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Antioxidant capacity of *Rosa* species: Optimised cyclic voltammetry method *versus in vitro* antioxidant properties and selected phytochemicals

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Abstract

The electrochemical method, cyclic voltammetry (CV), was used to examine the antioxidant properties of rosehip species. In order to optimise the CV method, scan speed (25, 50, and 75 mV/s) and pH (2, 4.5, and 7) were varied. Based on anodic current intensity, the optimal conditions were found to be 75 mV/s and pH = 4.5. Cyclic voltammograms were recorded in a potential range from 0 to 1,200 mV/s. The first and second anodic peak detected between 0.465 and 0.529 V, and between 0.707 and 0.782 V, could be attributed to oxidation of catechin-type flavonoids. The third peak, appearing between 0.951 and 1.056 V in the cyclic voltammograms of samples, corresponded to the oxidation of quercetin. A significant correlation was found between CV and *in vitro* antioxidant assays: FRAP (R^2 = 0.7793, p < 0.00001), CV and CUPRAC (R^2 = 0.7691, p < 0.00001), and between CV and total flavonoid content (R^2 = 0.7611, p < 0.00001), as well as between CV and total phenolic content (R^2 = 0.7080, p < 0.00001). The HPLC method was used for the identification of individual phenolic compounds. Principal component analysis (PCA) provided a classification of samples based on their individual phenolic content.

Introduction

According to recent estimations, food insecurity, undernourishment, and hunger are pressing problems of the modern world (UNICEF, 2023). Along with the complex political situation and human population growth, climate change and pronounced extreme weather conditions, significantly increase the problem. Due to severe floods, landslides, fires, and droughts, large areas of fertile land are being irretrievably lost. This way, traditional agricultural food production is being compromised. Therefore, discovering cultivation-undemanding, yet nutritionally rich alternative food sources, has become a necessity.

One of these is the deciduous bush rosehip - *Rosa* (fam. Rosaceae), which includes many species, grows spontaneously, and is widespread in areas with a continental climate. This plant has been used for ages in traditional medicine, and successfully cultivated in various climatic conditions. Rosehip fruits, alone or combined with other types of fruits, are often used in human nutrition for the preparation of teas, jams, marmalades, and sweets (Demir *et al.*, 2014). The addition of rosehip powder contributes to physicochemical and organoleptic properties, as well as microbiological stability of gingerbread (Ghendov-Mosanu *et al.*, 2020).

Numerous chemical compounds with beneficial effect on human health were identified in

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cultivated and wild rosehip species. Caffeic, ferulic, gallic, and chlorogenic acids, as well as a significant amount of ascorbic acid, were reported in rosehip fruits (Ercisli, 2007; Roman *et al*., 2013; Paunović *et al*., 2019; Fetni *et al*., 2020). Essential fatty acids - linoleic and α-linolenic, and flavonoids - quercitrin, quercetin, kaempferol, and their glycosides, catechin and epicatechin, were also found with notable differences in quantity among rosehip species (Ercisli, 2007; Nađpal *et al*., 2016). Due to the high content of lycopene, rosehips can be used as a good source of this phytochemical (Böhm *et al*., 2003). Depending on the cultivar, growing region, and climate, notable differences in chemical composition of rosehips have been observed (Fan *et al*., 2014). Additionally, the content of flavonoids and phenolic acids increase during ripening, and vary depending on the harvesting period (Elmastas *et al*., 2017). A concentration-dependent correlation was reported between reducing power and total phenolic content of rosehip (Kiliçgün and Altiner, 2010). In the same study, a strong scavenging effect of rosehip extracts against superoxide radical was also noted (Kiliçgün and Altiner, 2010).

In addition, some studies have also highlighted the potential value of not only the rosehip fruit, but also other parts of the plant. Rosehip's twigs and leaf extracts decreased lipid peroxidation level, and exhibited antiradical scavenging activity due to phenolic acids, flavonoids, essential amino acids, tocopherols, and water-soluble B vitamins (Kubczak *et al*., 2020). Based on the results of DPPH, FRAP, TEAC, and ORAC *in vitro* assays, Ouerghemmi *et al*. (2020) pointed out a significant antioxidant activity of twig extracts. The rosehip seeds are often removed during the processing of rosehip fruits, thus considered a waste. However, the seeds can be used for obtaining the rosehip oil, which is rich in carotenoids, minerals, and polyunsaturated fatty acids (Szentmihályi *et al*., 2002).

Standard and modern techniques, such as the spectrophotometric analysis, GC-MS, LC-DAD-ESI-MS, and HPLC-DAD-MS, were used for the comprehensive analysis of the rosehip plant (Ercisli, 2007; Paunović *et al*., 2019; Ouerghemmi *et al*., 2020; Fetni *et al*., 2020). However, to the best of our knowledge, the electrochemical method, cyclic voltammetry (CV), has not been used so far for antioxidant activity measurements of rosehip samples. The instrumentation for CV measurements is easy to handle, and does not require the use of reactive, volatile, or toxic substances. No preparation of additional regents is necessary, and CV measurements can be performed very quickly. Based on these, the aims of the present work were: (1) to optimise CV conditions for determining the antioxidant activity of rosehips; (2) to examine the antioxidant activity of rosehips using *in vitro* spectrophotometric assays; (3) to determine the HPLC profile of rosehip extracts, and (4) to establish correlations among the obtained results.

