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Total Phenolic Content and Antioxidant Activity of Different Extracts of *Aronia melanocarpa* L. Fruit

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ABSTRACT

Objective: *Aronia melanocarpa* L. (aronia) fruit contains anthocyanins, flavonoids, phenolic acids, and compounds from the polyphenol group. The fruits of this plant are the most abundant natural source of anthocyanins. The aim of this study is to determine the total phenolic compound amount of methanol and ethanol extract in the fruit of the aronia plant and to investigate its antioxidant effects.

Methods: Ethanol and methanol extracts from aronia fruits were obtained. The Folin–Ciocalteu Reagent (FCR) was used to determine the total phenolic component levels in the extracts of aronia. By using the 1,1-diphenyl-2-picrylhydrazyl (DPPH), iron ion reducing antioxidant power (FRAP), and Cu^{2+} ion reducing (CUPRAC) techniques, antioxidant activities were assessed. To calculate the extracts' equivalent antioxidant capacity, different reference sample concentrations ranging from 125 to 500 g/mL were prepared.

Results: Both ethanol and methanol aronia extracts showed the highest phenolic component at a concentration of 500 µL/mL. Similary, both extracts FRAP and CUPRAC (Trolox Eq g/mL) activities and DPPH radical scavenging capacity (inhibition %) were highest at the concentration of 500 µL/mL.

Conclusion: Aronia stands out as an antioxidant fruit and a potential natural therapeutic agents to alleviate oxidative stress. More research is needed to elucidate the exact mechanisms of action, optimum extraction method, optimal dosage, and potential side effects of the extracts.

Keywords: Antioxidant, *Aronia melanocarpa* L., ethanol, extract, methanol.

INTRODUCTION

Free radicals are substances produced in the body by the contaminated air we breathe throughout the day, toxic substances in ruined meals, additives, unconscious nutrition, and inactivity. Oxygen atoms, broken off by these harmful effects from outside, circulate freely in the body, breaking off hydrogen atoms and causing tissue damage. Free radicals specifically attack cells and the immune system. Molecules that minimize and block the effects of free radicals in the body and prevent chain reactions that can cause many diseases and premature aging are called "antioxidant" substances.[1] It is known that the living organism has an antioxidative protection system to protect it from the effects of free radicals. In some cases, the antioxidative protective system is not adequate to maintain oxidant–antioxidant balance, and free radicals increase in the body. This causes some damage in the body; as the amount of free radicals increases, first aging accelerates, cell death, then tissue death, and then damage to the brain vessels occurs.[2,3] Some plants contain more antioxidants than others. The most important of these are grape-like fruits. These fruits play an important role in the prophylaxis and treatment of many diseases due to the high amount of antioxidants they contain. One of these fruits is aronia.[4,5]

Aronia is a plant belonging to the Rosaceae family with fruits rich in bioactive compounds with antioxidant properties. Ripe fruits are used in the pharmaceutical industry to make medicines.[6,7] Aronia fruit contains anthocyanins, flavonoids, phenolic acids, and compounds from the polyphenol group. These plant fruits are the richest natural source of anthocyanins.[8] Numerous investigations have revealed that these substances not only possess potent antioxidant, anti-inflammatory, hypotensive, antibacterial, and antitumor properties but also decrease the content of plasma lipids.[9-13] Studies have also found that anthocyanin is beneficial in cardiovascular system diseases.[14,15] In a review study, it was reported that aronia fruits can be considered a promising component of foods designed for their antioxidant potential.[16] Aronias, which are recommended and used as hypertensive and antiatherosclerotic drugs in Russia and Eastern European countries, are among the most important plants that have gained popularity in this geography to meet the need for herbal medicine.[12]

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Antioxidants used in in vivo and in vitro studies where antioxidant activity is investigated are generally synthetically prepared substances by manufacturers. Therefore, the use of naturally obtained antioxidants in studies is rare. In this study, we used the standard antioxidants gallic acid and Trolox.[17,18]

In this study, it was aimed to determine the total phenolic compounds and measure the antioxidant activity of ethanol and methanol extracts of aronia fruit by copper ion reducing antioxidant capacity (CUPRAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP) methods, and to compare the obtained data according to the extracts.

METHODS

Plant Material

Aronia fruit was obtained from the Tver Oblast region of Russia in September 2018 and dried under suitable conditions (in a dry environment, away from sunlight, at room temperature). After the plant was dried, it was pulverized using liquid nitrogen and a porcelain mortar. The powdered aronia fruit was stored under suitable conditions until the experiment began.

