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COMPARATIVE ANALYSIS OF ANTIOXIDANT ACTIVITY AND PHENOLIC COMPOUNDS IN THE FRUITS OF *ARONIA* SPP.

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ABSTRACT

Chokeberry (*Aronia* Medik.) is a non-traditional fruit plant known as a rich source of biologically active compounds and inhibits the numerous biological activities. We compared the antioxidant activity and phenolic compounds of fruits between widely cultivated *Aronia mitschurinii* (AM-TCH, from Tchekhov district; AM-D, from Dmitrov district; AM-OZ, from Orekhovo-Zuevsky district of Moscow region, Russia) and introduced North American Aronia species (*Aronia arbutifolia* (AA-M), *A. melanocarpa* (AML-M), *A.* × *prunifolia* (AP-M), which have not been planted yet in the arboretum of Main Botanical Garden of the Russian Academy of Sciences (Moscow). Studying samples were collected in their secondary distribution range. Ethanolic extracts were determined for antioxidant capacity (antioxidant activity by DPPH and phosphomolybdenum methods, the total content of polyphenols, flavonoids, phenolic acids) and measured spectrophotometrically. As standards were used Trolox (TE) for antioxidant activities, gallic acid (GAE) for polyphenol content, quercetin (QE) for flavonoid content, caffeic acid (CAE) for phenolic acid content. The antioxidant activity by DPPH method in ethanol extracts of investigated plants was from 6.96 (AM-D) to 8.89 (AM-OZ) mg TE.g^{-1} DW. Reducing the power of investigated extracts exhibited activity from 151.47 (AM-OZ) to 297.8 (AA-M) mg TE.g^{-1} DW. The content of polyphenol compounds determined from 25.98 (AM-TCH) to 54.39 (AA-M) mg GAE.g^{-1} DW, phenolic acids content was from 7.76 (AP-M) to 11.87 (AM-D) mg CAE.g^{-1} DW and the content of flavonoids detected from 8.12 (AM-OZ) to 16.62 (AM-D) mg QE^{-1} DW. Obtained data showed a strong correlation between the content of polyphenol compounds and reducing the power of extracts (r = 0.700), between flavonoids and phenolic acids (r = 0.771) and also between phenolic acids and reducing power (r = 0.753) in Aronia ethanol extracts. Fruits of investigated species of Aronia can be propagated as a source of polyphenol compounds with antioxidant activity and obtained results may use for farther pharmacological study.

Keywords: *Aronia* spp.; antioxidant activity; polyphenols; flavonoids; phenolic acids

INTRODUCTION

The last studies demonstrated that fruit plants are rich in antioxidants and their use can promote human health **(Widén et al., 2012)**. *Aronia mitschurinii* A. K. Skvortsov and Maitul are one of the most famous sources of food polyphenols and antioxidants, its juice has long been used in clinical practice. The natural distribution range of Aronia is located in the eastern part of North America. According to a later nomenclature **(Hardin, 1973)**, the genus Aronia consists of three species: *A. arbutifolia* (L.) Rers., *A. melanocarpa* (Michx.) and their hybrid *A.* × *prunifolia* (Marshall) Rehder. All three species have been introduced in European gardens since the beginning of the XIX century. At the end of the XIX century, *A. melanocarpa* from Germany has been grown in the nursery of I. V. Mitchurin (Tambov province, Russia). There, by the method of "screening in three generations", a man-made *A. mitschurinii* was born **(Vinogradova and Kuklina, 2014)**. It is still unclear whether this taxon arose as a result of macro mutation, or is it a hybrid between *A. melanocarpa* and *Sorbus* spp. Undoubtedly, however, that *A. mitschurinii*, both by morphological and by genetic characteristics, differs so much from the parental *A. melanocarpa*, which is quite correctly described as an individual species **(Skvortsov and Maitulina, 1982; Skvortsov, Maitulina and Gorbunov, 1983)**. Also, based on the morphological properties of *A. mitchurinii* selection work with this species can be successful **(Vinogradova et al., 2017)**. At first, *A. mitschurinii* has been grown as a fruit crop enriched in vitamins and minerals. In the 1960s, the discovery of high content of P-vitamin substances in its berries led to the using of Aronia juice in medical institutions for the treatment of hypertension **(Vinogradova and Kuklina, 2012)**. Now this species began to be tested as a source of antioxidant activity due to the high content of polyphenols **(Mayer-Miebach, Adamiuk and Behsnilian, 2012; Bräunlich, 2013; Taheri et al., 2013; Žlabur Šic et al., 2017)**.