Materials and methods

Chemicals

For the preparation of all samples and standards, purified water (18 MΩ/cm) prepared by a MicroMed purification system (TKA Wasseraufbereitungssysteme GmbH, Niederelbert, Germany) was used. Ethanol and methanol were purchased from J.T. Baker (Deventer, The Netherlands). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Acros Organics (Morris Plains, New Jersey, USA). ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonate), DPPH (2-diphenyl-2-picrylhydrazyl hydrate), TPTZ ((2,4,6-tri(2-pyridyl)-S-tirazine), kaempferol, protocatechuic acid, gallic acid, (+)-catechin, rutin and quercetin (HPLC grade), neocuproine, and thiourea were purchased from Sigma Aldrich (Steinheim, Germany). Cyanidin-3-*O*-glucoside chloride was from ChromaDex (Irvine, CA, USA). Folin Ciocalteu's phenol reagent, $Na_2S_2O_8$, $FeCl_3$, $FeSO_4x7H_2O$, $CuCl_2$, NaOH, CH_3COONa, CH_3COONH_4, $NaNO_2$, Na_2CO_3, Na_2SO_4, $AlCl_3•6H_2O$, HCl, H_2SO_4, ascorbic acid, metaphosphoric acid, trichloroacetic acid, acetic acid, formic acid, and bromine water were purchased from Merck® (Darmstadt, Germany).

Samples and preparation of rosehip extracts

The samples were collected in the south-eastern part of Serbia, during the full maturity period of rosehips (Autumn 2022). *Rosa* species, sample marks, and sampling locations are given in Table 1. Due to rugged terrain, four points were chosen at each individual location, with a minimum distance of 500 m between the selected rosehip bushes. Approximately 300 - 500 g of rosehip were collected from every point. The fruit samples were stored at -20°C until analysis. Specimens were deposited at the

Herbarium of the Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš, under the vouchers no. given in Table 1. Plant material was identified by Dr. B.K. Zlatković. Dichotomous keys used for the taxa identification, including the nomenclature, follow the relevant literature for the study area (Vukićević, 1972; Dimitrov,1973).

Approximately 30 - 40 g of frozen fruit samples were dried by lyophilisation. After complete drying, the samples were ground to homogenised powder by a grinding mill. Several solvents of different polarity were chosen (water, methanol, acidified 80% methanol, ethanol, and 60% ethanol), and the extraction was performed following a previous study (Stanila *et al.*, 2015) with minor modifications: 1.0000 g of lyophilised sample was extracted with 20 mL of solvent on a laboratory shaker for 60 min and then centrifuged for 10 min (4,000 rpm). The procedure was repeated three times. All the extracts were combined and filtered through PTFE microfilter (0.45 μm), and evaporated to dryness under reduced pressure at 40 - 50°C. The dry residues were reconstituted in the matching solvent, transferred into a 25-mL flask, filled up to the mark, and analysed.

Table 1. *Rosa* species, sample marks, and sampling locations of analysed rosehips.

Variety	Sample mark	Location (GPS coordinates)	Voucher no.
Rosa myriacantha DC.	RM1, RM2, RM3, RM4	Selicevica, Donje Vlase 43° 22′ N, 21° 58′ E	18602
Rosa dumalis Bechst.	RDN1, RDN2, RDN3, RDN4	Nis, Gorica 43° 18′ N, 21° 53′ E	18603
Rosa corymbifera Borkh.	RCC1, RCC2, RCC3, RCC4	Crna Trava 42° 48′ N, 22° 18′ E	18608
Rosa agrestis Savi	RA1, RA2, RA3, RA4	Selicevica, Donje Vlase 43° 23′ N, 21° 51′ E	18610
Rosa corymbifera Borkh.	RCV1, RCV2, RCV3, RCV4	Vlasina, Vlasina Rid 42° 41′ N, 21° 19′ E	18609
Rosa dumalis Bechst.	RDV1, RDV2, RDV3, RDV4	Vlasotince 42° 57′ N, 22° 07′ E	18604
Rosa spinosissima L.	RS1, RS2, RS3, RS4	Rtanj mountain 43° 46′ N, 21° 56′ E	18612

Instruments

For absorbance measurements and spectra recording, an Agilent 8453 UV/Vis spectrophotometer (Agilent Technologies, Santa Clara, California, USA) was used, using optical cuvettes of 1 cm optical path. A pH-meter (Hanna Instruments, Smithfield, Rhode Island, USA) equipped with a glass electrode was used for pH measurements. The measurements were performed at ambient temperature.

Electrochemical analysis

CV measurements were carried out on a CHI760B instrument (CHInstruments, Austin, Texas, USA). The cell was equipped with a glassy carbon (GC) electrode, an accessory platinum electrode (Model CHI221), and an Ag/AgCl reference electrode (Model CHI111). The surface preparation of the glassy carbon electrode involved a gentle abrasion with 1.0, 0.3, and 0.05 μm alumina powder and degreasing in ethanol. Solutions of available standards, as well as the rosehip extracts, were mixed with 0.1 mol/L sodium acetate-acetic buffer (pH 4.5) at a ratio of 1:1 (mixing 2.5 mL of extract and the same volume of buffer, v/v) in water, and CV measurements were taken in the potential range between 0 and 1,200 mV at 2 mV intervals. Cyclic voltammograms were also recorded for Trolox in the concentration range of 2 - 80 μmol/L (Piljac-Žegarac *et al.*, 2010). The area below the voltammetric anodic peak, which spanned the entire potential range (Q_{1200}) *versus* concentration (c) obtained for this standard, was used to construct the calibration curve, and to calculate the TEAC (Trolox Equivalent Antioxidant Capacity) of the studied rosehip samples.

The HPLC analysis

The HPLC analysis of extracts was carried out on an Agilent-1200 series HPLC with the UV-Vis photodiode array detector (DAD). The column Agilent-Eclipse XDB C-18 (4.6 × 150 mm) was thermostated at 30°C. The flow rate was 0.3 mL/min, and the injection volume was 20 μL. The mobile phase consisted of aqueous 5% formic acid (eluent A) and 80% acetonitrile/5% formic acid (eluent B), at a flow rate of 0.3 mL/min, and injection volume of 20 μL. The elution program used was as follows: 0 - 28 min 0% B, 28 - 35 min 25% B, 35 - 40 min 50% B, 40 - 45 min 80% B, and finally for the last 10 min gradually decreased 80 - 0% B (Miletić *et al.*, 2022). Individual compounds were identified based on the retention times and spectral data of the available standards.

Total phenolic content (TPC)

The Folin-Ciocalteu procedure for total phenolic content was performed (Stratil *et al.*, 2006), with gallic acid used as the standard. The measurements were taken at 760 nm. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight of rosehip samples (mg GAE/g dw).

