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Polyphenol component and antioxidant activity of *Thymus* spp.

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ABSTRACT

This scientific work was aimed to evaluate the antioxidant potential of aromatic plants of *Thymus* spp. in the East of Ukraine. These plants are known as medicinal and food around the world. All antioxidant parameters were investigated spectrophotometrically: total content of polyphenols (TPC), the total content of phenolic acids (TPAC), the total content of flavonoids (TFC), molybdenum reducing power of extracts (MRP), and antioxidant activity by DPPH method (DPPH). Investigation of ethanolic extracts demonstrated that TPC varied from 57.89 to 123.67 mg/g gallic acid equivalent (GAE) DW for *Th. pulegioides*, from 61.43 to 168.18 mg GAE/g for *Th. serpyllum*, and from 47.36 to 115.67 mg GAE/g for *Th. vulgaris*. TPAC ranged from 27.36 to 50.22 mg/g caffeic acid equivalent (CAE) DW for *Th. pulegioides*, from 28.58 to 59.62 mg CAE/g for *Th. serpyllum*, and from 22.95 to 53.82 mg CAE/g for *Th. vulgaris*. TFC was determined in a range from 29.88 to 61.23 mg/g quercetin equivalent (QE) DW for *Th. pulegioides*, from 36.0 to 82.43 mg QE/g for *Th. serpyllum*, and from 24.59 to 55.41 mg QE/g for *Th. vulgaris*. MRP was detected in the range of 94.65 – 204.76 mg/g Trolox equivalent (TE) DW for *Th. pulegioides*, 96.06 – 219.0 mg TE/g for *Th. serpyllum*, and 87.56 – 215.43 mg TE/g for *Th. vulgaris*. The antioxidant activity of extracts by the DPPH method was 6.34 – 9.23 mg TE/g for *Th. pulegioides*, 8.11 – 9.21 mg TE/g for *Th. serpyllum*, and 4.97 – 9.53 mg TE/g for *Th. vulgaris*. It was established that polyphenol accumulation depended on the growth stage and species. For all species was found a strong correlation between TPC and TFC (r=0.938, 0.908, and 0.854). Investigated *Thymus* spp. are a valuable source of antioxidants that can be used in pharmacological studies and the food industry.

Keywords: *Thymus*, polyphenol content, antioxidant activity

INTRODUCTION

Plant raw such as above-ground part **[1]**, leaves **[2]**, **[3]**, flowers **[4]**, pollen **[5]**, fruits **[6]**, **[7]**, **[8]**, **[9]**, roots **[10]** from different plant families, vegetables **[11]** and food products **[12]**, **[13]** is one of the most valuable sources of biologically active compounds with high antioxidant activity. The Lamiaceae family includes numerous species used in cosmetics, perfumery, food, and pharmaceutical industries worldwide. It is still a popular group of plants with increasing interest among aromatic cultures **[14]**. They have been used in traditional and non-traditional medicine to treat different diseases **[15]**. Among *Lamiaceae* representatives, *Thymus* spp. known for its therapeutic properties from ancient times, is a rich source of biologically active compounds, among which phenolic such as rosmarinic, salvianolic acids, luteolin glycosides with numerous biological activities **[16]**, **[17]**. Also, the antibacterial and cytotoxic properties of *Thymus* representatives are known **[18]**, **[19]**, **[20]**. One of the most widespread species of *Thymus* is *Th. vulgaris* L. **[21]**. This species is also known as thyme and belongs, like others, to aromatic and medicinal plants from the Mediterranean. Existing varieties of thymes are based on various chemotypes. The most studied raw material of *Th. vulgaris* last time is an essential oil component such as *p*-cymene, γ-terpinene, linalool, thymol, carvacrol that depends on plant origin or genotype **[22]**, **[23]**. *Th. serpyllum* is a perennial plant that has been extensively used in official and folk medicine. Essential oil from this plant contains (E)-nerolidol, caryophyllene oxide, myrcene, (E)-β-caryophyllene, germacrene D **[24]**. *Th. vulgaris* raw possesses antimicrobial **[25]**, medicinal, astringent, anthelmintic, disinfectant, tonic capacities **[26]**. The herb of this species is used to prepare natural remedies,

syrups, teas, etc. It is known antiseptic, ethnoveterinary usage of this plant, as remedies from bronchitis, bronchial catarrh, whooping cough, etc. Plant raw material of *Th. serpyllum* possesses antioxidant, antimicrobial, antitumor, cytotoxic activities **[27]**. The study of six *Thymus* species showed that flavonoid content was 0.15 – 0.42%, tannins 0.77 – 1.59%, and procyanidins 0.21 – 0.70% **[28]**.

The essential oil composition of *Th. capitatus* is carvacrol, p-cymene, γ-terpinene, Linalyl acetate, 1,8-cineole, β-myrcene, terpinene-4-ol, and α-terpinene. In total, the essential oil of this species was identified with 27 compounds **[29]**. According to Borugă et al. **[30]**, the major components of *Th. vulgaris* essential oil is *p*-cymene, γ-terpinene, and thymol. Thymol and carvacrol possess solid antiseptic activity. As reported by Verma et al. **[31**], the growth period significantly affects the quantity and quality of thyme oil composition. *Th. vulgaris* and its oil are a good source of vitamin A and ascorbic acid **[21]**. Essential oil from the thyme possesses antimicrobial **[30]**, **[32]**, antioxidant **[33]**, **[34]**, anti-inflammatory activities **[21]**. The highest oil components, such as thymol and carvacrol, were obtained under mineral nutrition **[35]**. *Th. pulegioides* is a less critical commercial species, such as *Th. vulgaris*, but antioxidant and antibacterial activities characterize its plant raw. It was found valuable essential oil components such as geranial, neral, geraniol, and linalool **[36]**. Aqueous and hydro-ethanolic extracts of *Th. pulegioides* demonstrated antioxidant and antiproliferative activities **[37]**.

