The antioxidant capacity determination of *Rosa canina* L. fruit extracts using optimized cyclic voltammetry and spectrophotometric assays, and determination of vitamin C

Running title: Antioxidant capacity and vitamin C in *R. canina* extract

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ABSTRACT

The optimized electrochemical method, cyclic voltammetry, was applied to investigate the antioxidant capacity of water extracts of *Rosa canina* L. fruits. Scan speeds (25, 50, and 75 mV/s) and pH (2, 4.5, and 7) were varied and optimal conditions were found at 75 mV/s and pH 4.5. Cyclic voltammograms, recorded from 0 to 1200 mV, showed anodic peaks for catechin-type flavonoids and quercetin. Spectrophotometric tests (FRAP, CUPRAC, DPPH, ABTS) and determination of total polyphenols content (TPC), total flavonoids content (TFC), and vitamin C content were also conducted. FRAP and CUPRAC values ranged from 0.534 to 0.710 mmol Fe/g dw and from 103 to 174 mg TE/g dw, respectively. ABTS values were in the interval from 566 to 623 mg TE/g dw, while DPPH values ranged from 113 to 188 mg TE/g dw. Total polyphenols content and total flavonoids content ranged from 24.0 to 38.9 mg GAE/g dw, and from 12.7 to 21.5 mg CE/g dw, respectively. The concentrations of vitamin C were in the interval from 2.78 to 3.67 mg/g dw. Pearson's analysis showed good correlations between ABTS and DPPH ($R^2 = 0.87$), FRAP and DPPH ($R^2 = 0.85$), FRAP and ABTS ($R^2 = 0.86$), TPC and *in vitro* tests ($0.5 < R^2 < 0.77$), and TFC and *in vitro* tests ($0.47 < R^2 < 1$). For the purpose of classifying spectrophotometric tests for determining antioxidant activity, hierarchical cluster analysis was performed.

Keywords: *Rosa canina L., antioxidant activity, cyclic voltammetry, spectrophotometric assays, cluster analysis, Pearson correlation analysis*

Introduction

Ensuring a sufficient supply of nutritionally rich food represents one of the key global challenges of modern society. The growing world population, along with adverse environmental factors and changes in agroecosystems, increasingly affects the stability of food resources. Climate change, including frequent droughts, floods, and extreme weather events, significantly reduces agricultural yields and complicates the availability of essential nutrients. Consequently, investigating plant species rich in bioactive components has become crucial for finding sustainable solutions in the fields of nutrition and public health.

The fruits of plants from the genus *Rosa* (family *Rosaceae*), particularly rosehip (*Rosa canina* L.), have been extensively studied in scientific research because of their rich chemical composition, which includes high levels of vitamin C, polyphenols, minerals, fatty acids, and other bioactive compounds.

Rosehip has been traditionally used in folk medicine and nutrition. At the same time, modern research approaches have revealed their potential in the prevention and treatment of various diseases, including inflammatory conditions, cancer, and cardiovascular disorders (Larsen et al., 2003; Rein et al., 2004; Christensen et al., 2008; Fujii et al., 2009; Andersson et al., 2012). Numerous studies indicate that rosehip fruits contain significantly higher amounts of vitamin C compared to citrus fruits, classifying them among the most valuable natural sources of this essential nutrient (Demir and Özcan, 2001; Ercisli and Esitken, 2004). The concentration of ascorbic acid in rosehip varies from 300 to 4000 mg per 100 g of fruit, enabling its widespread application in the food and pharmaceutical industries, particularly in the production of teas, marmalades, and dietary supplements (Taneva et al., 2016). Besides being rich in vitamin C, rosehip fruits are also rich in flavonoids and phenolic acids, which contribute to their pronounced antioxidant, anticancer, and anti-inflammatory effects. These bioactive components play an essential role in nutrition and can contribute to health improvement by protecting cells from oxidative stress (Elmastaş et al., 2017). Furthermore, research has shown that rosehip consumption may positively influence blood sugar regulation, indicating its potential for diabetes prevention (Orhan et al., 2007).

Flavonoids are secondary metabolites of plants that exhibit a range of positive effects on human health, such as antioxidant and anti-inflammatory properties, antimutagenic, and anticancer properties. For this reason, they are widely used in various industries, such as pharmaceuticals and cosmetics (Panche et al., 2016). For example, rutin and quercetin, which are present in *R. canina* L. fruits, have protective effects against cardiovascular diseases, positive impacts on diabetes, and antimicrobial properties against certain infections (Elmastaş et al., 2017; Kumar et al., 2017; Semwal et al., 2021).

The composition of bioactive compounds in rosehip fruits varies depending on numerous factors, including environmental conditions, geographical origin, cultivation methods, as well as extraction and processing techniques (Taneva et al., 2016; Fascella et al., 2019). During the ripening process,

significant changes occur in the concentration of flavonoids and phenolic acids, which can influence their antioxidant capacity and nutritional value (Elmastaş et al., 2017).
The aim of this study was to determine the antioxidant activity of *Rosa canina* L., the most prevalent species from the Rosaceae family, collected from two different locations in the Republic of Serbia. The antioxidant activity of the water extracts of *Rosa canina* L. fruits was determined using the electrochemical method**,** cyclic voltammetry (CV), and *in vitro* spectrophotometric tests. Also, a correlations were established between the obtained results.