Previous reports about *Aronia melanocarpa* showed that its fruits contain powerful antioxidants (anthocyanidins,

phenolic acids, quercetin glycosides) with different pharmacological activities such as anti-cancer **(Jeong, 2008; Kulling and Rawel, 2008; Olas et al., 2008)**, cardio-protective **(Olas et al., 2010)**, antimutagenic, lipid-lowering, antihypertensive, hepatoprotective, gastroprotective, antimicrobial, radioprotective, immunomodulatory **(Kokotkiewicz, Jaremicz and Luczkiewicz, 2010)**, anti-inflammatory, anti-allergic, antiatherogenic, antidiabetic **(Banjari et al., 2017)**. Fruits of Aronia species contain flavonoids anthocyanins **(Brand et al., 2017)**. In the fresh fruits of *Aronia* spp. was identified carbohydrates (sorbitol, fructose, glucose, sucrose), organic acids (quinic acid, malic acid, ascorbic acid, shikimic acid, citric acid, etc.) **(Sidor and Gramza-Michałowska, 2019)**, polyphenols (neochlorogenic acid, chlorogenic acid, epicatechin, quercetin-3-rutinoside, quercetin, etc.) **(Šnebergrová et al., 2014; Denev et al., 2018)**. Biochemical composition of fresh fruits it's also sugars (10 – 18%), pectin (0.6 – 0.7%), fat (0.14%), ash (0.44%), mineral compounds (high content of K, Zn, Na, Ca, Mg, Fe) **(Kokotkiewicz, Jaremicz and Luczkiewicz, 2010)**. Some results showed that the fruits of *Aronia* spp. rich in antioxidants such as polyphenols, flavonoids, anthocyanins **(Widén et al., 2012; Kapci et al., 2013)**. According to **Kapci et al (2013)**, chokeberry fruits contain four major anthocyanins such as cyanidin-3-galactoside, cyanidin-3-arabinoside, cyanidin-3-glucoside and cyanidin-3-xyloside. Polyphenols in *A. mitschurinii* 'Viking' have been found to consist of cyaniding anthocyanins, proanthocyanidins, flavonols, chlorogenic acid, and neochlorogenic acid **(Oszmianski and Wojdylo, 2005; Slimestad et al., 2005; Koponen et al., 2007)**.

The study of **Suvajdžić et al. (2017)** demonstrated that *Aronia* × *prunifolia* juice and ethanol extracts showed antialgal activity. Moreover, a higher concentration of selected biochemical compounds was identified in the extracts than in juice. The juice from fruits of *A. mitschurinii* has an antimutagenic activity **(Gasiorowski et al., 1997)**, gastroprotective effect **(Matsumoto et al., 2004)**, hepatoprotective activity **(Valcheva-Kuzmanova and Belcheva, 2006)**, cardioprotective and anti-diabetes effect **(Kulling and Rawel, 2008; Denev et al., 2012; Gralec, Wawer and Zawada, 2019)**, anticancer activity **(Sharif et al., 2012)**, anti-inflammatory effect **(Martin et al., 2014)**, antiatherogenic activity **(Daskalova et al., 2015)**. In addition, fruits of *A. melanocarpa* characterized by antioxidant, antimicrobial, and neutrophil-modulating activity **(Denev et al., 2019)**. Production containing chokeberry fruits also characterized. As reported by **Nguyen and Hwang (2016)**, yogurt with *Aronia melanocarpa* juice had high antioxidant activity.

However, there is little information on the polyphenol contents in the other wild species of Aronia. As reported **Denev et al. (2018)**, in *A. melanocarpa* fruits identified organic acids (quinic, malic, ascorbic, shikimic, citric, oxalic, succinic), polyphenols (neochlorogenic acid, chlorogenic acid, epicatechin, quercetin-3-rutiniside, quercetin-3-glucoside, quercetin, anthocyanins, proanthocyanidins), carbohydrates (the main component is sorbitol).