However, numerous studies concerning the antioxidant capacity of *Thymus* spp. describe the effect of several factors on polyphenol composition in above-ground parts, first of all, growth conditions. This work aimed to assess the antioxidant capacity of three *Thymus* species grown in the East of Ukraine during vegetation and evaluate the peculiarities of polyphenol accumulation.

The specific aim was to evaluate polyphenol compounds accumulation in plant raw material of *Thymus* spp. during vegetation.

Scientific Hypothesis

Antioxidant properties of plant raw material of *Thymus* spp. during the vegetation from the East of Ukraine will depend on the plant part, the species, and the growth stage.

MATERIAL AND METHODOLOGY

Samples

The plant material of *Thymus pulegioides* L., *Thymus serpyllum* L., and *Thymus vulgaris* L. (Figure 1) took from a 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) in 2018 – 2019. Plant raw material (leaves, buds, inflorescences, fruits, herb, i.e., all above-ground parts were prepared at the budding stage, flowering, and fruitage).

In this study, accumulation and distribution of polyphenols and antioxidant activity of ethanol extracts of *Th. pulegioides*, *Th. serpyllum* and *Th. vulgaris* from Ukraine during vegetation were studied. Plant samples were dried at 45 °C for three 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 of analytical grade quality and were purchased from Reachem (Slovakia) and Sigma Aldrich (USA).

Animals and Biological Material

The animal and biological materials weren't used in this research.

Instruments

The centrifuge Rotofix 32 A (Hettich, Germany), spectrophotometer Jenway, 6405 UV/Vis (England), vortex shaker (IKA VORTEX 3, Germany) were used in this research.

Laboratory Methods

Total polyphenol content (TPC) of extracts were measured by the spectrophotometric method with the Folin-Ciocalteu reagent **[38]**; the total content of phenolic acids (TPAC) was determined using Farmakopea Polska (1999) **[39]**; procedure of total flavonoid content (TFC) was conducted by a spectrophotometric method based on the formation of aluminum-flavonoid complex **[40]**, **[41]**; the reducing power of extracts (molybdenum reducing antioxidant power, MRP) was determined by the phosphomolybdenum method **[42]**; the antioxidant activity of samples was conducted by DPPH method **[43]**.

