



Polyphenolic composition and antioxidant activity of fruits of some wild fruit species

Milena Taseska-Gjorgijevski¹ , Roze Dzoljevska Milenkovska¹ , Filip Selamovski² , Dushko Nedelkovski¹ , Ana Selamovska¹ , Viktor Gjamovski¹

¹ Ss. Cyril and Methodius University in Skopje, Institute of Agriculture, Skopje, North Macedonia

² Plekhanov Russian University of Economics, Higher School of Economics and Business, Moscow, Russia

Abstract

The aim of this work was to determine the antioxidant activity of fruits from several wild fruit species: *Malus sylvestris* (L.) Mill., *Cornus mas* L., *Prunus spinosa* L., and *Rosa canina* L. depending on the content of vitamin C, total phenols, total anthocyanins, and flavan 3-ols. The content of vitamin C was examined by the volumetric method, total phenols according to the Folin-Ciocalteu method, total anthocyanins according to the acid-ethanol method, and p-dimethylaminocinnamaldehyde (p-DMACA) was used for the quantification of flavan-3-ols. Determining of the antioxidant activity was performed spectrophotometrically as an anti-radical activity against the stable product DPPH (2,2-diphenyl-1-picrylhydrazil). In terms of the parameters examined, statistically significant differences were determined between the examined species. The highest content of vitamin C (356 mg/100 gFW), total phenols (38799 mg/kgFW), and antioxidant activity (89.2% inhibition) were found in the fruits of *Rosa canina* L. The highest content of anthocyanins (1270 mg/kgFW) was found in the fruits of *Cornus mas* L. The highest presence of flavan-3-ols was found in the fruits of *Prunus spinosa* (2121 mg/kgFW) and *Malus sylvestris* (1589 mg/kgFW). The lowest content of vitamin C (14.8 mg/100 gFW), total phenols (2028 mg/kgFW), total anthocyanins, and antioxidant activity (50.7% inhibition) were found in the fruits of *Malus sylvestris*. The antioxidant activity in the fruit of the species under study was in a strong or weaker positive correlation with vitamin C (0.796), total phenols (0.516), and total anthocyanins

(0.144), but in a very strong negative correlation with flavan-3-ols (-0.841). The greatest contribution to the antioxidant capacity of the fruits of *Malus sylvestris* was made by flavanols, in *Cornus mas* L by vitamin C and total anthocyanins, in *Rosa canina* L by total phenols and vitamin C, while in *Prunus spinosa* L. by total phenols.

Key words: Vitamin C, Total phenols, Total anhtocyanins, Flavan-3-ols, Antioxidant activity

Introduction

Wild fruit species are plants that survive in natural conditions, adapted to the abiotic and biotic factors in the environment they grow in. They are found in the hilly and mountainous areas of Macedonia, where extensive agriculture is still practiced. These fruit species are very well adapted to local soil and climatic conditions and they are more vital and have greater resistance to various abiotic and biotic factors compared to cultivated species. They grow in nature without human intervention, they do not need appropriate chemical treatment to protect them against diseases and pests. For these reasons, they represent a natural, health-safe, medicinal, unpolluted and at the same time easily accessible source of food for humans and other living organisms. Due to their adaptability and resilience, the promotion of wild fruit species can be considered as one of the most promising efforts for climate change mitigation. On the other hand, by collecting fruits and other plant parts, and their processing, significant economic gains can be achieved, especially for people in rural areas. Their fruits, leaves, seeds, flowers, and roots can be used in different forms (fresh, dried, or processed) and thus find application in various industries. They can be used as nutrients, dietary supplements, or functional foods. The fruits represent a safe food with high nutritional value, rich in bioactive compounds such as polyphenols (anthocyanins, flavonols, phenolic acids), vitamins, carotenoids, tannins, proteins, carbohydrates, organic acids, minerals, plant fibres, unsaturated fatty acids, amino acids, proteins, and other constituents that have a structural, energetic, and protective function in the human body. Bioactive compounds play a synergistic and cumulative role in promoting human health, they have potent antioxidant activity and exhibit several health-promoting properties, they have anti-inflammatory, antimicrobial, anti-hyperglycemic, and hypo-cholesterolemic effect, reduce the incidence of cancer and coronary heart diseases, and other activities. For most of these chemically significant substances, wild plants contain them in larger quantities compared to cultivated plants (Jakobek and Barron, 2016; Lončarić et al., 2023). Wild apple fruits (*Malus sylvestris* (L.) Mill) are a good source of polyphenolic compounds. Some epidemiological studies have indicated that eating more apple fruits and their

related products could reduce the risk of various chronic human diseases. The fruits of *C. mas* L. are good resources of polyphenols (phenolic acids, hydrolysable tannins, anthocyanins, flavonols), iridoids, vitamin C, dietary fibres, hydrolysable tannins, and unsaturated fatty acids, making it a "superfood". The plant is widely used in folk medicine for the treatment and prevention of gastrointestinal and circulatory disorders, diabetes, sore throat, digestive ailments, measles, chicken pox, anaemia, rickets, liver and renal diseases, diarrhoea, haemorrhoids, and flu. Ethnopharmacological studies have highlighted the use of *P. spinosa* L. fruits in treating conditions such as urinary tract infections, diabetes, bronchitis, diarrhea, and external inflammations of the mouth and throat (Marčetić et al., 2022). Various parts of *R. canina* have been traditionally used for medicinal and culinary purposes for centuries. European folk medicine uses rosehip in the treatment of gastrointestinal disorders, infections, and fever, although its fruits exert a positive influence on the osteoarthritis, anxiety, depression, recognition memory, urinary and reproductive systems disorders, and neutrophil respiratory burst.