Experimental

Chemicals

For the preparation of samples and standards, deionized water (0.05 μS/cm) was used (MicroMed purification system, TKA Wasseraufbereitungssysteme GmbH, Niederelbert, Germany). Ethanol and methanol from J.T. Baker (Deventer, Netherlands) were used for the preparation of standards. Chemical reagents, including ABTS (2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonate), DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate), TPTZ (2,4,6-tri(2-pyridyl)-S-triazine), as well as flavonoids such as (+)-catechin, kaempferol, rutin, and quercetin (HPLC grade), phenolic compounds such as gallic acid and protocatechuic acid, along with neocuproine and thiourea, were purchased from Sigma-Aldrich (Steinheim, Germany). Cyanidin-3-O-glucoside chloride was obtained from ChromaDex (Irvine, California, USA), while trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Acros Organics (Morris Plains, New Jersey, USA). Folin–Ciocalteu reagent, as well as various salts and acids used in the analyses, including aluminum chloride hexahydrate, ammonium acetate, copper(II) chloride, iron(III) chloride, iron(II) sulfate heptahydrate, sodium acetate, sodium nitrite, sodium carbonate, sodium hydroxide, sodium sulfate, sodium persulfate, as well as acetic acid, ascorbic acid, bromine water, hydrochloric acid, formic acid, metafosforic acid, nitric acid, sulfuric acid, and trichloroacetic acid, were obtained from Merck® (Darmstadt, Germany).

Sample preparation for analysis

Rosa canina L. fruit samples were collected from two locations in the southeastern region of Serbia during October 2023. At each location, sampling was conducted at four different points, with a maximum distance of 500 m between them. Between 300 and 500 g of fruits were collected and stored at -20 °C until sample preparation for analysis. Herbarium specimens were registered and are preserved housed in the collection of the Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš. The voucher numbers, along with details of the sampling locations, are provided in Table 1 (Milenković et al., 2024). Approximately 30 g of frozen samples were taken and completely dried by lyophilization (Freeze-dryer Alpha 1-2 LDplus, Osterode am Harz, Germany). After full moisture removal, the samples were homogenized using a laboratory grinder. For extraction, 20 ml of deionized water were added to 1 g of lyophilized powder, mixed and treated on a laboratory shaker for 60 minutes. All the samples

were centrifuged at 4000 rpm (10 minutes). This procedure was repeated three times. All extracts were filtered through a PTFE microfilter (0.45 μm), combined, and evaporated to dryness under reduced pressure at 40–50 °C. The dry extract residues were dissolved in the deionized water and transferred into a volumetric flask with a final volume of 25 mL.

Table 1. Geographical characteristics of *Rosa canina* sampling sites and corresponding voucher numbers

Sample mark	Locality	Latitude (N)*	Longitude (E)*	Altitude (A)	Voucher No.
S1-S4	Senokos, Dimitrovgrad	43° 8'35.73"	22°55'27.27"	938 m	18613
S5-S8	Visočka Ržana, Pirot	43° 9'26.45"	22°48'59.04"	741 m	18614

*the geographical coordinates are given in decimal degrees (°N, °E)

Electrochemical analysis

One of electrochemical techniques, cyclic voltammetry (CV), was used to examine the redox properties of the investigated compounds. In this study, measurements were conducted using a CHI 760B electrochemical instrument (CH Instruments, Austin, Texas, USA). The experimental electrochemical cell consisted of a glassy carbon (GC) electrode as the working electrode, a platinum electrode as the auxiliary electrode (Model CHI221), and a reference electrode made of silver-silver chloride (Ag/AgCl) (Model CHI111). Before measurements, the working electrode was mechanically treated by polishing with aluminum oxide powder of different grain sizes (1.0, 0.3, and 0.05 μm) and subsequently degreased in ethanol to remove impurities and ensure optimal surface conductivity. Rosehip extracts and standard compound solutions were prepared by mixing them with sodium acetate-acetate buffer at pH 4.5, and with phosphate buffer at pH 7 in a 1:1 volume ratio, while pH 2 was adjusted by adding hydrochloric acid. All measurements were performed at room temperature. The potential range for measurements was set from 0 to 1200 mV, while the scan rate varied at 25, 50, and 75 mV/s, with an increment of 2 mV. Additionally, voltammograms were recorded for trolox within a concentration range of 2 to 80 μmol/L, following previous studies (Piljac-Žegarac et al., 2010; Veljković et al., 2013). The area under the dominant anodic peak (Q600), depending on the concentration of the standard compound, was used to obtain the calibration curve. The antioxidant capacity of the analyzed rosehip samples was determined as the trolox equivalent antioxidant capacity (TEAC).

Spectrophotometric analysis

The absorbance of the samples was measured using an Agilent 8453 UV/Vis spectrophotometer (Agilent Technologies, Santa Clara, California, USA), utilizing optical cuvettes with a path length of 1 cm.

Total polyphenolic content (TPC)

The TPC was determined following the method outlined by Prior et al. (2005). Measurements were performed at 760 nm, and the results were reported as gallic acid equivalents (GAE) per gram of dry weight sample (mg GAE/g dw).

Total flavonoid content (TFC)

The TFC was determined using the method developed by Yang et al. (2004). Absorbance was measured at 520 nm, and the total flavonoid content was quantified as milligrams of catechin equivalents (CE) per gram of dry weight sample (mg CE/g dw).