Total flavonoid content (TFC)

The well-established $AlCl_3$-spectrophotometric method was performed for flavonoid content measurements at 510 nm (Zhishen *et al.*, 1999). Catechin was used as the standard, and the results were expressed as milligrams of catechin equivalents (CE) per gram of dry weight of rosehip samples (mg CE/g dw).

Vitamin C content

Vitamin C content was determined according to Khan *et al.* (2006). Rosehip samples were homogenised with a mixture of metaphosphoric/ acetic acid. Bromine water was used to oxidise ascorbic to dehydroascorbic acid. The excess of bromine was removed by adding a 10% thiourea solution. Then, 2,4-dinitrophenylhydrazine was added to the reaction mixture. After the addition of 85% sulphuric acid, the absorbance of red coloured solution was measured at 521 nm (Khan *et al.*, 2006).

Antioxidative assays

Brand-Williams *et al.* (1995) developed the DPPH method, based on the discoloration of the violet solution of DPPH radical. A slightly modified procedure was conducted as follows: a solution of DPPH (1×10^{-4} mol/L) was prepared in methanol. The total volume of 5.0 mL of this solution and 100 μL of rosehip extract were mixed in a 10-mL volumetric flask, filled with methanol to the mark, and kept at room temperature for 30 min. The absorbance was measured at 520 nm. The Trolox calibration curve was plotted as a function of the decrease in absorbance of DPPH radical scavenging activity. The final results were expressed as milligrams of Trolox equivalents (TE) per gram of dry weight of rosehip samples (mg TE/g dw).

The ABTS antioxidant assay was performed according to Arts *et al*. (2004). A total volume of 100 μL of rosehip extract was mixed with 3.9 mL of diluted ABTS radical cation solution. The reduction in absorbance was measured at 734 nm after 6 min. The Trolox calibration curve was plotted as a function of the decrease in absorbance of ABTS radical cation scavenging activity. The results were expressed as milligrams of TE per gram of dry weight of rosehip samples (mg TE/g dw).

For the FRAP assay, the reduction of the Fe^{3+}-TPTZ complex to the ferrous form at pH = 3.6 was measured by the increase in absorbance at 595 nm. The method was described in detail by Benzie and Strain (1999). The FRAP values were expressed as millimoles of Fe^{2+} equivalents (FE) per gram of dry weight of rosehip samples (mmol FE/g dw).

The CUPRAC method is based on the capacity of antioxidants to reduce Cu^{2+} to Cu^{+} ions. The maximum absorbance of Cu^{+}-complex was spectrophotometrically measured at 450 nm. The assay was performed as described by Apak *et al.* (2007). The results were expressed as milligrams of TE per gram of dry weight of rosehip samples (mg TE/g dw).

Statistical analysis

All the measurements for TPC, TFC, Vit C, DPPH, ABTS, FRAP, and CUPRAC were performed in triplicate, and given as the mean ± standard deviation (SD). The statistical analysis was performed using a statistical package (Statistica 8.0, StatSoft, Tulsa, Oklahoma, USA). A probability of $p < 0.05$ was considered to be statistically significant (Miller and Miller, 2005).

Antioxidant composite index

The antioxidant composite index (ACI) is a parameter that assigns equal weight to all the antioxidant activity assays. It was calculated for each sample as score = (sample score / best score) × 100 (Seeram *et al*., 2008). An index value of 100 was assigned to the best score for each test, and index scores for all the other samples within the test were calculated. The average of the index scores obtained for all tests of antioxidant capacity was defined as its ACI (Piljac-Žegarac *et al*., 2010).

Results and discussion

Optimisation of CV conditions

In order to optimise CV conditions for the analysis of rosehip extracts, the pH value of the extracts (pH 2, 4.5, and 7) and the scan rate (25, 50, and 75 mV/s) were varied. The influence of the scan rate on the current intensity is presented in Figure 1. As can be seen, a higher current intensity was observed at faster scan rates due to the decrease of the diffusion layer (Bard and Faulkner, 2001).

Changes in the pH affect the voltammetric response (59 mV per pH unit shift in the potential, based on the Nernst equation). An increase in pH from 2 to 4.5 led to higher current intensity, and less positive values of the anodic peak potential (Figures 1a and 1b). As the pH further increased from 4.5 to 7, the current peak intensity and the anodic peak potential decreased (Figure 1c). Based on the results obtained, the anodic peak current at pH 7.0 was around 65% of the peak current recorded at pH 4.5 (Figures 1b and 1c), which implied slightly differentiated mechanisms of oxidation of polyphenols in relation to pH. The same was found by Filipiak (2001) and Giacomelli *et al.* (2002) during the oxidation of some polyphenolic compounds and caffeic acid, respectively. They found that the lower peak current observed at higher pH can be attributed to the combined effect of chemical instability, slower electron/proton exchange, and a modified reaction mechanism, all of which contributed to a decrease in the efficiency of the electrochemical process. Based on these observations, the chosen scan rate and pH for CV measurements were 75 mV/s and 4.5, respectively (Figure 1).

Cyclic voltammograms of some phenolic compounds were recorded in these conditions, and their oxidation potentials are given in Table 2.

