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Accumulation of Total Content of Polyphenol Compounds and Antioxidant Activity of *Echinacea* Moench Species

Olena Vergun[1]*, Liudmyla Svydenko[2], Oksana Shymanska[1], Liudmyla Hlushchenko[3], Vladimíra Horčinová Sedlačková[4], Eva Ivanišová[4], Ján Brindza[4]

[1]M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine, Kyiv, Ukraine
[2]Institute of Climate Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine, Kyiv, Ukraine
[3]Research Station of Medicinal Plants of the Institute of the Agroecology and Nature Management of the National Academy of Agrarian Sciences of Ukraine, Berezotocha, Ukraine
[4]Slovak University of Agriculture in Nitra, Nitra, Slovak Republic

Olena Vergun: https://orcid.org/0000-0003-2924-1580
Oksana Shymanska: https://orcid.org/0000-0001-8482-5883
Liudmyla Svydenko: https://orcid.org/0000-0002-4043-9240
Liudmyla Hlushchenko: https://orcid.org/0000-0003-2329-5537
Vladimíra Horčinová Sedlačková: https://orcid.org/0000-0002-5844-8938
Eva Ivanišová: https://orcid.org/0000-0001-5193-2957
Ján Brindza: https://orcid.org/0000-0001-8388-8233

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Echinacea Moench species (Asteraceae Bercht. & J. Presl) are one of the most known medicinal and ornamental plants with numerous pharmacological activities. The objects of this study were plant raw materials of *Echinacea angustifolia* DC. (Kherson Oblast, Ukraine) and *E. purpurea* (L.) Moench (Kherson and Poltava Oblast, Ukraine) was collected at the start of the vegetation, budding, flowering, and seed ripening period in 2021–2022. It was determined The total polyphenol content (TPC) by the Folin-Ciocalteu method, the total flavonoid content (TFC) by the aluminum chloride method, and the total phenolic acid content (TPAC) with Arnova reagent. The antioxidant activity of investigated plant extracts was conducted by the phosphomolybdenum method (molybdenum-reducing power, MRP) and DPPH method (free radical scavenging activity with 2,2-diphenyl-1-picrylhydrazyl radical). The TPC was determined in the amount of 21.15–78.34 mg GAE·g^{-1}, TFC in the amount of 8.23–47.98 mg QE·g^{-1}, and TPAC in the amount of 7.34–29.21 mg CAE·g^{-1} depending on species, stage, and region of growth. The MRP of investigated extracts was in the range of 54.32–161.34 mg TE·g^{-1} and FRSA in the range of 6.12–9.69 mg TE·g^{-1} depending on species, stage, and region of growth. The lowest content of TPC, TFC, and TPAC was determined in the extracts of *E. angustifolia* in all investigated periods. The highest content of the TPC, TFC, and TPAC was detected in the extracts of *E. purpurea* from the Kherson region of Ukraine. A positive strong correlation was found between investigated parameters in spring growth, budding, and flowering (r = 0.675–0.998). The negative weak correlation was found in the seed ripening period between TFC and MRP (r = -0.079). The obtained results showed that the accumulation of the total polyphenol content and antioxidant activity of ethanol extracts of *Echinacea* plants depended on species, period, and region of growth. It can be useful for further pharmacological and biochemical investigations.

Keywords: coneflower, flavonoids, phenolic acids, reducing power of extracts, correlation

***Corresponding Author:** Olena Vergun, M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine, Sadovo-Botanichna str. 1, 01014 Kyiv, Ukraine
olenavergun8003@gmail.com

Introduction

Plants from the genus *Echinacea* Moench belong to Asteraceae Bercht. & J. Presl family and native to mid-latitude North America. They have been known for a long time as unique medicinal plants with ethnobotanical importance for Native Americans (Tang et al., 2017).

The pharmacological studies of *Echinacea* spp. demonstrated numerous biological activities of their extracts such as antioxidant, anti-inflammatory (Merali et al., 2003), immunomodulatory (Noce et al., 2024), antiviral (Hudson and Vimalanathan, 2011), antimicrobial (Manayi et al., 2015), and antifungal (Burlou-Nagy et al., 2022). The antimicrobial activity of *E. angustifolia* was extremely higher through noisome encapsulation against *Klebsiella pneumoniae* than free extracts (Moghtaderi et al., 2021). The methanolic extracts of *E. pallida* were effective against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Marjhan and Mannan, 2024). Root extracts of *E. angustifolia*, *E. pallida*, and *E. purpurea* exhibited antioxidant activity (Hu and Kitts, 2000).

