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# Investigation of Phenolic, Flavonoid, Total Antioxidant Capacity, Vitamins, and Glutathione Contents of *Aronia Melanocarpa* (Michx.) Fruits

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## Abstract

The nutritional profile and antioxidant capacity of ripe aronia (*Aronia melanocarpa*) fruit were comprehensively analyzed using High-Performance Liquid Chromatography (HPLC) and Ultraviolet-visible (UV-vis) spectrophotometry. The content of fat-soluble vitamins was quantified as follows: vitamin A – 12.44 ± 0.69 µg/g, vitamin E – 19.51 ± 0.88 µg/g, β-carotene – 51.64 ± 2.44 µg/g, and lycopene – 5.85 ± 0.30 µg/g. Water-soluble vitamins were also abundant, with ascorbic acid (vitamin C) being the most prevalent at 412.92 ± 12.61 µg/g, followed by riboflavin (16.43 ± 0.83 µg/g), pantothenic acid (36.37 ± 1.02 µg/g), and others including thiamine, nicotinic acid, pyridoxine, folic acid, and cyanocobalamin. Glutathione levels, essential for redox balance, were measured as 706.32 ± 19.46 µg/g for reduced glutathione (GSH) and 133.67 ± 8.28 µg/g for oxidized glutathione (GSSG). The fruit also exhibited high polyphenol and flavonoid contents, with total phenolics at 81.28 ± 3.21 µg gallic acid equivalents per gram and flavonoids at 65.55 ± 2.50 µg quercetin equivalents per gram. Antioxidant capacity was evaluated using two methods: the DPPH assay yielded an $IC_{50}$ value of 23.17 ± 1.35 µg/mL, indicating strong radical scavenging activity, while the TEAC method demonstrated a total antioxidant capacity of 272.85 ± 10.91 µmol Trolox equivalents per gram. Ripe aronia fruit is rich in essential vitamins, glutathione, phenolic and flavonoid compounds, and possesses strong antioxidant capacity. These findings support its potential health benefits when consumed in appropriate amounts.

**Keywords:** *Aronia melanocarpa* (Michx.), Vitamins, Glutathione, Phenolic substance, Flavonoid substance, Total antioxidant capacity.

# Aronia Melanocarpa (Michx.) Meyvesindeki Fenolik, Flavonoid, Toplam Antioksidan Kapasitesi, Vitamin ve Glutatyon İçeriğinin Araştırılması

## Öz

Aronya meyvesindeki yağda ve suda gözünen vitaminler, glutatyon miktarları yüksek performanslı sıvı kromatografisi (HPLC) ile tayin edilmiştir. Toplam fenolik ve flavonoid bileşik miktarları ve toplam antioksidan kapasitesi spektrofotometre ile belirlenmiştir. Meyvedeki vitamin A, E, β-karoten ve likopen miktarları sırasıyla 12.44±0.40, 19.51±0.51, 51.64±1.41 ve 5.85±0.17 µg/g olarak bulundu. Aronya meyvesindeki suda çözünen vitaminlerden askorbik asit, tiamin, riboflavin, nikotinik asit, pantotenik asit, pridoksin, folik asit ve siyanokobalamin miktarları sırasıyla 412.92±7.29, 4.27±0.14, 16.43±0.48, 34.13±1.01, 36.37±0.59, 3.43±0.12, 14.0±0.55, 5.33±0.14 µg/g, iken redükte ve okside glutatyon (GSH, GSSG) miktarları ise 706.32±11.25, 133.67±4.79 µg/g olarak belirlendi. Meyvelerdeki toplam fenolik madde miktarı 81.28±1.86 µg Gallik Asit/g, flavonoid madde miktarı ise 65.55±1.45 µg Kuersettin/g olarak belirlenmiştir. Aronya meyvesindeki DPPH yöntemine göre belirlenen $IC_{50}$ değeri 23.17±0.78 µg/mL, TEAK yöntemine göre hesaplanan antioksidan kapasite değeri ise 272.85±6.31 µmol Troloks/g olarak bulunmuştur. Olgun aronya meyvesindeki vitaminler, glutatyon, toplam fenolik ve flavonoid madde miktarları ile toplam antioksidan kapasite bakımından uygun dozda tüketildiğinde sağlık için yararlı olduğu söylenebilir.