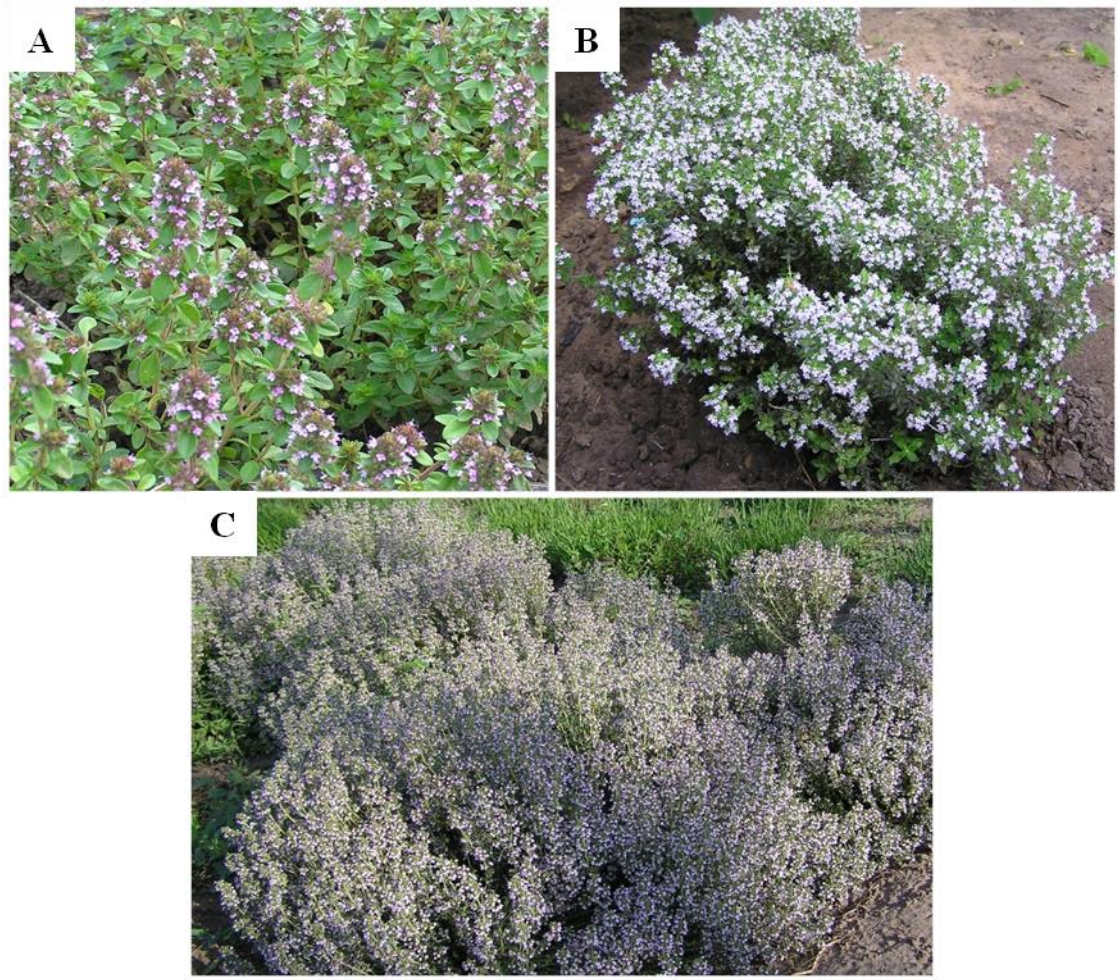

Figure 1 Plants of *Thymus pulegioides* L. (A), *Th. serpyllum* L. (B), and *Th. vulgaris* L. at the flowering period.

Description of the Experiment

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 for 10 min, the supernatant was used for the next measurements: antioxidant activity, polyphenols, and flavonoids. All data were expressed in mg of standard compound per gram of dry weight (DW).

Number of samples analyzed: we analyzed 27 samples.

Number of repeated analyses: all biochemical procedures were conducted in triplicate.

Number of experiment replication: 2 times.

Design of the experiment: Total polyphenol content of extracts was measured by the following procedure: 0.1 ml of each sample extract was mixed with 0.1 ml of the Folin-Ciocalteu, 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. Gallic acid (25 – 250 mg/l; R^2 = 0.996) was used as the standard, and the results were expressed in mg/g gallic acid equivalents. For phenolic acid content, 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. Caffeic acid 1 – 200 mg/l (R^2 = 0.999) was used as a standard. The results were expressed in mg/g caffeic acid equivalents (CAE). The procedure of total flavonoid content determination was conducted the following way: 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. Quercetin 0.01 – 0.5 mg/l (R^2 = 0.997) was used as the standard and the results were expressed in mg/g quercetin equivalents (QE). The reducing power of extracts was determined by the phosphomolybdenum method with slight modifications: the mixture of 1 ml of sample, 2.8 ml of monopotassium phosphate (0.1 M), 6 ml of sulfuric acid (1 M), 0.4 ml of ammonium heptamolybdate (0.1 M), and 0.8 ml of distilled water was incubated at 90 °C for 120 min, then rapidly cooled and detected by monitoring absorbance at 700 nm using the spectrophotometer. Trolox 10 – 1000 mg/l (R^2 = 0.998) was used as the standard, and the results were expressed in mg/g TE. The antioxidant activity of samples was measured

using 2,2-diphenyl-1-picrylhydrazyl (DPPH): the ethanol extract (1 ml) was mixed with 4 ml of DPPH solution (0.025 g of radical in 100 ml of ethanol). The absorbance of the sample extract was determined using the spectrophotometer at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) 10 – 100 mg/l (R^2 = 0.983) was used as a standard and the results were expressed in mg/g Trolox equivalents (TE).

Statistical Analysis

Significant differences (p<0.05) between means were evaluated by ANOVA and the Tukey–Kramer test. Correlation coefficients were calculated using Statistica version 13.0 software (StatSoft, Tulsa, OK, USA).

RESULTS AND DISCUSSION

In recent years, the interest in natural antioxidants has increased concerning phenolic compounds, including flavonoids and phenolic acids. Natural antioxidants are present in different plant raw materials **[44]**. Herbs from Lamiaceae exhibited the strong antioxidant potential and high content of polyphenol compounds in dry form as well as in fresh **[45]**.

The total content of polyphenol compounds (TPC)

The total content of polyphenol compounds in this study was from 57.89 to 123.67 mg GAE/g DW for *Th. pulegioides*, from 61.43 to 168.18 mg GAE/g DW for *Th. serpyllum*, and from 47.36 to 115.67 mg GAE/g DW for *Th. vulgaris* (Figure 2). As shown from Figure 2, the maximal content of total polyphenol compounds is determined in the buds for all species.