The plant raw of these species is a rich source of variable biologically active compounds (Dobrange et al., 2019). The aerial parts of *Echinacea* species contain polysaccharides, derivates of caffeic acid, chlorogenic acid, isochlorogenic acid, terpenoids, flavonoids, anthocyanins, etc. (Quynh et al., 2023). The root and herbal extracts of plants of *E. angustifolia* and *E. purpurea* have essential pharmacological importance and raw of them are best-selling in North America. In the root extracts found alkylamides, polysaccharides, volatile oils, flavonoids, glycoproteins, caffeic acid derivates, etc. (Ahmadi et al., 2024). *E. purpurea* aerial parts contain several metals such as Mn (71.32 mg·kg^{-1}), Fe (255.48 mg·kg^{-1}), Cu (8.07 mg·kg^{-1}), and Zn (37.74 mg·kg^{-1}) (Momchev et al., 2020). According to Fu et al. (2021), the main antioxidant agents of *Echinacea* species were chicoric acid, caftaric acid, and echinacoside.

Echinacea extracts can be used for the treatment of numerous diseases among which gynecological disorders. The extracts of *E. purpurea* are characterized by immunostimulative effects (Riemma et al., 2022).

These plants are used in the world as ornamental plants in different plant compositions for phytoremediation (Francini et al., 2022). According to Oniszczuk et al. (2019), *E. purpurea* can be used as a nutritional supplement for fish diseases. Some studies of *E. purpurea* showed an accumulation of proline in plant tissues that can demonstrate tolerance to stress salinity (Choirunnisa et al., 2021a).

Taking into account the useful properties of these plants, this study aimed to investigate the polyphenol content and antioxidant activity of *E. angustifolia* and *E. purpurea* from different regions (of Ukraine) and during selected stages of growth to evaluate the peculiarities of their accumulation.

Material and methodology

Biological material

The above-ground part (herb) of *Echinacea angustifolia* DC. and *E. purpurea* (L.) Moench from different regions was investigated in seasons 2021–2022. Plants were collected at the start of the vegetation, budding, flowering, and seed ripening periods. The plant samples were collected from Ukraine namely Kherson Oblast (KO) and Poltava Oblast (PO).

Biochemical analyses

All biochemical analyses were conducted at the Slovak University of Agriculture in Nitra (Slovak Republic). 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 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 the standard. The results were expressed as mg·g^{-1} DW gallic acid equivalent.

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}; R2 = 0.9977) was used as the standard. The results were expressed in mg·g^{-1} DW quercetin equivalent.

Total phenolic acid content

The content of phenolic acids (TPAC) was determined using Árvay et al. (2017). 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).

Molybdenum-reducing power of extracts

The 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 (10–1,000 mg·L^{-1}; R^2 = 0.998) was used as the standard and the results were expressed in mg·g^{-1} DM Trolox 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.

Statistical analysis

The results are expressed as mean values of three replications ± standard deviation (SD). Data were analyzed with the ANOVA test and differences between means were compared through the Tukey-Kramer test ($p < 0.05$).

Results and discussions

The study of the antioxidant activity of plant raw material and methods of its determination is the most abundant topic in modern biological science (Gulcin, 2020). The most diverse antioxidant in the plant world is a group of secondary metabolites with numerous biological activities called polyphenol compounds (Hano and Tungmunnithum, 2020). An accumulation of total polyphenol compounds depends on many factors such as species, genotypes, stage and conditions of growth, plant part, etc. (Vergun et al., 2023). Plants from Asteraceae species are characterized by high antioxidant activity of various plant parts (Shymanska et al., 2020). Most investigations with *Echinacea* species, as well-known representatives of Asteraceae, including antioxidant activity, are connected with *E. angustifolia* and *E. purpurea* (Pellati et al., 2004; Jukić et al., 2015). The content of the total polyphenol compounds, flavonoids, and phenolic acids in *E. purpurea* extracts was determined in amounts of 1.5%, <0.1%, and 0.84%, respectively (Petrova et al., 2023). Accumulation of polyphenol content of selected Asteraceae plant extracts and their antioxidant activity depended on species, genotypes, and period of growth (Vergun et al., 2023).

