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CONTENT OF POLYPHENOLIC COMPOUNDS AND BIOLOGICAL ACTIVITY OF BERRIES, LEAVES AND FLOWERS OF *CRATAEGUS* L.

– Research paper –

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Abstract: In this study, the berries, leaves and flowers of six species of hawthorn (*Crataegus* L.) were evaluated for bioactive compounds (polyphenols, flavonoids, procyanides, UPLC profile) and their antioxidant activity ($ABTS^{\bullet+}$, CUPRAC, iron ion chelation, scavenging $O_2^{\bullet-}$ and OH^- radicals). Most of the analyzes were performed for the first time for this material. The content of bioactive compounds differed significantly both between the species and morphological parts studied. In particular, the highest content of polyphenols was determined for hawthorn berries (301.65 to 387.16 mg/100 g d.w.), including the highest concentration for *C. x subsphaericea*. The polyphenolic profile of this species was dominated by flavan-3-ols, with procyanidin trimer, (-)-epicatechin and procyanidin dimer being the most numerous. Hawthorn berries were also characterized by the highest antioxidant activity, among which the species *C. laevigata x rhipidophylla x monogyna* showed the strongest antiradical activity (0.91 mmol TE/g and 294.96 µg/ml for tests with the $ABTS^{\bullet+}$ and OH^- radical, respectively) and chelating iron ions (700.28 µg/ml). In conclusion, the results explain the traditional use of hawthorn in folk medicine and indicate a potentially new wider application as a source of natural antioxidants in the design of functional foods.

Keywords: *Crataegus* L., antioxidants, oxygen free radicals, polyphenols compounds

INTRODUCTION

Great interest in polyphenolic compounds as active ingredients of food products, including mainly functional foods, has resulted in wider research into many species of plants that have so far been widely used in folk medicine so far. Among them, hawthorn (*Crataegus* L.) belongs to this category of plants. Hawthorn belongs to the rose family (*Rosaceae*) and the apple subfamily (*Maliodeae*). The estimated number of hawthorn species in the world ranges from 20, through 185 to even 1200. Interspecific hybridization also contributes to significant biodiversity (Edwards et al., 2012; Rigelsky & Sweet, 2002).

Hawthorn's natural habitats are sunny and forested areas, with a predominance of clay, alkaline and moderately dry soils. Hawthorn grows in the form of thorny, densely branched 2-5-meter bushes or small trees reaching a height of up to 5-10 meters. Hawthorn is resistant to low temperatures and its lifespan can exceed 200 years (Venskutonis, 2018). Leaves in terms of shape, size and color are characterized by high species variability. They are usually single with persistent stipules. The shape of the lamina is obovate, rhomboid to broadly elliptical with 3-7 deep lobes, about 1.5 to 6 cm long. The gills are glossy dark green on the top, dull and bluish-green on the underside (Sagaradze et al., 2021). Hawthorn blooms in May and June. The flowers are hermaphrodite, insect-pollinated, white, rarely pink, collected in characteristic dense inflorescences. The flowers have from 1 to 5 pistil necks, from 10 to 20 stamens with red anthers. The fruits of hawthorn shrubs and trees, collected in fructoses, reach about 1 to 3 cm in diameter, are characterized by fleshy, white-cream flesh, spherical, ellipsoidal or ovoid in shape. Depending on the species and degree of maturity, the color of the berries varies from yellow, through green and red, to dark purple and black. Hawthorn berries ripen at the turn of August and September, have from 1 to 5, usually single-seeded seeds (Alirezalu et al., 2018, 2020; Król, 2011; Rigelsky & Sweet, 2002).

Hawthorn is a plant with a long history of use in the treatment and prevention of many diseases. Before it was used by the people of Europe, it was first

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known in North America. North American Indian tribes used decoctions of berries, roots, shoots and bark to treat gastrointestinal ailments. Other traditional uses included the treatment of edema and insomnia. In the form of a rinse, it was used in inflammation of the mucous membrane of the throat, mouth and in the treatment of cough, bronchitis and asthma. In traditional Chinese medicine, hawthorn berries were used primarily to improve blood circulation, remove blood stagnation, treat hypertension, hyperlipidemia and abdominal pain, indigestion and diarrhoea. In Europe, berries, flowers and leaves have traditionally been used in the treatment of cardiovascular disorders, as an antispasmodic, hypotensive, cardiotonic and anti-atherosclerotic agent (Edwards et al., 2012; Nekkaa et al., 2021; Venskutonis, 2018). Currently, hawthorn is considered one of the most valuable and valuable plant materials used in the treatment of cardiovascular disorders (Furey et al., 2010; Trexler et al., 2018).

The above health-promoting properties of this plant are mainly attributed to polyphenolic compounds (Alirezalu et al., 2018, 2020; Yang & Liu, 2012). Several groups of polyphenols have been described in various parts of hawthorn, including procyanidins, flavanols, flavonols, phenolic acids, anthocyanins and lignans. In berries, the main polyphenolic compounds are oligomeric procyanidins and their glycosides, while in leaves and flowers flavonols and flavonol glycosides predominate (Dekić et al., 2020; Edwards et al., 2012; González-Jiménez et al., 2018; Yang & Liu, 2012).

Despite the above data and traditional knowledge about the high health-promoting potential of various morphological parts of *Crataegus* L., little is known about the profile of secondary metabolites and the biological activity of individual morphological parts collected from different species of this plant. Therefore, this study aimed to characterize the qualitative and quantitative profile of polyphenolic compounds of six hawthorn berries, leaves, and flowers by spectrophotometric and UPLC-PDA-ESI-TQD-MS/MS methods, together with the *in vitro* assessment of antioxidant activity ($ABTS^{\bullet+}$, CUPRAC, iron ion chelation, $O_2^{\bullet-}$ and OH^- radicals scavenging), making such a broad characterization for the first time. The obtained results can provide a scientific basis for further use of hawthorn berries, leaves and/or flowers not only in the pharmaceutical industry, but also in the food industry as an active ingredient in functional food, which is currently one of the fastest growing segments of the food market.

MATERIALS AND METHODS

Materials and Reagents

2-Deoxy-D-ribose, cyanidin chloride, EDTA, ferrozine, gallic acid, NADH, neocuproine, quercetin were purchased from Sigma-Aldrich (Steinheim, Germany).

Polyphenolic standard for UPLC analyses were purchased from Sigma-Aldrich (Darmstadt, Germany) and Extrasynthese (Lyon, France). Other chemicals were obtained from Chempur (Piekary Śląskie, Poland).

Plant Material

Material from six species of hawthorn (*Crataegus* L.) was used in the study: (C1) *C. monogyna*, (C2) *C. rhipidophylla*, (C3) *C. x subsphaericea*, (C4) *C. laevigata x rhipidophylla x monogyna*, (C5) *C. macrocarpa* and (C6) *C. laevigata*. Samples of flowers (F) and leaves (L) were plucked in May, whereas berries (B) in October, from the natural environment located in the Podkarpackie Voivodeship (Poland) in 2018.

The material delivered to the laboratory was lyophilized (ALPHA 1-2 LD plus, Martin Christ GmbH, Germany). Freeze-dried berries (after removing the seeds), leaves and flowers were ground and stored at -20 °C.

Preparation of Extract

The extracts were prepared on the basis of our previous reports Żurek et al. (2020) (Żurek et al., 2020). In brief, the ground material was extracted with 50% and 70% ethanol using an ultrasonic bath (50 Hz, 30 °C) for 30 minutes (Sonic 10, Polsonic, Poland). After this time, the solutions were centrifuged at 10 000 *x* g for 10 min (Centrifuge 5430, Eppendorf, Hamburg, Germany). The resulting extracts were filtered through a 0.20 μm hydrophilic PTFE membrane and used for the assays.