Antioxidant assays

The antioxidant activity of water extracts was determined using four spectrophotometric assays. The ABTS assay is based on 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), an organic compound with strong reducing power over phenolic compounds, acting as an electron donor. The method was conducted according to Re et al. (1999). In this assay, the reduction in absorbance is correlated with the concentration of antioxidants. Absorbance was measured at 734 nm, and the results were given as milligrams of trolox equivalents (TE) per gram of dry weight sample (mg TE/g dw). The DPPH method was used to determine the radical scavenging capacity according to Brand-Williams et al. (1995), employing a prepared DPPH solution with a concentration of 10^{-4} mol/L in methanol. After a 30-minute incubation, absorbance was recorded at 520 nm. Antioxidant activity determined by the DPPH method was quantified as milligrams of trolox equivalents per gram of dry weight sample (mg TE/g dw). The FRAP method relies on the ability of the extract to reduce Fe^{3+} ions to Fe^{2+} ions in the presence of 2,4,6-tripyridyl-s-triazine (TPTZ) under acidic conditions (Benzie et al., 1999). The reduction process was monitored by measuring changes in absorbance at 595 nm. The obtained results were expressed as millimoles of Fe^{2+} equivalents per gram of sample (mmol Fe/g). The CUPRAC assay determines antioxidant activity based on the ability to reduce Cu^{2+} ions to Cu^{+}. This method was first described by Apak et al. (2004). The mixture containing the CUPRAC reagent and the sample was thermostated at 25 °C for 30 minutes in the dark, after which absorbance was measured at 450 nm. The obtained antioxidant activity values were expressed as milligrams of trolox equivalents per gram of dry weight sample (mg TE/g dw).

Vitamin C content

The content of vitamin C was determined using the method outlined by Khan et al. (2006). Absorbance of the reaction mixture was recorded at 521 nm, and the results were expressed as milligrams of vitamin C per gram of dry weight sample (mg/g dw).

Results and Discussion

To achieve optimal conditions for determining the antioxidant activity of rosehip water extracts using cyclic voltammetry, the pH values and scan rates were varied. The samples were prepared in three series, with pH values of the extract set at 2.0, 4.5, and 7.0, while the scan rate was monitored at 25, 50, and 75 mV/s. The effect of the scan rate on the anodic current intensity is presented in Figure 1.

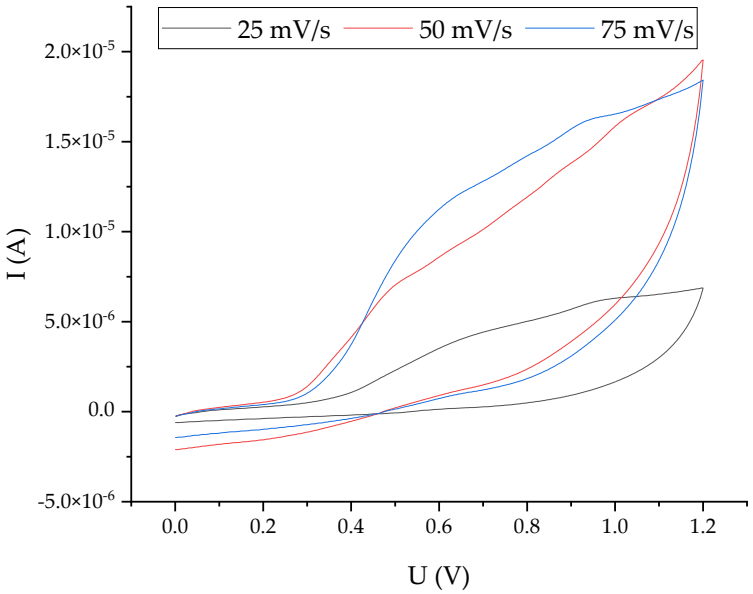

Figure 1. Effect of scan rate on anodic current intensity

As shown in Figure 1, a higher scan rate leads to a more intense anodic current, while a lower scan rate allows sufficient time for the stabilization of the double-layer charge on the glassy electrode. To adjust the pH of the aqueous rosehip extracts, acetate buffer (pH 4.5), phosphate buffer (pH 7), and hydrochloric acid (pH 2) were used. Regarding the effect of pH on the intensity of the anodic current, the most optimal current was observed at a pH value of 4.5, as presented in Figure 2. This finding is consistent with the study by Yakovlev et al. (2007), which reported that an increase in the pH of the electrolyte solution results in the shift of anodic potential (E_{pa}) towards the less positive values of potential, explained by the reduced degree of antioxidant protonation and a shift in the molecular charge toward more negative values.

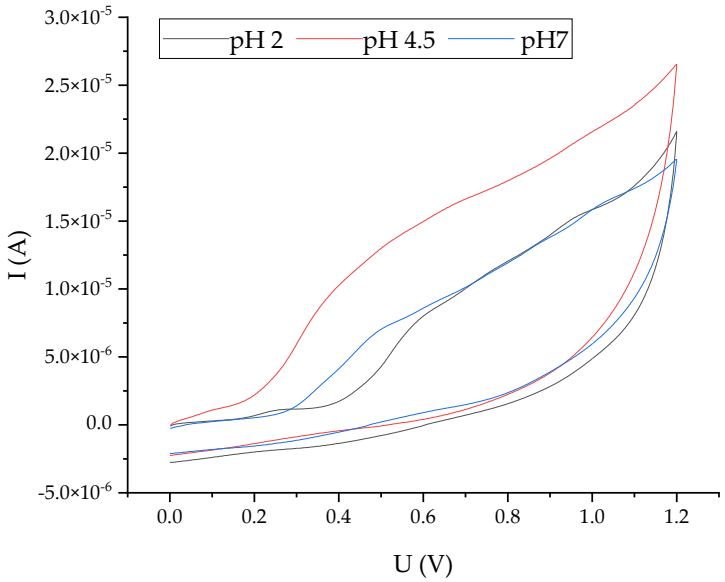

Figure 2. Effect of solution pH on anodic current intensity

Based on the obtained data, the optimal conditions used for cyclic voltammetry were a pH of 4.5 and a scan rate of 75 mV/s. Before recording the sample, standards were recorded under the optimal conditions, and their values of oxidation (E_{pa1}, E_{pa2}, E_{pa3} - potential of first, second, and third anodic pick) and reduction potentials (E_{pc1} and E_{pc2} - potential of first and second catodic peak) are presented in Table 2.