The analysis of the biochemical composition of the fruits of these wild fruit species *M. sylvestris* (L.) Mill., *C. mas* L., *P. spinosa* L., and *R. canina* L. can contribute to understanding their significance and importance in nutrition and how they help maintain human health. Although their fruits have long been used in traditional medicine, the results will provide support for their nutritional and medicinal value. Through comparative analyses of the antioxidant activity of fruits from several fruit species, recommendations can be made for individual species in the prevention or treatment of certain diseases.

Material and Methods

This paper provides comparative values for the antioxidant activity of fruits from different wild fruit species: *M. sylvestris* (L.) Mill., *C. mas* L., *P. spinosa* L., and *R. canina* L. The analyses included determination of the content of vitamin C, anthocyanins, flavan 3-ols, total phenols, and antioxidant activity of the fruits. The fruits of these species were taken from rural areas in the Skopje region at full maturity. The Skopje region is located at 41°57'N and 21°28'S and belongs to the continental-mediterranean climate-vegetation-soil region, with an average annual air temperature of 12 °C and an average annual precipitation of 507 mm. The drought index according to De Martonen averaged 19.7 (especially pronounced in the months of July, August and September), which indicates that the climate is semi-arid (Filipovski et al., 1996). In recent decades, global climate change has been observed in this area, expressed through reduced annual

precipitation, increased aridity, altered pluviometric regime, reduced temperature, especially in winter and periodic occurrences of heavy frosts.

The laboratory analyses were performed in the oenological laboratory at the Institute of Agriculture in Skopje. 30 fruits were taken in three replicates from each fruit species. The fruits were grown under natural conditions and harvested at botanical maturity. They had been stored in refrigerators under appropriate conditions for a short time until the samples were analysed.

The content of vitamin C was examined by the volumetric method (Sadasivam and Balasubramanian, 1987). Total phenols in the samples were determined according to the Folin-Ciocalteu method (Slinkard and Singleton, 1977). The content of total anthocyanins was performed according to the acid-ethanol method (Somers and Evans, 1977) and p-dimethylaminocinnamaldehyde (p-DMACA) was used for the quantification of flavan-3-ols in the samples (Di Stefano et al., 1989). Determining of the antioxidant activity was performed as an anti-radical activity against the stable product DPPH (2,2-diphenyl-1-picrylhydrazil). This analysis was performed spectrophotometrically, at a wavelength of 517 nm. The antioxidant activity, especially for vitamin C, as well as the inhibitory antioxidant activity of all concentrations are expressed in percentages (%).

To determine a significant difference from the mean values of the four parameters (vitamin C, antioxidant activity, total phenols, and total anthocyanins), the ANOVA and the Least Significant Difference (LSD) test at the $p < 0.05$ level were used for all fruit species samples. The correlation between the examined fruit species was determined using the Pearson correlation coefficient (r) (LaMorte, 2021). The use of this correlation coefficient (r) can be justified by the fact that it is a standard statistical method used to quantify the strength and direction of a linear relationship between variables. In the context of this research it enabled the measurement of how changes in polyphenolic composition correspond to variations in the antioxidant activity across the examined fruit species.

Results and Discussion

The results obtained in this study are presented in Table 1. The average vitamin C content in the studied species was 124.7 mg/100 g. The highest content of vitamin C was found in the fruits of *R. canina* 356.0 mg/100 g and the lowest in *M. sylvestris* (14.8 mg/100 g). The fruits of *R. canina* had about 3 times higher vitamin C content than *C. mas*, about 11 times more than *P. spinosa* and 24 times more than *M. sylvestris*. Statistically significant differences were found between species in terms of vitamin C content (Tables 2 and 3).

Tab. 1 - The content of vitamin C, total phenols, total anhtocyanins, flavan-3-ols, and antioxidant activity in the fruits of *M. sylvestris* (L.) Mill., *C. mas* L., *P. spinosa* L., and *R. canina* L.

Species	Vitamin C /mg/100 g	Total phenols /mgGAE/kg	Total anhtocyanins /mg/kg	Flavan-3-ols /mg/kg	Antioxidant activity /% inhib.
<i>M. sylvestris</i>	14.8	2 028	<1	1 589	50.7
<i>C. mas</i>	97.5	5 462	1 270*	994	84.8
<i>P. spinosa</i>	30.5	22 097	1 198	2 121*	60.6
<i>R. canina</i>	356.0*	38 799*	73	615	89.2*
Average	124.7	17 096.5	635.2	1 329.7	71.3

*The highest value

Tab. 2 - Summary statistics of the ANOVA Single Factor

Groups	Count	Sum	Average	Variance
Vitamin C/mg/100g	4	498.8	124.7	18797.8
Total phenols/mgGAE/kg	4	68386.0	17096.5	2.86E+08
Total anhtocyanins/mg/kg	4	2541.0	635.24	479754.3
Flavan-3-ols/mg/kg	4	5319.0	1329.74	438960.8
Antioxidant activity/% inhib.	4	285.3	71.32	347.2

Tab. 3 - The source of variation

Source of variation	Sum of squares (SS)	Degrees of freedom (df)	Mean square (MS)	F-statistic value	P-value	F-critical
Between groups	8.81E+08	4	2.20E+08	3.84	0.02	3.06
Within groups	8.61E+08	15	5.74E+07			
Total	1.74E+09	19				

The results of this study correspond to the literature data. *M. sylvestris* fruits have shown a higher vitamin C content than some old apple cultivars 9.37 mg/100 g (Selamovska et al., 2021–2022).