Determination of the content of polyphenolic compounds

a. Determination of Total Phenolic Content (TPC)

TPC was estimated using the method of Gao et al. (2000) (X. Gao et al., 2000). Distilled water (2.0 ml), Folin-Ciocalteau solution (0.2 ml) and sodium carbonate (1 ml, 20%) were added to the extracts. The absorbance was measured at 765 nm (UV–VIS

spectrometer, UV2900, Hitachi, Japan). The results were expressed in mg of gallic acid per 1 g of dry weight (mg GAE/g dm).

b. Determination of Total Flavonoid Content (TFC)

TFC was evaluated using the procedure of Chang et al. (2020) (Chang et al., 2020). Ethanol (1.5 ml), sodium acetate (0.1 ml, 1 M), aluminum chloride (0.1 ml, 10%) and distilled water (2.8 ml) were added to the extracts. The absorbance was measured at 415 nm. The results were expressed in mg of milligram of quercetin per 1 g of dry weight (mg QE/g d.w.).

c. Determination of Total Proanthocyanidin Content (TPA)

TPA was evaluated using the procedure of Żurek et al. (2022) (Żurek et al., 2022). Iron (III) ammonium sulfate (0.1 ml, 2%, in 2 M HCl) and *n*-BuOH (3 ml, in 35% HCl) were added to the extracts. After heating to 95°C for 45 minutes, the absorbance was measured at 550 nm. The results were expressed in mg of cyanidin chloride per 1 g of dry weight (mg CYE/g d.w.)

Determination of Antioxidant Activity

a. $ABTS^{\bullet+}$ Radical Scavenging Activity (ABTS)

The ABTS test was evaluated using the procedure of Re et al (1999) (Re et al., 1999). Solution of $ABTS^{\bullet+}$ radicals (0.03 ml, with an absorbance of 0.7) were added to the extracts. The absorbance was measured at 734 nm. The results were expressed in Trolox equivalents per 1 g of dry weight (mmol TE/g d.w.).

b. Determination of Copper Ion Reduction (CUPRAC)

The CUPRAC test was evaluated using the procedure of Apak et al. (2006) (Apak et al., 2006). Copper chloride (1 ml, 0.01 M), neocuproine solution (1 ml, 0.0075 M) and acetate buffer (1 ml, 1 M) were added to the extracts. The absorbance was measured at 450 nm. The results were expressed as mmol TE/g d.w.

c. Chelating Ability of Metals Ion (ChA)

The ChA test was evaluated using the procedure of Mosmann (1983) (Mosmann, 1983). Iron II sulfate (0.2 ml, 0.1 mM) and ferrozine (0.2 ml, 0.25 mM) were added to the extracts. The absorbance was measured at 562 nm. The result was calculated as the IC_{50}.

d. Superoxide Radical Scavenging Activity Assay ($O_2^{\bullet-}$)

The $O_2^{\bullet-}$ test was evaluated using the procedure of Robak and Gryglewski (1988) (Robak & Gryglewski, 1988).

Nitrotetrazolium blue chloride (1 ml) and 1.0 ml phenazine methosulfate (1 ml) were added to the extracts. The absorbance was measured at 560 nm. The result was calculated as the IC_{50}.

e. Hydroxyl Radical Scavenging Activity Assay (OH^-)

The OH- test was evaluated using the procedure of Żurek et al. (2022) (Żurek et al., 2022). Mixture of 2-deoxyribose, iron ammonium sulphate, EDTA, ascorbic acid and perhydrol (1 ml) were added to the extracts. After holding for 1 h at 37 °C, trichloroacetic acid (1 ml) and thiobarbituric acid (0.5 ml) were added. The absorbance was measured at 532 nm. The result was calculated as the IC_{50}.

Determination of Polyphenols Profile

Identification and analysis of the content of polyphenolic compounds was carried out using the Ultra-Performance Liquid Chromatography Array Detector (UPLC-Q-TOF-MS, Waters, Milford, MA, USA) using the guidelines developed by Żurek et al. (2021) (Żurek et al., 2021). A UPLC BEH C18 column (1.7 µm, 100 mm x 2.1 mm, Waters, Warsaw, Poland) maintained at 50°C was used to separate individual polyphenolic compounds. The flow rate and injection volume were 0.35 ml/min and 5 µl, respectively. The separation was carried out using a mobile phase consisting of water (solvent A) and 40% acetonitrile (solvent B). The operating parameters of the UPLC-Q-TOF-MS were as follows: voltage 30 V, desolvation gas flow 800 l/h, gas flow con 100 l/h, source and desolvation temperature 120°C and 350°C, respectively. The results are presented in mg/100 g d.w.

Statistical Analysis

The results obtained in triplicate were subjected to statistical analysis. Duncan's test ($p < 0.05$), Pearson's correlation ($p < 0.05$; $p < 0.01$) and principal component analysis (PCA) were analyzed using Statistica 13.3 (StatSoft, Krakow, Poland).

RESULTS AND DISCUSSION

Total Phenolic, Flavonoid and Proanthocyanidin Contents

Among the compounds with health-promoting effects polyphenolic compounds are of great importance. Polyphenols are a large group of bioactive compounds with sensory properties as well as physiological functions beneficial to human health, thanks to which they are a desirable active ingredient in the food, pharmaceutical or cosmetic industries. (Pandey & Rizvi, 2009). Their content in berries, leaves, and flowers of six hawthorn species was expressed as total phenols (TPC), flavonoids (TFC) and total proanthocyanidins (TPA). The obtained results are presented in table 1.

Among the examined morphological parts, the highest range of TPC and TPA content was noted for hawthorn leaves. The obtained values ranged from 40.84 to 60.23 mg GAE/g d.w. and from 8.59 to 10.76 mg CYE/g d.w., respectively. For both groups of compounds, the highest concentration was found in the leaves of the species *C. laevigata x rhipidophylla x monogyna* (CL4). These values for berries (CB4) and flowers (CF4) of the same hawthorn species were respectively 1.1 and 1.2 times lower for TPC and 2.0 and 2.1 times lower for TPA.

The obtained TPC results are lower than those reported by Alirezalu et al. (2018, 2020). Among the analyzed berries, leaves and flowers of hawthorn, the highest TPC concentration was also found for leaves, but these values were higher than in this analysis and ranged from 27.21 (*C. monogyna*) to 87.73 (*C. pseudomelanocarpa*) mg GAE/g d.w. (Alirezalu et al., 2018, 2020). On the other hand, comparable concentrations of TPC were shown by Lin et al. (2022) in analyzes of *C. pinnatifida* berries (Lin et al., 2022) and Froehlicher et al. (2009) in analyzes of *C. monogyna* flowers (Froehlicher et al., 2009). In previous studies of TPA content in hawthorn berries, significantly lower values were determined than in our own research. In the work of Froehlicher et al. (2009) in *C. monogyna* berries, the TPA content was estimated at 0.97 mg CYE/g d.w., and in the work of González-Jiménez et al. (2018) estimated only 0.85 mg CYE/g d.w. in *C. pubescens* berries (Froehlicher et al., 2009; González-Jiménez et al., 2018). However, higher concentrations of TPA were recorded in hawthorn flowers. In *C. monogyna* flowers, Froehlicher et al. (2009) determined 17.12 mg CYE/g d.w. TPA. In turn, in the flowers of *C. azarolus*, studied by Bahri-Sahloul et al. (2009), 5.20 to 9.25 mg CYE/g d.w. TPA (Bahri-Sahl et al., 2009). The available literature lacks data on the content of TPA in hawthorn leaves.

In turn, in the case of TFC, among the analyzed morphological parts, the highest content range, ranging from 9.81 to 15.42 mg QE/g d.w., was observed for hawthorn flowers. The highest concentration was found for flowers of the species *C. rhipidophylla* (CF2). This value was 1.4 times higher for berries of the same species of hawthorn (CB2) and 1.6 times higher than for leaves (CL2). The obtained results for the TFC content are confirmed in the literature data. Alizeralu et al. (2018, 2020), analyzing hawthorn berries, leaves, and flowers, also found the highest concentration of TFC for flowers. Estimated values ranged from 2.27 mg (*C. orientalis*) to 17.40 (*C. songarica*) mg QE/g d.w. and were similar to the results presented in this study (Alirezalu et al., 2018, 2020). On the other hand, in other studies on hawthorn, significantly lower results of TFC content were reported for berries(Lin et al., 2022), leaves (Bignami et al., 2003; Orhan et al., 2007) and flowers (Froehlicher et al., 2009) compared to our results.

The presented results indicate that the content of polyphenolic compounds, flavonoids and proanthocyanidins in total is significantly influenced by both the hawthorn species and the analyzed morphological part of the plant. This thesis has already been confirmed in hawthorn studies conducted by other authors (Alirezalu et al., 2018; Özyürek et al., 2012). Environmental conditions, including UV radiation, hydration, temperature, humidity and access to nutrients available in the soil, can also modify the composition of polyphenolic compounds in hawthorn shrubs and trees. Kirakosyan et al. (2003) analyzing the content of TPC and TFC in hawthorn leaves, noticed an increase in the level of these groups of compounds under the influence of stress caused by cold or drought (Kirakosyan et al., 2003). Several studies also report different ranges of polyphenolic compounds depending on the maturity of the morphological parts of hawthorn at the time of harvest. Luo et al. (2016), examining hawthorn berries, leaves, twigs and roots harvested in the period from May to October, showed significant differences between the harvest dates and the content of total polyphenols and flavonoids, while recording the highest levels for berries, leaves and twigs in September, and for roots in July (Luo et al., 2016). The same observations appear in the work of Liu et al. (2011) and Gao et al. (2013) (Z. Gao et al., 2013; P. Liu, Kallio, & Yang, 2011).