Table 2. Oxidation (E_{pa1}, E_{pa2}, E_{pa3}) and reduction (E_{pc1}, E_{pc2}) potentials of phenolic compounds

Phenolic compound	E_{pa1} (V)	E_{pa2} (V)	E_{pa3} (V)	E_{pc1} (V)	E_{pc2} (V)
Ellagic acid	0.355	0.704	-	0.519	-
(+)-Catechin	0.485	0.779	-	-	-
Quercetin	0.617	1.002	-	0.774	0.069
Rutin	0.526	0.886	-	-	-
(-)-Epicatehin	0.510	0.735	-	-	-
Protocatechuic acid	0.605	-	-	0.155	-
Caffeic acid	0.435	-	-	0.189	-
Galic acid	0.553	0.884	-	-	-

Abou Samra et al. (2011) evaluated the antioxidant potential of the investigated compounds based on the E_{pa} values of the first anodic peak. Lower E_{pa} values indicate a greater ability to donate electrons, meaning the compound exhibits stronger antioxidant activity. Comparing the anodic

peaks obtained for the standards to those obtained for the samples (Table 3), it can be concluded that the first anodic peak, with values ranging from 0.485 to 0.513 V, and the second anodic peak, ranging from 0.712 to 0.746 V, correspond to the oxidation of flavonoids-catechins. According to the study by Medvidović-Kosanović et al. (2010), the first oxidation peak for (+)-catechin, which is reversible, corresponds to the oxidation of the 3',4'-dihydroxyl substituent on the B-ring. The second oxidation peak is irreversible and has been attributed to the oxidation of the 5,7-dihydroxy substituent on the A-ring. Catechin and epicatechin exhibit similar electrochemical properties due to their nearly identical chemical structures and the same oxidation mechanism (Jara-Palacios et al., 2024). In the voltammograms for the epicatechin standard, the oxidation peak appeared at a potential of 0.510 V, corresponding to the oxidation of the catechol group. The second anodic peak was detected at 0.735 V, indicating the oxidation of the resorcinol group. However, according to some sources, this anodic peak may result from the irreversible oxidation of the hydroxyl group at the third position of the non-aromatic C-ring (Petrović, 2009; Rebelo et al., 2013). The values obtained for the third anodic peak of the samples ranged from 0.931 to 0.991 V, which may be attributed to the oxidation of quercetin. The oxidation mechanism of quercetin involves the first anodic peak and the corresponding cathodic peak, which corresponds to the reversible oxidation of the catechol group. In contrast, the second anodic peak indicates the oxidation of the resorcinol group (Jara-Palacios et al., 2024).

Table 3. Peak potential (E_p) and current (I_p) values of analyzed rosehip samples

Sample	E_{pa1} (V)	I_{pa1} (A)	E_{pa2} (V)	I_{pa2} (A)	E_{pa3} (V)	I_{pa3} (A)	E_{pc} (V)	I_{pc} (A)
S1	0.492	0.58	0.714	1.19	0.980	1.44	-	-
S2	0.513	1.05	0.746	1.63	0.988	1.68	-	-
S3	0.505	0.51	0.712	0.95	0.957	1.34	-	-
S4	0.502	0.66	0.743	1.36	0.989	1.60	-	-
S5	0.485	0.53	0.717	1.09	-	-	-	-
S6	0.495	0.46	0.713	0.88	0.931	1.14	-	-
S7	0.485	0.61	0.737	1.33	0.991	1.50	-	-
S8	0.485	0.61	0.744	1.30	0.972	1.42	-	-

The lowest value of TPC (24.0 mg GAE/g) was found in the fruits from the Senokos locality, while the highest value (38.9 mg GAE/g) was recorded in the samples from Visočka Ržana (Figure 3). The average TPC at the Senokos site was 30.65 mg GAE/g (24.00–37.50), whereas at Visočka Ržana, it was 35.49 mg GAE/g (30.55–38.90). The difference in TPC may be attributed to the environmental conditions (Ciornea et al., 2018), as well as to differences in altitude between the

two sites (Lacramioara and Rosu, 2021). The obtained results for TPC were lower than those reported by Paunović et al. (2019), who analyzed raw and dried samples of this species from Serbia. They were also lower than the TPC of *R. canina* L. fruits from Sicily, extracted with 80% methanol (Fascella *et al.*, 2019), but higher than results obtained for samples from Slovakia, extracted with 96% ethanol (Rovna et al., 2020). This suggests that, in addition to the origin of the sample, the choice of extraction solvent may significantly influence the total polyphenol content.

The TFC in the water extracts ranged from 12.7 to 21.5 mg CE/g. The average TFC in fruits at the Senokos site was 15.9 mg CE/g (12.7–20.5 mg CE/g), while in fruits at Visočka Ržana, it was 19.4 mg CE/g (16.4–21.5 mg CE/g). Similar to total polyphenols, the total flavonoid content was higher in fruits at the Visočka Ržana site, as shown in Figure 3. Paunović et al. (2019) reported a TFC (miligrame of quercetin equivalents, QE, per gram of raw and dried fruit sample) of 38.5 mg QE/g in raw and 26.47 mg QE/g in dried samples using a 70% ethanol extract. In contrast, Tahirović and Bašić (2017) found much lower values for aqueous extracts of samples from Bosnia and Herzegovina, reporting 0.214 mg QE/g, while the ethanol (50%) extract yielded 0.675 mg QE/g. Similarly, Nađpal et al. (2016) reported a total flavonoid content of 1.14 mg QE/g in dried *R. canina* fruits. The differences amoung TFC in the literature may be caused by the geographical origin of the samples, soil conditions, climate, as well as using different compounds as equivalents (quercetin or catechin) for the calculate of flavonoid contents (Taneva et al., 2016; Fascella et al., 2019).