Preparation of Plant Extracts

The powdered aronia fruit was extracted with ethanol and methanol in a shaking water bath at 50°C for 72 hours and filtered every 24 hours. Filtrates were combined, and the filtrate was evaporated in the evaporator at 50°C and stored in the refrigerator at +4°C for determination.

Determination of total phenolic content

The amounts of total phenolic compounds in aronia ethanol and methanol extracts were determined using the modified version of the method developed by Slinkard and Singleton.[19]

First, 50 mL of 7.5% Na_2CO_3 was prepared. Then, after weighing 25 mg of gallic acid for the standard, it was completed with methanol to 25 mL in a test tube. Finally, the Folin and Ciocalteu reagent was taken into a beaker for phenolic compound determination. Stock solutions were prepared, and the necessary dilutions were made. First, 40 µL of sample and 200 µL of Folin and Ciocalteu reagent were added to the plates and incubated for 5 minutes. Finally, 160 µL of Na_2CO_3 was added and incubated again for 30 minutes. After incubation, absorbance was measured at 765 nm. Using the standard graph prepared using gallic acid, the results were given as mg gallic acid equivalent (GAE)/g.

Determination of Antioxidant Capacity

1,1-Diphenyl-2-picrylhydrazyl *Radical Scavenging Capacity Assay*

The DPPH radical scavenging capacities of ethanol and methanol extracts obtained from aronia were determined according to the Brand Williams method.[20] The inhibitory response of samples to DPPH radicals is measured spectrophotometrically to determine antioxidant capacity. The DPPH solution loses its color during the reduction reaction in the presence of an antioxidant, and the decrease in color intensity makes it easier to measure in the spectrophotometer. After preparing DPPH solution, 210 µL of extract sample was pipetted into the plate wells, and then 70 µL of DPPH solution was added to each well. The plate was mixed with a stirrer for 1 minute and incubated for 30 minutes in the dark. Trolox was used as the standard antioxidant for the control sample. Then absorbance was measured at 517 nm, and the results were calculated as percent inhibition.

The Ferric-Reducing Antioxidant Power Assay

The method of determination of the antioxidant capacity of extracts obtained from aronia based on electron transfer was applied by Huang et al.[21] First, 300 mmol/L acetate buffer (pH 3.6) was prepared. 10 mM TPTZ was taken into a 100 mL flask, 40 mM HCl was added, and the final volume was made up to 100 mL. Finally, a 20 mmol/L $FeCl_3$ solution was prepared. A total of 30 mL of FRAP solution was obtained by taking 2.5 mL of TPTZ, 2.5 mL of $FeCl_3$, and 25 mL of acetate buffer from these prepared solutions. Ten microliters of the extracted sample and 200 µL of FRAP solution were pipetted into the plate wells and allowed to incubate for 30 minutes, and then the absorbance was measured at 593 nm.

Cupric Ion (Cu^{2+}) Reducing Antioxidant Capacity Assay

This method used by Apak et al is based on the conversion of the Cu(II) neocuproin complex to Cu(I) neocuproin using antioxidant compounds in the environment and the measurement of the absorbance of this complex at 450 nm wavelength.[22] To prepare the CUPRAC reagent (10 mM), 0.4262 gram of $CuCl_2 \cdot 2H_2O$ was weighed and dissolved in 250 mL of distilled water. To prepare the acetate buffer, 19.27 g of NH_4Ac was dissolved in 250 mL of water. 7.5 mM neocuproin solution was obtained by preparing 0.039 g neocuproin compound with 96% pure ethanol in a 25 mL flask. Afterward, solutions consisting of 60 µL $CuCl_2$, 60 µL acetate buffer, 60 µL neocuproin solution, and 66 µL extracts were mixed, and after 30 minutes of incubation, absorbances were measured at 450 nm wavelength. The standard antioxidant, Trolox, was used as a control sample. Calibration curves for the working range of 1-100 µg/mL, where the plot of absorbance versus concentration is linear, were derived.

RESULTS

Findings of Total Phenolic Compound Quantification

Total phenolic compound amounts of ethanol and methanol extracts prepared from aronia were determined by Folin–Ciocalteu Reagent (FCR). Gallic acid was used as the standard phenolic compound and was calculated as gallic acid equivalent from the equations obtained from the calibration curves of gallic acid (Figure 1).