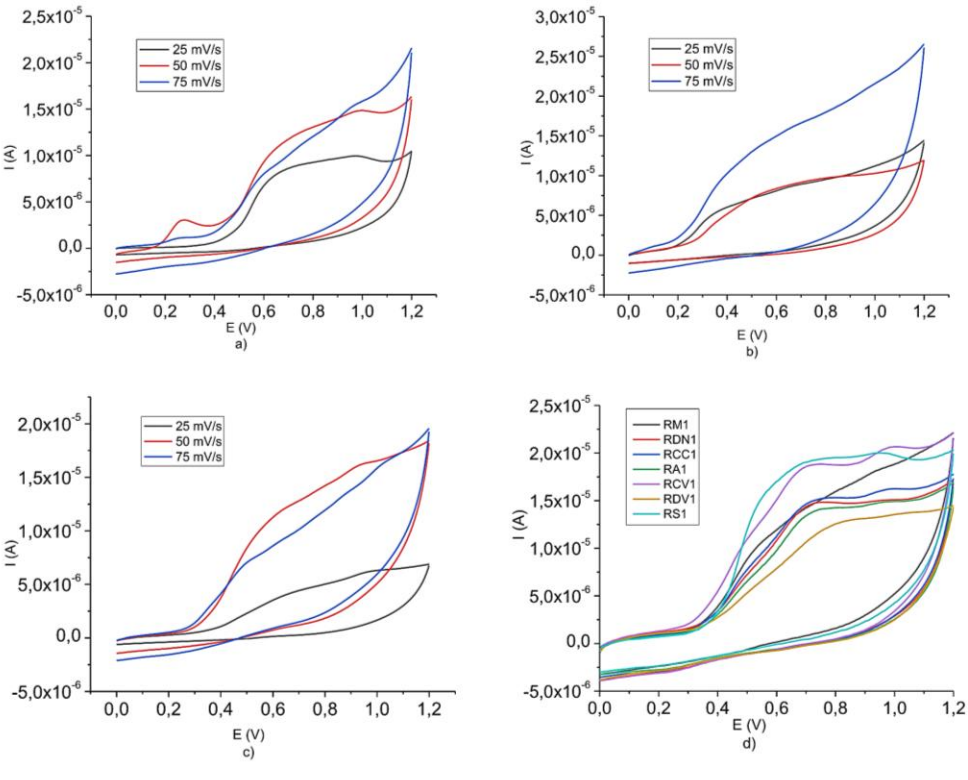

Figure 1. Cyclic voltammograms of rosehip extract at different scan rates and pH **(a)** 2, **(b)** 4.5, and **(c)** 7, and **(d)** cyclic voltammograms of one sample of each analysed rosehip species, in the following operating conditions: sodium acetate-acetic buffer in water (pH = 4.5); potential up to 1,200 mV; and scan rate 75 mV/s.

Table 2. Electrochemical data for phenolic compounds.

Compound*	E_{pa1} (V)	E_{pa2} (V)	E_{pc1} (V)	E_{pc2} (V)
(+)-Catehin	0.485	0.779		
Quercetin	0.617	1.002	0.774	0.069
Rutin	0.526	0.886		
(-)-Epicatechin	0.510	0.735		
Protocatechuic acid	0.605		0.155	
Gallic acid	0.553	0.884		

*c = 1 mmol/L; sodium acetate-acetic buffer in water; pH = 4.5; potential up to 1,200 mV; and scan rate 75 mV/s.

Electrochemical parameters of rosehip samples

Cyclic voltammograms of analysed samples were taken in the potential range of 0 – 1,200 mV, so that all groups of antioxidant compounds would be covered.

Gallic acid displayed two oxidation processes at E_{pa} = 0.553 and 0.884 V, and underwent irreversible oxidation since no reduction peak was observed (Table 2). The first anodic peak corresponded to the irreversible oxidation of gallic acid to semiquinone. Semiquinones are unstable and easily undergo dimerisation (Abdel-Hamid and Newair, 2011). The second anodic peak, near 0.884 V, is attributed to the further oxidation of the semiquinone radical to a quinone form. This process involves an additional electron and proton transfer, culminating in the complete two-step oxidation of gallic acid. Abdel-Hamid and Newair (2011) provide a detailed study of the gallic acid oxidation mechanism in aqueous media. They propose that the second oxidation step, occurring at a higher potential, is the rate-limiting step, and involves the transfer of a second electron and proton, leading to the formation of a stable quinone structure. Zagoraios *et al.* (2021) observe that the second anodic peak of gallic acid appeared around +0.8 V, and was attributed to the oxidation of the semiquinone radical to the quinone cation, which then underwent deprotonation to form the stable quinone form. Protocatechuic acid showed single peak, at 0.605 V. The oxidation of quercetin was described as a cascade process, closely related to the structure of the molecule (Brett and Ghica, 2003). Namely, quercetin displayed two anodic peaks (Table 2). The first peak at E_{pa} = 0.617 V was due to the oxidation of catechol groups on the B ring. These catechol groups are mainly responsible for the antioxidant activity of quercetin (Cosio *et al.*, 2006). Subsequently, the hydroxyl group at position 3 at ring C was oxidised, and the second peak at E_{pa} = 1.002 V appeared. Peak potentials (E_p) and currents (I_p) from the cyclic voltammograms of analysed rosehips (Figure 1d) are given in Table 3. The anodic peak between 0.554 and 0.567 V can be assigned to the oxidation of gallic acid. The first and second anodic peaks detected between 0.465 and 0.529 V, and between 0.707 and 0.782 V, could be attributed to the oxidation of catechin-type flavonoids. The electrochemical oxidation of (+)-catechin corresponded to the oxidation of 3',4'-dihydroxy substituent on the B ring, and included a transfer of two electrons and two protons. The mechanism involved the ionisation of (+)-catechin, and the formation of monoanionic species, which were further oxidised and formed a radical anion. The radical anion underwent a second reversible oxidation, and a dehydro-form of (+)-catechin was formed. The final oxidation product was *o*-quinone, which is obtained by the condensation of the A ring of one and the B ring of another (+)-catechin unit (Castaignede *et al.*, 2003). The third peak, appearing between 0.951 and 1.056 V in cyclic voltammograms of samples, corresponded to the oxidation of quercetin, as previously described.

The overall response in a cyclic voltammogram is the sum of the various species present. Within the *Rosa* species, the cyclic voltammograms were similar. The position of the peaks revealed many phenolics with the catechol group or non-flavonoids (gallic acid), in all the samples. Based on the peak potential (E_p) and current (I_p) from the cyclic voltammograms of analysed rosehip samples (Figure 1d), the peak of gallic acid (0.554 V) was detected in samples RM1 and RS1, the peak of catechin-type flavonoids (0.465 - 0.529 V and 0.707 - 0.782 V) was detected in samples RDN1, RCC1, RA1, RCV1, and RDV1, and the peak of quercetin was detected in sample RCC1, RCV1, and RS1. Also, rutin, similar to catechin and epicatechin, exhibited an anodic peak

Table 3. Peak potential (E_p), current (I_p), and TEAC values of analysed rosehip samples.