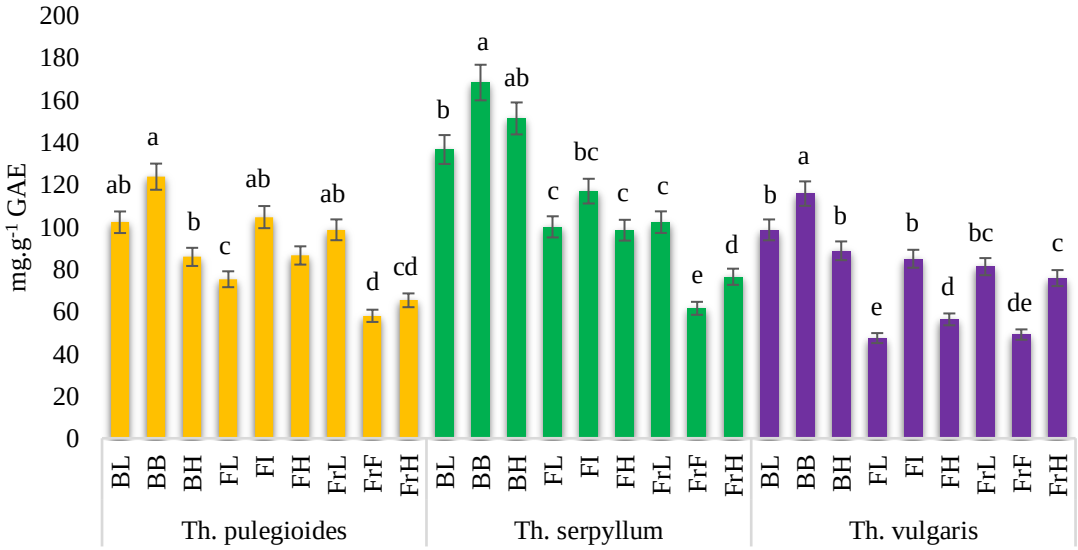

Figure 2 Content of polyphenol compounds in ethanol extracts of *Thymus* spp. depending on the stage of growth (Means in columns followed by different letters are different at p<0.05. Each value represents the mean of three independent experiments (±SD)).

Note: BL – leaves, budding; BB – buds, budding; BH – herb (above-ground part), budding; FL – leaves, flowering; FI – inflorescences, flowering; FH – herb, flowering; FrL – leaves, fruitage; FrF – fruits, fruitage; FrH – fruitage, herb; GAE – gallic acid equivalent.

As reported by Armatu et al. **[46]**, in methanol extracts of *Th. vulgaris* determined 32 mg GAE/g of TPC. The total polyphenol content of another species *Thymus capitatus* was 175.53 mg GAE/g **[29]**. Maximal value of TPC of Tunisian plants of *Th. capitatus* was 18.40 mg GAE/g DW in methanol extracts **[47]**. Plant ethanol extracts of other species *Th. riatarum* demonstrated 135.8 mg GAE/g DW of TPC **[48]**. According to Köksal et al. **[49]**, TPC of ethanol extracts of *Th. vulgaris* was 158 µg GAE/mg DW. However, some investigations demonstrated low content of TPC, namely 7.30 mg GAE/g DW of ethanol extracts **[50]**. Taghouti et al. **[37]** reported that *in vitro* hydro-ethanolic post-blooming extracts of *Th. pulegioides* demonstrated 155.38 mg GAE/g DW of TPC. Hydro-ethanolic extraction of freeze-dried raw showed TPC as 70.31 mg/g caffeic acid equivalent DW. Ethanol extracts of *Th. serpyllum* and *Th. vulgaris* from Slovakia showed 41.13 and 52.1 mg GAE/g DW of TPC, respectively **[51]**. The content of polyphenols and flavonoids in extracts of different *Thymus* spp. depended on the solvent. Methanol and acetone extracts contained higher polyphenol content than ethanol **[52]**. The methanol extracts of *Th. serpyllum* and *Th. vulgaris*, raw of which were shade dried at 25 °C, had 22.14 and 35.73 mg/g GAE of TPC, respectively **[53]**. Also, as reported in the study with *Th. vulgaris* and other

Lamiaceae herbs, the content of phenolic compounds and flavonoids depended on harvest time, so the best results were obtained at the first harvesting **[54]**.

As a result, it should be noted that maximal TPC accumulated at the budding stage for *Th. serpyllum* and *Th. vulgaris* (all parts of the plant), while for *Th. pulegioides* TPC accumulated unevenly.

The total content of phenolic acids (TCPA)

As reported in some studies, among phenolic compounds of *Thymus* spp. the most widespread are flavonoids and phenolic acids. The last group of polyphenols is represented by hydroxycinnamic or hydroxybenzoic structure **[55]**.

The total content of phenolic acids was in a range from 27.36 to 50.22 mg CAE/g DW for *Th. pulegioides*, from 28.58 to 59.62 mg CAE/g DW for *Th. serpyllum*, and from 22.95 to 53.82 mg CAE/g DW (Figure 3). TPAC for both investigated species was the highest in the bud's extracts (at the budding) and the lowest in the fruit's extracts (at the fruitage). This parameter decreased during vegetation in the above-ground part (herb) of *Th. serpyllum* and decreased from budding to flowering stage and increased from flowering to fruitage for *Th. vulgaris* plant extracts.