According to this study, the fruits of *C. mas* showed a higher vitamin C content than the available literature data, 43.78 to 76.75 mg/100 g (Güleriüz et al., 1998). A higher vitamin C content of 199-433 mg/100 g FW was found in the study by Cetkovská et al. (2015). The amount of these compounds and the antioxidant properties of this plant depend on its geographical location, growth stage, weather conditions, the time of its harvest, fruit maturity level, and the methods which are used to store and extract it (Ghazghazi et al., 2010). Rose hips

that grow in colder climates contain more vitamin C than those that grow in warmer climates. (Pyke and Melville, 1942). Ripe, red fruits contain a higher amount of vitamin C (417.5 mg/100 g), compared to green fruits (18.0 mg/100 g). Fruits of *Rosa canina* harvested from higher altitudes contain more vitamin C (360.22-1 480 mg/100 g) than fruits harvested from lower altitudes (112.20-470 mg/100 g) (Pyke and Melville, 1942).

The results of this study have shown the fruits of *Rosa canina* and *Cornus mas* have a higher vitamin C content compared to other fruit species such as blackberry (52.41 mg/100 g FW), red raspberry (92.17 mg/100 g FW), strawberry (90.13 mg/100 g FW), blueberry (73.21 mg/100 g FW), sweet cherry (62.42 mg/100 g FW) (de Souza et al., 2014), pomegranate (23.67 mg/100 g FW), and cherry 12.83 mg/100 g FW (Selamovska et al., 2021-2022), while the fruits of *M. sylvestris* and *P. spinosa* have a lower content. From the results obtained in this paper (Table 4), a strong to very strong positive correlation is evident between vitamin C and antioxidant activity (0.796) and total phenols (0.812). Vitamin C had a weak negative correlation with anthocyanins (-0.397) and a very strong negative correlation with flavan-3-ols (-0.824)..

The average content of total phenols in the studied wild species was 17 096.5 mg/kg. The highest content of total phenols was found in rose hips (38 799 mg/kg), and the lowest in wild apple (2 028 mg/kg). The fruits of *R. canina* contained about 1.5 times more total phenols than *P. spinosa*, about 7 times more than *C. mas* and 19 times more than *M. sylvestris*. We found statistically significant differences between the species in terms of the content of total phenols (Tables 2 and 3).

These results correspond to the literature data. Depending on the genotype, the average content of total polyphenols in apple fruits ranged between 66.2 to 211.9 mg/100 g FW according to Vrhovsek et al. (2004). The concentration of phenols was substantially higher in the peel than in the flesh (Jakobek et al., 2013). The content of total phenols in some old apple cultivars was between 2651.6-6860.2 mg/kg FW in the flesh and 5885.4-14002.2 mg/kg FW in the peel (Wolfe et al., 2003). However, it is important to keep in mind that the peel only represents 10% of the entire fruit. Polyphenols are the major group of compounds responsible for preventive and antioxidant properties of apples. The most important polyphenol compounds in apples are quercetin (in glycosylated forms), chlorogenic acid, catechin, and epicatechin, which are responsible for the astringent and bitter taste, as well as hydroxycinnic acid, flavonols. The synthesis of antioxidants depends on the genotype, developmental stage, growth periods, plant material, geographic locations influenced by environmental factors such as nutrient availability, temperature and in particular, light, and depends on the extraction method and solvents used. The extracts obtained by ultrasonic extraction using ethanol and distilled water as solvents contained the highest

amount of polyphenolic compounds and demonstrated the best antioxidant activity as well (Stojiljković et al., 2016). Some studies have shown that indigenous species of fruit contain higher levels of phenolic compounds, flavonols, phenolic acid and have higher antioxidant activity than most cultivated apple varieties, because they show good adaptability to the local environment and represent a valuable source of genetic variability for the crop resistance to various changes, as well as for quality properties (Jakobek and Barron, 2016; Stojiljković et al., 2016). The content of total phenols in wild apple fruit extracts originating from Serbia ranged from 172.91-1556.99 mg GAE/100 g DW (Stojiljkovic et al., 2016). According to Lončarić et al. (2023) and Jakobek et al. (2013), old apple cultivars also have a higher total phenolic content compared to commercial apple varieties. *C. mas* is a rich source of polyphenols (Tural and Koca, 2008). The seeds and bark of *C. mas* contain about 90% of the polyphenols of all different parts. The content of phenols in fruits varied from 2174-6143 mg/kg FW according to Cetkovská et al. (2015). Dogwood fruits with a mass of 0.39–1.03 g, fruit length between 14.24-22.20 mm, and 9.59-13.21 mm width contained between 2.81-5.79 mg/g of total phenols (Tural and Koca, 2008).