The total polyphenol content of ethanol extracts of investigated *Echinacea* spp. was 21.15–32.56 mg GAE·g^{-1} at the spring vegetation, 34.93–52.88 mg GAE·g^{-1} at the budding stage, 53.29–78.34 mg GAE·g^{-1} at the flowering stage, and 24.56–37.98 mg GAE·g^{-1} at the seed ripening stage depending on species and region of growth (Figure 1). In the spring vegetation, budding, and flowering periods the least content of total polyphenol content accumulated plants of *E. angustifolia* (Kherson Oblast). The ethanol extracts of *E. purpurea* from Poltava Oblast demonstrated the lowest value of the TPC during the seed ripening period. The maximum values of TPC were determined in extracts of *E. purpurea* (Kherson Oblast) in the budding, flowering, and seed ripening.

The study of root extracts of *E. purpurea* showed that in methanol was determined 14.1–47.1 mg GAE·g^{-1}, while, in ethanol 11.6–52.3 mg GAE·g^{-1} of TPC (Wu et al., 2007). The TPC of *E. purpurea* extracts obtained

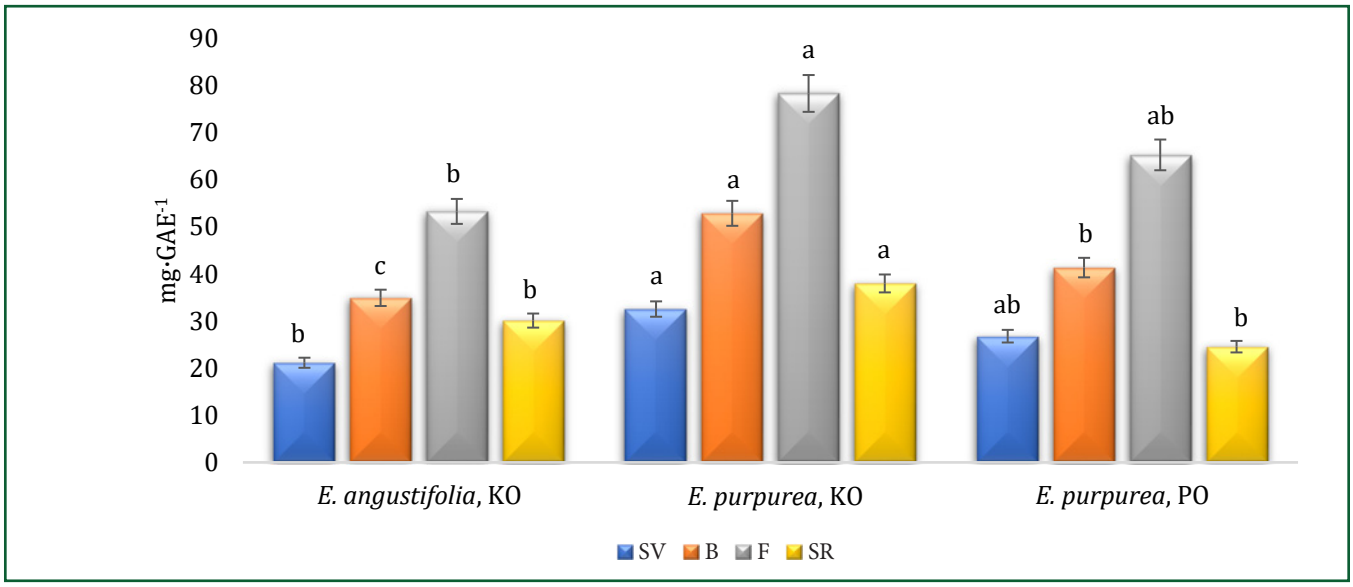

Figure 1 The total polyphenol content of ethanol extracts of *Echinacea* Moench species from different regions
GAE – gallic acid equivalent; SV – spring vegetation, B – budding, F – flowering stage, SR – seed ripening; KO – Kherson Oblast, PO – Poltava Oblast. Means in each column followed by different letters are significantly different ($p < 0.05$)

