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Comparison of Chemical Profiles of *Aronia melanocarpa* Fruit Extracts

Eda Sönmez Gürer[1,a,*], Ayşe Esra Karadağ[2,b], Ayhan Altıntaş[3,c]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Sivas Cumhuriyet University, 58140, Sivas, Türkiye*
[2]*Department of Pharmacognosy, School of Pharmacy, Istanbul Medipol University, 34810, Istanbul, Türkiye*
[3]*Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470, Eskisehir, Türkiye*
[*]*Corresponding author*

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ABSTRACT

The chokeberry plant, which is native to North America and the south of Canada, is a deciduous, shrub-shaped, berry-like plant belonging to the Rosaceae family, which survives for many years. It is seen as a plant that adapts easily to almost every climatic condition and soil and has many beneficial properties for health. Within the scope of this study, methanol, 70% ethanol, ethanol, ethyl acetate, hexane and water extracts were prepared from the fruits collected from the *Aronia melanocarpa* (Michx.) Elliott plant, which is cultivated in the Kırklareli region. The chemical contents of the obtained extracts were clarified by high performance liquid chromatography. It was determined that phenolic compounds such as ferulic acid, caffeic acid, quercetin, quercetin-3-galactoside, p-coumaric acid were found in the extracts, and ethyl acetate extract was found to have the richest phenolic substance profile. Malvin anthocyanin compound was detected only in methanol extract.

[a] *edagurer@cumhuriyet.edu.tr* *https://orcid.org/ 0000-0003-0319-6312*
[b] *aeguler@medipol.edu.tr* *https://orcid.org/ 0000-0002-3412-0807*
[c] *aaltinta@anadolu.edu.tr* *https://orcid.org/ 0000-0002-9413-0636*

Introduction

There are three different species named *Aronia melanocarpa* (Michx) Elliot (Black chokeberrey), *A. prunifolia* (Marsh) (Purple chokeberry) and *A. arbutifolia* (L.) Elliot (Red chokeberry) (Kulling and Rawel, 2008; Šnebergrová et al.,2014). Aronia plant is known as 'super fruit' because of its rich content of phenolic compounds, high antioxidant capacity and benefits for human health and nutrition (Kulling and Rawel, 2008; Šnebergrová et al., 2014). Aronia berries show significant antioxidant activity due to the rich phytochemical compounds it contains, and plays an active role in the prevention and treatment of heart diseases, cancer, and chronic diseases (Oszmiański and Lachowicz, 2016; Chrubasik et al., 2010; Graves, 2013; Tolic et al., 2015). In addition to its active use in the food and health sector, it is called functional food due to its pharmacological activities and due to this popularity in the health field, its use and culture in the world is becoming more widespread day by day. It is known that it is used in the food industry in different forms such as syrup, fruit juice, tea, sauce, jam and for the purpose of coloring foods due to its intense color (Graves, 2013; Tolic et al., 2015). As a result of the researches on the pharmacological effects of aronia, which is in the berry class, on human health, it has been determined that it is more valuable than other berry fruits due to the high ratio of phytochemical compounds with antioxidant effect in its content. As a result of studies, it has been revealed that aronia plays a protective role in cardiovascular system diseases, gastrointestinal system diseases and various cancer diseases by eating it regularly as food (Kulling and Rawel, 2008).

The fact that foods of natural origin are both medicinal and edible, contain antioxidants, antibiotics and structures that provide antineoplastic effects, have also become a subject of interest for many pharmacological activity studies (Sevindik et.al., 2017). As a result of the studies carried out; It has been found out that the consumption of various phenolic compounds found in natural foods can reduce the possibility of significant health problems in people due to their antioxidant effects as well as many

mechanisms (Akalın and Selamoğlu, 2019; Sevindik et al., 2017; Pehlivan et al., 2021). Among the most common product types in studies on products containing aronia; teas, yoghurts, nectars, juices, jellies, chewing gums, concentrated juices and chewable tablets (Engels and Brinkman, 2014). Due to its protective effects that prevent the occurrence of many diseases such as diabetes, cardiovascular diseases and cancer, fruits arouse curiosity especially by scientists who conduct cancer research. *A. melanocarpa* is a berry rich in anthocyanins with high antioxidant activity. *In vitro* and *in vivo* studies; It has been determined that they have activities on diseases such as stomach ulcers, colon cancer, diabetes (Jurikova et al., 2017; Bermúdez-Soto et al., 2007).