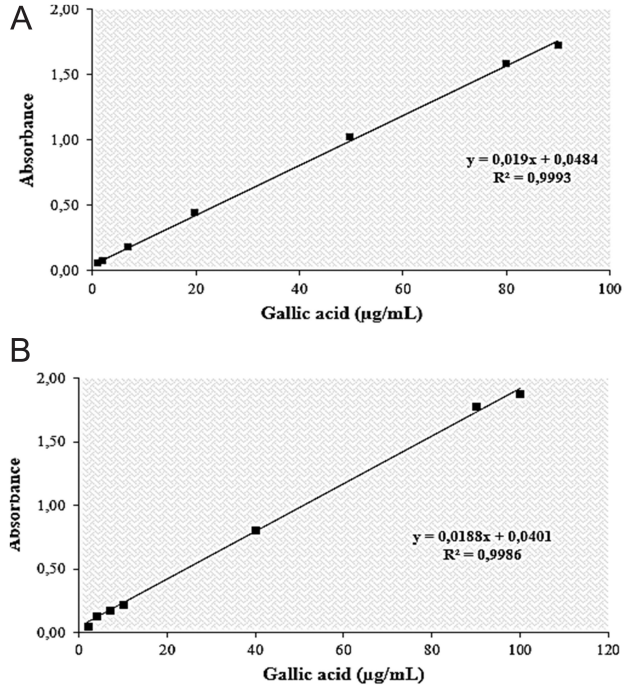

Figure 1. Calibration curves of gallic acid in different solvents. A. Ethanol. B. Methanol.

Table 1. Total Phenolic Compound Amounts of *Aronia melanocarpa* L. extracts

Concentration (µg/mL)	Total Phenolic Compound (µg GAE/mg Extract)	
	Ethanol Extract	Methanol Extract
125	1.06	6.55
250	3.37	8.48
500	6.02	11.19

GAE, gallic acid equivalent.

Table 2. Comparison of DPPH Free Radical Scavenging Capacities of Extracts at Different Concentrations

Concentration (µg/mL)	% Inhibition [Trolox (Eq µg/mL)]	
	Ethanol Extract	Methanol Extract
125	21.57	11.82
250	57.69	29.94
500	84.19	52.55

The total phenolic contents of ethanol and methanol extracts of aronia fruit at different concentrations were determined. Accordingly, the highest total phenolic content was determined to be at a concentration of 500 µg/mL (Table 1).

Antioxidant Capacity Findings

Findings from 1,1-Diphenyl-2-picrylhydrazyl radical scavenging studies

The DPPH radical scavenging activities of standard antioxidant compounds of ethanol and methanol extracts prepared from aronia were determined according to the Brand-Williams method.[20] The analyzed concentration range (1-100 µg/mL) was determined as a result of studies on standard antioxidant compounds. The DPPH radical scavenging activity of Trolox, a standard antioxidant, reached its highest value at a concentration of 90 µg/mL for both ethanol and methanol (Figure 2).

The DPPH radical scavenging capacities of methanol and ethanol extracts of aronia fruit in the range of (125-500 µg/mL) are shown in Table 2 as % inhibition.

Aronia fruit methanol extract showed the highest DPPH free radical scavenging effect at 500 µg/mL concentration.