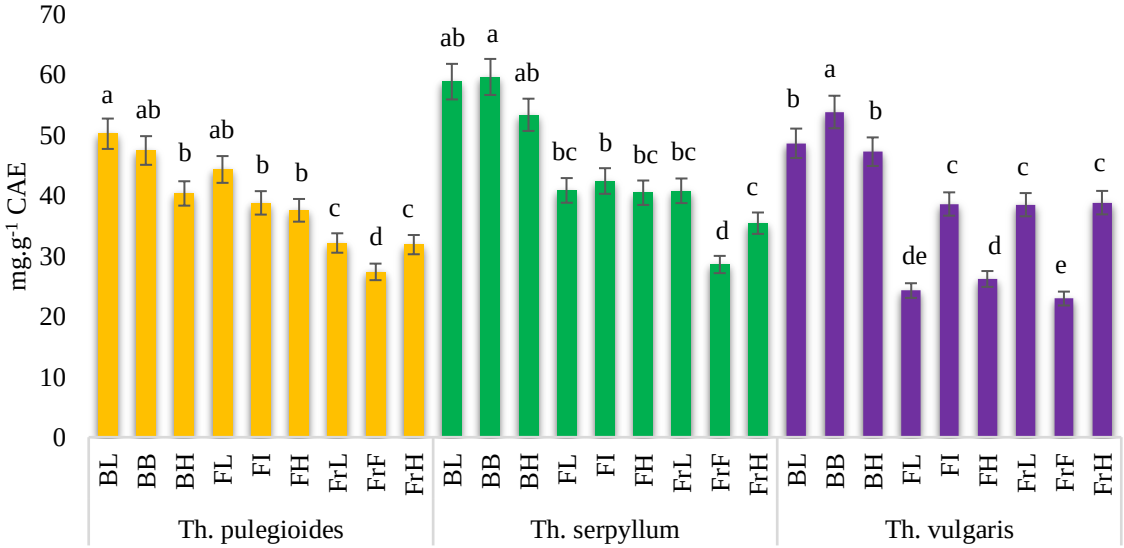

Figure 3 Content of phenolic acids in ethanol extracts of *Thymus* spp. depending on the stage of growth (Means in columns followed by different letters are different at $p<0.05$. Each value represents the mean of three independent experiments (±SD)).

Note: BL – leaves, budding; BB – buds, budding; BH – herb (above-ground part), budding; FL – leaves, flowering; FI – inflorescences, flowering; FH – herb, flowering; FrL – leaves, fruitage; FrF – fruits, fruitage; FrH – fruitage, herb; CAE – caffeic acid equivalent.

Taghouti et al. **[37]** reported that in vitro hydro-ethanolic extracts of *Th. pulegioides* exhibited 56.11 mg CAE/g DW of TPAC. Hydro-ethanolic extraction of freeze-dried post-blooming raw *Th. pulegioides* showed TPAC as 34.09 mg CAE/g DW. TPAC was the highest for *Th. serpyllum* and *Th. vulgaris* at the budding stage as well as TPC. Extracts of *Th. pulegioides* demonstrated maximal values at the budding besides herb extracts. In extracts of Slovakian samples of *Th. serpyllum* and *Th. vulgaris* from Slovakia showed 19.60 and 19.31 mg CAE/g DW, respectively, which was two times less than obtained result in this study for *Th. serpyllum* **[51]**.

The total content of flavonoids (TFC)

Flavonoids are secondary plant products and are an important component of the human diet due to their functions. From the polyphenol compounds, flavonoids received the most attention due to their biological activities, distribution in natural products **[56]**. The most important classes of flavonoids are flavones, flavanones, isoflavones, flavonols, anthocyanidins, etc. **[44]**.

The total content of flavonoids was in a range from 29.88 to 61.23 mg QE/g DW for *Th. pulegioides*, from 36.0 to 82.43 mg QE/g DW for *Th. serpyllum*, and from 24.59 to 55.41 mg QE/g DW (Figure 4).

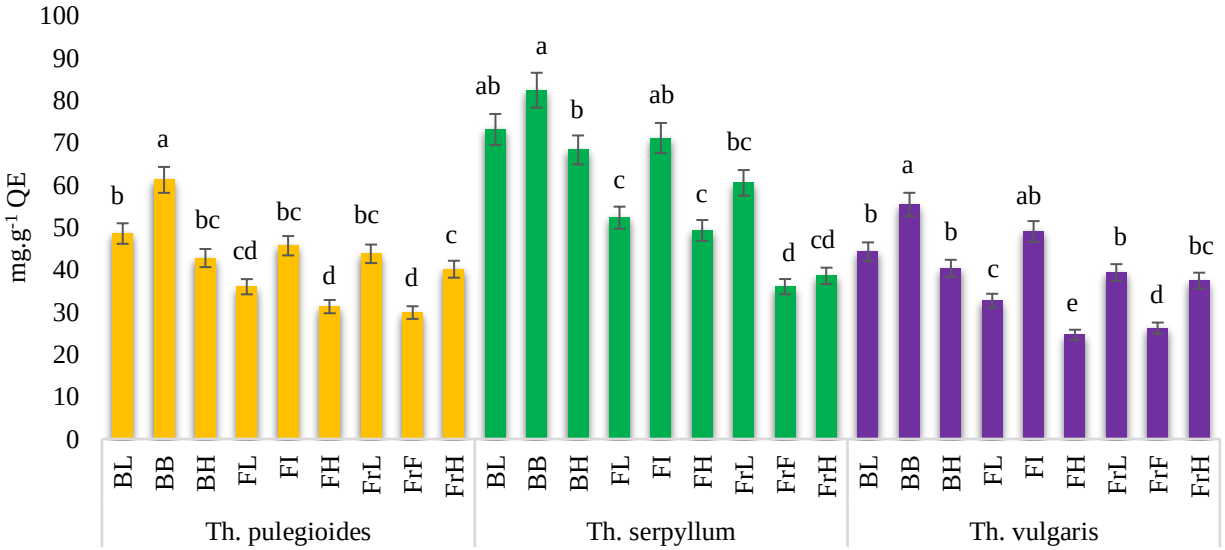

Figure 4 Content of flavonoids in ethanol extracts of *Thymus* spp. depending on the stage of growth (Means in columns followed by different letters are different at $p<0.05$. Each value represents the mean of three independent experiments (±SD)).
Note: BL – leaves, budding; BB – buds, budding; BH – herb (above-ground part), budding; FL – leaves, flowering; FI – inflorescences, flowering; FH – herb, flowering; FrL – leaves, fruitage; FrF – fruits, fruitage; FrH – fruitage, herb; QE – quercetin equivalent.