**Anahtar Kelimeler:** *Aronya melanocarpa* (Michx.), Vitaminler, Glutatyon, Fenolik madde, Toplam antioksidan kapasite

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## 1. Introduction

As the incomes and living standards of people's increase, they have higher expectations about food they consume and health benefits. Therefore, the market demand for healthy foods containing bioactive ingredients (Gironés-Vilaplana et al., 2014) increase every year. At the same time, the increasing interest in natural products in the health and food industry also pushes the importance of products obtained from medicinal and aromatic plants (İşbilir et al., 2023). *Aronia melanocarpa* (Michx.) is considered a medicinal and aromatic plant, including Elliot (black and red aronia) and *Aronia arbutifolia* (L.) (purple aronia), which is a hybrid aronia species (Denev et al., 2018; Shahin et al., 2019).

*Aronia melanocarpa* has been reported to exhibit positive health benefits due to the presence and high content of various bioactive components such as vitamins, minerals, and polyphenolic compounds (Szopa et al., 2017). Aronia fruit is rich in polyphenols with antioxidant and antimicrobial properties (Denev et al., 2018).

There are many studies conducted on the antioxidant activity of Aronia berries, as well as their anti-inflammatory, antidiabetic, anticancer, anti-mutagenic, and antibacterial activities, and their effects and therapeutic potential on various diseases such as obesity, cardiovascular diseases, and autoimmune diseases (Kulling et al., 2008; Oszmiański and Lchowicz, 2016; Jurikova et al., 2017).

Vitamins have a regulatory role in the living system and act as catalysts in metabolic events; they are organic substances that help nutrients to be used efficiently. Water and fat-soluble vitamins must be consumed with the diet (Asensi-Fabado and Munne-Bosch, 2010; Kennedy, 2016). Lycopene, a major carotenoid found in fruits and vegetables, is reported to be a powerful antioxidant and effective in the treatment of many diseases (Ono et al., 2015).

GSH is a powerful antioxidant that decreases with aging and is an essential scavenger of reactive oxygen species (ROS). It also participates in the detoxification of hydrogen peroxide by glutathione peroxidases; GSH and GSSG are the central redox couple that determines the antioxidant capacity of cells (Gaucher et al., 2018; Oliveira and Laurindo, 2018).

Phenolic compounds, an indispensable part of our nutrition, are organic substances found mainly in plant foods and constitute a large group of antioxidants. They effectively prevent cardiovascular and degenerative diseases due to their antioxidant properties (Karakaya, 2004; Cartea et al., 2011). Flavonoids, which constitute the most phenolic group in plants, are essential in the functions of some enzymes and are reported to have anti-oxidative, anti-inflammatory, anticancer, and anti-mutagenic properties (Panche et al., 2016). Fruits, in particular, attract special attention because they contain high amounts of antioxidants. Therefore, it is essential to study the antioxidant

capacity of many molecules naturally found in food and biological systems (MacDonald-Wicks et al., 2006).

As in all medicinal and aromatic plants, the Aronia plant's and fruit contents may change due to cultural processes such as climate, soil, irrigation, fertilization, harvest time, etc. Therefore, the aim was to determine the level of vitamins, total phenolic and flavonoid substance, total antioxidant capacity, and the amount of glutathione in the ripe fruits of the Aronia plant grown in the Malatya region.

## 2. Material and Methods

### 2.1. Materials

Aronia seedlings obtained from Konya province were used to create an aronia garden in the Merdivenler neighborhood of Battalgazi district of Malatya three years ago. Aronia plants were grown in completely organic conditions and absolutely no pesticides were used for the soil or the plant. Aronia fruit samples were collected when it was reached to sufficient maturity in October 2024.

### 2.2. Methods

#### 2.2.1. Determination of vitamins A, E, β-carotene, and lycopene

Aronia fruits were analyzed according to Ali et al. (2024a) by High Performance Liquid Chromatography (HPLC).