Across different studies the total phenolic content (TPC) of blackthorn (*P. spinosa*) fruits ranged from 152.22 to 321.36 mg GAE/100 g (Marčetić et al., 2022), 1851 to 3825 mg/100 g FW (Ruiz-Rodríguez et al., 2014); 30.74 mg/(GAE)/g (Kotsou et al., 2023), 21.30 mg/g to 732.34 mg/g (Pinacho et al., 2015). According to Pinacho et al. (2015), the total phenolic content depends on the solvent and plant material. Mikulic-Petkovsek et al. (2016) examined the phenolic composition of four wild *Prunus* species (*P. mahaleb*; *P. padus*; *P. spinosa*; *P. avium*). The total phenolic content varied from 2 373 mg/kg (*Prunus avium*) to 11 053 mg/kg (*Prunus padus*). The amount of total phenols in *Rosa canina* depends on the genotype, growth stage, geographical location, weather conditions, the time of harvest and the methods that are used to store and extract it (Ghazghazi et al., 2010). The total polyphenols content in eight rose hip (*Rosa canina*) varieties from wild Transylvania populations varied from 326 mg/100 g to 575 mg/100 g of frozen pulp (Roman et al., 2013), in two Bulgarian commercial forms of *Rosa canina* the content was from 12.07 to 37.17 mg GAE/g DW (Mihaylova et al., 2015). In rose hip samples of the *R. pisocarpa* species, Butkevičiūtė et al. (2022) found 26.49 mg GAE/g total phenols. Compared to other fruit species such as blackberry (850.52 mg GAEs/100 g FW), red raspberry (357.83 mg GAEs/100 g FW), strawberry (621.92 mg GAEs/100 g FW), blueberry (305.38 mg GAE/100 g FW), and sweet cherry (314.45 mg GAE/100 g FW) (de Souza et al., 2014), according to this study, *P. spinosa* and *R. canina* L. fruits showed a higher content of total phenols, while *M. sylvestris* showed a lower content of total phenols. *C. mas* fruits had a higher content than red raspberry, blueberry, and sweet cherry fruits.

Tab. 4 - Matrix of correlation (the Pearson correlation method)

	Vitamin C /mg/100g	Total phenols /mgGAE/kg	Total anthocyanins mg/kg	Flavan-3-ols /mg/kg	Antioxidant activity /% inhib.
Vitamin C /mg/100 g	1.000	-	-	-	-
Total phenols /mg/kg	0.812	1.000	-	-	-
Total anthocyanins /mg/kg	-0.397	-0.205	1.000	-	-
Flavan-3-ols /mg/kg	-0.824	-0.344	0.341	1.000	-
Antioxidant activity/% inhib.	0.796	0.516	0.144	-0.841	1.000

From the results presented in Table 4, a very strong positive correlation of total phenols with vitamin C (0.812) can be seen, but a weak negative correlation with total anthocyanins (-0.205) and flavan-3-ols (-0.344). Total phenols showed a moderate positive correlation with antioxidant activity (0.516). In comparison with our results, Cetkovská et al. (2015) found a positive correlation between total polyphenol and antioxidant activity in fruits of *Cornus mas*, Pinacho et al (2015), Marčetić et al. (2022), and Veličković et al. (2020) in fruits of *P. spinosa*, while Butkevičiūtė et al. (2022), Roman et al. (2013), and Mihaylova et al. (2015) in fruits of *Rosa canina*. This indicates that in fruits of *Cornus mas*, *P. spinosa*, and *Rosa canina*, total phenols have a large share in antioxidant activity. In fruits of *P. spinosa*, Veličković et al. (2020) found a strong negative correlation between phenols and flavan-3-ols.

The average content of total anthocyanins in the studied species was 635.2 mg/kg. The highest content of total anthocyanins was found in the fruits of *Cornus mas* (1 270 mg/kg), while in the fruits of *M. sylvestris* no anthocyanins were found. A high content of anthocyanins was also found in the fruits of *P. spinosa* (1198 mg/kg). We found statistically significant differences between the species in terms of the content of total anthocyanins (Tables 2 and 3).

The results of our study correspond to the available literature data. In the study of Jakobek and Barron (2016) flavanols, dihydrochalcones, and phenolic acids were identified in the flesh of apple varieties studied (including the crab apple), while anthocyanins were less abundant. Comparing to other apples, wild apple contained much higher amounts of flavanol and phenolic acids in the flesh, while the amount of these compounds in peel was similar. Jakobek et al. (2013) found the presence of anthocyanins ranging from 79.4 to 761 mg/kg FW in fruits

with red skin, representing 1-7% of the total polyphenol content. According to these authors, epicatechin represented more than 85% of constitutive units. The main pigment responsible for the red colour is cyanidin-3-galactoside (idaein). Similar results were obtained by Vrhovsek et al. (2004). The anthocyanin concentration of apple peel changes according to internal plant conditions, fruit maturity and plant hormones, environmental factors, (light, temperature), nutrients, and the cultivar. Dogwood fruits with the mass from 0.39-1.03 g, fruit length ranging from 14.24 to 22.20 mm, fruit width 9.59–13.21 mm contained 1.12-2.92 mg/g total anthocyanins (Tural and Koca 2008), and the total anthocyanins ranged from 0.12 to 4.2 mg/g FW in Turkey (Tural and Koca, 2008), 0.061-0.347 mg/g FW in Czech Republic (Cetkovská et al., 2015). Additionally, anthocyanins depend on the storage temperature processes. The most abundant anthocyanins in cornelian cherry fruits are cyanidin 3-O-glucoside, cyanidin 3-O-robinobiside, cyanidin 3-O-rutinoside, peonidin-3-O-glucoside, pelargonidin 3-O-glucoside, pelargonidin 3-O-robinobiside, and pelargonidin 3-O-galactoside. The predominant anthocyanin in cornelian cherry fruits is pelargonidin 3-glucoside (Tural and Koca, 2008). The fruits of *P. spinosa* L. contained 0.68-14.38 mg/100 g anthocyanins (Marčetić et al., 2022), 9 -23 µg/g DW (Veličković et al., 2020), 2335.56 mg/kg FW (Mikulic-Petkovsek et al., 2016). Compared to the literature data, in this study the fruits of *P. spinosa* showed a lower content of anthocyanins. In the ethanol and acetone extracts of *Prunus spinosa* fruits, Veličković et al. (2020) identified 4 anthocyanin compounds: delphinidin (Dp), cyanidin (Cy), malvidin (Mv), and pelargonidin (Pg), while in the aqueous extract there was no presence of pelargonidin (Pg). The most dominant anthocyanin was cyanidin (Cy). The content of total anthocyanins ranged from 9 to 23 µg/g, the highest in the sample with acetone. Compared to other fruit species blackberry (58.61mg/100 g FW), red raspberry (14.69 mg/100 g FW), strawberry (16.03 mg/100 g FW), blueberry (29.72 mg/100 g FW), and sweet cherry (26.72 mg/100 g FW) (de Souza et al., 2014), the fruits of *C. mas* and *P. spinosa* analysed in this study showed a higher content of total anthocyanins.