Table 1. Total content of polyphenols, flavonoids and proanthocyanidins in extracts from berries, leaves, and flowers of six hawthorn species.

Sample symbol	TPC	TFC	TPA
	(mg GAE/g d.w.)	(mg QE/g d.w.)	(mg CYE/g d.w.)
CB1	47.21 ± 0.26^{d}	10.70 ± 0.06^{b}	5.21 ± 0.03^{g}
CB2	34.76 ± 0.28^{a}	11.08 ± 0.01^{c}	4.31 ± 0.01^{d}
CB3	39.83 ± 0.27^{b}	12.53 ± 0.00^{d}	4.65 ± 0.02^{e}
CB4	55.57 ± 0.10^{f}	12.35 ± 0.00^{d}	5.40 ± 0.00^{h}
CB5	43.64 ± 0.11^{c}	10.36 ± 0.01^{b}	5.93 ± 0.01^{i}
CB6	42.93 ± 0.13^{c}	11.32 ± 0.01^{c}	4.91 ± 0.01^{f}
CL1	57.77 ± 0.13^{f}	10.07 ± 0.06^{b}	10.19 ± 0.02^{m}
CL2	48.66 ± 0.39^{d}	9.82 ± 0.01^{a}	9.47 ± 0.01^{l}
CL3	50.65 ± 0.24^{d}	11.28 ± 0.17^{c}	9.41 ± 0.01^{l}
CL4	60.23 ± 0.10^{g}	12.77 ± 0.04^{d}	10.76 ± 0.02^{n}
CL5	40.84 ± 0.04^{b}	12.36 ± 0.04^{d}	8.59 ± 0.01^{k}
CL6	49.56 ± 0.01^{d}	9.45 ± 0.04^{a}	9.38 ± 0.01^{l}
CF1	52.96 ± 0.09^{e}	13.62 ± 0.01^{e}	5.84 ± 0.04^{i}
CF2	55.62 ± 0.50^{f}	15.42 ± 0.02^{f}	7.00 ± 0.01^{j}
CF3	48.37 ± 0.07^{d}	15.02 ± 0.23^{f}	3.79 ± 0.07^{c}
CF4	52.62 ± 0.36^{e}	10.16 ± 0.25^{b}	5.21 ± 0.01^{g}
CF5	43.09 ± 0.35^{c}	9.81 ± 0.09^{a}	2.71 ± 0.00^{b}
CF6	36.36 ± 0.23^{a}	13.57 ± 0.01^{e}	2.39 ± 0.26^{a}

C, *Crataegus* L.; B, berries; L, leaves; F, flowers; 1-6, hawthorn species. Results are expressed as mean ± SD. The values in the columns marked with different letters indicate statistically significant differences ($p < 0.05$).

Phytochemicals Constituents Profile of *Crataegus* L. Berries, Leaves and Flowers Extracts

Identification of Polyphenolic Compounds

Identification of polyphenolic compounds in berries, leaves and flowers of six hawthorn species was carried out using UPLC-PDA-ESI-MS. The results are presented in Table 2.

In total, 21 polyphenolic compounds were detected, including 17 compounds in berries, 11 compounds in leaves and 10 in hawthorn flowers. In the group of all 21 identified compounds, 4 were assigned to the group of anthocyanins, 6 to the group of flavan-3-ols, 4 to the group of phenolic acids, and 7 compounds to the group of flavones and flavonols.

The first group, anthocyanins, was identified only in hawthorn berries. In each of the analyzed extracts, four compounds belonging to this group were detected. Peaks **1** and **3** had a fragment ion at *m/z* 287, which is reported in the literature to be cyanidin (Song et al., 2011). On this basis, these compounds were assigned to cyanidin derivatives as 3-O-glucoside (m/z 449) and 3-O-arabinoside (m/z 419), respectively. Peak no. **2** was identified as pelargonidine 3-*O*-rutinoside with a pseudomolecular peak with of *m/z* of 579 and fragment ions of *m/z* 271 and 433 (corresponding to loss of rutinose and rhamnose). The another peak (**4**) with pseudomolecular ion *m/z* 463 and fragmentation ion 301 *m/z* was assigned to peonidine 3-*O*-glucoside based on available standards. The anthocyanins listed above have already been described in the analysis of the polyphenolic composition of berries: *C. almaatensis* (Bekbolatova et al., 2018), *C. monogyna* (Mraihi et al., 2015; Wyspiańska et al., 2017) and *C. pinnatifida* (S. Liu et al., 2016).

Another group of polyphenolic compounds identified in hawthorn were flavan-3-ols. Six flavan-3-ols were detected in the berries, four in the leaves and two in the flowers. Compounds **5**, **6** and **9** had an MS/MS fragmentation ion at *m/z* 289 representing epicatechin (Gu et al., 2003). These compounds were identified as trimer (*m/z* 865), dimer (*m/z* 577) and tetramer (*m/z* 1442) procyanidin type B, respectively. Peak **7** and **8** showing the molecular ion *m/z* 289 were assigned to (+)-catechin and (-)-epicatechin, as a result of available patterns. Peak no. **10**, representing the ion at *m/z* 619 and *m/z* 169, 301, 305 fragments, was identified as epigallocatechin gallate 4''-glucoside based on literature data (Çoklar & Akbulut, 2016). The detected procyanidins, (+)-catechin, (-)-epicatechin and epigallocatechin gallate have already been presented in papers describing the polyphenolic profile of the species: *C. almaatensis* (Bekbolatova et al., 2018), *C. pinnatifida* (Lin et al., 2022), *C. pubescens* (González-Jiménez et al., 2018), *C. azarolus* (Mraihi et al., 2015) and *C. orientalis* (Çoklar & Akbulut, 2016).

The third main group of polyphenolic compounds identified in hawthorn were phenolic acids. Three

phenolic acids have been identified in berries, leaves and flowers. Peak no. **11** had fragmentation ions typical of coumaric acid (*m/z* 163, 119). Based on literature data, it has been identified as 3-*O*-*p*-coumaroylquinic acid (Jaiswal & Kuhnert, 2011). Compounds **12** and **13** representing quinic acid derivatives with a pseudomolecular peak of *m/z* 353, during fragmentation broke up into two fragments with *m/z* 191 and 173. These are typical mass ions for caffeoylquinic acid, hence these compounds were identified as 4-*O*-caffeoylquinic acid and 3-*O*-caffeoylquinic acid, respectively (Jaiswal & Kuhnert, 2011; Plazonić et al., 2009). Compound number **14**, also in this category, was identified as 3,4-*O*-dicaffoylquinic acid (515 *m/z*), with a fragmentation ion at *m/z* 353. Our own results regarding the identification of phenolic acids are consistent with previously published works for species: *C. almaatensis* (Bekbolatova et al., 2018), *C. pinnatifida* (Lin et al., 2022), *C. pubescens* (González-Jiménez et al., 2018) and *C. azarolus* (Mraihi et al., 2015).

The last major group of compounds were flavones and flavonols. Four compounds were detected in berries and leaves, and five in flowers. Four quercetin derivatives have been identified, such as 3-*O*-rutinoside (**15**, *m/z* 609), 3-*O*-glucoside (**16**, *m/z* 463), 3-*O*-galactoside (**17**, m/z 463) and 3-*O*-acetyl hexoside (**18**, *m/z* 505). These compounds had the same fragmentation ion of *m/z* 301 characteristic of quercetin aglycone. The analyzed compounds **19** and **20** with *m/z* 577 and 431 ions characteristic of apigenin derivatives were identified as apigenin 7-*O*-rutinoside and apigenin 8-*C*-glucoside, respectively. The assignment was based on the fragmentation spectrum and comparison with previously published data (Belkhir et al., 2013). Based on the characteristic fragmentation ion m/z 317, peak no. **21** (*m/z* 463) were assigned myricetin 3-*O*-rhamnoside. The compounds listed above have been described in the species literature: *C. almaatensis* (Bekbolatova et al., 2018), *C. pinnatifida* (Lin et al., 2022), *C. monogyna* (Mraihi et al., 2015) and *C. azarolus* (Mraihi et al., 2015).