Sample	Ep_{a1} (V)	Ip_{a1} (μA)	Ep_{a2} (V)	Ip_{a2} (μA)	Ep_{a3} (V)	Ip_{a3} (μA)	Ep_c (V)	Ip_c (μA)	Q_{1200} (μC)	TEAC
RM1	0.554	1.09	0.943	1.82	-	-	-	-	9.63	16.39
RM2	0.508	0.75	0.712	1.37	0.951	1.63	-	-	9.74	16.58
RM3	0.525	1.19	0.680	1.75	0.961	2.21	-	-	5.27	8.97
RM4	0.512	0.89	0.695	1.55	0.983	1.94	-	-	12.98	22.08
RDN1	0.481	0.65	0.734	1.48	0.975	1.51	-	-	11.53	19.62
RDN2	0.467	0.72	0.709	1.67	0.968	1.51	-	-	10.97	22.90
RDN3	0.480	0.52	0.741	1.12	1.005	1.38	-	-	13.45	29.62
RDN4	0.511	0.69	0.736	1.62	0.980	1.47	-	-	6.45	10.98
RCC1	0.486	0.72	0.752	1.53	0.986	1.63	-	-	7.39	12.58
RCC2	-	-	0.782	0.85	1.056	1.12	-	-	15.55	26.47
RCC3	0.531	0.58	0.756	1.96	0.993	1.13	-	-	6.23	10.60
RCC4	0.470	0.92	0.696	1.74	0.979	1.97	0.245	-0.31	19.45	33.10
RA1	0.486	0.60	0.737	1.40	0.969	1.49	0.232	-0.26	9.99	17.00
RA2	0.465	0.64	0.736	1.66	0.973	1.59	0.236	-0.30	11.60	19.75
RA3	0.508	0.57	0.808	1.35	1.039	1.39	0.234	-0.27	5.66	9.63
RA4	-	-	0.778	0.92	1.045	1.04	-	-	13.55	23.07
RCV1	0.484	1.05	0.723	1.88	1.002	2.07	0.225	-0.30	17.38	29.58
RCV2	0.518	0.64	0.725	1.15	0.954	1.46	0.225	-0.27	12.54	21.34
RCV3	0.487	0.78	0.707	1.61	0.988	1.85	0.215	-0.34	20.58	35.03
RCV4	0.485	0.83	0.714	1.61	0.989	1.79	0.225	-0.27	24.07	40.97
RDV1	0.529	0.61	0.870	1.31	1.049	1.38	0.218	-0.27	16.97	28.88
RDV2	0.506	0.56	0.739	1.23	0.988	1.37	0.218	-0.27	9.84	16.75
RDV3	-	-	0.812	0.95	1.056	1.04	-	-	15.50	26.38
RDV4	0.497	0.77	0.727	1.60	0.973	1.52	0.218	-0.27	10.00	17.01
RS1	0.563	1.58	0.763	1.94	0.956	2.00	-	-	4.69	7.99
RS2	0.555	1.81	-	-	0.975	2.25	-	-	12.95	22.04
RS3	0.513	1.46	0.748	2.07	0.972	2.19	-	-	16.06	27.33
RS4	0.567	1.75	-	-	0.979	1.80	-	-	18.10	30.81

at approximately 0.465 - 0.529 V, due to the oxidation of the *ortho*-dihydroxy-phenol group in its molecular structure. Based on the pronounced anodic peak observed between 0.951 and 1.056 V in the cyclic voltammograms, along with the HPLC analysis, quercetin was identified as one of the most abundant compounds in the analysed rosehip samples.

The area under the anodic peak corresponded to the charge up to a potential of 1,200 mV (Q_{1200}), and was used for the estimation of antioxidant content in rosehip samples. Based on the calibration curve for Trolox and obtained Q_{1200} values from the cyclic voltammograms of the samples, the TEAC values were calculated (Table 3).

Total phenolic content (TPC), total flavonoid content (TFC), and vitamin C content

Individual antioxidants contribute differently to the total antioxidant activity; therefore, a single test cannot be sufficient to obtain reliable data regarding the quantity of antioxidants in the tested samples. Thus, in addition to the CV method, a spectrophotometric analysis of rosehip samples was performed. Based on the obtained results regarding the total phenolic content (TPC), total flavonoid content (TFC), vitamin C content, and antioxidant activity, the most efficient solvents for the extraction of bioactive compounds from rosehip samples were found to be water and acidified methanol. The polarity of extraction solvents influences the extraction efficiency of phenolic compounds. In general, alcohol, acetone, and water are often used for the extraction of bioactive compounds from plant materials (Zhang *et al*., 2018). The solvents accepted for use in pharmaceutical industry are water, ethanol, and glycerol (Grodowska and Parczewski, 2010). The use of water as a cheap, non-toxic extraction solvent simplifies the entire process, and provides the production of edible extracts without the need for solvent evaporation. It also lowers the costs, and reduces the environmental impact (Lakka *et al*., 2021). Therefore, the results regarding water extracts will be further discussed in more detail. The TPC, TFC, and Vit C contents are given in Table 4.

The TPC varied from 57 to 269 mg GAE/g. The average TPC among species decreased in the following order: *R. corymbifera* (V) > *R. dumalis* (V) > *R. spinosissima* > *R. corymbifera* (CT) > *R. agrestis* > *R. myriacantha* > *R. dumalis* (N). It is worth mentioning that notable differences in TPC exist within the same species grown in different locations, as seen herein in *R. dumalis* samples. According to Fetni *et al*. (2020), TPC determined by the Folin-Ciocalteu method in *R. canina* ethanol/water extracts from Algeria was 354.46 mg/g, but according

Table 4. Total phenolic content (TPC), total flavonoid content (TFC), vitamin C content (Vit C), and antioxidant activity of rosehip extracts (c_{sr} ± SD; *n* = 3).