In Europe, these species are not yet cultivated and are only available in collections of botanical gardens **(Vinogradova and Kuklina, 2014)**, although, according to the latest data, they possess economically valuable traits **(Kokotkiewicz, Jaremicz, and Luczkiewicz, 2010)**. In the USA, native *A. melanocarpa*, *A. arbutifolia,* and *Aronia* × *prunifolia* have unique polyphenol profiles warranting further investigation of their comparative nutraceutical and commercial values **(Taheri et al., 2013)**.

Antioxidant properties of *Aronia* spp. can be useful as well in human life as an animal. Investigation of **Bolser et al. (2013)** showed that autumn-migrating birds selected certain of wild-growing plant fruits among which were *Aronia* × *prunifolia* and *A. melanocarpa*. These two species, along with others, had a high concentration of antioxidants as reported in this study.

The purpose of this study was to investigate the antioxidant capacity of Aronia fruits of different origins.

Scientific hypothesis

The first, we assumed (based on the Vavilov's Law about homological rows) that the closely related North American species of the genus Aronia will have the same high levels of antiradical activity as the widely cultivated *A. mitschurinii*. The second, we assumed that native species (from North America) have the same polyphenol contents as plants in the secondary distribution range (from Moscow).

MATERIAL AND METHODOLOGY

Plant materials

In this study, we investigated fruits of plants of *Aronia arbutifolia* (L.) Pers. (AA-M), *A. melanocarpa* (Michx.) (AML-M), *Aronia* × *prunifolia* (Marshall) Rehder (AP-M) in the arboretum of Main Botanical Garden of the Russian Academy of Sciences (Moscow, Russia), which were brought from the USA in the 1980s. Also, we used to study samples of cultivated *A. mitschurinii* A. K. Skvortsov and Maitul from the Tchekhov (AM-TCH) and *A. mitschurinii* from Dmitrov (AM-D) districts of the Moscow region and one sample of naturalized *A. mitschurinii* from the Orekhovo-Zuevsky (AM-OZ) district of the Moscow region.

Chemicals

All chemicals were analytical grade and were purchased from Reachem (Slovakia) and Sigma Aldrich (USA).

Sample preparation

0.2 g of dried plant raw material was extracted with 20 mL of 80% ethanol for 2 hours. After centrifugation at 4000 g (Rotofix 32 A, Hettich, Germany) for 10 min, the supernatant was used for the next measurements: antioxidant activity, polyphenols, and flavonoids.

Antioxidant activity

Radical scavenging assay

The radical scavenging activity of samples was measured using 2.2-diphenyl-1-picrylhydrazyl (DPPH) **(Sanchez-Moreno, Larrauri and Saura-Calixto, 1998)**. The extracts (0.5 mL) were mixed with 3.6 mL of radical solution (0.025 g of DPPH in 100 mL ethanol). The absorbance of the sample extract was determined using the

spectrophotometer Jenway (6405 UV/VIS, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10 – 100 mg.L^{-1}; R^2 = 0.988) was used as the standard and the results were expressed in mg.g^{-1} Trolox equivalents.

Molybdenum reducing power

Reducing the power of extracts was determined by the phosphomolybdenum method of **Prieto, Pineda and Aguila (1999)** with slight modifications. The mixture of a sample (1 mL), monopotassium phosphate (2.8 mL, 0.1 M), sulfuric acid (6 mL, 1 M), ammonium heptamolybdate (0.4 mL, 0.1 M) and distilled water (0.8 mL) incubated at 90 °C for 120 min, then rapidly cooled and detected by monitoring absorbance at 700 nm using the spectrophotometer Jenway (6405 UV/VIS, England). Trolox (10 – 1000 mg.L^{-1}; R^2 = 0.998) was used as the standard and the results were expressed in mg.g^{-1} Trolox equivalents.

Total polyphenol content

Total polyphenol content extracts were measured by the method of **Singleton and Rossi (1965)** using Folin-Chiocalteu reagent. 0.1 mL of each sample extract was mixed with 0.1 mL of the Folin-Chiocalteu reagent, 1 mL of 20% (w/v) sodium carbonate and 8.8 mL of distilled water. After 30 min. in darkness the absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/VIS, England). Gallic acid (25 – 250 mg.L^{-1}; R^2 = 0.996) was used as the standard and the results were expressed in mg.g^{-1} gallic acid equivalents.