Table 2. Polyphenolic compounds identified in extracts from berries, leaves, and flowers of six hawthorn species.

No.	Identified compound	λ_{max}	[M-H] *m/z*		Morphological part		
		nm	MS	MS/MS	B	L	F
Anthocyanins							
1	Cyanidin 3-*O*-glucoside	278, 514	449^+	287	+		
2	Pelargonidine 3-*O*-rutinoside	279, 517	579^+	271, 433	+		
3	Cyanidin 3-*O*-arabinoside	274, 510	419^+	287	+		
4	Peonidine 3-*O*-glucoside	278, 516	463^+	301	+		
Flavan-3-ols							
5	Procyanidin trimer (type B)	278	865	289	+	+	
6	Procyanidin dimer (type B)	279	577	289	+	+	+
7	(+)-catechin	281	289	-	+		
8	(-)-epicatechin	274	289	-	+	+	+
9	Procyanidin tetramer (type B)	278	1442	720, 577, 289	+		
10	Epigallocatechin gallate 4"-glucoside	268, 377	619	169, 305, 301	+	+	
Phenolic acids							
11	3-*O*-*p*-coumaroylquinic acid	309	337	163, 119	+	+	+
12	4-*O*-caffeoylquinic acid	299sh, 324	353	191, 173	+	+	
13	3-*O*-caffeoylquinic acid	299sh, 321	353	191, 173	+		+
14	3,4-*O*-caffeoylquinic acid	299sh, 327	515	353		+	+
Flavones and flavonols							
15	Quercetin 3-*O*-rutinoside	289sh, 353	609	301, 300, 271			+
16	Quercetin 3-*O*-glucoside	255, 355	463	301	+	+	+
17	Quercetin 3-*O*-galactoside	255, 352	463	301	+	+	+
18	Quercetin 3-*O*-acetyl hexoside	260, 352	505	301			+
19	Apigenin 7-*O*-rutinoside	266, 338	577	296, 112			+
20	Apigenin 8-*C*-glucoside	266, 338	431	311, 341, 289	+	+	
21	Myricetin 3-*O*-rhamnoside	281	463	317	+	+	

$[M-H]^-$, negative ion values; *m/z*, mass-to-charge ratio; B, berries; L, leaves; F, flowers; +, compound identified.

Quantification of Polyphenolic Compounds

The results of the content of polyphenolic compounds in hawthorn berries, leaves and flowers are presented in Figure 1 and in Tables 3-5. The highest content of polyphenolic compounds was found in hawthorn berries. Estimated values ranged from 301.65 to 387.16 mg/100 g d.w. The highest content of polyphenolic compounds was found in hawthorn berries *C. x subsphaericea* (CB3), of which 59.6% of all polyphenols were flavan-3-ols. The content of this group of compounds was 232.98 mg/100 g d.m. The dominant compounds were: procyanidin trimer (85.78 mg/100 g d.w.), (-)-epicatechin (68.63 mg/100 g d.w.) and procyanidin dimer (59.80 mg/100 g d.w.). Other groups of compounds occurred in the following order: phenolic acids (24.0%) > flavonols (16.4%) > anthocyanins (0.4%).

Polyphenol profile studies conducted by Lin et al. (2022), Wen et al. (2015) and Liu et al. (2011), showed a dominant share of procyanidins in *C. pinnatifida* berries, the concentration of which ranged from 10.00 to 240.00 mg/100 g d.w. The highest concentration was found in procyanidin B2 (Lin et al., 2022; P. Liu, Kallio, Lü, et al., 2011; Wen et al., 2015). In own research, the sum of all procyanidins ranged from 226.75 to 240.68 mg/100 g d.w, and the predominant compound was mainly procyanidin trimer (type B). A different view was presented in their analyzes by Bekbolatova et al. (2018) and Froehlicher et al. (2009), who recognized quercetin 3-*O*-galactoside as the main compound found in hawthorn berries (*C. almaatensis* and *C. monogyna*), the content of which was estimated at the level of 37.6 to 70.00 mg/100 g d.w. (Bekbolatova et al., 2018; Froehlicher et al., 2009). In turn, in the study by Froehlicher et al. (2009) on *C. monogyna* berries, it was found that the dominant polyphenolic compounds also include (-)-epicatechin, the concentration of which ranged from 32.4 to 136.6 mg/100 g d.w. (Froehlicher et al., 2009). The same thesis was put forward by Gonzalez-Jimenez et al. (2018), assessing the content of (-)-epicatechin as one of the main polyphenolic compounds of *C. pubescens* berries at the level of 4.32 mg/100 g d.w. (González-Jiménez et al., 2018). In own research, the content of (-)-epicatechin ranged from 68.47 to 82.61 mg/100 g d.w.

The content of polyphenolic compounds in hawthorn leaves ranged from 248.30 to 365.38 mg/100 g d.w. The leaves of the species *C. monogyna* (CL1) were characterized by the highest content of polyphenolic compounds, where about 55.2% of all polyphenols were phenolic acids. Their amount was 201.80 mg/100 g d.w. In this group, the dominant compounds were: 3,4-*O*-dicaffeoyl-quinic acid (171.31 mg/100 g d.w.), 3-*O*-*p*-coumaroylquinic acid (23.12 mg/100 g d.w.) and 4-*O*-caffeoylquinic acid (7.38 mg/100 g dm). Other groups of compounds occurred in the following order: flavan-3-ols (34.7%) > flavonols (10.0%).

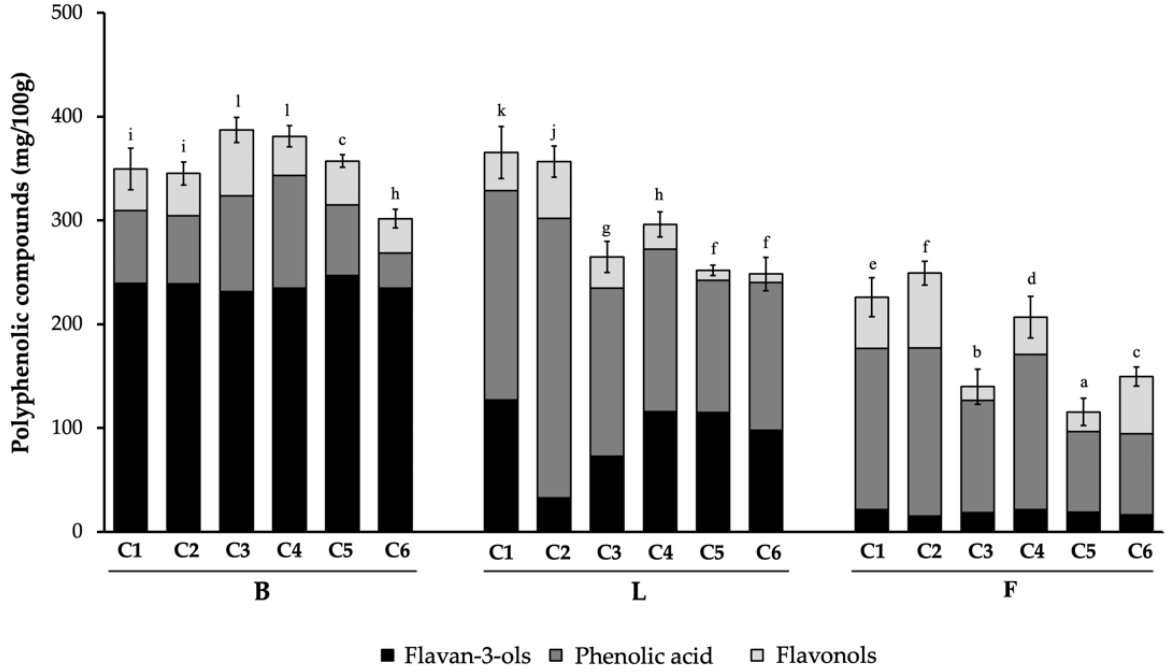

Figure 1. Concentration of polyphenolic compounds (mg/100 g) detected in fruits, leaves and flowers of six hawthorn species. Mean ± SD values are shown in the graph. Values marked with different letters indicate statistically significant differences ($p < 0.05$). Abbreviations: B, berries; L, leaves; F, flowers; C1–C6, tested species hawthorn.

Table 3. Content of polyphenolic compounds (mg/100 g dw) in extracts from the berries of six hawthorns (*Crataegus* L.) species (CB1 - CB6).