Phenolic compounds; they are known for their hydroxyl groups and aromatic rings in their chemical structure (Pehlivan et al., 2018; Mohammed et al., 2020). According to their phenolic structures; They consist of 5 main groups: phenolic acids, stilbenes, flavonoids (flavonols or catechins, flavonols, flavones, flavonones, isoflavonoids, anthocyanins), tannins and lignans (Paredes-López et al., 2010). Flavonols are mainly associated with quercetin, chemferol and myricetin, of which quercetin is the most popular (Özgen et al., 2016; Shahidi and Ambigaipalan, 2015).

As revealed in previous studies, the antioxidant effects of purple-red fruit or flowering anthocyanin-rich plants are high (Pehlivan et.al., 2018; Mohammed et al., 2020). The main flavanols in the content of aronia fruits are procyanidins and the amount of procyanidin varies between 0.66% and 5.18% of the dry weight of the fruit, the second largest group of phenolic compounds is anthocyanins, and the dry weight of the fruit varies between 0.60% and 2.00%. It has been concluded that it has a high concentration (Wu et al., 2004; Kokotkiewicz et al., 2010). Phytochemical content of aronia fruits; It forms polymeric proanthocyanins mainly consisting of (-) epicatechin and represents the main polyphenolic compound class (66%). Anthocyanins constitute the second group of phenolic compounds and represent approximately 25% of the total polyphenols (Oszmiański and Wojdylo, 2005). The anthocyanins in *A. melanocarpa* are essentially a mixture of four cyanidin glycosides: cyanidin 3-galactoside, cyanidin 3-glucoside, cyanidin 3-arabinoside, and cyanidin 3-xyloside. Of these, cyanidin 3-galactoside is found in the highest concentration. Within the scope of this study, methanol, 70% ethanol, ethanol, ethyl acetate, hexane and water extracts of the fruits of the *A. melanocarpa* plant, which is cultivated in the Kırklareli region, were prepared in August 2021 and chemical extracts were determined according to certain standards by high performance liquid chromatography (HPLC) method of each extract. contents were evaluated.

Materials and Methods

Plant material

A. melanocarpa plant fruits, which were collected from the culture gardens of the breeder company in Kırklareli province, were procured at the end of August 2021. The plants were photographed in their natural habitat, collected fresh, dried in the shade and prepared as study material (Figure 1).

Figure 1. General view of the fruit of the aronia plant

Figure 2. High performance liquid chromatography device (Agilent 1100 HPLC)

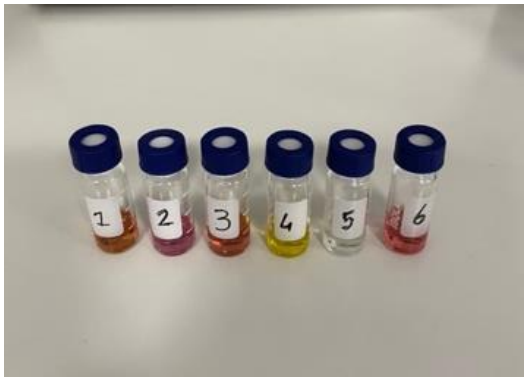

Figure 3. Extract samples prepared for HPLC device

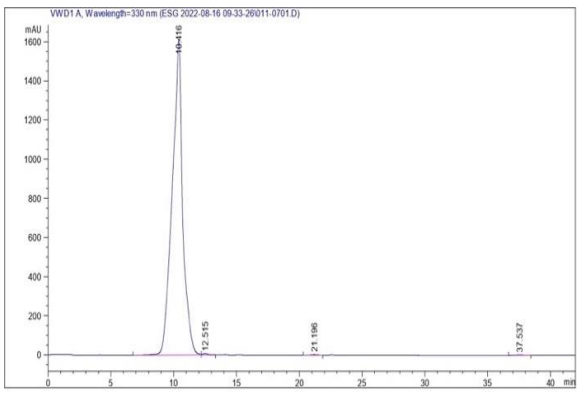

Figure 4. Ferulic acid HPLC chromatogram (R.T.: 10.416)