From the results obtained (Table 4), a very weak positive (or no association) correlation can be seen between total anthocyanins and antioxidant activity (0.144) and a weak positive correlation with flavan-3-ols (0.341), but a weak negative correlation with the content of vitamin C (-0.397) and total phenols (-0.205). The results of this study correspond to the literature data. Veličković et al. (2020) established that anthocyanins contributed weakly to the antioxidant activity of *P. spinosa* fruits.

According to this study, the average content of flavan-3-ols in the species under study was 1 329.7 mg/kg. The highest content of flavan-3-ols was found in *P. spinosa* (2 121 mg/kg) and the lowest in *R. canina* L. (615 mg/kg). The

fruits of *M. sylvestris* also had a high content of flavan-3-ols (1589 mg/kg). There were statistically significant differences between the species in terms of the content of flavan-3-ols (Table 2 and 3). The results of the study correspond to the available literature data.

The most common polyphenols in apples consist of flavan-3-ols both in a monomeric form ((+)-catechin and (-)-epicatechin) and in an oligomeric form (proanthocyanidins), flavonols, phenolic acids, dihydrochalcones, and anthocyanidins (Alonso-Salces et al., 2004). Jakobek et al. (2013) researched polyphenol compounds of thirteen old apple varieties grown in local areas from the Southeastern European region. Flavan-3-ol monomers were quantified in different old apple varieties, usually in the form of epicatechin and the content was higher in the peel (58.2-541 mg/kg FW) than in the flesh (35.6-259.2 mg/kg FW). Epicatechin represented more than 85% of constitutive units. Proanthocyanidins were the major class of polyphenols in flesh and peel (> 70% of total polyphenols), phenolic acids were mainly distributed in the flesh (6-25%); flavonols (1-13%) and dihydrochalcones (1-10%) in the peel, and anthocyanins (1-7%) in the peel of red coloured apples (Jakobek et al. 2013). Flavonols and anthocyanins in peels, and hydroxycinnamic acids and flavan-3-ols in pulps were present in higher concentrations in ripe fruits (Alonso-Salces et al., 2005). The content of flavan-3-ols in the flesh and peel of two apple varieties (wild apple and Slavonska srčika) was 590.4 mg/kg and 260.1 mg/kg FW (Jakobek et al., 2017). The content of proanthocyanidin oligomers in the whole fruits of different apple varieties ranged from 522.4 to 2033 mg/kg of the whole apple (Vrhovsek et al., 2004), whereas in the flesh of some old apple varieties it ranged from 672 to 3041 mg/kg FW (Alonso-Salces et al., 2005). The concentration of total flavonoids in wild apple extracts originating from Serbia ranged from 3.97 to 182.22 mg RE/100 g DW (Stojiljkovic et al., 2016).

Flavonoids are the main group of phenolic compounds in the fruits of *P. spinosa* L (Pinacho et al., 2015; Kotsou et al., 2023). Pinacho et al. (2015) identified three phenolic acids, two coumarins, fourteen flavan-3-ols, and six flavonols. The total flavonoid content (TFC) in the fruits of *P. spinosa* was from 35.51 to 554.82 mg/g (Pinacho et al., 2015), 3.23 mg rutin equivalent (RtE)/g (Kotsou et al., 2023).

Compared to other fruit species such as blackberry (87.03 mg CE/100 g FW), red raspberry (9.61 mg CE/100 g FW), strawberry (38.17 mg CE/100 g FW), blueberry (47.53 mg CE/100 g FW), and sweet cherry 59.92 mg CE/100 g FW (de Souza et al., 2014), according to this study, the fruits of *M. sylvestris*, *C. mas*, and *P. spinosa* showed a higher content of flavan-3-ols.

Based on Table 4, it was found that flavan-3-ols were in a very strong negative correlation with vitamin C (-0.824) and antioxidant activity (-0.841) and

a weak negative correlation with total phenols (-0.344). They were in a weak positive correlation with anthocyanins (0.341).

Veličković et al. (2020) found a strong negative correlation of flavan-3-ols with total phenols, total anthocyanins (which was unusual), and antioxidant activity in *P. spinosa* fruits. Pinacho et al. (2015) also found a negative correlation of flavanols with antioxidant activity in *P. spinosa* fruits.

Regarding the antioxidant activity of the fruits, statistically significant differences were found between the species in terms of antioxidant activity (Tables 2 and 3). The highest antioxidant activity was found in the fruits of *R. canina* (89.2% inhibition) and *C. mas* (84.8% inhibition). The fruits of *P. spinosa* (60.6% inhibition) and *M. sylvestris* (50.7% inhibition) had a lower antioxidant activity than the average. The results of our study correspond to the available literature data.