Identified compound		***Crataegus* L. species**					
		CB1	**CB2**	**CB3**	**CB4**	**CB5**	**CB6**
Anthocyanins							
1	Cyanidin 3-*O*-glucoside	1.08 ± 0.27c	0.83 ± 0.03a	0.98 ± 0.15c	0.98 ± 0.05c	0.91 ± 0.05b	0.98 ± 0.25c
2	Pelargonidine 3-*O*-rutinoside	0.20 ± 0.03a	0.21 ± 0.00a	0.20 ± 0.00a	0.21 ± 0.01a	0.25 ± 0.00b	0.20 ± 0.09a
3	Cyanidin 3-*O*-arabinoside	0.22± 0.03a	0.21 ± 0.04a	0.20 ± 0.05a	0.22 ± 0.00a	0.19 ± 0.03a	0.21 ± 0.02a
4	Peonidine 3-*O*-glucoside	0.23 ± 0.01a	0.24 ± 0.04a	0.22 ± 0.00a	0.22 ± 0.02a	0.23 ± 0.05a	0.24 ± 0.01a
	Sum	**1.74 ± 0.28c**	**1.48 ± 0.09a**	**1.64 ± 0.26b**	**1.65 ± 0.26b**	**1.61 ± 0.22b**	**1.64 ± 0.20b**
Flavan-3-ols							
5	Procyanidin trimer (type B)	79.68 ± 15.90b	80.11 ± 15.24b	85.78 ± 18.82c	82.92 ± 10.13b	87.75 ± 10.61c	76.62 ± 4.12a
6	Procyanidin dimer (type B)	68.36 ± 9.08c	63.84 ± 9.06b	59.80 ± 3.96a	63.33 ± 3.95b	68.63 ± 3.91c	62.89 ± 7.07b
7	(+)-catechin	6.88 ± 3.20b	7.58 ± 21.16b	9.81 ± 0.24c	9.93 ± 4.65c	6.68 ± 0.41b	4.72 ± 2.59a
8	(-)-epicatechin	77.32 ± 9.07c	73.80 ± 8.97b	68.63 ± 1.18a	68.47 ± 0.72a	74.37 ± 1.43b	82.61 ± 0.89d
9	Procyanidin tetramer (type B)	2.52 ± 0.08a	2.93 ± 0.90a	2.72 ± 0.46a	3.54 ± 1.63b	3.25 ± 0.27b	2.87 ± 0.21a
10	Epigallocatechin gallate 4"-glucoside	2.87 ± 0.33a	9.29 ± 0.59c	2.98 ± 0.92a	4.79 ± 1.31b	4.58 ± 0.49b	3.28 ± 0.57a
	Sum	**237.63 ± 37.66c**	**237.56 ± 55.92c**	**229.73 ± 25.59a**	**232.98 ±22.41b**	**245.26 ± 17.13d**	**233.00 ± 15.45b**
Phenolic acids							
11	3-*O*-*p*-coumaroylquinic acid	21.51 ± 2.37c	18.66 ± 13.79b	18.50 ± 0.98b	20.38 ± 2.77c	33.91 ± 0.33d	14.79 ± 3.86a
12	4-*O*-caffeoylquinic acid	23.08 ± 0.27b	32.07 ± 4.01c	22.94 ± 4.39b	33.29 ± 1.64c	19.18 ± 2.89a	18.04 ± 0.78a
13	3-*O*-caffeoylquinic acid	25.60 ± 3.37c	14.82 ± 0.36b	51.06 ± 8.42d	55.00 ± 2.94d	15.01 ± 1.52b	0.94 ± 0.08a
	Sum	**70.19 ± 6.01b**	**65.55 ± 18.16b**	**92.50 ± 13.79c**	**108.67 ± 7.36d**	**68.10 ± 4.74b**	**33.78 ± 5.32a**
Flavones and flavonols							
16	Quercetin 3-*O*-glucoside	29.42 ± 0.15a	34.79 ± 1.67c	45.30 ± 0.69d	31.63 ± 0.80b	34.87 ± 0.24c	29.70 ± 1.20a
17	Quercetin 3-*O*-galactoside	6.27 ± 0.49c	1.98 ± 0.86a	15.82 ± 2.26d	3.87 ± 0.59b	4.58 ± 0.06b	1.29 ± 0.81a
20	Apigenin 8-*C*-glucoside	2.74 ± 0.54b	2.68 ± 0.67b	2.17 ± 0.13a	2.29 ± 0.05a	2.66 ± 0.03b	2.24 ± 0.00a
21	Myricetin 3-*O*-rhamnoside	1.55 ± 0.05b	1.15 ± 0.01a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Sum	**39.98 ± 4.23c**	**40.61 ± 4.21c**	**63.29 ± 3.07d**	**37.79 ± 2.44b**	**42.12 ± 0.33c**	**33.24 ± 2.01a**
	Total content (mg/100 g dw)	**349.54 ± 48.18b**	**345.19 ± 78.38b**	**387.16 ± 42.71d**	**381.08 ±32.47d**	**357.11 ± 22.46c**	**301.65 ± 23.04a**

Values are expressed as mean ± SD. Means with different letters in the same row (between species) indicate statistically significant differences ($p < 0.05$), as assessed by Duncan's test.

Table 4. Content of polyphenolic compounds (mg/100 g dw) in leaves extracts of six hawthorns (*Crataegus* L.) species (CL1 - CL6).

Identified compound		***Crataegus* L. species**					
		CL1	**CL2**	**CL3**	**CL4**	**CL5**	**CL6**
Flavan-3-ols							
5	Procyanidin trimer (type B)	12.56 ± 0.51c	8.5 ± 0.37a	12.52 ± 0.66c	16.11 ± 0.26e	14.45 ± 0.49d	10.47 ± 0.28b
6	Procyanidin dimer (type B)	27.85 ± 0.48b	13.28 ± 0.44a	14.16 ± 0.23a	81.38 ± 0.44d	33.83 ± 0.22c	68.32 ± 0.05e
8	(-)-epicatechin	16.34 ± 0.12c	9.24 ± 0.09a	13.30 ± 0.03b	17.41 ± 0.00c	18.30 ± 0.10d	17.53 ± 0.06c
10	Epigallocatechin gallate 4"-glucoside	70.20 ± 0.23e	1.53 ± 0.05b	32.87 ± 0.19c	0.66 ± 0.03a	48.29 ± 0.41d	1.20 ± 0.03b
	Sum	**126.95 ± 0.99e**	**32.56 ± 0.81a**	**72.85 ± 0.90b**	**115.56 ± 0.71d**	**114.87 ± 0.72d**	**97.54 ± 0.33c**
Phenolic acids							
11	3-*O*-*p*-coumaroylquinic acid	23.12 ± 1.36e	13.99 ± 0.97bc	14.87 ± 0.88c	19.03 ± 1.30d	13.40 ± 0.44b	9.36 ± 0.19a
12	4-*O*-caffeoylquinic acid	7.38 ± 0.67e	1.36 ± 0.00b	4.45 ± 0.00d	10.60 ± 0.00f	2.47 ± 0.00c	0.51 ± 0.00a
14	3,4-*O*-caffeoylquinic acid	171.31 ± 0.01e	254.18 ± 0.10f	142.49 ± 0.04d	127.09 ± 0.18b	111.56 ± 0.23a	132.93 ± 0.14c
	Sum	**201.80 ± 2.04e**	**269.54 ± 1.08f**	**161.83 ± 0.92d**	**156.72 ± 1.49c**	**127.43 ± 0.68a**	**142.82 ± 0.33b**
Flavones and flavonols							
16	Quercetin 3-*O*-glucoside	23.96 ± 1.14d	5.55 ± 0.79b	5.86 ± 0.09b	14.01 ± 0.06c	5.62 ± 0.06b	3.35 ± 0.37a
17	Quercetin 3-*O*-galactoside	10.67 ± 0.68e	12.61 ± 0.14f	6.43 ± 0.63c	7.57 ± 0.24d	2.03 ± 0.41b	1.25 ± 0.01a
20	Apigenin 8-*C*-glucoside	0.25 ± 0.06a	4.40 ± 0.12c	2.87 ± 0.07b	0.25 ± 0.09a	0.25 ± 0.04a	0.31 ± 0.02a
21	Myricetin 3-*O*-rhamnoside	0.30 ± 0.01a	30.87 ± 1.12d	13.09 ± 0.01c	0.44 ± 0.03a	0.30 ± 0.02a	0.67 ± 0.03b
	Sum	**36.62 ± 1.82f**	**54.54 ± 2.17e**	**30.00 ± 0.81d**	**23.90±0.33c**	**9.46 ± 0.47b**	**7.95 ± 0.43a**
	Total content (mg/100 g dw)	**365.38 ± 4.86e**	**356.64 ± 4.06b**	**264.68 ± 2.63c**	**296.19 ± 2.53d**	**251.77 ± 1.87a**	**248.30 ± 1.09a**

Values are expressed as mean ± SD. Means with different letters in the same row (between species) indicate statistically significant differences ($p < 0.05$), as assessed by Duncan's test.