#### 2.2.2. Analysis of Water Soluble Vitamins

Water-soluble vitamins were determined on homogenized aronia samples using the procedures by Ali et al. (2024b) using HPLC.

#### 2.2.3. Determination of Glutathione in Aronia berries

The amounts of GSH and GSSG in homogenized aronia fruits were determined using HPLC, according to Ali et al. (2024a).

### 2.2.4. Total Antioxidant Capacity

Obtaining the extract from the aronia fruit and determining total phenolic and flavonoid substances and total antioxidant capacity were done according to the methods applied by Çakmak et al. (2023).

### 2.3. Statistical Analysis

All measurements were made in three parallels, and the results were given as Mean ± Standard deviation.

## 3. Findings and Discussion

Studies conducted in recent years have shown that the potential of consuming medicinal plants and fruits to reduce the risks of degenerative diseases is related to their bioactive compounds, mainly phenolic substances, which have antioxidant properties. The increasing interest in healthy nutrition and functional foods increases the demand for the production and use of these plants (Karadag, 2019).

The amount of fat-soluble vitamins and lycopene in Aronia fruit is given in Figure 1.

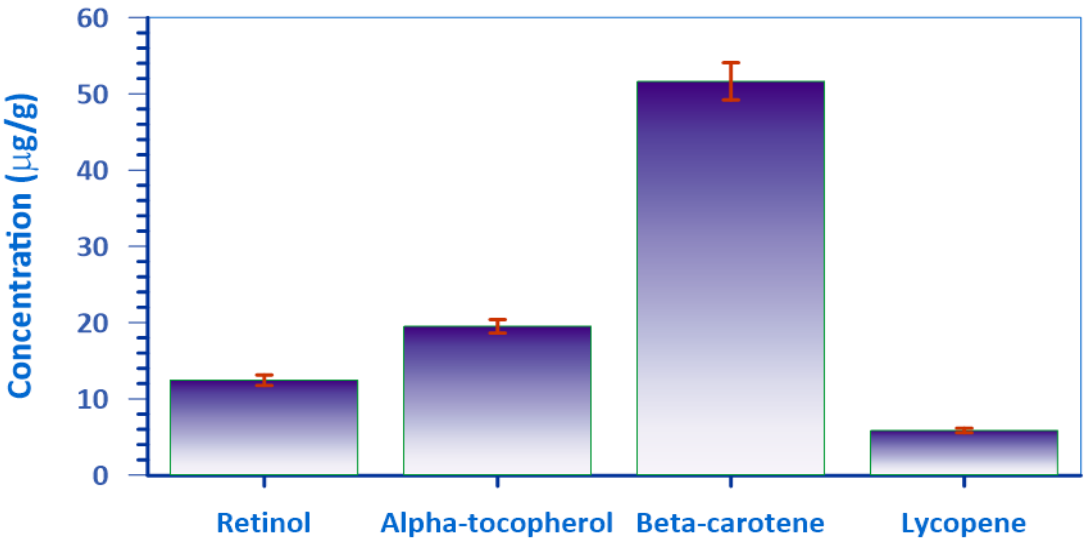


**Figure 1.** Fat-soluble vitamins and lycopene concentration in aronia berries

Vitamins are vital micronutrients that are obtained through the diet. Fat-soluble vitamins complement numerous physiological processes, including vision, bone health, immunity, and coagulation. Vitamin A, a singlet oxygen scavenger, is essential for maintaining mucous membranes

and normal vision. Vitamin E is an antioxidant that protects polyunsaturated fatty acids in cell membranes from oxidative damage and is also reported to maintain membrane fluidity and stability (Andrès et al., 2024). β-carotene is a precursor of vitamin A and is easily converted to vitamin A in metabolism. It also prevents eye diseases (Grune et al., 2010). Lycopene is a carotenoid with antioxidant properties and has been reported to have many pharmacological benefits, such as anticancer, cardio-protective, and antihypertensive effects (Khan et al., 2021).