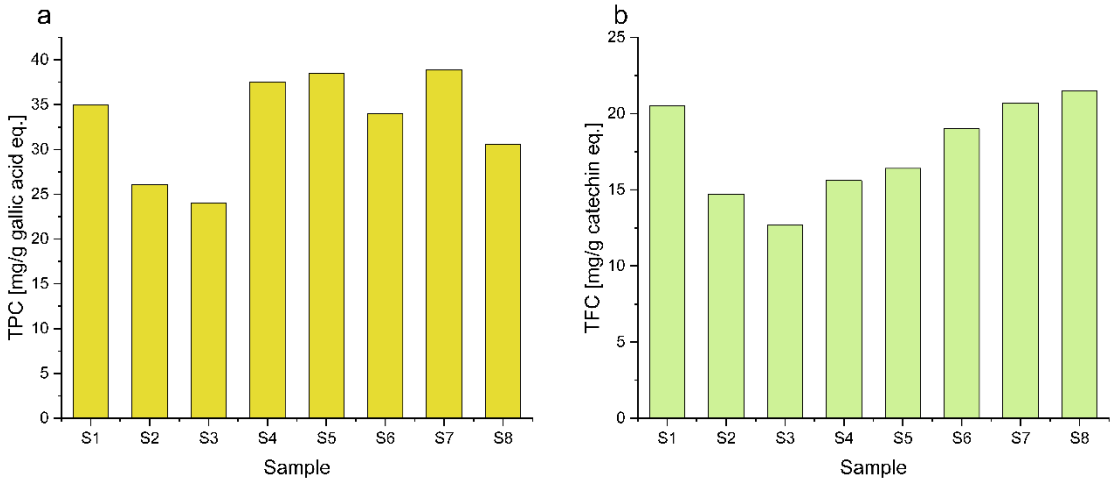

Figure 3. Total polyphenol and flavonoid content in the analyzed water extracts of *R. canina* L. fruits

Rosa canina L. is a good source of antioxidants, which is one of the reasons for its widespread application. The analysis of the antioxidant activity of the aqueous extract of *R. canina* L. fruits using the DPPH assay showed that the average value for samples from the Senokos locality was 127 μmol TE/g (113–144 μmol TE/g), while for the samples from the Visočka Ržana locality, the

average value was 141 μmol TE/g (120–188 μmol TE/g). These results are consistent with those found in the literature, for example, 250 μmol TE/g for the ethanol (70%) extracts of dried fruits and 320 μmol TE/g for the fresh samples (Paunović et al., 2019), as well as 382.3 μmol TE/g for the aqueous extracts (Tahirović and Bašić, 2017). The average ABTS values for the water extracts of fruits from Senokos and Visočka Ržana were 589 mmol TE/g and 592 mmol TE/g, respectively, with ranges of 566–609 and 580–623 mmol TE/g. Unlike the values for TPC, TFC, and DPPH, the results obtained for ABTS antioxidant activity of samples from two locations were very similar. Taneva et al. (2016) analyzed the antioxidant activity of *R. canina* L. aqueous extract using the ABTS assay and reported a value of 313.1 mmol TE/g, while for the ethanol (50%) extract, the ABTS value was 368.4 mmol TE/g. The average FRAP test value for the fruit extracts from Senokos was 0.61 mmol Fe/g (0.53–0.69), while for the fruit extracts from Visočka Ržana, it was 0.66 mmol Fe/g (0.61–0.71). The mean CUPRAC test values were 129 mg TE/g (103–166) and 157 mg TE/g (133–174 mg TE/g) for the extracts of fruits from two locations, respectively. The FRAP test results were similar to those obtained for the aqueous extract of *Rosa* species investigated by Tahirović and Bašić (2017). Adamczak et al. (2012) emphasized that vitamin C is highly stable and exhibits significant bioavailability in the human body, as organic acids and flavonoids prevent its oxidation. The vitamin C concentrations in the analyzed samples ranged from 2.78 to 3.67 mg/g of dry sample. As shown in Table 4, the content of vitamin C were slightly higher in the samples from Visočka Ržana. The obtained results are in good agreement with the findings of Barros et al. (2011) and Nađpal et al. (2016), while Demir et al. (2014) reported significantly lower vitamin C concentrations in similar samples. The effect of ripeness on vitamin C content was investigated by Nojavan et al. (2008), who found that *R. canina* rosehips contain the highest concentration of vitamin C in their fully ripe stage-six times higher than in oranges. All previous studies confirm the traditional use of rosehips as a rich source of vitamin C.

For the purpose of classifying spectrophotometric tests for determining antioxidant activity, hierarchical cluster analysis was performed using Euclidean distance and Ward's clustering method on standardized data for these methods. The resulting dendrogram is presented in Figure 4.