Table 5. Content of polyphenolic compounds (mg/100 g dw) in flower extracts of six hawthorns (*Crataegus* L.) species (CF1 - CF6).

Identified compound		***Crataegus* L. species**					
		CF1	**CF2**	**CF3**	**CF4**	**CF5**	**CF6**
Flavan-3-ols							
6	Procyanidin dimer (type B)	12.15 ± 0.58b	8.21 ± 0.58a	10.36 ± 0.57ab	11.93 ± 0.58b	9.92 ± 0.58ab	8.55 ± 0.58a
8	(-)-epicatechin	9.05 ± 0.12d	6.67 ± 0.35a	8.15 ± 0.23c	9.20 ± 0.29	8.83 ± 0.83cd	7.94 ± 0.74b
	Sum	**21.21 ± 0.70d**	**14.91 ± 0.93a**	**18.51 ± 0.81c**	**21.14 ± 0.41d**	**18.75 ± 0.81c**	**16.50 ± 0.53b**
Phenolic acids							
11	3-*O*-*p*-coumaroylquinic acid	5.53 ± 2.45d	0.23 ± 0.12a	2.61 ± 0.32c	5.81 ± 1.22d	1.12 ± 0.09b	0.25 ± 0.09a
13	3-*O*-caffeoylquinic acid	119.07 ± 10.58d	113.84 ± 10.58c	76.95 ± 10.57b	114.80 ± 10.57c	54.92 ± 10.58a	53.13 ± 10.58a
14	3,4-*O*-caffeoylquinic acid	30.99 ± 3.46cd	48.02 ± 3.46d	28.41 ± 3.45c	29.18 ± 3.45c	21.73 ± 3.45a	24.35 ± 3.45b
	Sum	**155.59 ± 15.32e**	**162.09 ± 1.73f**	**107.98 ± 0.69c**	**149.79 ± 0.89d**	**77.77 ± 2.50b**	**77.75 ± 1.49a**
Flavones and flavonols							
16	Quercetin 3-*O*-glucoside	18.21 ± 0.86f	1.96 ± 0.23a	5.71 ± 0.22b	10.69 ± 0.06d	7.10 ± 0.23c	15.75 ± 0.13e
17	Quercetin 3-*O*-galactoside	2.65 ± 1.48c	18.68 ± 0.98d	0.52 ± 0.02b	0.47 ± 0.07b	0.35 ± 0.15b	0.08 ± 0.02a
18	Quercetin 3-*O*-acetyl hexoside	2.20 ± 0.38b	4.36 ± 1.25d	0.35 ± 0.08a	2.17 ± 0.12b	0.82 ± 0.05a	3.14 ± 0.39c
19	Quercetin 3-*O*-rutinoside	1.22 ± 0.98a	2.36 ± 0.29c	3.46 ± 0.38d	1.13 ± 0.38a	2.08 ± 0.87c	1.19 ± 0.09a
20	Apigenin 8-*C*-glucoside	24.82 ± 11.03c	44.77 ± 3.00e	3.24 ± 0.05a	21.49 ± 0.83c	8.58 ± 1.97b	35.16 ± 2.26d
	Suma	**49.10 ± 3.34d**	**72.13 ± 13.52e**	**13.28 ± 1.98a**	**35.97 ± 3.27c**	**18.93 ± 2.20b**	**55.33 ± 3.99d**
	Total content (mg/100 g dw)	**225.90 ± 18.47d**	**249.13 ± 17.21e**	**139.78 ± 7.98b**	**206.90 ± 11.34c**	**115.45 ± 4.05a**	**149.57 ± 5.12b**

Values are expressed as mean ± SD. Means with different letters in the same row (between species) indicate statistically significant differences ($p < 0.05$), as assessed by Duncan's test.

Quantitative studies of the polyphenol profile by Bekbolatov et al. (2018) and Belkhir et al. (2013) showed that the dominant polyphenol in hawthorn leaves is quercetin 3-*O*-galactoside. The content of this metabolite in *C. almaatensis* leaves was 219.00 mg/100 g d.w. (Bekbolatova et al., 2018; Belkhir et al., 2013). Quercetin 3-*O*-galactoside content in leaves of *C. pinnatifida* assessed by Gao et al. (2013) was much lower and amounted to 1.00 mg/100 g d.w. (Z. Gao et al., 2013). In own research, the content of this metabolite ranged from 1.25 to 12.61 mg/100 g d.w. In turn, in the study by Gao et al. (2013), the main compounds found in leaves of *C. pinnatifida* included vitexin 2-*O*-rhamnoside, whose estimated concentration ranged from 448.00 to 561.00 mg/100 g d.w. (Z. Gao et al., 2013). Vitexin 2-*O*-rhamnoside was also one of the two dominant compounds among the polyphenolic profile of hawthorn leaves analyzed by Alirezalu et al. (2018). For the species the highest level was found (425.00 mg/100 g d.w.), and *C. curvisepala* the lowest (3.00 mg/100 g d.w.) (Alirezalu et al., 2018). The presence of this compound was not found in our own research.

The content of polyphenolic compounds in hawthorn flowers ranged from 115.45 to 249.13 mg/100 g d.w. The highest concentration of polyphenolic compounds was found in flowers of the species *C. rhipidophylla* (CF2), where the most numerous groups were phenolic acids (162.09 mg/100 g d.w.), constituting 65.1% of all polyphenols. In this group of compounds, the highest concentration was found in: 3-*O*-*p*-coumaroylquinic acid (113.84 mg/100 g d.w.) and 3,4-*O*-dicaffeoylquinic acid (48.02 mg/100 g d.w.). The order of the remaining categories of polyphenolic compounds was as follows: flavonols (28.9%) > flavan-3-ols (6.0%).

According to the available literature on the polyphenolic composition of hawthorn flowers, their main polyphenolic compound is quercetin 3-*O*-galactoside (Nabavi et al., 2015). Bekbolatova et al. (2018) and Bhri-Sahloul et al. (2009) confirmed this thesis for flowers of *C. almaatensis* and *C. azarolus*, assessing the content of this compound at the level of 24.70 to 412.20 mg/100 g d.w. (Bahri-Sahl et al., 2009; Bekbolatova et al., 2018). In own research, the content of quercetin 3-*O*-galactoside ranged from 0.08 to 18.68 mg/100 g d.w. Other results were obtained by Alirezalu et al. (2018) and Froehlicher et al. (2009), who showed that the dominant compound in the polyphenolic composition was chlorogenic acid. Its concentration ranged from 49.00 (*C. pseudoheterophylla*) to 1267.00 (*C. pseudomelanocarpa*) mg/100 g d.w. (Bekbolatova et al., 2018; Froehlicher et al., 2009). In own research, chlorogenic acid derivatives dominated in the preparations obtained.

Antioxidant Activity

The multifaceted nature of the antioxidant activity of the plant matrix requires the use of several different methods for its assessment, adapted to the specificity of the tested material and taking into account the reaction mechanisms. Therefore, in our study, the antioxidant activity of hawthorn berries, leaves and flowers was assessed by five *in vitro* methods, such as reactive oxygen species (ROS) scavenging activity (superoxide radical ($O_2^{\cdot-}$), hydroxyl radical (OH^-)), $ABTS^{\bullet+}$ (ABTS), iron ion chelation (ChA) and copper ion reducing capacity (CUPRAC). The obtained results are presented in Table 3.

Among the analyzed morphological parts of hawthorn, the highest ABTS radical scavenging activity and the ability to reduce copper ions (CUPRAC) were noted for hawthorn berries. The obtained values ranged from 0.56 to 0.91 mmol TE/g d.w. and from 0.66 to 0.83 mmol TE/g d.w., respectively. For both methods, the highest activity was assessed for *C. laevigata x rhipidophylla x monogyna* berries (CB4). These values were higher for leaves and flowers of the same hawthorn species by 1.5 and 2.3 times, respectively, for the ABTS method and 1.2 and 1.4 times for the CUPRAC method.