As reported Köksal et al. **[49]**, in the ethanol extracts of *Th. vulgaris* was determined 36 μg QE/mg DW of TCF. Taghouti et al. **[37]** reported that *in vitro* hydro-ethanolic post-blooming extracts of *Th. pulegioides* showed TFC as 61.75 mg/g catechin equivalent DW. After hydro-ethanol extraction of freeze-dried plant, raw TFC of this species was 36.22 mg/g catechin equivalent DW. According to Aouam et al. **[48]**, in ethanol extracts of *Th. riatarum* was detected 120.6 mg RE/g DW (rutin equivalent) of TFC. Also, Slovakian samples of *Th. serpyllum* showed two times less TFC than in our study **[51]**. Evidently, TPC, TFC, and TPAC of *Th. serpyllum* have depended on conditions of growth. Methanol extracts of *Th. serpyllum* and *Th. vulgaris* had TFC 4.36 and 8.70 mg QE/g, respectively **[53]**.

Considering peculiarities of TFC accumulation, it's seen that flavonoids distributed during vegetation unevenly, but for all species were characterized the highest value in the buds. The lowest TFC was found in fruit extracts of *Th. pulegioides*, *Th. serpyllum* and in herb extracts of *Th. vulgaris*.

The molybdenum reducing power of extracts (MRP)

Exist numerous assays to determine the antioxidant capacity of plant raw material, among which DPPH scavenging activity and the reducing power of extracts **[44]**. The phosphomolybdenum method of antioxidant activity determination is based on the reduction of molybdate ions **[57]**.

The molybdenum reducing power of ethanol extracts was from 94.65 to 204.76 mg TE/g DW for *Th. pulegioides*, from 96.06 to 219.0 mg TE/g DW for *Th. serpyllum*, and from 87.56 to 215.43 mg TE/g DW for *Th. vulgaris* (Figure 5). The study of MRP didn't show definite patterns in the manifestation of this activity during vegetation, but for *Th. vulgaris* extracts identified maximal values in the buds (at the budding), inflorescences (at the flowering), and herb (at the fruitage). *Th. serpyllum* extracts demonstrated the highest MRP in the leaves (at the budding and fruitage) and herb (at the flowering).

According to Armatu et al. **[46]**, methanol extracts of *Th. vulgaris* demonstrated significant antioxidant activity by phosphomolybdenum method at 5 mg/mL (ascorbic acid equivalent). Another *Lamiaceae* species *Scutellaria baicalensis* Georgi from the same region, demonstrated MRP as 260.24 mg TE/g DW **[58]**. According to Mňahončaková et al. **[51]**, MRP of ethanol extracts of Slovakian samples of *Th. serpyllum* and *Th. vulgaris* was 125.44 and 132.49 mg TE/g DW, respectively that differed from our result for both species.