Table 4. Antioxidant activity of rosehip extracts and vitamin C content (mean ± standard deviation, number of repetitions -3)

Sample	ABTS (mg TE/g)	DPPH (mg TE/g)	CUPRAC (mg TE/g)	FRAP (mmol Fe/g)	Vitamin C (mg/g)
S1	609±6	132±9	166±1	0.63±0.01	2.78±0.06
S2	566±5	113±3	119±1	0.53±0.01	3.02±0.07
S3	581±4	120±9	103±2	0.59±0.01	3.11±0.06
S4	598±6	144±4	126±1	0.69±0.01	3.27±0.06
S5	582±8	120±9	133±2	0.61±0.01	3.67±0.06
S6	581±6	125±4	154±1	0.63±0.01	3.57±0.07
S7	623±5	188±9	168±2	0.71±0.01	3.33±0.07
S8	580±50	130±1	174±1	0.62±0.01	3.43±0.07

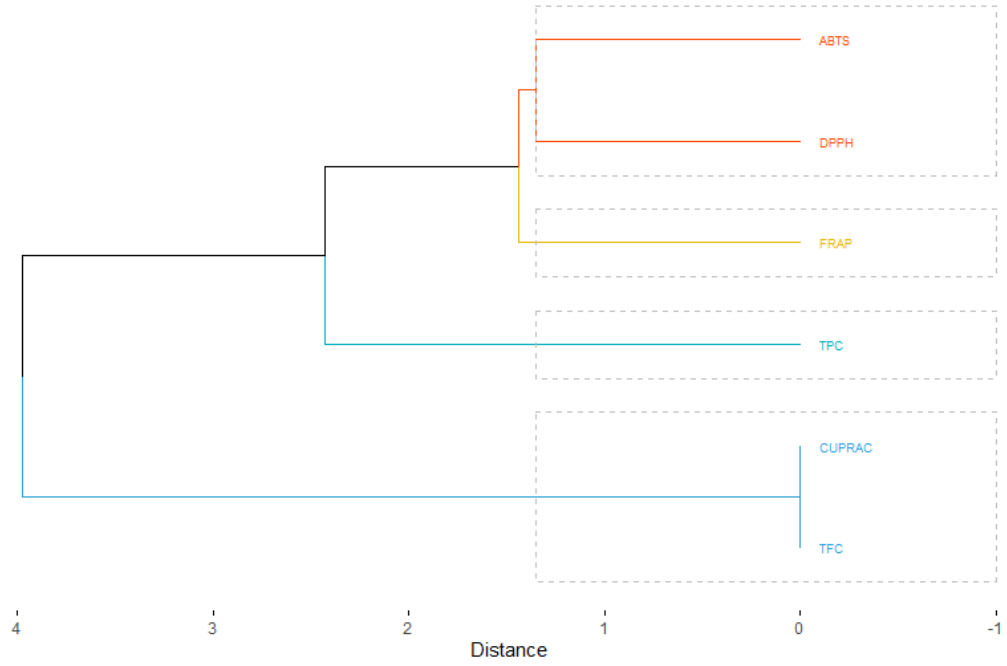

Figure 4. Classification of spectrophotometric tests

In the first cluster, CUPRAC and TFC are grouped together, indicating their interrelation in determining the extract's total antioxidant activity. This can be explained by flavonoids' ability to

reduce copper in the CUPRAC assay, as confirmed by Apak et al. (2004). They developed a method known as "cupric reducing antioxidant capacity."

Regarding the second cluster, TPC is grouped into a separate subcluster, suggesting that it is not a decisive factor in determining antioxidant activity; however, it remains closely associated with other antioxidant analysis methods. In another subcluster, FRAP is separated from the DPPH and ABTS methods, which can be explained by its significantly different mechanisms of action. ABTS and DPPH are grouped in a single subcluster, which is expected since both analytical methods measure the ability of compounds to neutralize free radicals through electron or hydrogen transfer.

To explore potential correlations between the spectrophotometric tests for determining antioxidant activity, total phenol and flavonoid content, and vitamin C content, Pearson correlation analysis was performed among these parameters, and the results are presented graphically in Figure 5.

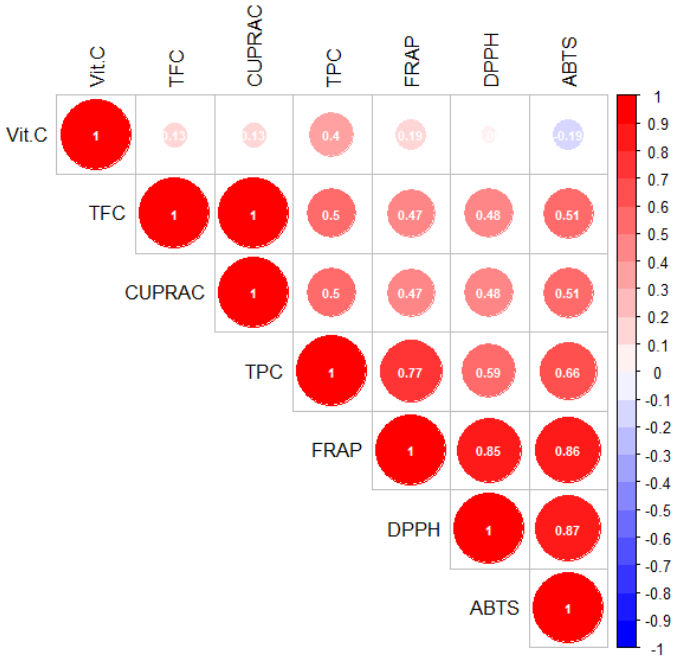

Figure 5. Correlations between the applied methods for determining TPC, TFC, antioxidant activity, and vitamin C content

As shown in Figure 5, there is a strong correlation between the antioxidant tests themselves, as well as between the antioxidant tests and the total phenol and flavonoid content. This can be explained by the fact that phenolic compounds, including flavonoids, exhibit significant antioxidant effects (Rice-Evans et al., 1997).