The obtained results for the scavenging potential of $ABTS^{\cdot+}$ radicals by berries, leaves and flowers are much higher than the results presented by other authors. Froehlicher et al. (2009) and Luo et al. (2016) in the study of *C. monogyna* and *C. pinnatifida* berries, obtained values ranging from 0.05 to 0.28 mmol TE/g d.w. (Froehlicher et al., 2009; Luo et al., 2016). Luo et al. (2016) for leaves of *C. pinnatifida* obtained values in the range from 0.20 to 0.34 mmol TE/g d.w. (Luo et al., 2016). In turn, Bahri-Sahl et al. (2009) in the study of *C. azarolus* flowers, obtained results ranging from 0.001 to 0.02 mmol TE/g d.w. (Bahri-Sahl et al., 2009). On the other hand, the CUPRAC test has so far been described in one study in the evaluation of hawthorn leaves and flowers. Value obtained by Ozyurek et al. (2012) ranged from 0.03 (*C. meyeri*) to 0.38 (*C. pentagyna*) mmol TE/g d.w., respectively (for leaves) and from 0.05 (*C. pontica*) to 0.37 (*C. monogyna*) (for flowers) and were significantly lower compared to the results obtained in this study (Özyürek et al., 2012).

The highest iron ion chelating capacity expressed as the IC_{50} value was also assessed for hawthorn berries. The obtained values were in the range of 700.28 - 903.29 µg/ml. The highest activity was

assessed for *C. laevigata x rhipidophylla x monogyna* berries (CB4). It was higher than leaves (CL4) and flowers (CF4) of the same hawthorn species by 1.1 and 1.3 times, respectively. So far, the ability to chelate iron ions has been assessed only for the berries and leaves of two species of hawthorn. Ebrahimzadeh (2009) in the study of *C. pentaegyna* berries, they obtained a value of 1840 µg/ml (Ebrahimzadeh, 2009), and Kallassy et al. (2017) for leaves of *C. azarolus* obtained values in the range from 500.00 to 1500.00 µg/ml (Kallassy et al., 2017). These values are much higher compared to the results obtained for hawthorn berries and leaves in our own research. On the other hand, there are no reports on the ability of hawthorn flowers to chelate iron ions.

The highest scavenging activity of reactive oxygen species $O_2^{\cdot-}$ and OH^- were estimated for leaves (479.29-722.67 µg/ml) and berries (294.96 - 622.22 µg/ml) of hawthorn, respectively. In the case of scavenging reactive oxygen species $O_2^{\cdot-}$, the leaves of *C. laevigata x rhipidophylla x monogyna* (CL4) had the highest activity. This activity was 1.2 and 1.4 times higher for berries (CB4) and flowers (CF4) of the same hawthorn species. However, for the OH^- method, the highest activity was found in *C. laevigata x rhipidophylla x monogyna* berries (CB4). The estimated value was 1.9 and 1.5 times higher for leaves (CL5) and flowers (CF5) of the same hawthorn species. Comparable results are given by Lin et al. (2022), where for the $O_2^{\cdot-}$ and OH^- radicals scavenging test, the values for *C. pinnatifida* berries were >400 and 139.03 µg/ml, respectively (Lin et al., 2022). The scavenging capacity of $O_2^{\cdot-}$ and OH^- radicals was first assessed in this study for hawthorn leaves and flowers.

In order to fully analyze the antioxidant activity of extracts from hawthorn berries, leaves and flowers, five tests were used, based on different mechanisms of action. The ABTS and CUPRAC methods are used to monitor redox reactions between antioxidants and a synthetic cation radical (for the ABTS test) and metal ions (Cu^{2+}) bound in colored complexes (for the CUPRAC test). The iron ion chelation test is used to assess the ability of antioxidants to form complexes with transition metal ions, the excess of which increases the formation of reactive oxygen species (ROS) (Chelliah & Oh, 2022). On the other hand, the superoxide anion radical scavenging test, which is a precursor of more active oxidizing species, such as singlet oxygen ($^1O_2$) and the hydroxyl radical scavenging test, which show the highest reactivity in biological systems, are one of the most important methods for the actual assessment of the ability of the tested extract to protect against ROS. ROS are highly damaging to cells. They can damage important molecules such as proteins, DNA and lipids (Valko et al., 2007). Their excess leads to redox imbalance, contributing to the development of a number of chronic diseases such as cancer, atherosclerosis, heart disease, as well as neurodegenerative disorders (Dalle-Donne et al., 2006; Forman & Zhang, 2021). For this reason, there is growing interest in the natural antioxidants from medicinal plants that can prevent oxidative damage (Forman et al.,2014; Lobo et al,2010).

Table 6. Antioxidant activity of ethanol extracts from berries, leaves, and flowers of six hawthorn species.

Sample symbol	ABTS	CUPRAC	ChA	$O_2^{\cdot-}$	OH^-
	(mmol TE/g d.w.)			IC_{50} (µg/ml)	
CB1	0.75 ± 0.00^{f}	0.75 ± 0.09^{i}	713.45 ± 0.15^{a}	599.03 ± 0.52^{e}	399.10 ± 0.21^{c}
CB2	0.57 ± 0.01^{de}	0.66 ± 0.16^{f}	903.29 ± 0.26^{k}	722.71 ± 0.52^{m}	515.34 ± 0.24^{i}
CB3	0.56 ± 0.03^{d}	0.67 ± 0.10^{f}	809.31 ± 0.51^{g}	648.20 ± 0.07^{j}	408.92 ± 1.25^{d}
CB4	0.91 ± 0.04^{g}	0.83 ± 0.14^{h}	700.28 ± 0.67^{a}	592.35 ± 0.06^{d}	294.96 ± 2.14^{a}
CB5	0.57 ± 0.00^{de}	0.70 ± 0.04^{g}	774.55 ± 0.10^{d}	589.63 ± 0.50^{c}	308.59 ± 0.73^{b}
CB6	0.77 ± 0.02^{f}	0.73 ± 0.05^{g}	774.72 ± 0.05^{d}	630.71 ± 0.04^{i}	622.22 ± 1.73^{n}
CL1	0.56 ± 0.10^{d}	0.61 ± 0.01^{e}	804.65 ± 0.23^{f}	554.11 ± 0.48^{b}	537.61 ± 1.33^{j}
CL2	0.57 ± 0.20^{de}	0.63 ± 0.01^{e}	951.24 ± 0.12^{m}	599.94 ± 0.50^{f}	547.98 ± 1.68^{k}
CL3	0.52 ± 0.30^{c}	0.57 ± 0.51^{d}	848.16 ± 0.53^{h}	616.69 ± 0.44^{h}	677.61 ± 0.31^{o}
CL4	0.60 ± 0.40^{e}	0.67 ± 0.01^{f}	776.23 ± 0.74^{e}	479.29 ± 0.00^{a}	405.76 ± 2.81^{d}
CL5	0.42 ± 0.50^{b}	0.46 ± 0.04^{b}	882.69 ± 0.18^{j}	722.67 ± 0.04^{m}	574.81 ± 1.60^{m}
CL6	0.49 ± 0.60^{c}	0.53 ± 0.02^{c}	875.15 ± 0.01^{i}	607.08 ± 0.07^{g}	558.84 ± 0.29^{l}
CF1	0.39 ± 0.02^{b}	0.59 ± 0.03^{d}	758.76 ± 0.22^{c}	633.98 ± 0.55^{i}	449.46 ± 1.11^{f}
CF2	0.41 ± 0.02^{b}	0.61 ± 0.06^{e}	722.69 ± 0.69^{b}	580.63 ± 0.49^{c}	431.77 ± 0.20^{e}
CF3	0.39 ± 0.02^{b}	0.51 ± 0.02^{c}	974.41 ± 0.61^{n}	705.96 ± 0.51^{l}	685.50 ± 0.36^{g}
CF4	0.39 ± 0.00^{b}	0.58 ± 0.06^{d}	941.45 ± 0.01^{l}	657.34 ± 0.07^{k}	453.28 ± 3.14^{p}
CF5	0.29 ± 0.04^{a}	0.42 ± 0.01^{b}	1022.61 ± 0.21^{o}	734.27 ± 0.04^{n}	449.30 ± 1.25^{f}
CF6	0.29 ± 0.06^{a}	0.35 ± 0.01^{a}	1014.78 ± 0.13^{o}	764.24 ± 0.09^{o}	475.23 ± 1.46^{h}

C, *Crataegus* L.; B, berries; L, leaves; F, flowers; 1-6, hawthorn species. Results are expressed as mean ± SD Means with different letters in the same row (between species) indicate statistically significant differences ($p < 0.05$), as assessed by Duncan's test.