The amounts of vitamin A, E, β-carotene and lycopene in aronia fruit were found to be 12.44±0.69, 19.51±0.88, 51.64±2.44 and 5.85±0.30 µg/g, respectively. Vitamin A in aronia berries is reported to be 0.77 mg/100 g (Lancrajan, 2012). The amounts of vitamin E, β-carotene, and lycopene in aronia fruit were reported to be 1.35–1.47 mg/100 g, 495–887 µg/100 g, and 16.7 mg/kg, respectively) (Sidor and Gramza-Michałowska, 2019).

Lancrajan (2012) reports that the amounts of vitamin A, α-tocopherol, and β-carotene in aronia berries are 0.77, 1.35-1.47 mg/100 g, and 495-887 µg/100 g, respectively.

The amount of vitamins A and E, β-carotene, lycopene in wild black myrtle fruit is reported to be 2.15±0.06, 186.96±7.05, 7.00±0.12 and 9.34±0.31 µg/g dw, respectively (Çakmak et al., 2023).

*Aronia melanocarpa* fruits which are rich in vitamins. It has been reported that vitamin C, an antioxidant, is necessary for the human body to prevent scurvy and gum disease and strengthen the immune system (Lupascu and Sîrbu, 2020).

B vitamins, which play a role in catabolic and anabolic metabolism, serve as coenzymes in enzymatic processes in addition to their primary functions in the brain and nervous system. They have also been reported to play a role in energy production, DNA/RNA synthesis/repair, genomic and non-genomic methylation, and the synthesis of many neurochemical and signaling molecules. It is stated that any vitamin B deficiency can negatively affect the mitochondrial metabolism of amino acids, glucose, and fatty acids via the citric acid cycle and electron transport chain (Kennedy, 2016; Hanna et al., 2022).

The amounts of water-soluble vitamins in Aronia fruit are given in Figure 2.