Considering the antioxidant activity in wild apple fruit extracts originating from Serbia, the FRAP value ranged from 2.13 to 7.65mM Fe²⁺ and %AOA from 57.73% to 95.32 %AOA (Stojiljković et al., 2016). Some researchers (Jakobek and Barron, 2016) have shown that the majority of wild varieties showed better antioxidant potential than cultivated apple species. The consumption of apple pulp may provide 48-78% of the antioxidant capacity; however, if the peel is ingested, it may increase to 79-89%. The type of an apple variety and the parts ingested are factors that must be taken into consideration in consumption. Also, old apple cultivars have a higher antioxidant activity compared to commercial cultivars (Lončarić et al., 2023). The antioxidant activity in two Ukrainian and two Austrian cultivars of cornelian cherry (*C. mas* L.) widely grown in Czech Republic, determined by EPR and DPPH radical scavenging assay, ranged from 29.5% to 67.2% (Cetkovská et al., 2015). Dogwood fruits with a mass of 0.39-1.03 g, fruit length between 14.24 and 22.20 mm, and fruit width ranging from 9.59 to 13.21 mm, had an iron reducing antioxidant power (FRAP) between 16.21 mmol/g and 94.43 mmol/g (Tural and Koca, 2008).

Compared to other fruit species, *C. mas* L had a higher antioxidant activity than in blackberry (13.23 ABTS lmol/g FW; 2142.42 EC50 gFW/g DPPH), red raspberry (6.27 ABTS lmol/g FW; 4960.58 EC50 gFW/g DPPH), strawberry (7.87 ABTS lmol/g FW; 3778.94 EC50 g FW/g DPPH), blueberry (5.88 ABTS lmol/g FW; 7775.45 EC50 g FW/g DPPH), and sweet cherry (8.83 ABTS lmol/g FW; 6065.68 EC50 g FW/g DPPH)(de Souza et al., 2014). The antioxidant activity of *P. spinosa* fruits was 146.09 µmol ascorbic acid equivalent (AAE)/g according to the FRAP (Ferric Reducing Antioxidant Power) assay, and 18.56 µmol AAE/g according to the DPPH radical scavenging activity assay (Kotsou et al., 2023). The species *P. spinosa* L showed a significantly higher antioxidant

activity than *Prunus domestica* and *Prunus cerasifera*, but lower than *Prunus padus* (Mikulic-Petkovsek et al., 2016).

Compared to other fruit species, *R. canina* fruits had a higher antioxidant activity than some indigenous fruit varieties of pomegranate 81.58%, apple 60.37%, cherry 43.36% (Selamovska et al., 2021–2022), *Ribes nigrum* 86.2% *Morus alba* 71.6%, *Aronia melanocarpa* 76.4%, *Rubus fruticosus* 78.6%, and *Punica granatum* 86.3% (Selamovska et al., 2024).

In our research (Table 4), it can be seen that the antioxidant activity of the fruits of the studied species was in a strong or weaker positive correlation with vitamin C (0.796), total phenols (0.516), and total anthocyanins (0.144), but in a very strong negative correlation with flavan-3-ols (-0.841). The greatest contribution to the antioxidant capacity of the fruits of *Malus sylvestris* was made by flavanols, in *C. mas* L. by vitamin C and total anthocyanins, in *Rosa canina* L. by total phenols and vitamin C, while in *P. spinosa* L. by total phenols. Similar results were also obtained by Veličković et al. (2020), who determined a high correlation between the content of phenols and antioxidant activity in the fruits of *P. spinosa* L., while Pinacho et al. (2015) and Cetkovská et al. (2015) found a positive correlation between the total phenolic content and antioxidant activity of dogwood fruits.

Conclusion

The highest content of vitamin C, total phenols, and antioxidant activity have been found in the fruits of *Rosa canina* L. In addition, *C. mas* L. had the highest content of total anthocyanins, while *P. spinosa* L. had the highest presence of flavan-3-ols. The fruits of *M. sylvestris* (L.) Mill. had the lowest content of vitamin C, total phenols, absence of total anthocyanins, and the lowest antioxidant activity. Statistically significant differences were determined between the tested species in relation to all examined parameters. The antioxidant activity of the fruits of the examined species was in a strong or weak positive correlation with vitamin C, total phenols, and total anthocyanins, but in a very strong negative correlation with flavan-3-ols. Total phenols were in a weak negative correlation with total anthocyanins and flavan-3-ols, while flavan-3-ols were in a very strong negative correlation with ascorbic acid and antioxidant activity. The antioxidant activity of *M. sylvestris* fruits mainly resulted from flavan-3-ols, in *C. mas* it was due to total anthocyanins and vitamin C, in *P. spinosa* because of total phenols, while in *R. canina* total phenols and vitamin C contributed to this activity.

Acknowledgement

This research was conducted within the framework of the project “Ethnobotanical Research of Wild Fruit Species”, funded by the integrative funds of Ss. Cyril and Methodius University in Skopje, implemented by the Institute of Agriculture, Skopje.