Sample	TPC (mg GAE/g)	TFC (mg CE/g)	Vit C (mg/g)	ABTS (mg TE/g)	DPPH (mg TE/g)	CUPRAC (mg TE/g)	FRAP (mmol FE/g)
RM1	128 ± 2	50 ± 1	6.6 ± 0.2	346 ± 3	118 ± 3	317 ± 2	1.195 ± 0.008
RM2	116 ± 1	50 ± 1	6.77 ± 0.06	296 ± 3	105 ± 3	325 ± 2	1.20 ± 0.01
RM3	100 ± 1	38 ± 1	6.3 ± 0.3	219 ± 2	88 ± 2	241 ± 2	0.963 ± 0.002
RM4	106 ± 1	48 ± 1	6.13 ± 0.06	420 ± 3	129 ± 3	340 ± 10	1.528 ± 0.007
RDN1	114 ± 1	67 ± 1	3.4 ± 0.1	399 ± 3	116 ± 2	413 ± 4	0.847 ± 0.007
RDN2	86 ± 1	37 ± 1	3.35 ± 0.09	280 ± 3	76 ± 1	230 ± 2	1.041 ± 0.009
RDN3	94 ± 3	44 ± 1	3.4 ± 0.1	296 ± 3	79 ± 3	329 ± 2	1.202 ± 0.006
RDN4	97 ± 1	48 ± 1	3.25 ± 0.05	329 ± 2	89 ± 3	371 ± 2	1.321 ± 0.007
RCC1	91 ± 1	39 ± 1	8.25 ± 0.06	296 ± 3	62 ± 2	262 ± 3	1.162 ± 0.006
RCC2	108 ± 1	52 ± 1	7.95 ± 0.05	348 ± 3	108 ± 3	398 ± 3	1.41 ± 0.01
RCC3	57 ± 1	26 ± 1	8.8 ± 0.2	227 ± 3	83 ± 1	221 ± 1	0.819 ± 0.002
RCC4	219 ± 3	89 ± 1	8.7 ± 0.2	779 ± 11	243 ± 5	659 ± 6	2.79 ± 0.02
RA1	110 ± 1	40 ± 1	11.47 ± 0.03	330 ± 3	118 ± 3	265 ± 3	0.975 ± 0.009
RA2	103 ± 1	44 ± 1	12.3 ± 0.2	275 ± 2	81 ± 2	358 ± 3	1.295 ± 0.006
RA3	105 ± 1	40 ± 1	12.63 ± 0.03	239 ± 1	79 ± 3	302 ± 1	1.117 ± 0.006
RA4	151 ± 1	56 ± 1	12.7 ± 0.2	388 ± 3	111 ± 3	361 ± 4	1.420 ± 0.009
RCV1	175 ± 1	68 ± 1	7.9 ± 0.2	417 ± 3	121 ± 3	527 ± 3	1.777 ± 0.006
RCV2	218 ± 1	62 ± 1	7.58 ± 0.06	568 ± 5	185 ± 8	530 ± 10	2.01 ± 0.01
RCV3	188 ± 3	75 ± 2	7.87 ± 0.06	761 ± 8	237 ± 8	477 ± 7	1.97 ± 0.02
RCV4	269 ± 2	87 ± 1	7.7 ± 0.2	716 ± 8	179 ± 3	668 ± 5	2.65 ± 0.01
RDV1	217 ± 1	82 ± 1	2.83 ± 0.06	457 ± 5	121 ± 3	621 ± 3	2.24 ± 0.01
RDV2	99 ± 1	59 ± 1	2.72 ± 0.03	323 ± 2	102 ± 1	408 ± 2	1.437 ± 0.007
RDV3	203 ± 2	66 ± 1	3.50 ± 0.05	462 ± 5	140 ± 3	600 ± 4	2.07 ± 0.01
RDV4	92 ± 1	43 ± 1	3.85 ± 0.05	305 ± 1	92 ± 3	337 ± 3	1.170 ± 0.008
RS1	129 ± 1	50 ± 1	6.77 ± 0.06	385 ± 5	129 ± 5	313 ± 5	1.17 ± 0.01
RS2	105 ± 1	49 ± 1	7.57 ± 0.08	257 ± 1	104 ± 2	392 ± 1	1.344 ± 0.008
RS3	165 ± 2	62 ± 1	6.83 ± 0.06	364 ± 3	145 ± 5	446 ± 5	1.615 ± 0.002
RS4	142 ± 1	62 ± 1	7.83 ± 0.06	316 ± 2	140 ± 5	570 ± 3	2.06 ± 0.01

to Nađpal *et al*. (2018), TPC of water extracts varied from 20.0 to 84.0 mg/g for *R. dumalis* and *R. sempervirens*, respectively. Demir *et al*. (2014) studied five different rosehip species grown in Turkey, and reported 52.94 mg/g of TPC in *R. dumalis*, and 31.08 mg/g of TPC in *R. canina* water extracts. According to Fascella *et al*. (2019), the TPC varied in the range of 5,732.52 – 6,784.55 mg GAE/100g dw for *R. myrcanta* and *R. canina* methanol/HCl extracts, respectively; these values were lower than those reported in the present work. Besides variety, fruit maturity, fertiliser, the climate, and geographic location obviously affect the amount of polyphenolics in rosehips, and the obtained results support this claim (Scalzo *et al*., 2005; Heimler *et al*., 2017).

The TFC was found to be in the range of 26 to 87 mg CE/g. Similar to TPC, the RCV and RDV samples contained the highest amount of TFC. The results obtained were higher than the ones previously reported in the literature (Demir *et al*., 2014; Nađpal *et al*., 2016; 2018).