Total flavonoid content

Determination of total flavonoids content was conducted using the modified method of **Shafii et al. (2017)**. 0.5 mL of sample extract was mixed with 0.1 mL of 10% (w/v) ethanolic solution of aluminum chloride, 0.1 mL of 1 M sodium acetate and 4.3 mL of distilled water. After 30 min. in darkness the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/VIS, England). Quercetin (0.01 – 0.5 mg.L^{-1}; R^2 = 0.997) was used as the standard and the results were expressed in mg.g^{-1} quercetin equivalents.

The total phenolic acid content

Determination of total phenolic acid content of extracts was carried out using the method of **Farmakopea Polska (1999)**. 0.5 mL of sample extract was mixed with 0.5 mL of 0.5 M hydrochloric acid, 0.5 mL Arnova reagent, 0.5 mL of 1 M sodium hydroxide (w/v) and 0.5 mL of distilled water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid (1 – 200 mg.L^{-1}, R^2 = 0.999) was used as a standard and the results were expressed in mg.g^{-1} caffeic acid equivalents.

Statistical analysis

The statistically treated data are given in tables as the arithmetical mean values and their standard errors. Data were submitted ANOVA and differences between means compared through the Tukey-Kramer test (α = 0.05). Correlation analysis was performed using Pearson's criterion.

RESULTS AND DISCUSSION

Numerous biologically active compounds act as powerful antioxidants, among which different groups of polyphenol compounds that are determined in fruits and berries **(Szopa et al., 2017; Aly, Maraei and El-Leel, 2019; Rodriguez-Werner, Winterhalter and Esatbeyoglu, 2019)**.

Antioxidant activity

As reported **Oszmiański and Wojdylo (2005)**, antioxidant activity level decreased in the following order: juice, fruits, pomace. There are numerous methods to determine antioxidant activity in plant extracts among them radical scavenging assay by DPPH and phosphomolybdenum method (reducing power of extracts) **(Alam, Bristi and Rafiquzzaman, 2013; Moharram and Youssef, 2014; Pisoschi et al., 2016)**. DPPH scavenging activity is the most popular assay to determine antioxidant capacity **(Gralec, Wawer and Zawada, 2019)**.

Radical scavenging assay by DPPH

Our preliminary data show that the DPPH scavenging activity of extracts for all specimens of Aronia was 83.25 – 93.30% (methanol extracts), 78.07 – 93.23% (ethanol extracts) and 59.87 – 88.36% (aqueous extracts). Alcoholic and aquatic extracts of fruits have almost equal antioxidant activity. The lowest antioxidant activity in alcohol extracts was shown by cultivated *A. mitschurinii*, and the highest one by invasive plants of *A. mitschurinii*. Conversely, aqueous extracts have the lowest antioxidant activity in invasive plants of *A. mitschurinii* and the highest one in cultivated samples **(Vinogradova et al., 2018)**. Also, in another study, the Aronia extract was tested for various antioxidative potentials and inhibitory effects. Thus, high polyphenol and flavonoid content suggests that Aronia extracts may be useful for the prevention or treatment of allergic disease **(Jeong, 2008)**. In study **Jakobek et al. (2012)** antiradical activity of wild chokeberries was higher than cultivars (besides one cultivar).

In our study radical scavenging activity of the ethanol extracts of investigated Aronia species was screened against DPPH radical which is the most used to determine the antiradical activity of several natural compounds and was from 6.96 (AM-D) to 8.89 (AM-OZ) mg TE.g^{-1} (Figure 1). **Strugała and Gabrielska (2014)** determined that significantly higher antioxidant activity was found at the start of storage for Japanese quince and chokeberry extracts than the same of hawthorn and quince. **Tolić et al. (2015)** found that antioxidant activity by DPPH method in dried fruits of *A. melanocarpa* was 183.52 – 191.31 mg TE.L^{-1}. Antioxidant activity by DPPH-method, according to **Wangensteen et al. (2014)**, in methanol extracts of *A. melanocarpa* cultivars and *Aronia* × *prunifolia* was higher than in ethanol extracts. **Yang, Kim and Shin (2019)** measured antioxidant activity by DPPH method of fruit extracts with ascorbic acid (AA) as equivalent and obtained values 4144.44 – 4565.28 mg AA.100 g^{-1} FW.