References

- Alonso-Salces, R.M., Herrero, C., Barranco, A., Berrueta, L.A., Gallo, B., & Vicente, F. (2005). Classification of apple fruits according to their maturity state by the pattern recognition analysis of their polyphenolic compositions. *Food Chemistry*, 93,1, 113-123. <https://doi.org/10.1016/j.foodchem.2004.10.013>
- Cetkovská, J., Diviš, P., Vespalcová, M., Porízka, J., & Rezníček, V. (2015). Basic nutritional properties of cornelian cherry (*Cornus mas* L.) cultivars grown in the Czech Republic. *Acta Alimentaria*, 44(3), 357–364. <https://doi.org/10.1556/aalim.2014.0013>.
- De Souza, V.R., Pereira, P.A.P., da Silva, T.L.T., de Oliveira Lima, L.C., Pio, R., & Queiroz, F. (2014). Determination of the bioactive compounds, antioxidant activity and chemical composition of Brazilian blackberry, red raspberry, strawberry, blueberry and sweet cherry fruits. *Food Chemistry* 156, 362-368. <https://doi.org/10.1016/j.foodchem.2014.01.125>.
- Di Stefano, R., Cravero, M.C., & Gentilini, N. (1989). Methods for the study of wine polyphenols. *The winemaker*, 5, 83-90. https://www.researchgate.net/publication/254864043_Metodi_per_lo_studio_dei_polifenoli.
- Filipovski, Gj., Rizovski, R., & Ristevski, P. (1996). Characteristics of climatic-vegetation-soil zones (regions) in the Republic of Macedonia. *Macedonian Academy of Sciences and Arts*, Skopje, pp 177 (Mac).
- Ghazghazi, H., Miguel, M.G., Hasnaoui, B., Sebei, H., Ksontini, M., Figueiredo, A.C., Pedro, L.G., & Barroso, J.G. (2010). Phenols, essential oils and carotenoids of *Rosa canina* from Tunisia and their antioxidant activities. *African Journal of Biotechnology*, 9(18),2709-2716. <https://doi.org/10.5897/AJB09.1612>.
- Guleryuz, M., Bolat, I., & Pirlak, L. (1998). Selection of table cornelian cherry (*Cornus Mas* L.) types in Coruh valley. *Turkish Journal of Agriculture and Forestry*, 22, 4, 357–364. <https://www.acarindex.com/turkish-journal-of-agriculture-and-forestry/selection-of-table-cornelian-cherry-cornus-mas-l-types-in-coruh-valley-466088>.
- Jakobek, L., Villalba, R.G., & Tomás-Barberán, F.A. (2013). Polyphenolic characterisation of old local apple varieties from Southeastern European region. *Journal of Food Composition and Analysis*, 31(2),199–211. <https://doi.org/10.1016/j.jfca.2013.05.012>.

- Jakobek, L., & Barron, A.R. (2016). Ancient apple varieties from Croatia as a source of bioactive polyphenolic compounds. *Journal of Food Composition and Analysis*, 45, 9-15. <https://doi.org/10.1016/j.jfca.2015.09.007>
- Kotsou, K., Stoikou, M., Athanasiadis, V., Chatzimitakos, T., Mantiniotou, M., Sfougaris, A.I., & Lalas, S.I.(2023). Enhancing Antioxidant Properties of *Prunus spinosa* Fruit Extracts via Extraction Optimization. *Horticulturae*, 9(8), 942. <https://doi.org/10.3390/horticulturae9080942>.
- LaMorte, W.W.MD. (2021). All Rights Reserved Date last modified: April 21, 2021. <https://sphweb.bumc.bu.edu/otlt/MPH/Modules/PH717-QuantCore/PH717-Module9/Correlation.Regression/PH717-Module9/Correlation-Regression4.html>
- Lončarić, A., Flanjak, I., Kovač, T., Tomac, I., Gotal Skoko, A.M., Skendrović Babojelić, M., Fruk, G., Zec Zrinušić, S., Čiček, D., Babić, J., & Jozinović, A. (2023). Unveiling Apple Diversity: The Quality of Juice Produced from Old vs. Commercial Apple Cultivars. *Plants* (Basel), 12(21), 3733. <https://doi.org/10.3390/plants12213733>.
- Marčetić, M., Samardžić, S., Ilić, T., Božić, D.D., & Vidović, B. (2022). Phenolic Composition, Antioxidant, Anti-Enzymatic, Antimicrobial and Prebiotic Properties of *Prunus spinosa* L. fruits. *Foods*, 11(20), 3289. <https://doi.org/10.3390/foods11203289>
- Mihaylova, D., Georgieva, L., & Pavlov, A. (2015). Antioxidant activity and bioactive compounds of *Rosa canina* L. herbal preparations. *Scientific Bulletin, Series F. Biotechnologies*, XIX, 160-165. <https://www.researchgate.net/publication/295074110>.
- Mikulic-Petkovsek, M., Stampar, F., Veberic, R., & Sircelj, H. (2016). Wild *Prunus* Fruit Species as a Rich Source of Bioactive Compounds. *Journal of Food Science*, 81, 8, 1928-1937. <https://doi.org/10.1111/1750-3841.13398>
- Pinacho, R., Caverro, R.Y., Astiasarán, I., Ansorena, D., & Calvo, M.I. (2015). Phenolic compounds of blackthorn (*Prunus spinosa* L.) and influence of *in vitro* digestion on their antioxidant capacity. *Journal of Functional Foods*, 19, Part A, 49-62. <https://doi.org/10.1016/j.jff.2015.09.015>
- Pyke, M., & Melville, R. (1942). Vitamin C in rose hips. *Biochem J.*, 36, 3-4, 336-9. <https://doi.org/10.1042/bj0360336>.
- Roman, I., Stănilă, A., & Stănilă, S. (2013). Bioactive compounds and antioxidant activity of *Rosa canina* L. biotypes from spontaneous flora of Transylvania. *Chemistry Central Journal*, 7(1), 73. <https://doi.org/10.1186/1752-153X-7-73>.
- Sadasivam, S., & Balasubramanian Y. (1987). Practical Manual in Biochemistry. Tamil Nadu Agricultural University, Coimbatore, India, 14.
- Selamovska, A., Gjamovski, V., Korunoska, B., Taseska-Gjorgijevski, M., Beleski, K., Nedelkovski, D., Bandjo Orešković, K., Djoljevska-Milenkovska, R., & Rajčin, V. (2021–2022). Comparative research on antioxidant content in the fruits of select indigenous varieties of fruits, grapes and vegetables. *Contributions, Section of Natural, Mathematical and Biotechnical Sciences*,