The correlation between vitamin C content and the antioxidant tests is considerably weaker, which is consistent with the findings of Rice-Evans et al. (1997), who reported that polyphenols are more effective antioxidants *in vitro* than vitamin E and vitamin C. Consequently, polyphenols play a more significant role in protecting the body against oxidative stress than these vitamins.

Conclusion

The results of this study indicate that the antioxidant properties of *R. canina* L. fruit extracts can be determined using an optimized electrochemical method, cyclic voltammetry. Based on the anodic peaks recorded for both standards and samples, it was established that the antioxidant activity of this species is significantly influenced by the presence of catechin, epicatechin, and quercetin. Furthermore, the results obtained from spectrophotometric assays show a significant correlation with the total polyphenol and flavonoid content but not with the results of vitamin C determination, confirming that this vitamin is a weaker antioxidant compared to polyphenols.

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Conflict-of-Interest Statement

The authors declare that they have no conflict of interest.

References

Abou Samra, M., Chedea, V. S., Economou, A., Calokerinos, A., & Kefalas, P. (2011). Antioxidant/prooxidant properties of model phenolic compounds: Part I. Studies on equimolar mixtures by chemiluminescence and cyclic voltammetry. *Food Chemistry*, *125*(2), 622-629.

Adamczak, A., Buchwald, W., Zieliński, J., & Mielcarek, S. (2012). Flavonoid and organic acid content in rose hips (Rosa L., sect. Caninae dc. Em. Christ.). *Acta Biologica Cracoviensia s. Botanica*, *54*(1), 105-112.

Andersson, U., Berger, K., Högberg, A., Landin-Olsson, M., & Holm, C. (2012). Effects of rose hip intake on risk markers of type 2 diabetes and cardiovascular disease: a randomized, double-blind, cross-over investigation in obese persons. *European Journal of Clinical Nutrition*, *66*(5), 585-590.

Apak, R., Güçlü, K., Özyürek, M., & Karademir, S. E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in

the presence of neocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry*, *52*(26), 7970-7981.

Barros, L., Carvalho, A. M., & Ferreira, I. C. (2011). Exotic fruits as a source of important phytochemicals: Improving the traditional use of *Rosa canina* fruits in Portugal. *Food Research International*, *44*(7), 2233-2236.

Benzie, I. F., & Strain, J. J. (1999). Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology,* 299(2), 15-27.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, *28*(1), 25-30.

Ciornea, E. T., Dumitru, G., Zaharia, M., Drochioiu, G., Sandu, I. (2018). Heavy metal pollution affects the antioxidant potential of *Rosa canina* L. species. *Revista de Chimie*, *69*(2), 449-452.

Christensen, R., Bartels, E. M., Altman, R. D., Astrup, A., & Bliddal, H. (2008). Does the hip powder of *Rosa canina* (rosehip) reduce pain in osteoarthritis patients? a meta-analysis of randomized controlled trials. *Osteoarthritis and Cartilage*, *16*(9), 965-972.

Demir, F., & Özcan, M. (2001). Chemical and technological properties of rose (*Rosa canina* L.) fruits grown wild in Turkey. *Journal of Food Engineering*, *47*(4), 333-336.

Demir, N., Yildiz, O. K. T. A. Y., Alpaslan, M., & Hayaloglu, A. A. (2014). Evaluation of volatiles, phenolic compounds and antioxidant activities of rose hip (*Rosa* L.) fruits in Turkey. *Lwt-Food Science and Technology*, *57*(1), 126-133.

Elmastaş, M., Demir, A., Genç, N., Dölek, Ü., & Güneş, M. (2017). Changes in flavonoid and phenolic acid contents in some *Rosa* species during ripening. *Food Chemistry*, *235*, 154-159.

Ercişli, S., & Eşitken, A. (2004). Fruit characteristics of native rose hip (*Rosa* spp.) selections from the Erzurum province of Turkey. *New Zealand Journal of Crop and Horticultural Science, 32*(1), 51–53.

Fascella, G., D'Angiolillo, F., Mammano, M. M., Amenta, M., Romeo, F. V., Rapisarda, P., & Ballistreri, G. (2019). Bioactive compounds and antioxidant activity of four rose hip species from spontaneous Sicilian flora. *Food Chemistry*, *289*, 56-64.

Fujii, T., & Saito, M. (2009). Inhibitory effect of quercetin isolated from rose hip (*Rosa canina* L.) against melanogenesis by mouse melanoma cells. *Bioscience, Biotechnology, and Biochemistry*, *73*(9), 1989-1993.

Jara-Palacios, M. J., Begines, E., Heredia, F. J., Escudero-Gilete, M. L., & Hernanz, D. (2024). Effectiveness of cyclic voltammetry in evaluation of the synergistic effect of phenolic and amino acids compounds on antioxidant activity: Optimization of electrochemical parameters. *Foods*, *13*(6), 906.

Khan, M. M. R., Rahman, M. M., Islam, M. S., & Begum, S. A. (2006). A simple UV-spectrophotometric method for the determination of vitamin C content in various fruits and vegetables at Sylhet area in Bangladesh. *Journal of Biological Sciences*, *6*(2), 388-392.

Kumar, R., Vijayalakshmi, S., & Nadanasabapathi, S. (2017). Health benefits of quercetin. *Defence Life Science Journal*, *2*(2), 142-151.