Molybdenum reducing the power of extracts

We determined the reducing power of Aronia extracts by phosphomolybdenum method that is also widely used to determine the antioxidant capacity **(Alam, Bristi and Rafiquzzaman, 2013)**. We found that reducing the power of ethanol extracts of Aronia samples in our study was from 151.47 (AM-OZ) to 297.8 (AA-M) mg TE.g^{-1} (Figure 2).

It is very difficult to compare data with other reviews because were used different methods of measure and various units. So, **Rugină et al. (2012)** determined reducing the power of extracts by the FRAP method and found a correlation between this parameter and total flavonoid content. The same method of reducing power determination used by **Tolić et al. (2015)** and a strong correlation found between reducing power and flavonoid content. **Oszmiański and Lachowicz (2016)** also used the FRAP method for the determination of reducing the power of extracts and determined a strong correlation between phenolic acids and FRAP and total phenolics and this parameter. Our preliminary results about another species *Asimina triloba* (L.) Dunal. showed molybdenum reducing the power of extracts from 97.25 to 275.41 mg TE.g^{-1} DW **(Brindza et al., 2019)**.

Total polyphenol content

The polyphenols contained in fruits are of great interest for their antioxidant and anti-inflammatory properties **(Chrubasik, Li and Chrubasik, 2010; Montrose et al., 2011)**. Previous data showed that Aronia pomace had higher content of polyphenols than juice and fruits. Polymeric proanthocyanins are the major class of phenolic compounds of fruits of these species **(Oszmiańsky and Wojdylo, 2005)**. As emphasized by **Jurikova et al. (2017)**, the content of total polyphenol compounds and their selected groups of *A. melanocarpa* depends on some factors, among which is the temperature of storage. So, after six months of storage at 3 ℃ content of polyphenols decreased by 30%.

The antioxidant capacity of plant extracts has been attributed to their phenolic contents that were determined by Folin-Ciocalteu reagent. The amount of total polyphenol content of investigated extracts was from 25.98 (AM-TCH) to 54.39 (AA-M) mg GAE.g^{-1} (Table 1).

According to **Denev et al. (2018)**, total polyphenol content was found from 1022.4 to 1795.5 μmol TE.g^{-1} FW. HPLC analysis identified total polyphenol compounds in crude extracts of *A. melanocarpa* fruits 53.5 mg.g^{-1} **(Denev et al., 2019)**. As reported **Gao et al. (2018)**, *A. melanocarpa* polyphenols showed no cytotoxic effect. Polyphenol compounds from *A. melanocarpa* demonstrated immunomodulatory and anti-inflammatory functions **(Ho et al., 2014)**. The main group of *A. melanocarpa* polyphenols is procyanidins **(Gralec, Wawer and Zawada, 2019)**.

According to **Brand et al. (2017)**, the highest concentration of total phenolic compounds identified in fruits of *Aronia* × *prunifolia*, whereas, for *A. arbutifolia* and *A. melanocarpa* this parameter was less. Comparing obtained data and previous results of yogurt with chokeberry juice (*Aronia melanocarpa*), it's should be noted that total polyphenol content of 1%, 2%, and 3% yogurts was 28.70, 41.30 and 54.05 mg GAE.g^{-1} DW respectively **(Nguyen and Hwang, 2016)**. Also, dried fruits of *A. melanocarpa* showed total phenolics content in the range from 2,000 to 8,000 mg per 100 g **(Kokotkiewicz, Jaremicz and Luczkiewicz, 2010)**. In our case, the content of polyphenol content of investigated fruits of *A. melanocarpa* was 40.06 mg GAE.g^{-1} DW. In the study of **Jakobek et al. (2007)** total polyphenol content of *A. melanocarpa* fruits was 10637.20 mg GAE.kg^{-1} and total flavanol content by HPLC method was 76.43 mg.kg^{-1}. Different cultivars of *A. melanocarpa* had polyphenol content from 11721.7 to 14350.3 mg GAE.kg^{-1} FW **(Jakobek et al., 2012)**. As identified by **Jurikova et al. (2017)**, the total polyphenol content of *A. melanocarpa* can vary from 690 to 2560 mg GAE.100g^{-1} FW. In the study of **Rugină et al. (2012)** total phenolic values were 1586.5 to 2059.5 mg GAE.100 g^{-1} FW.