The Vit C values varied from 2.72 to 12.63 mg/g with a nearly 5-fold difference among the tested species. The highest content of Vit C was found in RA samples, and the lowest in RDV samples. The obtained results were in agreement with or higher than the ones reported earlier for Polish and Serbian water extracts (Adamczak *et al*., 2012; Nađpal *et al*., 2016; 2018) and Sicilian methanol/HCl extracts (Fascella *et al*., 2019). Demir *et al.* (2014) reported that the concentrations of ascorbic acid in water extracts ranged between 65.75 (*R. dumalis*) - 101.38 (*R. canina*) mg/100 g dw, which were lower than the results obtained in the present work. Roman *et al.* (2013) found a strong positive correlation (R^2 = 0.8022) between altitude and ascorbic acid content in rosehip fruits. The degradation of the ascorbic acid in plants decreases due to decrease in oxygen content at higher altitudes (Guneş and Dölek, 2010).

Antioxidative activity

The results of ABTS, DPPH, FRAP, and CUPRAC *in vitro* techniques for the determination of antioxidant activity are also given in Table 4. The ABTS and DPPH methods are based on reactions with stable radicals, and primarily involve single electron transfer (SET) and/or hydrogen atom transfer (HAT) mechanisms. In these assays, antioxidants act by neutralising radicals ($ABTS^{+\bullet}$ or $DPPH^{\bullet}$), resulting in a colour change of the solution, which is then quantified spectrophotometrically. These methods simulate radical stress similar to that occurring in biological systems. On the other hand, the CUPRAC and FRAP methods rely exclusively on the single electron transfer (SET) mechanism. In these assays, metal ions present in the system (Cu^{2+} in CUPRAC and Fe^{3+} in FRAP) are reduced in the presence of antioxidants (to Cu^{+} and Fe^{2+}, respectively), leading to the formation of coloured complexes that are also detected spectrophotometrically. These methods assess the overall reducing capacity of the tested substances, but do not involve radicals as reactants (Shivakumar and Yogendra Kumar, 2018; Munteanu and Apetrei, 2021). These methods have been used to estimate the antioxidant activity of rosehip species (Montazeri *et al*., 2011; Ousaaid *et al*., 2020). The ABTS and DPPH assays, as mentioned earlier, are based on a similar reaction mechanism, and measure the relative activity of antioxidants in scavenging free ABTS or DPPH radicals. The results obtained for the ABTS assay were up to three times higher than for the DPPH assay, and ranged from 219 to 761 mg TE/g for ABTS, and from 62 to 237 mg TE/g for DPPH. FRAP and CUPRAC assays are based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+}, and Cu^{2+} to Cu^{+}, and the results ranged from 0.819 to 2.79 mmol FE/g, and from 221 to 659 mg TE/g, respectively (Table 4).

Antioxidant composite index

To determine the antioxidant activity of polyphenols, a widely accepted standardised method has not yet been identified. Therefore, in their studies of antioxidant activity of different samples, the researchers employ a range of assays, each of which has certain advantages and limitations, because antioxidants have varying contributions to the total antioxidant capacity. In order to scale the data from the different assays to relative percentages, ACI were calculated as the mean of five antioxidant assays, and the results are given in Table 5. The ACI parameter provides a simple way of integrating the data obtained from several antioxidant capacity methods into one value, and facilitates the comparison of antioxidant capacity in a large group of samples. Within the species, RCV samples stood out with the highest ACI values, while the lowest average ACI values were observed in RM and RA samples.

Table 5. Antioxidant potency composite index (ACI) of rosehip samples calculated from five antioxidant capacity measures scaled to relative percentages

Sample	$ABTS_{index}$	$DPPH_{index}$	$CUPRAC_{index}$	$FRAP_{index}$	$Q_{600index}$	ACI
RM1	44.4	48.6	47.5	42.8	40.0	44.7
RM2	38.0	43.2	48.6	43.0	40.5	42.7
RM3	28.1	36.2	36.1	34.5	21.9	31.4
RM4	53.9	53.1	50.9	54.8	53.9	53.3
RDN1	51.2	47.7	61.8	30.4	47.9	47.8
RDN2	35.9	31.3	34.4	37.3	55.9	39.0
RDN3	38.0	32.5	49.3	43.1	72.3	47.0
RDN4	42.2	36.6	55.5	47.3	26.8	41.7
RCC1	38.0	25.5	39.2	41.6	30.7	35.0
RCC2	44.7	44.4	59.6	50.5	64.6	52.8
RCC3	29.1	34.1	33.1	29.3	25.9	30.3
RCC4	100	100	98.6	100	80.8	95.9
RA1	42.4	48.6	39.7	34.9	41.5	41.4
RA2	35.3	33.3	53.6	46.4	48.2	43.4
RA3	30.7	32.5	45.2	40.0	23.5	34.4
RA4	49.8	45.7	54.0	50.9	56.3	51.3
RCV1	53.5	49.8	78.9	63.7	72.2	63.6
RCV2	72.9	76.1	79.3	72.0	52.1	70.5
RCV3	97.7	97.5	71.4	70.6	85.5	84.5
RCV4	91.9	73.4	100	95.0	100	92.1
RDV1	58.7	49.8	93.0	80.3	70.5	70.5
RDV2	41.5	42.0	61.1	51.5	40.9	47.4
RDV3	59.3	57.6	89.8	74.2	64.4	69.1
RDV4	39.1	37.9	50.4	41.9	41.5	42.2
RS1	49.4	53.1	46.9	41.9	19.5	42.2
RS2	33.0	42.8	58.7	48.2	53.8	47.3
RS3	46.7	59.7	66.8	57.9	66.7	59.6
RS4	40.6	57.6	85.3	73.8	75.2	66.5

Correlations between methods

Via the regression analysis, correlation coefficients among the content of TPC, TFC, Vit C, *in vitro* antioxidant assays, and CV results were obtained and given in Table 6. As expected, due to a similar mechanism, a very strong positive correlation existed between the ABTS and DPPH results (R^2 = 0.9250, $p < 0.00001$). A high positive correlation was also observed between the FRAP and CUPRAC results (R^2 = 0.9353, $p < 0.00001$). A significant correlation was found between TPC and *in vitro* assays ($0.7833 < R^2 < 0.8969$), and TFC and *in vitro* assays ($0.7853 < R^2 < 0.9291$).