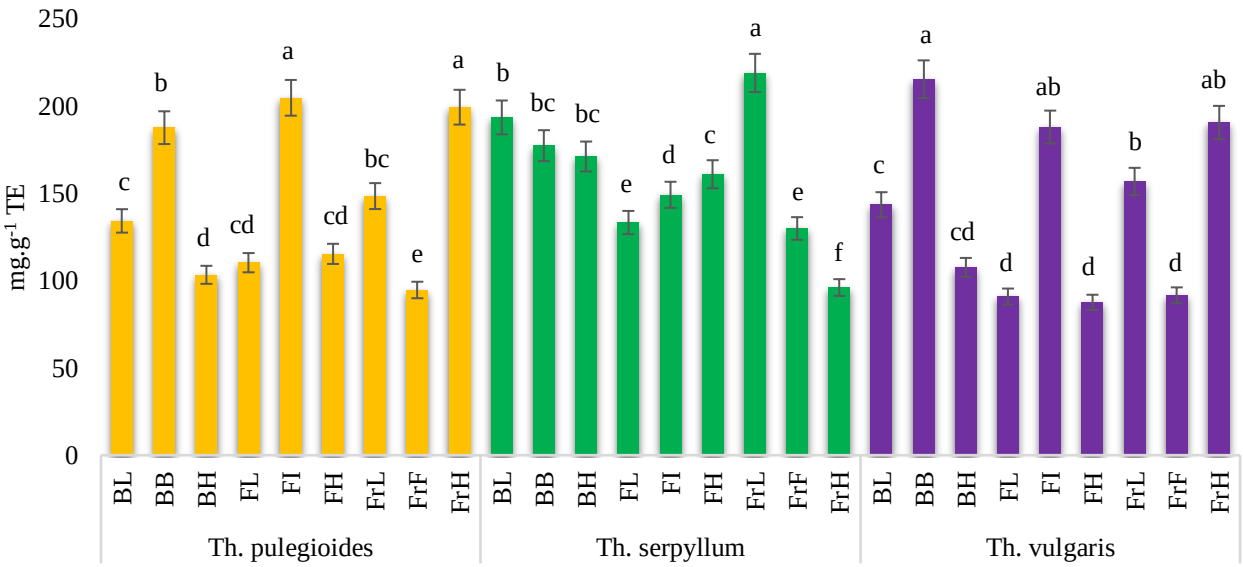

Figure 5 Molybdenum reducing power of ethanol extracts of *Thymus* spp. Depending on the stage of growth (The means in columns followed by different letters are different at $p<0.05$. Each value represents the mean of three independent experiments (±SD)).
Note: BL – leaves, budding; BB – buds, budding; BH – herb (above-ground part), budding; FL – leaves, flowering; FI – inflorescences, flowering; FH – herb, flowering; FrL – leaves, fruitage; FrF – fruits, fruitage; FrH – fruitage, herb; TE – Trolox equivalent.

The antioxidant activity of extracts by the DPPH method (DPPH)

One of the most widespread methods of antioxidant activity determination is the DPPH method, characterized by simple, accurate, and based on electron transfer that manifests on the discoloration of radical solution **[59]**. There are results not only about an investigation of antioxidant activity of medicinal plant extracts including Lamiaceae representatives such as *Thymus* spp. **[60]**, **[61]**, **[62]** but also products (juices) with herb addition **[63]**.

The antioxidant activity of ethanol extracts by the DPPH method was from 6.34 to 9.23 mg TE/g DW for *Th. pulegioides*, from 8.11 to 9.21 mg TE/g DW for *Th. serpyllum*, and from 4.97 to 9.53 mg TE/g DW for *Th. vulgaris* (Figure 6). These results demonstrated that maximal values of antioxidant activity by DPPH were found in the buds extracts of *Th. pulegioides*, *Th. vulgaris* and in leaf extracts of *Th. serpyllum*.

According to Chizzola et al. **[64]**, leaves of *Th. vulgaris* showed in 60 and 96% ethanol antioxidant activity by DPPH method 55.9 and 26.5 mg TE/g DW. *Scutellaria baicalensis* from the same region had antioxidant activity by DPPH method 8.83 mg TE/g DW **[58]**. Results obtained by Mňahončaková et al. **[51]** for *Th. serpyllum* and *Th. vulgaris* didn't differ from our concerning DPPH antioxidant activity and was 8.25 and 8.41 mg TE/g DW. The study of *Th. vulgaris* demonstrated that antioxidant activity was less in aqueous extracts than in ethanol extracts of leaves **[65]**. The antioxidant activity of *Th. serpyllum* extracts depended on extraction parameters and chosen assay of determination **[66]**, **[67]**.

The Pearson's correlation coefficients between investigated parameters of antioxidant activity are represented in Table 1. In the extracts of *Th. pulegioides* was found a very strong correlation between TPAC and antioxidant activity by DPPH ($r=0.840$, $p<0.01$), TPC and TFC ($r=0.854$, $p<0.01$).

We found a very strong positive correlation between TPC and the following parameters: TPAC ($r=0.963$, $p<0.01$) and TFC ($r=0.938$, $p<0.01$) for *Th. serpyllum* extracts. TPAC correlated with TFC also strong ($r=0.902$, $p<0.01$). A moderate correlation was found between molybdenum reducing the power of extract and polyphenol compounds, flavonoids, while between antioxidant activity by the DPPH method and all polyphenol compounds a negative correlation. A very strong correlation we determined between TPC and the following parameters: TPAC ($r=0.980$, $p<0.01$), TFC ($r=0.908$, $p<0.01$) and with MRP was found strong correlation ($r=0.758$, $p<0.05$) for *Th. vulgaris* extracts.

Also, a very strong correlation we found between TPAC and TFC ($r=0.865$, $p<0.01$), TFC and MRP ($r=0.834$, $p<0.01$). Compared with *Th. serpyllum* extracts, antioxidant activity by DPPH method of *Th. vulgaris* had a strong ($r=0.647$, $r=0.707$, $p<0.05$) or moderate ($r=0.521$, $p<0.05$) correlation between tested parameters. For all species found a weak correlation between MRP and antioxidant activity by the DPPH method. Antioxidant activity of *Th. serpyllum* by DPPH had a negative correlation with all polyphenol compounds.

Chizzola et al. **[64]** found a strong correlation between antioxidant activity by the DPPH method and TPC (r=0.946). Adámková et al. **[45]** determined a stronger correlation between antioxidant activity by DPPH and polyphenol compounds in dried herbs than in fresh. The reducing activity of extracts of *Th. sibthorpii* correlated with total polyphenols, as reported by Kontogiorgis et al. **[68]**.