In another study, represented that the total phenolic content of dried fruits of *A. melanocarpa* was 1954 – 2466 mg GAE.100 g^{-1} **(Tolić et al., 2015)**. Investigation of **Veljković et al. (2014)** demonstrated that *A. melanocarpa* tea diffusions, as well as dry or fresh fruits, had a high antioxidant activity due to the content of polyphenol content such as flavonoids and phenolic acids. **Wangensteen et al. (2014)** determined that the polyphenol content of *A. melanocarpa* cultivars was 98 – 148 mg GAE.g of extract and 1079 – 1921 mg GAE.g FW, and for *Aronia* × *prunifolia* this parameter was 175 mg GAE.g and 2996 mg GAE.g FW. **Yang, Kim and Shin (2019)** found that the phenolic content of fruits of Aronia cultivars was 3955.28 – 4393.50 mg GAE.100 g^{-1} FW.

The total phenolic acid content

Phenolic acids are a group of polyphenol compounds that are generally described as phenolic compounds that have one carboxylic acid group and possess higher antioxidant activity than vitamins and present in food mostly in bound form. These compounds exhibited the following activities: antioxidant, antimicrobial, anticancer, anti-inflammatory, anti-diabetic, neuroprotective **(Kumar and Goel, 2019)**. They determined in various berries and fruits such as saskatoon chokeberry, blueberry, dark plum, elderberry, cherry **(Mattila, Hellström and Törrönen, 2006; Jakobek and Seruga, 2012; Lachowicz et al., 2017; Horčinová Sedlačková et al., 2018)**.

The phenolic acid content of Aronia samples in our study determined from 7.76 (AP-M) to 11.87 (AM-D) mg CAE.g^{-1} (Table 2). *A. melanocarpa* is known as a rich source of anthocyanins and phenolic acids **(Oszmiańsky and Lachowicz, 2016)**. The phenolic acid concentration was higher in juice than in pomace of this species whereas total phenolic content is higher in pomace than in fruits and juice **(Oszmiańsky and Wojdylo, 2005)**. In selected cultivars of *A. melanocarpa* identifies high content of chlorogenic, caffeic, ferulic acids. During the process of pasteurization, the most unstable was hydroxycinnamic acid (**Jurikova et al., 2017**). According to **Jakobek and Seruga (2012)**, the phenolic acid content in chokeberry fruits was 266.9 mg.kg^{-1}.

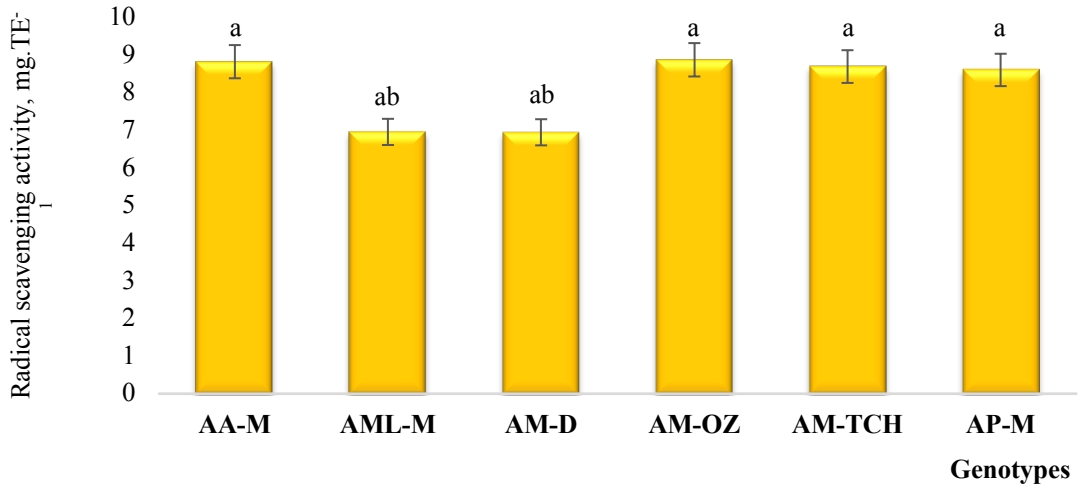

Figure 1 Antioxidant activity by DPPH-method of ethanol extracts of *Aronia* spp. Note: means in columns followed by different letters are different at $p = 0.05$; each value represents the mean of three independent experiments (±SD).