Multivariate Analysis

Many reports have proven that hawthorn has a strong antioxidant effect, which is mainly shaped by the content and profile of individual groups of polyphenols. The relationship between the content of biologically active compounds and antioxidant activity has been repeatedly described in the literature (Abubakar & Haque, 2020; Li et al., 2022; Venskutonis, 2018). The presented values of correlation coefficients show that the factor shaping the antioxidant potential of hawthorn berries is mainly the content of total polyphenols (TPC vs CUPRAC, $r > 0.995$, $p < 0.05$; TPC vs ChA, $r > 0.994$, $p < 0.05$, TPC vs $O_2^{\cdot-}$, $r > 0.998$, $p < 0.01$) and total procyanidins (TPA vs $O_2^{\cdot-}$, $r > 0.998$, $p < 0.01$). The content of polyphenols (TPC vs ABTS, $r > 0.999$, $p < 0.01$; TPC vs $O_2^{\cdot-}$, $r > 0.952$, $p < 0.05$), total proanthocyanidins (TPA vs ABTS, $r > 0.998$, $p < 0.01$, TPA vs CUPRAC, $r > 0.998$, $p < 0.01$, TPA vs $O_2^{\cdot-}$, $r > 0.994$, $p < 0.01$) and flavan-3-ols (flavan-3-ols vs ABTS, $r > 0.997$, $p < 0.01$) contributed to the formation of the antioxidant activity of hawthorn leaves extracts. In turn, for hawthorn flowers, the antioxidant activity was mainly dependent on total proanthocyanidins (TPA vs CUPRAC, $r > 0.997$, $p < 0.01$; TPA vs ChA, $r > 0.992$, $p < 0.01$; TPA vs $O_2^{\cdot-}$, $r > 0.992$, $p < 0.05$).

In our own research, it was shown that the antioxidant potential of the tested extracts from hawthorn berries, leaves and flowers mainly increases with the increase in the content of proanthocyanidins. In the available literature, you can find reports of a strong correlation between the high content of proanthocyanidins and the anti-radical properties of, among others, chocolate and cocoa products (Gu et al., 2006), red grapes (Monrad et al., 2010), peaches and plums (Goto et al., 2021), rosehip fruit (Patel, 2017) and chokeberry (Denev et al., 2018; Oszmiański & Wojdylo, 2005). Similar observations apply to the assessment of the polyphenol profile and antioxidant activity of hawthorn. In the work of Bahri-Sahloul et al. (2009) found a strong positive correlation between the antioxidant activity and the total content of proanthocyanidins, isoquercetin and procyanidin B2 identified in *C. monogyna* flowers (Bahri-Sahl et al., 2009). Also Bardakci et al. (2019) and Simirgiotis et al. (2013), found that the antioxidant activity of berries was strongly correlated with the content of procyanidin B2 (Bardakci et al., 2019; Simirgiotis, 2013). Of the procyanidins identified in berries, leaves and flowers, Froehlicher et al. (2009), found (-)-epicatechin to be the most effective antioxidant compound in hawthorn (Froehlicher et al., 2009).

In order to assess the relationship between the extracts obtained from the berries, leaves and flowers of six hawthorn species, and the estimated polyphenolic profile and antioxidant activity, principal component analysis (PCA) was performed. The data obtained are presented in Figure 2. For hawthorn berries, the PCA plot (Fig. 2A) represented 61.16% of the total variance, with PC1 and PC2 explaining 37.89% and 23.27% of the between-group variance, respectively. Two main groups can be distinguished in the presented PCA chart. The first included *C. laevigata x rhipidophylla x monogyna* berries (CB4) and total polyphenol content, ABTS, CUPRAC and ChA tests. The second group included fruits of *C. macrocarpa* (CB5) and the content of total proanthocyanidins, flavan-3-ols and the $O_2^{\cdot-}$ and OH^- radical scavenging test.

For hawthorn leaves, the PCA plot (Fig. 2B) represented 70.60% of the total data variance, with PC1 and PC2 explaining 42.0% and 28.60% of between-group variance, respectively. Two main groups can be distinguished in the presented PCA chart. The first group included *C. laevigata x rhipidophylla x monogyna* leaves (CL4) and total polyphenol content, total proanthocyanidin content, ABTS, CUPRAC, ChA, $O_2^{\cdot-}$ and OH^- radical scavenging test. The second group included leaves of *C. rhipidophylla* (CL2) and the content of phenolic acids and flavonols.

The analysis of the main components of hawthorn flowers is presented in Figure 2C. The PCA plot represented 93.39% of the total variability, where PC1 and PC2 explained 64.85% and 28.54% of the intergroup variance, respectively. Based on the data presented in the PCA chart, two main groups were distinguished. The first group was positively correlated with *C. monogyna* (CF1) flowers, total polyphenol content, total proanthocyanidin content, phenolic acid concentration and the ABTS, CUPRAC, ChA, $O_2^{\cdot-}$ radical scavenging test. The second group included flowers of *C. rhipidophylla* (CF2) and total flavonoid content, OH^- radical scavenging test.

The use of PCA analysis made it possible to show that the extracts obtained from the fruits and leaves of *C. laevigata x rhipidophylla x monogyna* (CB4, CL4) and *C. monogyna* flowers (CF1) are strongly correlated with most parameters of the assessed content of polyphenolic compounds and antioxidant activity.

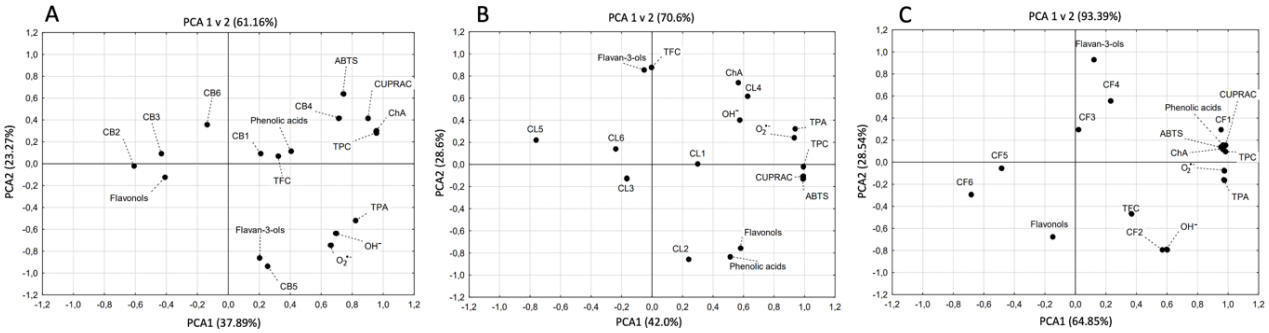

Figure 2**.** PCA charts for berries (**A**), leaves (**B**) and flowers (**C**) showing the relationship between antioxidant activity and the content of polyphenolic compounds identified in preparations of the fruits of six hawthorn species.

CONCLUSIONS

The analyzed morphological parts of six hawthorn species significantly differed in the content of bioactive components and antioxidant activity. In total, 21 polyphenolic compounds were detected, including 17 in berries, 11 in leaves and 10 in flowers. The dominant groups of compounds were flavan-3-ols (procyanidin trimer, (-)-epicatechin) and phenolic acids (3-*O*-*p*-coumaroylquinic acid, 3,4-*O*-dicaffeoylquinic acid). In terms of the profile and level of these compounds, the berries of the species *C. x subsphaericea*, the leaves of the species *C. monogyna* and the flowers of the species *C. rhipidophylla* stood out.

Also, the antioxidant activity was conditioned by the analyzed morphological parts and strongly correlated with the content of mainly procyanidins. The strongest antioxidant properties through various reaction mechanisms were assessed for berries, mainly of the species *C. laevigata x rhipidophylla x monogyna*. In turn, flowers were statistically the weakest in terms of antioxidant potential.

The obtained results allow to conclude that after further necessary tests, such as sensory tests, determination of bioavailability and digestibility of hawthorn bioactive compounds, cytotoxicity, interactions with food ingredients, these raw materials could be used in the composing of nutraceuticals and/or functional food, as a preparation preventing oxidative damage of products, and also as a preparation in the prevention and treatment of inflammatory diseases.

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