Larsen, E., Kharazmi, A., Christensen, L. P., & Christensen, S. B. (2003). An antiinflammatory galactolipid from Rose hip (*Rosa canina*) that inhibits chemotaxis of human peripheral blood neutrophils *in vitro*. *Journal of Natural Products*, *66*(7), 994-995.

Lacramioara, O., & Rosu, C. M. (2021). Total polyphenols, flavonoids contents and antioxidant activity of *Rosa* sp. genotipes from different altitude of Romanian regions. *Journal of Experimental and Molecular Biology*, *22*(1), 15-26.

Medvidović-Kosanović, M., Šeruga, M., Jakobek, L., & Novak, I. (2010). Electrochemical and antioxidant properties of (+)-catechin, quercetin and rutin. *Croatica Chemica Acta*, *83*(2), 197-207.

Milenković, K., Mrmošanin, J., Petrović, S., Mitov, D., Zlatković, B., Mutić, J., & Pavlović, A. (2024). Elemental composition of Rosa L. fruits: Optimization and validation procedure of an ICP AES method. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *52*(4), 13959.

Nađpal, J. D., Lesjak, M. M., Šibul, F. S., Anačkov, G. T., Četojević-Simin, D. D., Mimica-Dukić, N. M., & Beara, I. N. (2016). Comparative study of biological activities and phytochemical composition of two rose hips and their preserves: *Rosa canina* L. and *Rosa arvensis* Huds. *Food Chemistry*, *192*, 907-914.

Nojavan, S., Khalilian, F., Kiaie, F. M., Rahimi, A., Arabanian, A., & Chalavi, S. (2008). Extraction and quantitative determination of ascorbic acid during different maturity stages of *Rosa canina* L. fruit. *Journal of Food Composition and Analysis*, *21*(4), 300-305.

Orhan, D. D., Hartevioğlu, A., Küpeli, E., & Yesilada, E. (2007). In vivo anti-inflammatory and antinociceptive activity of the crude extract and fractions from *Rosa canina* L. fruits. *Journal of Ethnopharmacology*, *112*(2), 394-400.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of Nutritional Science*, *5*, e47.

Paunović, D., Kalušević, A., Petrović, T., Urošević, T., Djinović, D., Nedović, V., & Popović-Djordjević, J. (2019). Assessment of chemical and antioxidant properties of fresh and dried rosehip (*Rosa canina* L.). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *47*(1), 108-113.

Petrovic, S. C. (2009). Correlation of perceived wine astringency to cyclic voltammetric response. *American Journal of Enology and Viticulture*, *60*(3), 373-378.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, *53*(10), 4290-4302.

Piljac-Žegarac, J., Valek, L., Stipčević, T., & Martinez, S. (2010). Electrochemical determination of antioxidant capacity of fruit tea infusions, *Food Chemistry*, *121*, 820-825.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9-10), 1231-1237.

Rebelo, M. J., Rego, R., Ferreira, M., & Oliveira, M. C. (2013). Comparative study of the antioxidant capacity and polyphenol content of Douro wines by chemical and electrochemical methods. *Food Chemistry*, *141*(1), 566-573.

Rein, E., Kharazmi, A., & Winther, K. (2004). A herbal remedy, Hyben Vital (stand. powder of a subspecies of *Rosa canina* fruits), reduces pain and improves general wellbeing in patients with osteoarthritis—a double-blind, placebo-controlled, randomised trial. *Phytomedicine*, *11*(5), 383-391.

Rice-Evans, C., Miller, N., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science*, *2*(4), 152-159.

Rovná, K., Ivanišová, E., Žiarovská, J., Ferus, P., Terentjeva, M., Kowalczewski, P. Ł., & Kačániová, M. (2020). Characterization of *Rosa canina* fruits collected in urban areas of Slovakia. Genome size, iPBS profiles and antioxidant and antimicrobial activities. *Molecules*, *25*(8), 1888.

Semwal, R., Joshi, S. K., Semwal, R. B., & Semwal, D. K. (2021). Health benefits and limitations of rutin-A natural flavonoid with high nutraceutical value. *Phytochemistry Letters*, *46*, 119-128.

Taneva, I., Petkova, N., Dimov, I., Ivanov, I., & Denev, P. (2016). Characterization of rose hip (*Rosa canina* L.) fruits extracts and evaluation of their *in vitro* antioxidant activity. *Journal of Pharmacognosy and Phytochemistry*, *5*(2), 35-38.

Tahirović, A., & Bašić, N. (2017). Determination of phenolic content and antioxidant activity of *Rosa canina* L. fruits in different extraction systems. *Works of the Faculty of Forestry University of Sarajevo*, *47*(1), 47-59.

Veljković, J., Pavlović, A., Mitić, S., Tošić, S., Stojanović, G., Kaličanin, B., Stanković, D., Stojković, M., Mitić, M., & Brcanović, J. (2013). Evaluation of individual phenolic compounds and antioxidant properties of black, green, herbal and fruit tea infusions consumed in Serbia: spectrophotometrical and electrochemical approaches. *Journal of Food Nutrition and Research*, 52(1), 12-24.

Yang, J., Meyers, K. J., Van Der Heide, J., & Liu, R. H. (2004). Varietal differences in phenolic content and antioxidant and antiproliferative activities of onions. *Journal of Agricultural and Food Chemistry*, *52*(22), 6787-6793.

Yakovleva, K. E., Kurzeev, S. A., Stepanova, E. V., Fedorova, T. V., Kuznetsov, B. A., & Koroleva, O. V. (2007). Characterization of plant phenolic compounds by cyclic voltammetry. *Applied Biochemistry and Microbiology*, *43*, 661-668.