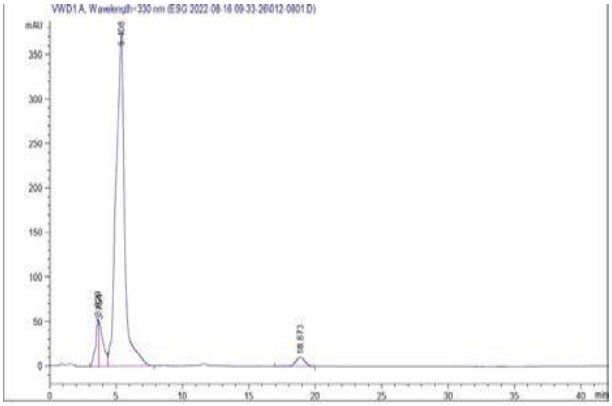

Figure 5. Caffeic acid HPLC chromatogram (R.T.: 5.408)

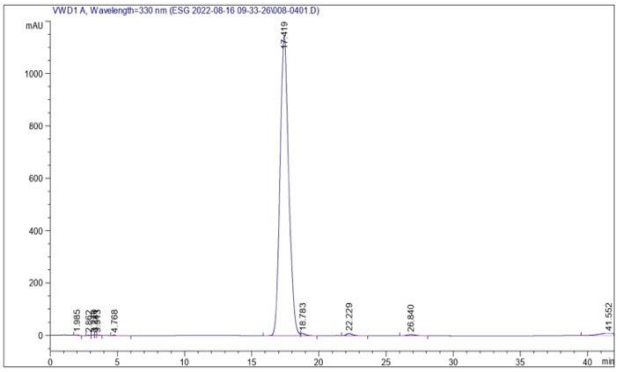

Figure 6. Quercetin HPLC chromatogram (R.T.: 17.419)

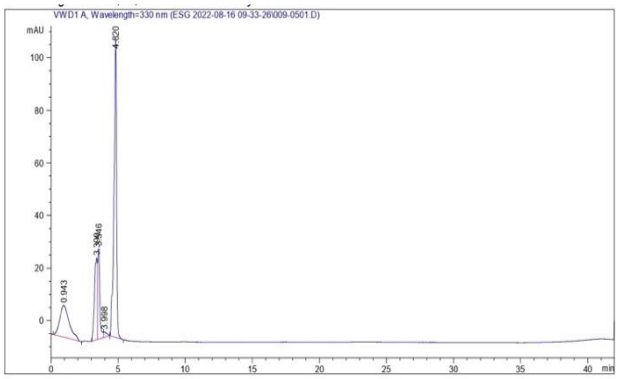

Figure 7. Quercetin-3-galactoside HPLC chromatogram (R.T.: 4.620)

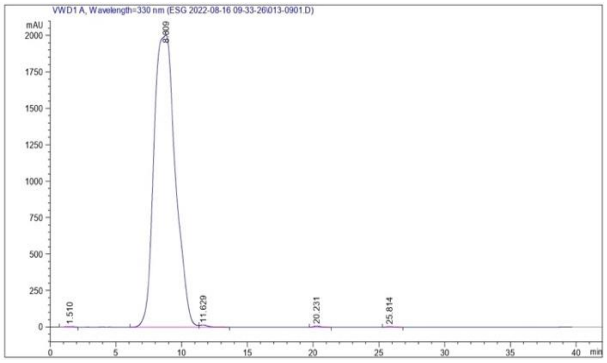

Figure 8. p-Coumaric acid HPLC chromatogram (R.T.: 8.809)

Extraction

Dried fruits were crushed and ground into coarse powder. 20 g of plant material was weighed and taken into separate flasks, 100 mL of methanol, 70% ethanol, ethanol, ethyl acetate and hexane were added to each of them, respectively, and left to maceration at room temperature with the mouth closed. After 24 hours, the macerates were filtered off and 100 ml of fresh solvent was added to the plant materials. After the maceration process, which lasted for 3 days, the collected macerates were removed from their solvents by using vacuum at low temperature (40°C) in a rotary evaporator. It was combined in dark-colored glass containers and kept in a refrigerator at -20°C until used in experimental studies by calculating the % yield. For the extract containing 70% ethanol, evaporation was first performed in a rotary evaporator, and then it was placed in a lyophilizer in order to completely remove the water in it.

Infusion was performed for the water extract. For this, 20 g of herbal material was weighed and poured into flasks, 100 mL of hot distilled water was added to it, and it was kept at room temperature for 10-15 minutes with the mouth closed, shaking occasionally. At the end of the period, the extract was filtered and 100 mL of hot distilled water was added to the herbal materials again. At the end of the process repeated 3 times, a lyophilizer was used to completely remove the water in the collected extracts.

