal et al. (2017) reported that the content of flavonoids in ethanol extracts of *Thymus vulgaris* was 36.6 mg QE/mg.

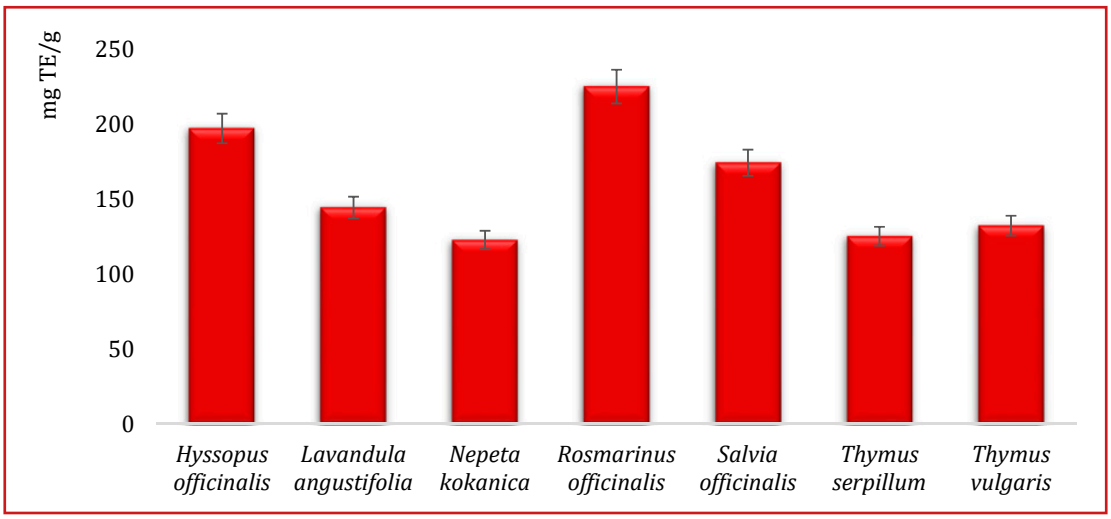

Figure 4 Antioxidant activity by phospomolybdenum method of ethanol extracts of Lamiaceae herbs in the period of flowering

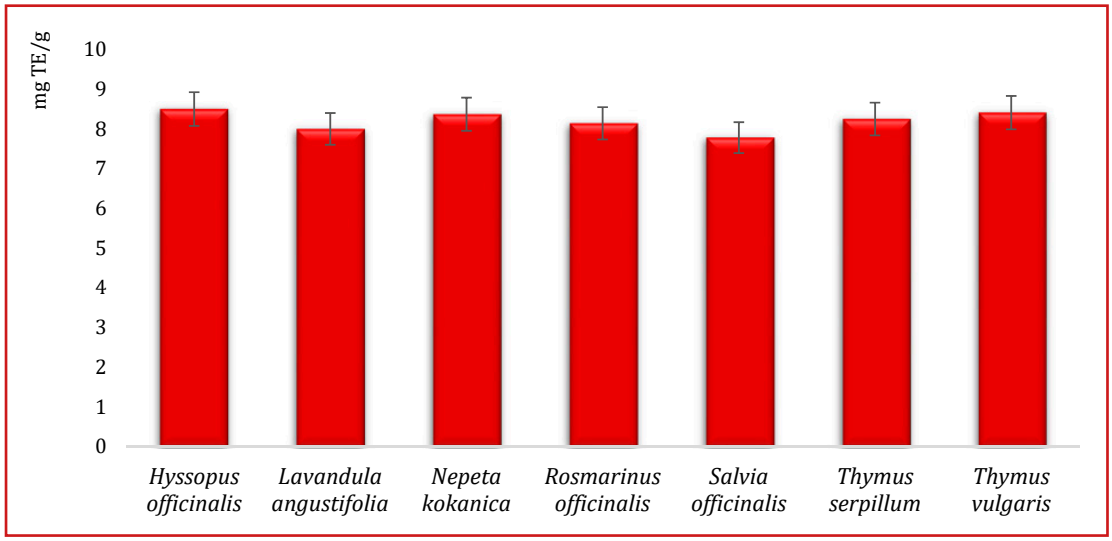

Figure 5 Antioxidant activity by DPPH-method of ethanol extracts of Lamiaceae herbs in the period of flowering

Exist numerous methods of measuring antioxidant activity for the screening of new plant antioxidants such as DPPH, DMPD, ABTS, ORAC, nitric oxide method, etc. (Gupta and Sharma, 2006). Antioxidant activity of investigated plants by phosphomolybdenum method was from 122.92 (*Nepeta kokanica*) to 225.36 (*Rosmarinus officinalis*) mg TE/g DW (Figure 4).

Antioxidant activity by phosphomolybdenum method, which also called reducing power of extract, in our previous study with *Scutellaria baicalensis* showed a strong correlation with all investigated groups of phenolic compounds (Vergun et al., 2019).

The antioxidant activity of ethanol extracts of investigated plants was from 7.78 (*Salvia officinalis*) to 8.50 (*Hyssopus officinalis*) mg TE/g (Figure 5). Amamra et al. (2018) found a correlation between antioxidant activity by DPPH and other methods and content of polyphenols and flavonoids (from 0.73 to 0.98) in different extracts of *Thymus vulgaris*.

Conclusions

Taking into account the results of this study and results obtained from other researchers should be concluded that a group of investigated Lamiaceae species is a valuable source of antioxidant compounds that can be recommended for use in the food and pharmacological industry. It should be noted that *Salvia officinalis* ethanol extracts were the leader of containing the polyphenols, phenolic acids and flavonoids among other investigated species. The study of plant raw for the screening of antioxidant compounds gives the possibility to identify new species for human use considering also the origin of plants.

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