by classical extraction techniques showed results of 60.02 mg GAE·g^{-1} of extract and by ultrasound extraction of 46.8 mg GAE·g^{-1} of extract (Stanisavljević et al., 2009). The whole plant extracts of *E. purpurea* from Taiwan exhibited 22.3 mg GAE·g^{-1} of TPC (Lee et al., 2010). *E. purpurea* flower extracts demonstrated a TPC from 176.33 to 183.08 mg caffeic acid equivalent per gram (Chen et al., 2015). In another study, the flower extracts showed 195.69 mg GAE·g^{-1} of TPC (Chiou et al., 2017). According to Russo et al. (2019), the TPC of *E. angustifolia* varied from 17.42 to 48.81 mg GAE·g^{-1} depending on the method. The herb ethanolic extracts of *E. purpurea* from Indonesia had TPC from 397.9 to 507.6 mg GAE·g^{-1} of extract (Sidhiq et al., 2020). Noce et al. (2024) studied the TPC of oral food supplements based on *E. angustifolia*, propolis, and three more components was 43.98 mg GAE·g^{-1}. According to Önder et al. (2023) determined in leaf extracts 10.97 mg GAE·g^{-1} of TPC.

Flavonoids is a large group of polyphenols and natural compounds with beneficial effects on health and distributed in different plant parts, wines, and teas (Panche et al., 2016). They include flavonols, flavones, isoflavonoids, anthocyanins, and chalcones and exhibit

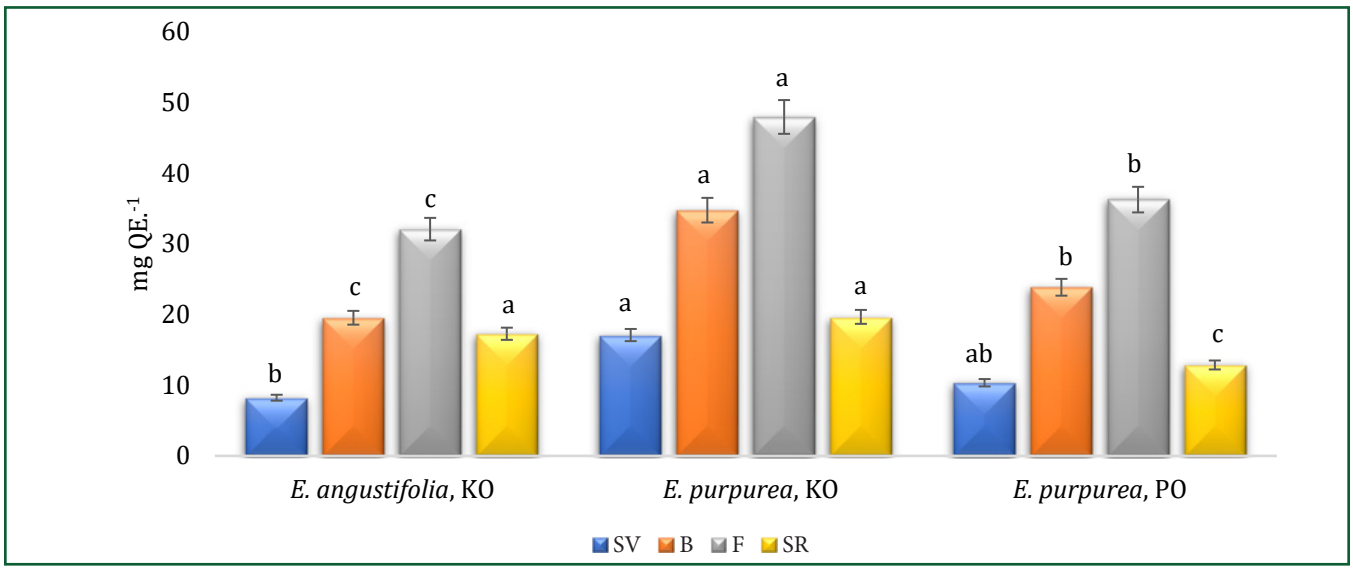

Figure 2 The total flavonoid content of ethanol extracts of *Echinacea* Moench species from different regions
QE – quercetin equivalent; SV – spring vegetation, B – budding, F – flowering stage, SR – seed ripening; KO – Kherson Oblast, PO – Poltava Oblast. Means in each column followed by different letters are significantly different ($p < 0.05$)