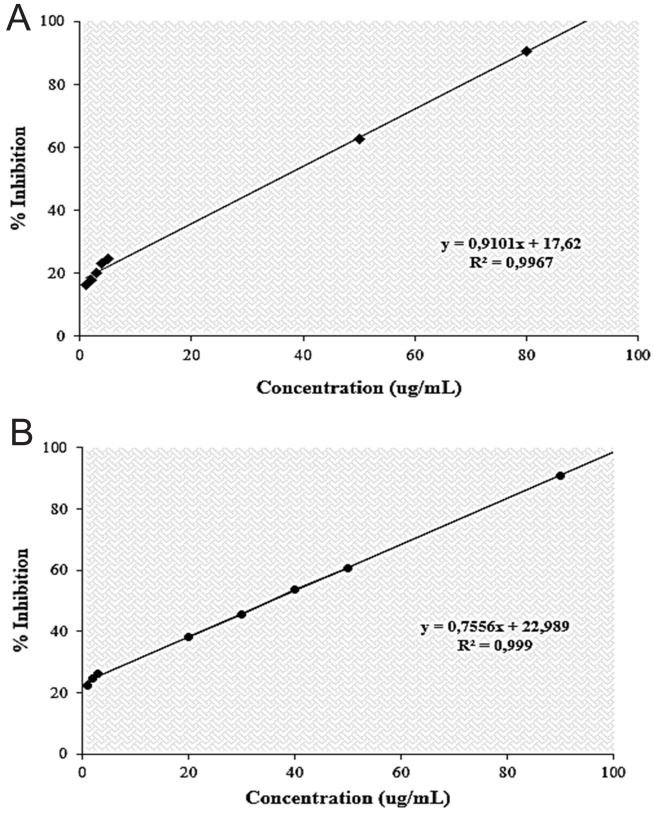

Figure 2. Concentration % inhibition graph of Trolox. A. Ethanol. B. Methanol.

Findings of the Copper Ion Reducing Antioxidant Capacity Determination Method

The conversion of ethanol and methanol extracts prepared from aronia and standard antioxidant compounds of Cu(II) neocuproin complex at 450 nm to Cu(I) neocuproin by means of compounds with antioxidant effects in the medium was done by measuring the absorbance at 450 nm. The concentration range to be analyzed (1-100 µg/mL) was determined as a result of studies on standard antioxidant compounds (Figure 3, Table 3)

Findings of Iron Ion Reducing Antioxidant Power

Spectrophotometric measurements of iron (III) reduction/antioxidant equivalent absorbances of aronia fruits, ethanol, methanol extract, and standard antioxidant compounds were made at 593 nm. Since the antioxidant power capacity of Trolox, one of the standard antioxidant compounds, reaches its maximum level at a concentration of 100 µg/mL in ethanol extract and 70 µg/mL in methanol extract, the study was carried out in the range of 1-100 µg/mL (Figure 4).

Comparison of the iron (III) reduction/antioxidant powers of aronia fruit ethanol and methanol extracts in terms of µg/mL Trolox equivalent antioxidant capacity (TEAC) using the spectrophotometric method at 593 nm are given in Table 4.

It was determined that the ethanol and methanol extracts prepared from aronia fruits had iron ion reducing antioxidant power

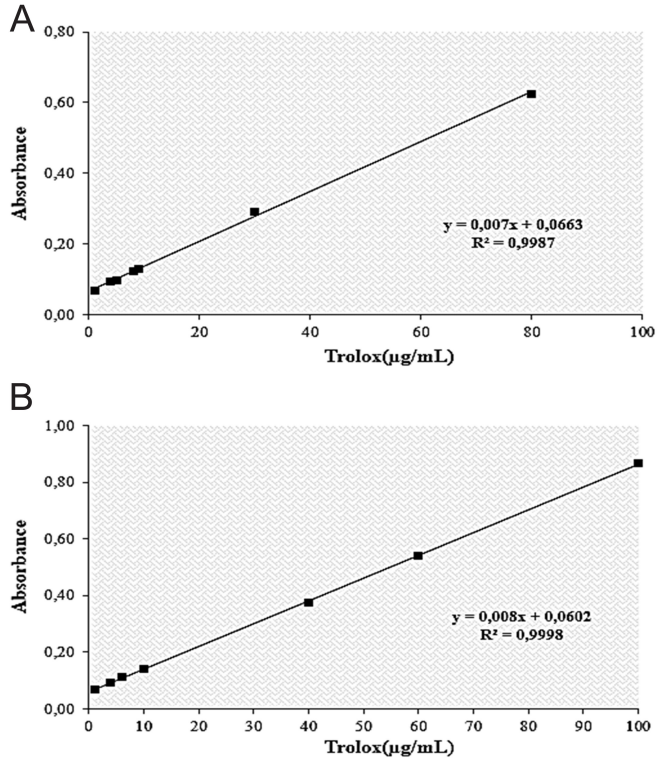

Figure 3. Trolox standard graph. A. Ethanol. B. Methanol.

Table 3. Comparison of the Conversion of the Extracts from Cu (II) Neocuproin Complex to Cu (I) Neocuproin at Different Concentrations in Terms of µg Trolox Equivalent Antioxidant Capacity

Concentration (µg/mL)	Trolox Equivalent (µg/mL)	
	Ethanol extract	Methanol extract
125	8.76	10.72
250	18.25	22.66
500	67.42	43.34

capacity; the concentration of 500 µg/mL was high in both extracts, and the results were close to each other.