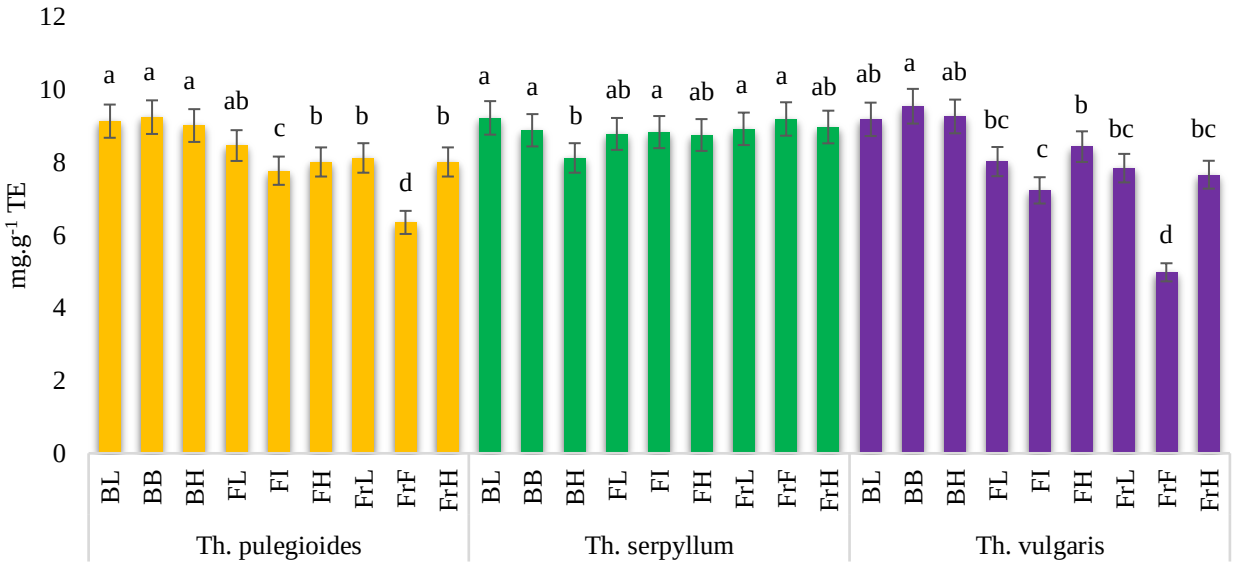

Figure 6 Antioxidant activity of ethanol extracts of *Thymus* spp. by DPPH method depending on the stage of growth (Means in columns followed by different letters are different at p<0.05. Each value represents the mean of three independent experiments (±SD)).
Note: BL – leaves, budding; BB – buds, budding; BH – herb (above-ground part), budding; FL – leaves, flowering; FI – inflorescences, flowering; FH – herb, flowering; FrL – leaves, fruitage; FrF – fruits, fruitage; FrH – fruitage, herb; TE – Trolox equivalent.

Table 1 The correlation coefficients between the different parameters of antioxidant activity of investigated plants of *Thymus* spp.

Parameters	Polyphenols	Phenolic acids	Flavonoids	MRP
		Thymus pulegioides		
Phenolic acids	0.656*	1	-	-
Flavonoids	0.854**	0.622*	1	-
MRP	0.456*	0.077*	0.601*	1
DPPH	0.657*	0.840**	0.707*	0.176*
		Thymus serpyllum		
Phenolic acids	0.963**	1	-	-
Flavonoids	0.938**	0.902**	1	-
MRP	0.561*	0.575*	0.657*	1
DPPH	-0.362*	-0.267*	-0.135*	0.375*
		Thymus vulgaris		
Phenolic acids	0.980**	1	-	-
Flavonoids	0.908**	0.865**	1	-
MRP	0.758*	0.688*	0.834**	1
DPPH	0.657*	0.707*	0.521*	0.266*

Note: MRP – molybdenum reducing power of extracts; DPPH – antioxidant activity by DPPH method; ** – correlation is significant at the level of 0.01; * – correlation is significant at the level of 0.05.

CONCLUSION

Plant raw material from *Thymus* spp. (*Th. pulegioides*, *Th. serpyllum*, and *Th. vulgaris*) from the Eastern region of Ukraine is a promising source of antioxidants, which can be used for their health properties in food products. The content of polyphenol compounds was statistically significant for all investigated species. It should be noted that an accumulation of polyphenol compounds, phenolic acids, and flavonoids was uneven and depended on the stage of growth, part of a plant, and species. The highest content of polyphenols (168.18 mg GAE/g), phenolic acids (59.62 mg CAE/g), and flavonoids (82.43 mg QE/g) was determined for *Th. serpyllum* extracts at the budding stage in buds. Molybdenum reducing power was maximal in ethanol extracts of *T. serpyllum* at the fruitage in leaves (219.0 mg TE/g) and antioxidant activity by DPPH in the buds of *Th. vulgaris* (9.53 mg TE/g). The minimal values of all investigated parameters were detected for *Th. vulgaris* extracts. So, the least polyphenol content (47.36 mg GAE/g) found in the leaf extracts at the flowering period, the least total phenolic acid content (22.95 mg CAE/g) found in the fruit extracts, the least content of flavonoids (24.59 mg QE/g) detected in the herb extracts of this species at the flowering period. Also, the fruit extracts and herb extracts (flowering) demonstrated the lowest values of antioxidant activity by the DPPH method (4.97 mg TE/g) and molybdenum reducing power (87.56 mg TE/g), respectively. A very strong and strong correlation was found between the accumulation of different polyphenol compounds for all species. A strong correlation between flavonoids and molybdenum reducing power of the extract was found for all investigation species (r=0.834 (*Th. vulgaris*), r=0.657 (*Th. serpyllum*), r= 0.601 (*Th. pulegioides*)). Obtained results can be used in further deep biochemical, pharmacological studies and selective work in nutritional, food, and horticultural practice.

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The authors declare no conflict of interest.

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This article does not contain any studies that would require an ethical statement.

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