- MASA, 42-43, 1-2, 45-52.
<https://repository.ukim.mk/server/api/core/bitstreams/ebd8304e-5e2b-463>.
- Selamovska, A., Miskoska-Milevska, E., Taseska- Gjorgijevski, M., Iljovski, I. (2024). Influence of the genotype on the polyphenolic composition and antioxidant activity of some berry fruits. *Genetika*, 56,1,143-155.
<https://doi.org/10.2298/GENSR2401143S>
- Slinkard, K., & Singleton, V.L. (1977). Total Phenol Analysis: Automation and Comparison with Manual Methods. *Am. J. Enol. Vitic.*, 28, 49-55.
<https://doi.org/10.5344/ajev.1977.28.1.49>
- Somers, C., & Evans, M.E. (1977). Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO₂, "chemical age". *Journal of the Science of Food and Agriculture*, 28(3), 279-287.
<https://doi.org/10.1002/jsfa.2740280311>.
- Stojiljković, D., Arsić, I., & Tadić, V. (2016). Extracts of wild apple fruit (*Malus sylvestris* (L.) Mill., Rosaceae) as a source of antioxidant substances for use in production of nutraceuticals and cosmeceuticals. *Industrial Crops and Products*, 80, 165-176, <https://doi.org/10.1016/j.indcrop.2015.11.023>
- Tural, S., & Koca I. (2008). Physico-chemical and antioxidant properties of cornelian cherry fruits (*Cornus mas* L.) grown in Turkey. *Scientia Horticulturae*, 116(4), 362–366.
<https://doi.org/10.1016/j.scienta.2008.02.003>.
- Veličković, I., Žižak, Ž, Rajčević, N., Ivanov, M., Soković, M., Marin, P., & Grujić, S. (2020). Examination of the polyphenol content and bioactivities of *Prunus spinosa* L. fruit extracts. *Arch Biol Sci.*, 72(1),105-115.
<https://doi.org/10.2298/ABS191217004V>.
- Vrhovsek, U., Rigo, A., Tonon, D., & Mattivi, F. (2004). Quantitation of polyphenols in different apple varieties. *Journal of Agricultural and Food Chemistry*, 52, (21), 6532–6538. <https://doi.org/10.1021/jf049317z>
- Wolfe, K., Wu, X., & Liu, R.H. (2003). Antioxidant Activity of Apple Peels *J. Agric. Food Chem.*, 51 (3), 609–614. <https://doi.org/10.1021/jf020782a>.

Полифенолни састав и антиоксидантна активност плодова неких врста дивљег воћа

Милена Тасеска-Гјорѓијевски¹, Розе Џолевска Миленковска¹, Филип Селамовски²,
Душко Неделковски¹, Ана Селамовска¹, Виктор Гјамовски¹

¹ Универзитет Св. Кирило и Методије у Скопљу, Пољопривредни институт,
Скопље, Сјеверна Македонија

² Руски универзитет економије „Г. В. Плеханов“, Висока школа за економију и
бизнис, Москва, Русија

Сажетак

Циљ овог рада био је одређивање антиоксидативне активности плодова неколико дивљих воћних врста: *Malus sylvestris* (L.) Mill., *Cornus mas* L., *Prunus spinosa* L. и *Rosa canina* L. у зависности од садржаја витамина С, укупних фенола, укупних антоцијанина и флаван-3-ола. Садржај витамина С испитан је волуметријском методом, укупни феноли према Folin-Ciocalteu методи, укупни антоцијани према киселинско-етанолну методу, а за квантификацију флаван-3-ола коришћен је р-диметиламиноцинамалдехид (p-DMACA). Одређивање антиоксидативне активности извршено је спектрофотометријски као антирадикалска активност против стабилног производа DPPH (2,2-дифенил-1-пикрилхидразил). У погледу испитиваних параметара, утврђене су статистички значајне разлике између испитиваних врста. Највећи садржај витамина С (356 mg/100 gFW), укупних фенола (38799 mg/kgFW) и антиоксидативна активност (89,2% инхибиција) пронађени су у плодовима *Rosa canina* L. Највећи садржај антоцијанина (1270 mg/kgFW) пронађен је у плодовима *Cornus mas* L. Највеће присуство флаван-3-ола пронађено је у плодовима *Prunus spinosa* (2121 mg/kgFW) и *Malus sylvestris* (1589 mg/kgFW). Најмањи садржај витамина С (14,8 mg/100 gFW), укупних фенола (2028 mg/kgFW), укупних антоцијанина и антиоксидативна активност (50,7% инхибиција) пронађени су у плодовима *Malus sylvestris*. Антиоксидативна активност плодова проучаване врсте била је у јакој или слабијој позитивној корелацији са витамином С (0,796), укупним фенолима (0,516) и укупним антоцијанинима (0,144), али у веома јакој негативној корелацији са флаван-3-олима (-0,841). Највећи допринос антиоксидативном капацитету плодова јабуке (*Malus sylvestris*) дали су флаваноли, код *Cornus mas* L. витамин С и укупни антоцијанини, код *Rosa canina* L. укупни феноли и витамин С, а код *Prunus spinosa* L. укупни феноли.

Кључне ријечи: Витамин С, укупни феноли, укупни антоцијани, флаван-3-оли, антиоксидативна активност

Corresponding author: Milena Taseska-Gjorgijevski
E-mail: m.taseska@zeminst.ukim.edu.mk

Received: June, 16, 2026
Accepted: July 31, 2026