DISCUSSION

In this study, ethanol and methanol extracts prepared from aronia fruits were evaluated in terms of antioxidant activity. It was determined that the ethanol extract was rich in CUPRAC and DPPH radical-scavenging activity. (Table 2,3) The methanol extract had high antioxidant activity in terms of FRAP and total phenolic compounds (Table 1,4). This feature may be due to the compounds contained in aronia fruits. It is thought that the various antioxidant activity differences observed in the extracts are due to the differences in the level and chemical structures of polyphenolic compounds transferred to the solvent used. For this reason, data obtained from in vitro studies on plant-derived natural antioxidants, which are preferred over synthetic antioxidants, will form the basis of in vivo studies.

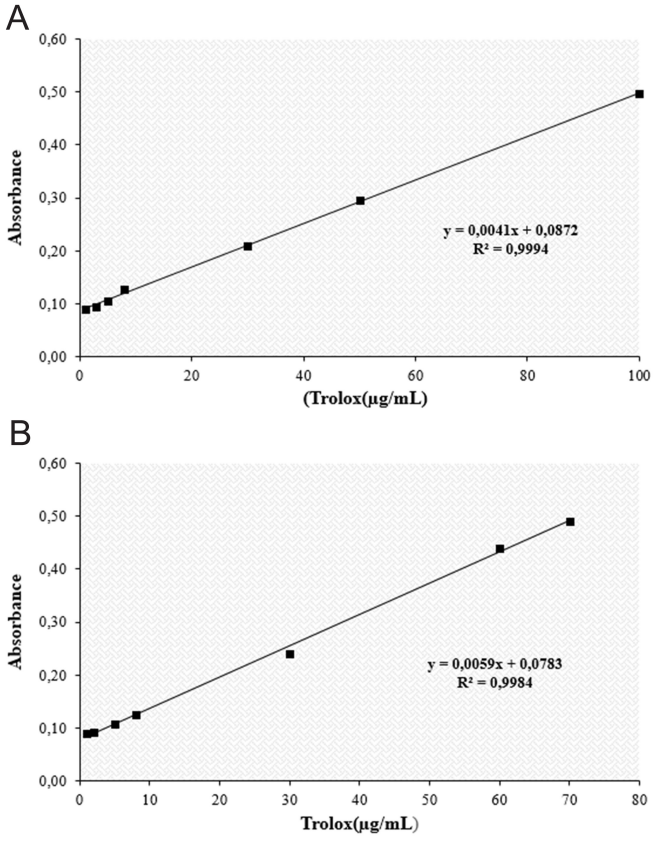

Figure 4. Trolox standard graph. A. Ethanol. B. Methanol.

Table 4. Comparison of İron (III) Reducing/Antioxidant Power of Extracts at Different Concentrations in µg Trolox Equivalent Antioxidant Capacity

Concentration (µg/mL)	Trolox Equivalent (µg/mL)	
	Ethanol Extract	Methanol Extract
125	4.44	4.72
250	7.67	10.03
500	18.65	18.74

In recent years, many studies have been conducted on ways to prevent, delay, and completely eliminate diseases. It has been proven as a result of research that many diseases are caused by the accumulation of free radicals in our bodies or as a result of environmental factors.[23-25] Preventing the formation of radicals or neutralizing the radicals before they damage the metabolism may delay the emergence of some diseases or positively affect the prognosis of the disease. One of the best ways to neutralize free radicals, which we ingest from external sources through various means, is to consume a diet rich in antioxidants.[23]

Since methods for determining antioxidant activity depend on various parameters and are affected by environmental factors, no single standard method is used to determine the antioxidant activity of a compound. Therefore, many methods are used to measure antioxidant activity. The different results in the literature may be due to the different methods and growing and drying conditions of the plants. For scientists working in the field of food, antioxidants are a very interesting topic due to their protective role against oxidative degradation in food products and pathological processes mediated by oxidative stress in the body. Effective investigation of natural antioxidant sources and the design of new antioxidant compounds require reliable methods for antioxidant activity assessment.[26]

There is still a need for traditional methods to quantify antioxidant activity, and certain methodological protocols are intricate and time-consuming to test. The pH is one of the most important selection factors in antioxidant testing. There are tests that function in alkaline (Folin–Ciocalteu), neutral (CUPRAC), or acidic (FRAP) environments. Another crucial element is the applicability of antioxidant testing to both lipophilic and hydrophilic antioxidants. Although both hydrophilic and lipophilic antioxidants can be measured using the ABTS and CUPRAC assays, certain techniques (FRAP and Folin–Ciocalteu) solely assess hydrophilic antioxidants, while others are limited to hydrophobic systems (DPPH).[27]