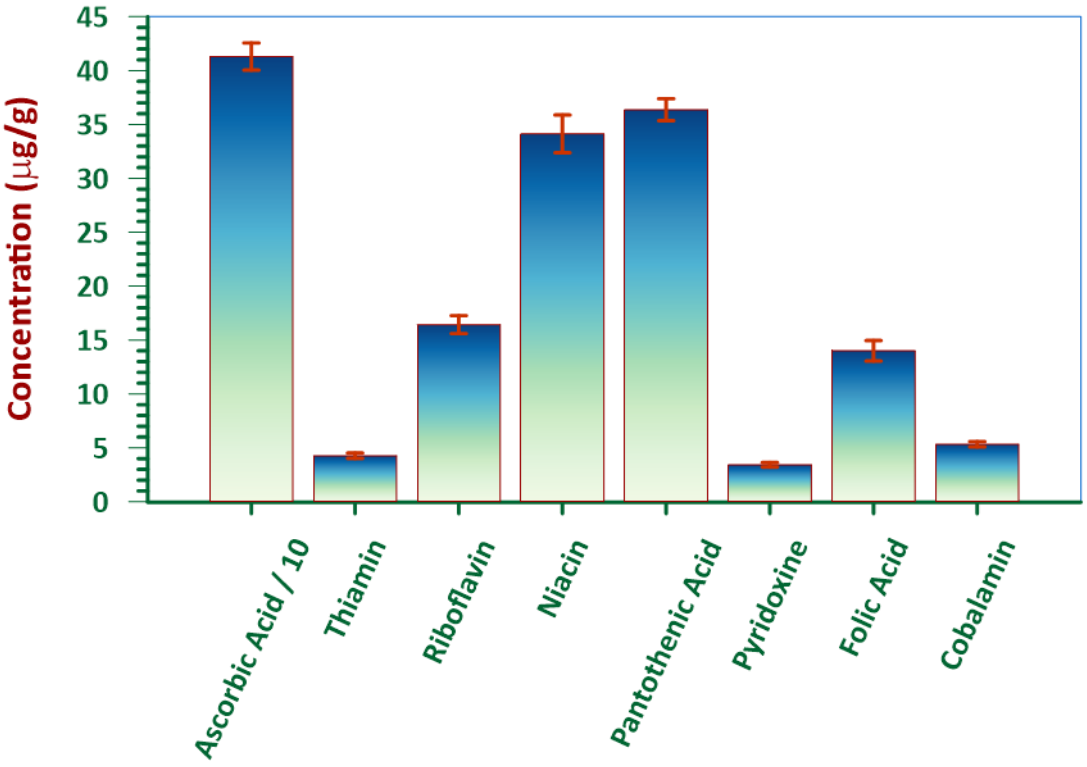


**Figure 2.** Water-soluble vitamin contents of Aronia berries

The amounts of ascorbic acid, thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, folic acid, and cyanocobalamin in aronia fruit were found to be 412.92±12.61, 4.27±0.25, 16.43±0.83, 34.13±1.75, 36.37±1.02, 3.43±0.21, 14.0±0.95, 5.33±0.25µg/g, respectively. The thiamine, riboflavin, and pyridoxine vitamins in aronia berries were reported to be 180, 200, and 280 µg/kg, respectively (Tanaka and Tanaka, 2001). Zhang et al. (2021) reported the amounts of vitamin C, thiamine, riboflavin, niacin, and folate in different aronia cultivars as 137, 0.18, 0.20, 0.03, and 3 mg/kg fresh weight, respectively.

It has been reported that vitamin C in aronia fruit varies between 2.3–31 mg/100 g, vitamin B1 varies between 0.017–0.019 mg/100 g, vitamin B2 varies between 0.016–0.027 mg/100 g, vitamin B3 varies between 0.27–0.34 mg/100 g, B5 varies between 0.225–0.382 mg/100 g, vitamin B6 varies between 0.024–0.029 mg/100 g, and B9 varies between 0.002–0.004 mg/100g (Sidor and Gramza-Michałowska, 2019).

It is stated that the amounts of ascorbic acid, thiamine, riboflavin, pyridoxine, niacin, and pantothenic acid in aronia fruit were reported to be 14, 0.02, 0.02, 0.03, 0.3, 0.3 mg/100 g, respectively. In contrast, the amount of folate was 20 µg/100 g (Jurendi´c and Š˘cetar, 2021).

The amounts of vitamins C, B1, B2, B3, B6, B9 and B12 in wild black myrtle fruit were reported to be 1323.0±31.9, 78.72±3.03, 118.20±3.03, 344.27±14.60, 40.80±1.36, 5190.0±55.2 and 71.39±2,059.34±0.31 µg/g dw, respectively (Çakmak et al., 2023).

Glutathione is found in GSH and GSSG forms in living beings and is a tripeptide that affects the intracellular redox status with antioxidant properties. Drugs are usually excreted from the body by conjugation with GSH, which is effective in detoxification. Glutathione is reported to be associated with anti-inflammatory, Parkinson's, Alzheimer's, chronic obstructive pulmonary disease, asthma, autoimmune diseases, hypertension, myocardial infarction, atherosclerosis, cholesterol oxidation, cataracts, and glaucoma (Pizzorno and Katzinger, 2012; Georgiou-Siafis and Tsiftsoglou, 2023).

The amounts of reduced and oxidized glutathione in aronia fruit are given in Figure 3.

The GSH and GSSG contents in aronia fruit were 706.32±19.46 and 133.67±8.28 µg/g, respectively. The amounts of GSH and GSSG in wild black myrtle fruit were reported to be 806.10±8.83 and 190.10±6.27 µg/g dw, respectively (Çakmak et al., 2023). It is stated that the amounts of GSH and GSSG in *Viburnum opulus* L. fruit grown in different regions vary between 206.78 -334.75 and 19.16-23.40 µg/g, respectively (Boyacı et al., 2016). Cerit et al. (2020) reported the GSH amounts in orange, lemon, grapefruit, mango, papaya, strawberry, and cardamom fruits as 5.0±11, 5±0, 13±3, 59±6, 136±12, 39±8 and 112±2 nM/g, respectively.