High Performance Liquid Chromatography (HPLC)

HPLC Analysis of Phenolic Compounds

HPLC analyzes of flavone derivatives and phenolic compounds were performed with a DAD detector connected to the Agilent 1100 HPLC system (Figure 2). C18 column (250*4.6mm*10µm) is used as stationary phase, while gradient with A: acetonitrile:water:formic acid (10:89:1), B: water:acetonitrile:formic acid (89:10:1) as mobile phase flow was studied. The flow rate was set to 0.7 mL/min. The peaks were detected at 330 nm (Karadağ et al., 2019). From the extracts prepared at a concentration of 10 mg/mL as stock; After dissolving methanol, 70% ethanol, ethanol and water with their solvents, ethyl acetate with ethanol and hexane DMSO, they were analyzed by filtration through a 0.22-micron membrane filter (Figure 3). Injection volume was 20 µL, column temperature was set to 40°C.

HPLC Analysis of Anthocyanin Compounds

DAD detector connected to Agilent 1100 HPLC system was used. While using C18 column (250*4.6mm*10µm) as stationary phase, A: Water:formic acid:acetic acid (100:0.7:0.9) as mobile phase, B: 10% to 90% between 0-30 minutes with Acetonitrile the linear gradient to the B concentration was studied as a flow. 30-35. within minutes, it was returned from 90% B to 10% B. The flow rate was set to 0.5 mL/min. The peaks were detected at 530 nm (Farina et al., 1995). The studied extracts were studied at a concentration of 10 mg/mL and from the extracts; after dissolving methanol, 70% ethanol, ethanol and water with their solvents, ethyl acetate with ethanol and hexane DMSO, they were analyzed by filtration through a 0.22-micron membrane filter. Injection volume was 20 µL, column temperature was set to 40°C.

Results and Discussion

It was determined that the yields of aronia fruit extracts varied between 68.6% g and 1.25% g. It was observed that the highest yield was with methanolic solvent and the lowest yield was with hexane solvent (Table 1).

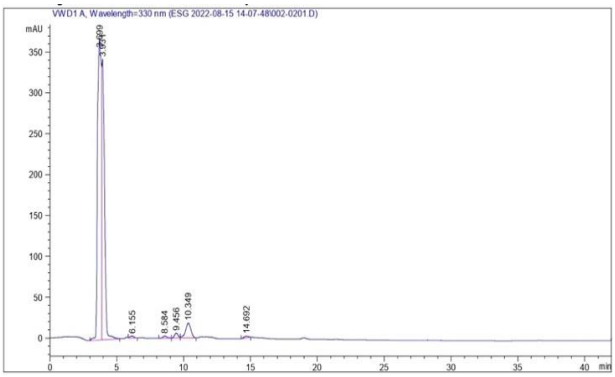

Figure 9. HPLC chromatogram of methanol extract (p-coumaric acid R.T.: 8.594; Ferulic acid R.T.: 10.349)

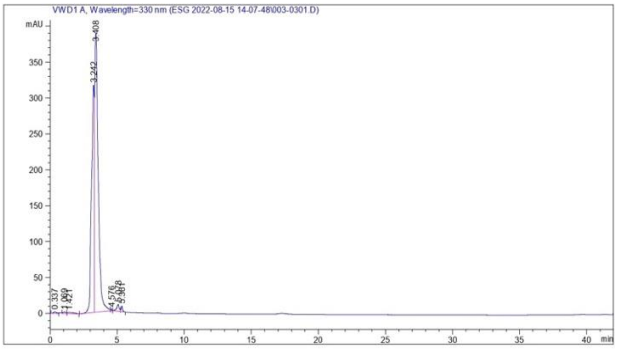

Figure 10. HPLC chromatogram of 70% ethanol extract (quercetin-3-galactoside R.T.:4.576; caffeic acid R.T.: 5.381)