Aronia fruits are used in functional fruit juices and teas thanks to their high anthocyanin content, and in various products such as fruit juices, fruit juice concentrates, jams, and marmalade due to their high pectin content. It is also evaluated as a food supplement, in fresh or processed form, to improve the taste, color, and antioxidant properties of foods.[28-30]

In a study, the total phenolic, flavonoid, and anthocyanin contents of fresh and dried fruits of *Aronia* were examined, and it was determined that there was a 28%-60% decrease in the phenolic substance content as a result of all drying methods applied.[31]

Previous studies have shown that aronia, which has a rich nutritional content, is especially rich in terms of phenolic compounds.[32]

Jurikova et al[13] have determined in their study on preventing chronic diseases of aronia fruit that aronia fruit has high antioxidant activity, its phenolic compounds are very valuable, and the importance of anthocyanins and procyanidins. They reported that the phenolic component content of aronia fruit varies depending on the maturity period of the fruit, variety, and ecology.

Benvenuti et al[14] investigated total polyphenols, total anthocyanins, and reduced ascorbic acid in fruits belonging to the *Rubus*, *Ribes*, and *Aronia* genera using spectrophotometric and high-performance liquid chromatography techniques. The 2,2-diphen yl-1-picrylhydrazyl radical scavenging activity of fruit extracts was tested, and the results showed that the tested fruits were good natural sources of antioxidants.[14]

Arancibia-Avila et al[33]extracted freeze-dried aronia fruits with water, acetone, and hexane twice at room temperature for 3 hours, and the resulting fruit extracts were found to have 57.4, 2.84, and 0.89 µM TE/g values, respectively, by the DPPH method. In the same study, as a result of antioxidant measurement with the FRAP and CUPRAC methods, water, acetone, and hexane extracts were found to have values of 87.12, 3.55, and 1.19 µM TE/g, respectively, with the FRAP method and 219.9, 7.16, and 3.12 µM TE/g, respectively, with the CUPRAC method. Based on the DPPH, FRAP, and CUPRAC techniques, it was shown that water extract produced the greatest results.[33]

Berries of the aronia plant, or chokeberries, are an extremely rich source of various compounds that have positive effects on health. These compounds, which include proanthocyanidins, anthocyanins, flavonoids, and phenolic acids, are primarily polyphenols, which have antiviral, antioxidative, anti-inflammatory, anticancer, antiatherosclerotic, hypotensive, antiplatelet, and antidiabetic qualities. In a study, it was emphasized that there are currently not enough studies on the effects of consuming different aronia products on the human body during occupational and environmental exposure to toxic compounds and on people poisoned by these substances. Aronia products and aronia-occurring polyphenolic compounds, however, are useful in counteracting the toxicity of various xenobiotics in experimental studies. This suggests that these compounds may also be effective in humans, and well-planned epidemiological studies are required to investigate this further. These studies should first look into subjects who have been exposed to the substances for which aronia effectiveness has been demonstrated in animal models.[34]

Our study revealed that the methanol and ethanol extracts obtained from the aronia fruit, which were analyzed in support of other studies, were rich in antioxidant activity and phenolic content. When the results obtained from the FCR, DPPH, FRAP, and CUPRAC methods of aronia fruit extract obtained from ethanol and methanol extracts were compared with each other, the effectiveness of different extracts in different methods was generally determined. The superiority of the extracts over each other could not be determined.

Methanol and ethanol extracts obtained from aronia fruit were rich in antioxidant activity and phenolic content. Therefore, the studied species can be considered an important source of natural antioxidants. As a result, it was determined that the ethanol and methanol extracts of the aronia plant were not superior to each other. It is thought that this study will contribute to other studies on aronia fruit.

Ethics Committee Approval: Ethical approval was not required as this study was conducted *in vitro*.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – N.B., L.D.; Design – N.B., L.D.; Supervision – N.B.; Resources – N.B.; Materials – N.B., L.D.; Data Collection and/or Processing – N.B., L.D., M.S.; Analysis and/or Interpretation – L.D., M.S.; Literature Review – M.S.; Writing – L.D., M.S.; Critical Review – N.B., L.D, M.S.

Declaration of Interests: The authors declare that they have no competing interest.

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