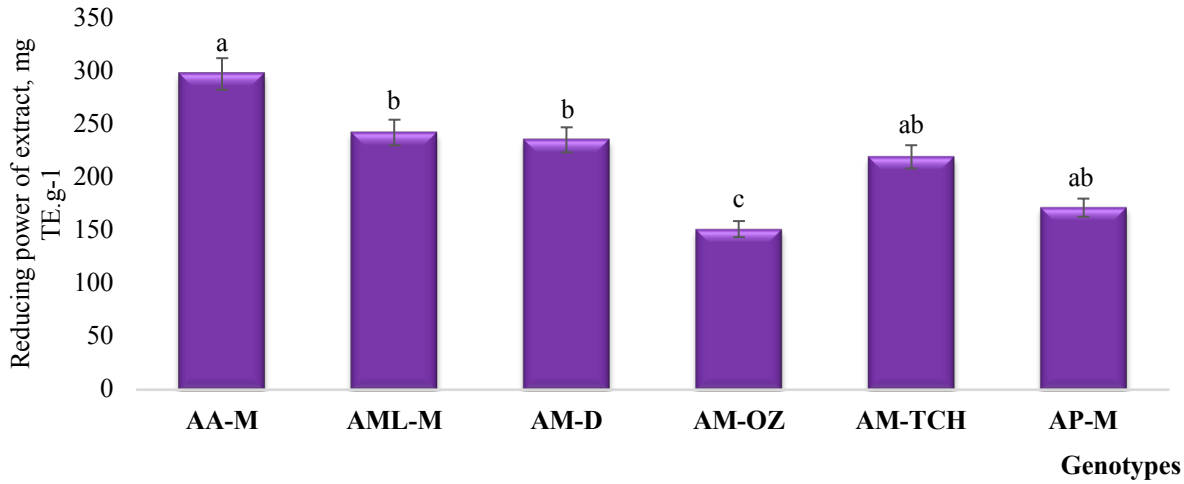

Figure 2 Reducing power of ethanol extracts by phosphomolybdenum method of *Aronia* spp. Note: means in columns followed by different letters are different at $p = 0.05$; each value represents the mean of three independent experiments (±SD).

Table 1 Total polyphenol, flavonoid and phenolic acid content in the fruits of Aronia specimens.

Plant sample	Total polyphenol content, mg GAE.g^{-1}	Total phenolic acids, mg CAE.g^{-1}	Total flavonoid content, mg QE.g^{-1}
A. arbutifolia **(AA-M)**	54.39 ±1.05a	11.06 ±0.79ab	10.88 ±0.41ab
A. melanocarpa **(AML-M)**	40.06 ±1.61b	11.54 ±0.09ab	13.16 ±0.15b
A. mitschurinii **(AM-TCH)**	25.98 ±1.28c	8.79 ±0.42b	11.05 ±0.21ab
A. mitschurinii **(AM-D)**	38.91 ±4.56ab	11.87 ±0.23a	16.62 ±0.50a
A. mitschurinii **(AM-OZ)**	28.80 ±2.75c	8.69 ±0.47b	8.12 ±0.17b
A.* × *prunifolia **(AP-M)**	40.39 ±1.78b	7.76 ±0.31c	10.02 ±0.56c

Note: Means in columns followed by different letters are different at $p = 0.05$; each value represents the mean of three independent experiments (±SD); GAE – gallic acid equivalents; CAE – caffeic acid equivalents; QE – quercetin equivalents.

Table 2 Coefficient of correlation between investigated parameters of *Aronia* spp. extracts.