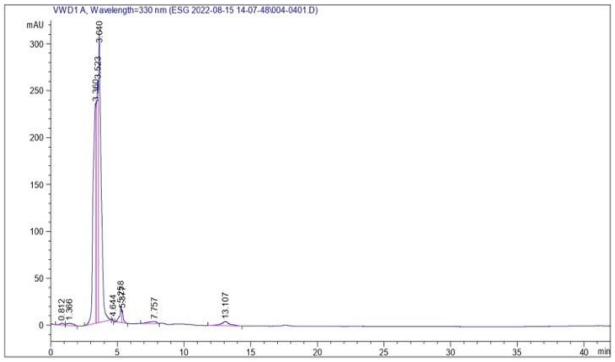

Figure 11. HPLC chromatogram of ethanol extract (quercetin-3-galactoside R.T.:4.644; caffeic acid R.T.: 5.371)

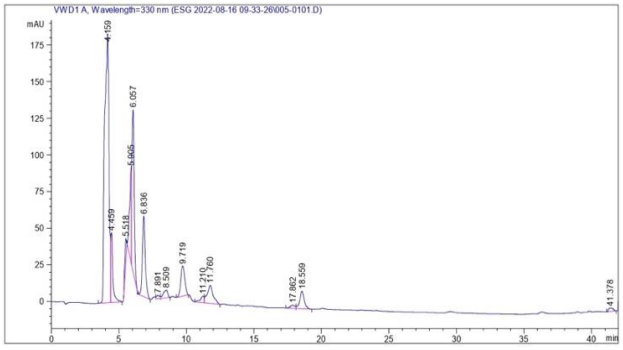

Figure 12. HPLC chromatogram of ethyl acetate extract (quercetin-3-galactoside R.T.:4.644; caffeic acid R.T.: 5.371; p-coumaric acid R.T.:8.509; quercetin R.T.:17.862)

According to the HPLC analysis results of 6 different extracts studied; standard phenolic compounds (Ferulic acid, caffeic acid, quercetin, quercetin-3-galactoside, p-coumaric acid) (Figure 4, Figure 5, Figure 6, Figure 7, Figure 8) analyzed as references were detected in methanol, 70% ethanol, ethanol and ethyl acetate extracts (Figure 9, Figure 10, Figure 11, Figure 12). It was revealed that the extract richest in phenolic compounds was the ethyl acetate extract and it is indicated in Table 2.

According to the results of HPLC analysis of 6 different extracts prepared for the experiments; The available standard anthocyanin compounds (malvin and malvidin-3-o-glucoside) were analyzed as reference and malvin compound was detected only in the methanol extract from 6 different extracts (Figure 13, Figure 14). However, according to the results obtained, it was observed that 70% ethanol, ethanol and water extracts also contained different anthocyanin compounds.

Aronia plants, which easily adapt to almost every climate condition and soil, are also known as 'super fruit' due to their rich content of phenolic compounds, high antioxidant capacity, and benefits for human health and nutrition. It is known to be therapeutic against colds, digestive system diseases, liver, gall bladder and various cardiovascular system problems. *A. melanocarpa* is a berry rich in anthocyanins with high antioxidant activity. In both *in vitro* and *in vivo* studies; It has been determined that it has activities on diseases such as stomach ulcer, colon cancer, diabetes.

In a study by Jakobek et al; Total polyphenol, total anthocyanin amounts and antioxidant activities of black aronia (*A. melanocarpa*) fruits were evaluated and it was determined that they contain high amounts of polyphenols and anthocyanins. In the same study, the amount of flavonols (quercetin, chemferol, myricetin) and anthocyanins in the content of aronia were determined in detail using the HPLC method. As a result; cyanidin derivatives of the highest amount of anthocyanins; cyanidin-3-galactoside (68.9%) and cyanidin-3-arabinoside; It was determined that quercetin (24.5%) constituted 93.07% of the total flavonol amount, chemferol level was low (6.93%), and myricetin could not be observed (Jakobek et al., 2007). In an HPLC study conducted on green fruits, it was observed that the fruit extract had a very high rate of chlorogenic acid (Zielinska et.al., 2020). In this study, chlorogenic acid was sought but not found. This shows that the compounds present in the fruits can be quite different before and after ripening. In addition, while cyanidin derivatives were generally detected as anthocyanins in previous studies, malvin and malvidin-3-o-glucoside were investigated in this study (Meng et.al., 2019). It is thought that the extract may also be rich in cyanidin, but cyanidin references in HPLC studies could not be studied in this study. In the study published by Lee et al., while different quercetin derivatives were detected in A. melanocarpa fruits, quercetin-3-galactoside was found in this study (Lee at.al., 2014). However, in another study, quercetin-3-galactoside detected in this study was isolated from fruits and its structure was clarified (Slimestad et.al., 2005).