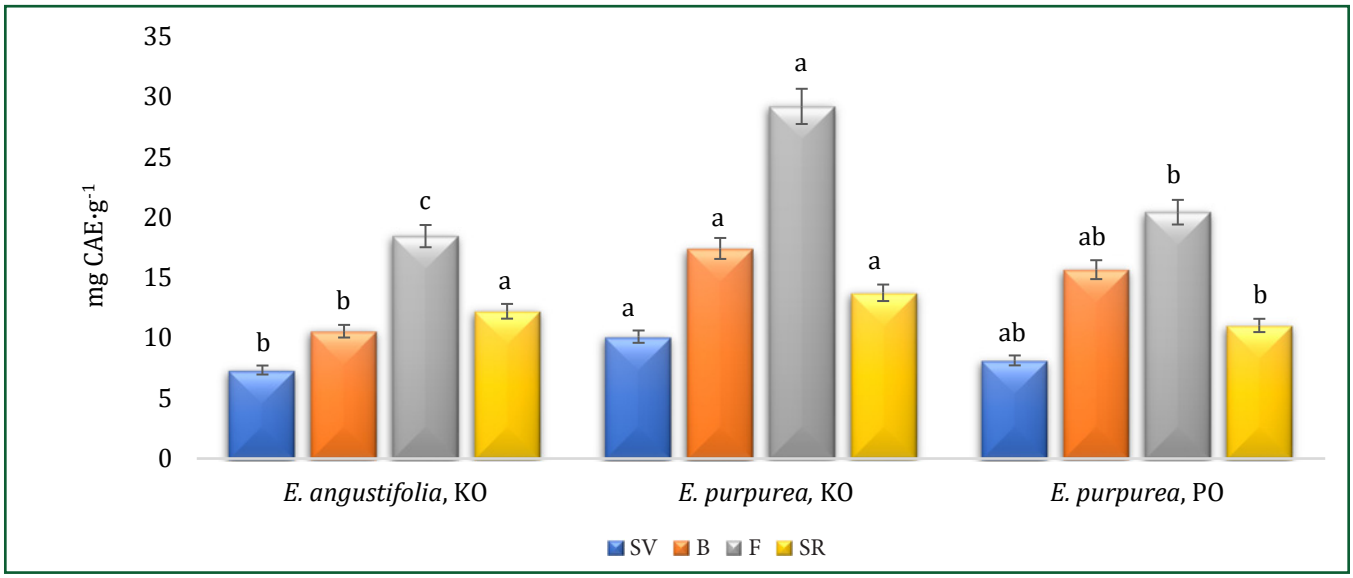

Figure 3 The total phenolic acid content of ethanol extracts of *Echinacea* Moench species from different regions
CAE – caffeic acid equivalent; SV – spring vegetation, B – budding, F – flowering stage, SR – seed ripening; KO – Kherson Oblast, PO – Poltava Oblast. Means in each column followed by different letters are significantly different (p <0.05)

antioxidant, inflammatory, antimicrobial, antiviral, and cardiovascular activities (Chen et al., 2023).

Also, the lowest content of total flavonoid compounds was found for extracts of *E. angustifolia* (Kherson Oblast) in the spring vegetation, budding, and flowering and *E. purpurea* (Poltava Oblast) in the seed ripening. The highest values of TFC were determined in the extracts of *E. purpurea* (Kherson Oblast) during the vegetation period. At all, the TFC in the spring vegetation was 8.23–17.10 mg QE·g^{-1}, in the budding of 19.54–34.78 mg QE·g^{-1}, in the flowering of 32.09–47.98 mg QE·g^{-1}, and in the seed ripening period of 12.86–19.67 mg QE·g^{-1} depending on species and region of growth (Figure 2).