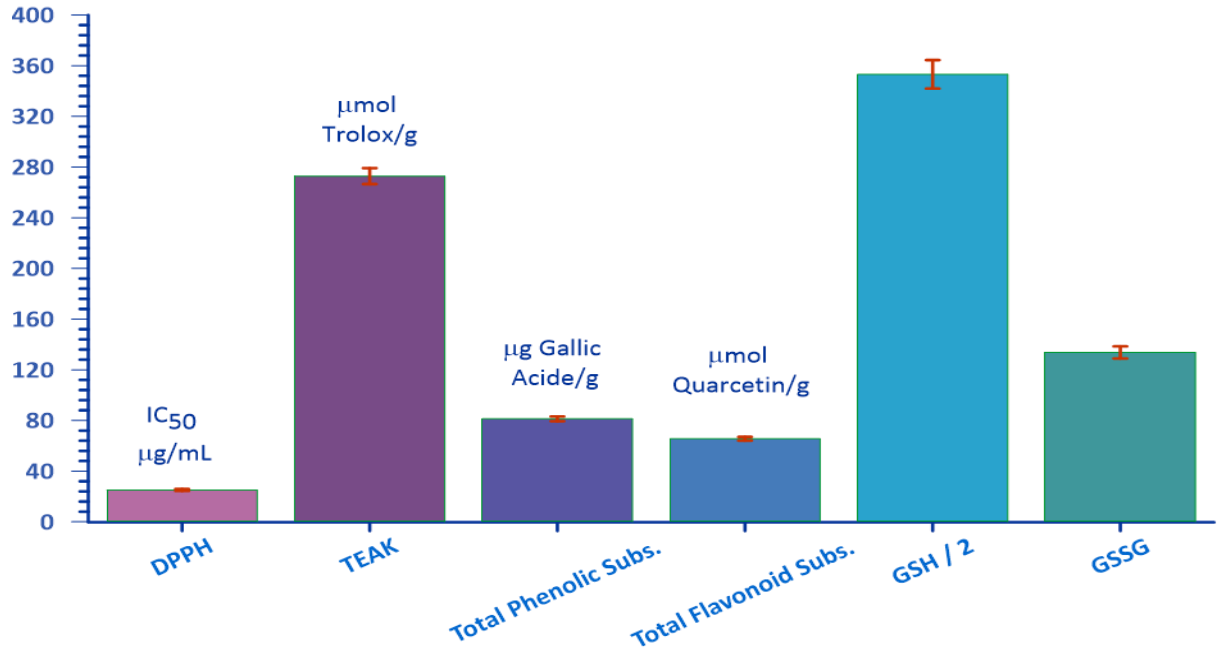


**Figure 3.** Glutathione, total phenolic and flavonoid substance, $IC_{50}$, and TEAC values of aronia fruits

Phenolic compounds are secondary metabolites in many plants' growth and development processes. Known as natural antioxidants, they are reported to have numerous biological activities such as antibacterial, antitumor, antidiabetic, and anti-inflammatory activities (Rahman et al., 2022).

Flavonoids are natural compounds found in plants with low molecular weight that positively affect human health. Flavonoids are notable for their antioxidant, anti-mutagenic, antiproliferative,

antitumor, antiviral, and anti-inflammatory properties. Epidemiological studies have shown the importance of flavonoid compounds in reducing the risk of cardiovascular diseases and cancer (Garcia-Lafuente et al., 2009; Sghaiera et al., 2011). The total phenolic and flavonoid content of aronia fruit is given in Figure 3.

While the amount of total phenolic substance in aronia fruit was determined as 81.28±3.21 µg Gallic Acid/g, the total flavonoid substance was determined as 65.55±2.50 µg Quersetin/g.

Zhang et al. (2021) reported that the total phenolic and flavonoid substance in different aronia cultivars as 110.92 and 21.94 g/kg, respectively. Najda and Łabuda (2013) reported that the total amount of flavonoids in aronia was 18.31 mg Quersetin equivalent (QE) /100 g fresh weight.