In this study; methanol, 70% ethanol, ethanol, ethyl acetate, hexane and water extracts of *A. melanocarpa* fruits, which are cultivated in Kırklareli region, were prepared.

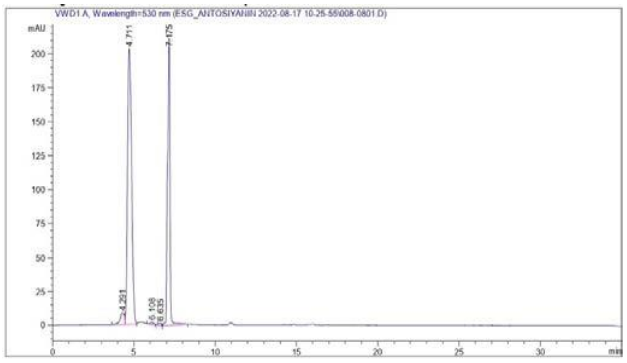

Figure 13. HPLC chromatogram of anthocyanin standard mixture (malvin R.T.: 4.711; malvidin-3-o-glucoside R.T.:7.175)

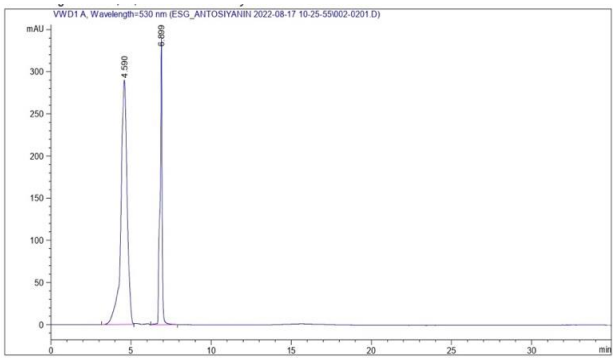

Figure 14. Methanol extract HPLC chromatogram (malvin R.T.: 4,590)

Table 1. Extract yields of aronia fruits prepared with different solvents

Extract	% Extraction yield
Methanol	%68.60
70% Ethanol	%36.84
Ethanol	%5.72
Ethyl acetate	%2.15
Hexane	%1.25
Water	%30.85

Table 2. Phenolic compounds of *Aronia melanocarpa* fruit extracts (R.T.: Retention time)

Extract	Ferulic acid R.T.: 10.416	Caffeic acid R.T.: 5.408	Quercetin R.T.: 17.419	Quercetin-3-galactoside R.T.: 4.620	p-Coumaric acid R.T.: 8.809
Methanol	10.349	-	-	-	8.594
70% Ethanol	-	5.381	-	4.576	-
Ethanol	-	5.371	-	4.644	-
Ethyl acetate	-	5.371	17.862	4.644	8.509
Hexane	-	-	-	-	-
Water	-	-	-	-	-

Yield calculations of the obtained extracts were made and their chemical contents were determined by high performance liquid chromatography. According to the results of HPLC analysis of 6 different extracts prepared for the experiments; determined from standard phenolic compounds analyzed as reference in methanol, 70% ethanol, ethanol and ethyl acetate extracts. The extract richest in phenolic compounds is ethyl acetate extract; It has been revealed that it contains quercetin-3-galactoside, caffeic acid, p-coumaric acid and quercetin compounds and is indicated in Table 2. According to the results of HPLC analysis of 6 different extracts prepared for the experiments; standard anthocyanin compounds analyzed as reference only in the methanol extract; malvin and malvidin-3-o-glucoside were detected.

Conclusion

It is known that there are hundreds of compounds in plant extracts in general and it is very important for pharmacognostic studies to understand which of these components causes real biological activities. However, it is not possible in terms of economy and time management to initiate advanced pharmacognostic testing processes without distinguishing all plants and performing preliminary studies. In this study, the aronia plant fruit extracts investigated in terms of phenolic compounds and anthocyanins were evaluated in the presence of some standards and their chemical profiles were revealed. In this respect, it can be said that it is an initial study that can guide future studies. Evaluation of different plant parts of the aronia plant in terms of content and biological activity capacities in the following processes will also be useful in terms of contributing to the literature and determining the potential of the plant to be converted into products with high added value.

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