Root extracts of *E. purpurea* showed that TFC in methanol was 3.3–32.8 mg GAE·g^{-1} and ethanol 1.5–32.4 mg GAE·g^{-1} (Wu et al., 2007). The TFC of *E. purpurea* extracts obtained by classical extraction techniques showed results of 32.3 mg·g^{-1} (rutin equivalent) of extract and by ultrasound extraction of 27 mg·g^{-1} (rutin equivalent) of extract (Stanisavljević et al., 2009). The whole extracts of Taiwan *E. purpurea* demonstrated 86 mg QE·g^{-1} of TFC (Lee et al., 2010). The TFC of Indonesian plants of *E. purpurea* was from 290.95 to 313.87 mg QE·g^{-1} of extracts (Sadhig et al., 2020). The study of morphological properties of *Echinacea purpurea* grown under salinity conditions showed a high accumulation of flavonoid content (Choirunnisa et al., 2021b). According to Önder et al. (2023), leaf extracts of *E. purpurea* had 11.88 mg of catechin equivalent per gram of TFC.

Phenolic acids are natural antioxidants, an important class of dietary polyphenols with essential roles in plant organisms such as plant growth, development, and defense from different stresses (Kumar and Goel, 2019; Kiokias et al., 2020).

The accumulation of the total phenolic acids in the investigated extracts was the following: 7.34–10.11 mg CAE·g^{-1} in the spring vegetation, 10.56–17.43 mg CAE·g^{-1} in the budding stage, 18.45–29.21 mg CAE·g^{-1} in the flowering, and 11.04–13.75 mg CAE·g^{-1} in the seed ripening period (Figure 3).

The total content of caffeic acid derivates in the flowering extracts of Taiwan *E. purpurea* was 78.42 mg·g^{-1} (Chiou et al., 2017). The study of Momchev et al. (2020) demonstrated phenolic acid accumulation from 14.13 to 206.2 µg.ml-1 of chlorogenic acid equivalent.

The molybdenum-reducing power of investigated extracts was 54.32–75.38 mg TE·g^{-1} in the spring vegetation, 76.89–88.14 mg TE·g^{-1} in the budding period, 98.03–120.16 mg TE·g^{-1} in the flowering period, and 109.31–161.34 mg TE·g^{-1} in the seed ripening period (Figure 4).

The study of *Artemisia balchanorum* Krasch. × *A. taurica* Willd showed the MRP of extracts was 4,143–209.93 mg TE·g^{-1} depending on the period of growth (Vergun et al., 2023). In another study, the reducing power by DPPH and CUPRIC methods showed higher values in the leaf extracts (Önder et al., 2023).

The free-radical scavenging activity of investigated extracts was in the following range: from 6.12