The total antioxidant capacity in blackberry, black currant, strawberry, red grape, and golden strawberry fruits were reported as 55.7 ± 14.7, 56.7 ± 13.5, 20.6 ± 2.3, 32.6 and 17.0 µmol of Trolox equivalent (TE) /g fresh weight, respectively (Kulling and Rawel, 2008).

Total antioxidant capacity ($IC_{50}$ and TEAC) values in aronia fruit are given in Figure 3. The IC50 value in aronia fruit determined by the DPPH method was found to be 23.17±1.35 µg/mL, and the antioxidant capacity value calculated by the 2,2'-azinobis (3-etil-bezotiazolin 6 sulfonat) [ABTS] method was found to be 272.85±10.91 µmol Trolox/g.

The EC50 value is expressed as the amount of extract that inhibits the radical (DPPH) concentration by 50%; that is, the low value indicates that the antioxidant activity of that plant is higher (Karadag, 2019). Aytuna Çerçi et al. (2024) reported the IC50 value in aronia as 0.71 ± 0.12 µg/mL.

Determining total antioxidant capacity (TAC) in biological systems is one of the most frequently used strategies to assess the free radical-antioxidant balance. The antioxidant capacity of blood is considered to be an indicator of the ability of the ingested food to act as an antioxidant in the living organism. Dietary interventions change the antioxidant capacity of the blood. The fruit's antioxidant power is closely related to the presence of adequate oxygen radical scavengers such as vitamin C and phenolic compounds (Fraga et al., 2014; Prenesti et al., 2021).

Özdemir and Eroğlu (2020) reported that the total flavonoid content in aronia was 5.3±0.2 g catechin/kg fresh weight, the $EC_{50}$ value was 1.8±0.3 mg/mL, and the ABTS value was 11.0±0.04 g TE /kg. The $IC_{50}$ and TEAC values in wild myrtle fruit were reported to be 39.21±1.25 µg/mL and 295.08±12.5 µmol trolox/g dw, respectively (Çakmak et al., 2021).

Tolić et al. (2017) reported that *Aronia melanocarpa* has a very complex chemical composition that can vary depending on many factors, such as soil composition, fertilization, climatic conditions, fruit maturity, harvesting method, and storage conditions.

Aronia's fruit is considered a medicinal plant, rich in vitamins, glutathione, total phenolic and flavonoid substances, and total antioxidant capacity. However, our findings are consistent with some

literature values; conversely, they are higher and lower than our findings in general. The reason for these differences can be explained by Tolić et al. (2017).

## 4. Conclusions

The fruit was found to be rich in both fat-soluble vitamins—A ($12.44 \pm 0.69$ µg/g), E ($19.51 \pm 0.88$ µg/g), β-carotene ($51.64 \pm 2.44$ µg/g), and lycopene ($5.85 \pm 0.30$ µg/g) and water-soluble vitamins, including ascorbic acid ($412.92 \pm 12.61$ µg/g), riboflavin, pantothenic acid, and others in notable amounts. Glutathione levels were particularly high, with GSH at $706.32 \pm 19.46$ µg/g and GSSG at $133.67 \pm 8.28$ µg/g. Additionally, the total phenolic content was measured at $81.28 \pm 3.21$ µg gallic acid equivalents per gram, while total flavonoids reached $65.55 \pm 2.50$ µg quercetin equivalents per gram. The fruit showed strong antioxidant capacity, with a DPPH $IC_{50}$ value of $23.17 \pm 1.35$ µg/mL and a TEAC value of $272.85 \pm 10.91$ µmol Trolox/g. These findings highlight aronia fruit as a potent source of bioactive compounds with potential health benefits, particularly due to its antioxidant properties. Regular consumption of aronia, in appropriate amounts, may support nutritional and therapeutic applications related to oxidative stress and immune health. In addition to other biochemical parameters of this fruit, different preservation methods can be suggested for further studies so that this fruit can be used outside the harvest period.

## Authors' Contributions

All authors contributed equally to the study.

## Statement of Conflicts of Interest

Authors declare that they have no known conflict of interest

## Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

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