Characters	Polyphenols	Phenolic acids	Flavonoids	Antioxidant activity (DPPH)	Molybdenum reducing power
Phenolic acids	0.495*	1			
Flavonoids	0.210*	0.771*	1		
Antioxidant activity (DPPH)	-0.098	-0.738	-0.870	1	
Molybdenum reducing power	0.700	0.753*	0.480*	-0.266	1

Note: Significant according to the t-test (p <0.05).

Total flavonoid content

The main groups of flavonoids of *A. melanocarpa* were anthocyanins, proanthocyanins, flavonols and flavanols **(Jurikova et al., 2017)**. Total flavonoid content was determined in the range from 8.12 (AM-OZ) to 16.62 (AM-D) mg QE.g^{-1}. Investigation of **Kapci et al. (2013)** reported that among different products from chokeberry dried fruits had the highest total flavonoids content and antioxidant activity values. But total phenolics and anthocyanins were found in chokeberry pomace. According to **Gralec, Wawer and Zawada (2019)**, the content of flavonoids of *A. melanocarpa* was from 7 to 11 g.100^{-1} DW. The study of **Rugină et al. (2012)** showed that the content of flavonoids ranged from 47.67 to 64.04 mg QE.100 g^{-1} FW. As reported by **Slimestad et al. (2005)**, the black chokeberries contained up to 71 mg flavonols per 100 g FW. **Tolić et al. (2015)** determined total flavonoids content in dried fruits of *A. melanocarpa* 867 – 1394 mg GAE.L^{-1}. According to **Yang, Kim and Shin (2019)**, the flavonoid content of three cultivars of Aronia was 3175.52 to 3577.7 mg CE.100 g^{-1} FW (catechin equivalent).

The antioxidant activity of investigated raw can be caused by the presence of different groups of compounds such as phenolics. In this case, we determined correlations between of investigated parameters. Correlation analysis found a strong correlation between the content of polyphenol compounds and reducing the power of extracts (r = 0.700), between flavonoids and phenolic acids (r = 0.771) and also between phenolic acids and reducing power (r = 0.753) of Aronia ethanol extracts (Table 2). The moderate correlation found between the accumulation of flavonoids and reducing the power of extracts (r = 0.480) and between polyphenol content and phenolic acids (r = 0.495). Weak relation was determined between the accumulation of polyphenol content and flavonoids of investigated extracts. Between rest, parameters were identified as a negative correlation.

As reported by **Gralec, Wawer and Zawada (2019)**, antioxidant activity by the DPPH test of *Aronia melanocarpa* positively correlated with the content of polyphenol compounds of both ripe and unripe fruits. **Yang, Kim and Shin (2019)** indicated a very strong correlation between flavonoid and phenolic content in fruit extracts (r = 0.991), between flavonoids and antioxidant activity by DPPH method (r = 0.997), between total phenolics and chlorogenic acid (r = 0.940). **Tolić et al. (2015)** found that the correlation between reducing power and total phenolic content of chokeberry juice was higher than between total antioxidant capacity and phenolic content.

CONCLUSION

Aronia species are non-traditional fruit plants, which are a rich source of polyphenol compounds with high antioxidant activity. In this study, cultivated plant species had the least values of polyphenol compounds as opposed to naturalized plants. However, the content of flavonoids and phenolic acids varied for all species. It was found that maximal values of polyphenol compounds were determined for *A. arbutifolia*, and total flavonoid and phenolic content for *A. mitschurinii* (from Dmitrov region). Thus, naturalizing plants of Aronia species (*Aronia arbutifolia*, *A. melanocarpa*, *Aronia* × *prunifolia*) can be an alternative source of antioxidants to widely cultivated species (*A. mitschurinii*). Thus, in the secondary distribution range, introduced species retain their biochemical characteristics.

Apparently, plants accumulate fewer biologically active substances in a comfortable culture environment than during forced adaptation to unfavorable ecological conditions. The determination of the polyphenol contents in the closely related species of the genus Aronia is necessary for the further use of the nutrient properties of this small fruit crop. Wild, still exploitable Aronia species could be selectively used to improve the nutritional and functional properties of cultivated *A. mitschurinii* fruits. In addition, interest in functional foods and food additives, which may support the protective mechanisms against diseases caused by oxidative stress, is increased currently.

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