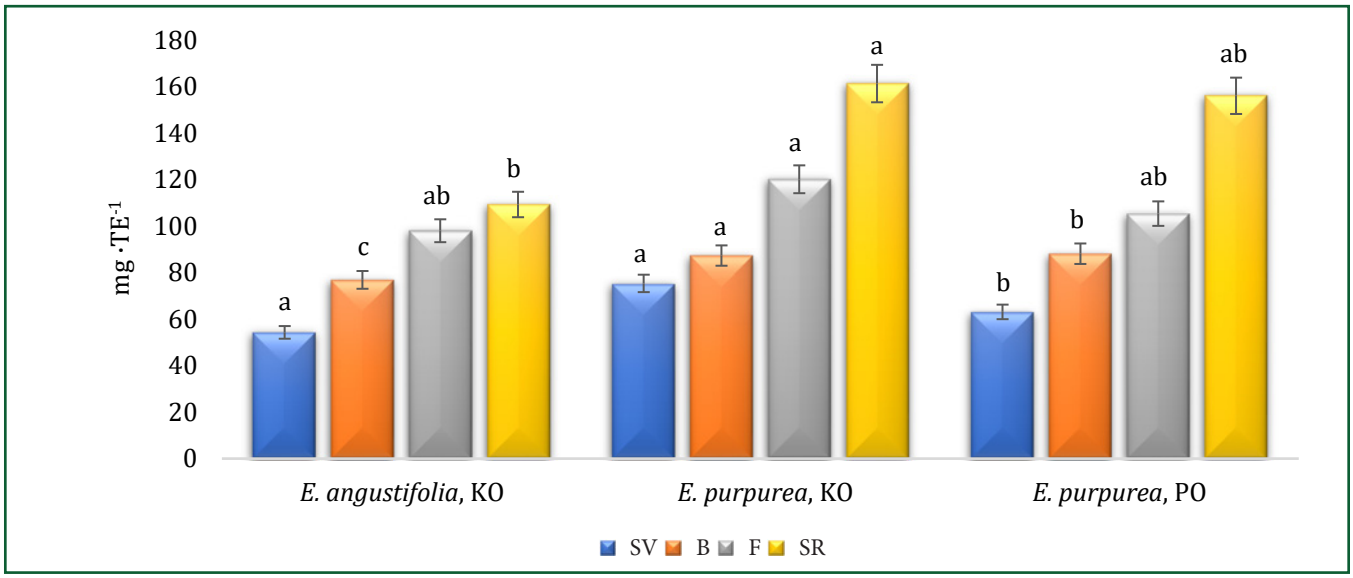

Figure 4 The molybdenum-reducing power of ethanol extracts of *Echinacea* Moench species from different regions
TE – Trolox equivalent; SV – spring vegetation, B – budding, F – flowering stage, SR – seed ripening; KO – Kherson Oblast, PO – Poltava Oblast. Means in each column followed by different letters are significantly different ($p < 0.05$)

(*E. angustifolia*) to 7.89 (*E. purpurea*, Kherson Oblast) mg TE·g^{-1} in the spring vegetation period, from 7.54 (*E. angustifolia*) to 8.52 (*E. purpurea*; Kherson Oblast) mg TE·g^{-1} in the budding stage, from 8.13 (*E. purpurea*; Poltava Oblast) to 9.69 (*E. purpurea*; Kherson Oblast) mg TE·g^{-1} in the flowering stage, and from 8.04 (*E. angustifolia*) to 9.65 (*E. purpurea*; Kherson Oblast) mg TE·g^{-1} in the seed ripening stage (Figure 5).

The studies of polyphenol compound accumulation demonstrated usually a strong positive correlation with different methods of antioxidant activity (Stagos et al., 2012).

A very strong correlation was found between all investigated antioxidant parameters in the spring vegetation period (Table 1). A very strong correlation in this period was also determined between the two methods of antioxidant activity determination. At the budding stage, a very strong correlation was detected between TPC and TFC, TPC and TPAC, TPC and FRSA, TFC and TPAC, TFC and FRSA, and TPAC and both studied antioxidant activity methods. The rest investigated parameters in that period demonstrated a strong correlation. All parameters in the flowering stage, besides TPC and FRSA, showed a strong correlation.

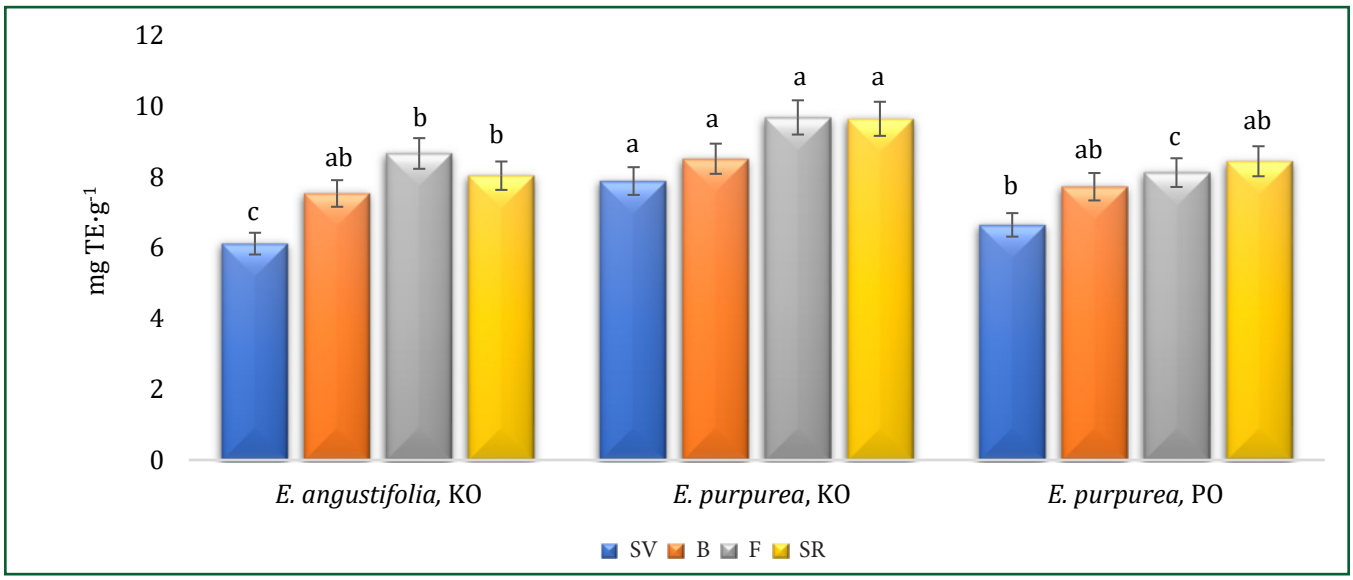

Figure 5 The free-radical scavenging activity of ethanol extracts of *Echinacea* Moench species from different regions
TE – Trolox equivalent; SV – spring vegetation, B – budding, F – flowering stage, SR – seed ripening; KO – Kherson Oblast, PO – Poltava Oblast. Means in each column followed by different letters are significantly different ($p < 0.05$)

Table 1 Correlation between antioxidant parameters of *Echinacea* Moench species.

Parameter	TPC	TFC	TPAC	MRP	FRSA
Spring vegetation					
TPC	1.000				
TFC	0.959*	1.000			
TPAC	0.973*	0.999**	1.000		
MRP	0.996**	0.980**	0.990**	1.000	
FRSA	0.976*	0.998**	0.998**	0.991**	1.000
Budding					
TPC	1.000				
TFC	0.997**	1.000			
TPAC	0.906**	0.868**	1.000		
MRP	0.733*	0.675*	0.952**	1.000	
FRSA	0.984**	0.995**	0.817**	0.601*	1.000
Flowering					
TPC	1.000				
TFC	0.971**	1.000			
TPAC	0.948**	0.997**	1.000		
MRP	0.986**	0.998**	0.988**	1.000	
FRSA	0.662*	0.823**	0.867**	0.778*	1.000
Seed ripening					
TPC	1.000				
TFC	0.964**	1.000			
TPAC	0.998	0.969**	1.000		
MRP	0.190*	-0.079	0.170*	1.000	
FRSA	0.783*	0.589*	0.770*	0.760*	1.000

Note: TPC – total polyphenol content; TFC – total flavonoid content; TPAC – total phenolic acid content; MRP – molybdenum-reducing power of extract; FRSA – free-radical scavenging activity of extracts; ** – correlation is significant at the level of 0.01; * – correlation is significant at the level of 0.05

In the seed ripening period, the very weak negative correlation between TFC and MRP (r = -0.079) was noted. A weak correlation was found between MRP and rest parameters in this period. A moderate correlation was found between TFC and FRSA in the seed ripening period. The rest parameters showed a strong or very strong correlation in this period.

A strong correlation between polyphenol content and antioxidant activity by the DPPH method was found for different varieties and organs of *E. purpurea* (Lin et al., 2023).

Conclusions

Thus, the obtained data demonstrated the accumulation of different groups of total polyphenol compounds and the antioxidant activity of *E. angustifolia* and *E. purpurea* plant raw material during the vegetation period. So, the ethanol extracts exhibited during the vegetation period, the TPC in the range of 21.15–78.34 mg GAE·g^{-1}, TFC in the range of 8.23–47.98 mg QE·g^{-1}, TPAC in the range of 7.34–29.21 mg CAE·g^{-1} depending on species, stage, and region of growth. The minimum values of these parameters were fixed for *E. angustifolia* (at the spring vegetation, budding, and seed ripening) and *E. purpurea*, Poltava Oblast (seed ripening period). The maximum values of these parameters were fixed for *E. purpurea*, Kherson Oblast (all investigated stages of growth). The MRP of investigated extracts was 54.32–161.34 mg TE·g^{-1}. The FRSA of extracts was 6.12–9.69 mg TE·g^{-1} during vegetation. The lowest values of antioxidant activity by both methods were found for *E. angustifolia* and the highest for E.purpurea, Kherson Oblast. The results of this study can be useful for further biochemical, ecological, and pharmaceutical investigations and breeding work.

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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