As for the electrochemical method, a significant correlation was found between CV and FRAP (R^2 = 0.7793, $p < 0.00001$), CV and CUPRAC (R^2 = 0.7691, $p < 0.00001$), CV and TFC (R^2 = 0.7611, $p < 0.00001$), and CV and TPC (R^2 = 0.7080, $p < 0.00001$). These data indicated that 3',4'-dihydroxy substituents on the B ring of polyphenolic compounds in rosehips significantly contributed to their antioxidant properties. The lowest correlation was observed between CV and the ABTS assay (R^2 = 0.6903) and CV and the DPPH assay (R^2 = 0.6345).

However, no correlation was observed between Vit C content and TPC, TFC, and antioxidant assays,

Table 6. Correlation coefficients (R^2) between *in vitro* antioxidant assays (ABTS, DPPH, CUPRAC, and FRAP) and cyclic voltammetry (CV), total phenolic content (TPC), total flavonoid content (TFC), and vitamin C content (Vit C).

	ABTS	DPPH	CUPRAC	FRAP	CV	TPC	TFC	Vit C
ABTS	1.0000	0.9250	0.7437	0.8167	0.6903	0.8390	0.8410	0.0090
DPPH		1.0000	0.7134	0.7780	0.6345	0.7833	0.7853	0.0821
CUPRAC			1.0000	0.9353	0.7691	0.8873	0.9291	-0.1298
FRAP				1.0000	0.7793	0.8969	0.8747	-0.0212
Q_{600}					1.0000	0.7080	0.7611	-0.0599
TPC						1.0000	0.8913	0.0200
TFC							1.0000	-0.1469
Vit C								1.0000

which can suggest that Vit C did not contribute dominantly to antioxidant activity of analysed samples. A similar finding was reported earlier for organically cultivated rosehips from Lithuania (Medveckiene *et al*., 2020). Furthermore, Prior *et al*. (1998) and Kalt *et al*. (1999) concluded that, since only 0.6 - 2.3% of antioxidant capacity was attributed to ascorbic acid and ascorbates, these compounds do not contribute greatly to the antioxidant capacity of blueberry, strawberry, and raspberry fruits.

HPLC analysis

Based on the results of the HPLC analysis (Table 7), rosehip species have quantitative similarities with certain qualitative differences. Cyanidin-3-glucoside and procyanidin B2 were present in all analysed samples, and ranged from 1.36 to 4.69 µg/g, and from 15.2 to 31.9 µg/g, respectively. Hydroxybenzoic acids - gallic and protocatechuic acid, ranged from n.d. to 9.52 µg/g, and from n.d. to 8.55 µg/g. The content of (-)-epicatechin was higher than the (+)-catechin content in all samples, and varied from n.d. to 30.1 µg/g, and from n.d. to 10.1 µg/g, respectively. Even though rutin was identified in only 57% of analysed samples, its content was very equable (from 3.19 to 3.99 µg/g). Rutin was not identified in RM, RCV, and RS samples. Kaempferol content varied from n.d. to 6.96 µg/g. Quercetin was present in 86% of samples, and varied from n.d. to 62 µg/g. Quercetin displays numerous health-beneficial effects, including anticancer, antiviral, antimicrobial, and anti-inflammatory activities, and also reduces blood pressure and cholesterol level (Aghababaei and Hadidi, 2023).

Principal component analysis (PCA)

In order to classify the samples according to the similarities in individual phenolic compounds, principal component analysis (PCA) was performed, and the results are illustrated in Figure 2. Two principal components were selected since they had Eigen-values higher than 1, according to the Kaiser criterion (Kaiser, 1960). The first component, PC1 (Eigen-value 2.62) and the second component, PC2 (Eigen-value 1.25) described 77.36% of variance of all the data. In the first quadrant were RCC samples with low positive loadings on PC1 (0.75) and PC2 (1.30). These were the samples where kaempferol and gallic acid were not detected. RM samples were grouped in the second quadrant, with negative loadings on PC1 and positive loadings on PC2. These samples stood out since rutin, catechin, and epicatechin were not detected. RCV and RS samples were in the third quadrant, where RCV had low negative loadings on PC1 (-0.70) and PC2 (-1.20), and RS had the highest negative loadings on PC1 (-3.22) and PC2 (-2.41). RDN and RDV samples were in the fourth quadrant, with positive loadings on PC1, and low negative and close to zero loadings on PC2 (Figure 2).

Conclusion

The results of the present work showed that cyclic voltammetry can be used for the estimation of antioxidant properties of rosehips. A very strong positive correlation was noted between the ABTS and DPPH results, as well as between the FRAP and CUPRAC results. A significant correlation between

Table 7. Contents (c_{sr} ± SD; *n* = 3; µg/g dw) of identified phenolics in rosehip extracts.

Sample	Cyanidine-3-glucoside	Quercetin	Rutin	Kaempferol	Catechin	Procyanidin B2	Epicatechin	Protocatechuic acid	Gallic acid
RM1	3.04 ± 0.04	55 ± 2	n.d.	6.19 ± 0.08	n.d.	17.1 ± 0.3	n.d.	4.55 ± 0.06	5.07 ± 0.06
RM2	2.85 ± 0.03	62 ± 2	n.d.	6.15 ± 0.08	n.d.	24.0 ± 0.6	n.d.	6.34 ± 0.08	3.29 ± 0.04
RM3	2.60 ± 0.03	57 ± 2	n.d.	5.58 ± 0.07	n.d.	17.3 ± 0.3	n.d.	4.52 ± 0.06	5.51 ± 0.06
RM4	1.36 ± 0.02	61 ± 2	n.d.	6.41 ± 0.08	n.d.	18.7 ± 0.3	n.d.	3.96 ± 0.05	2.56 ± 0.03
RDN1	1.81 ± 0.02	n.d.	3.42 ± 0.04	6.31 ± 0.08	7.30± 0.09	22.3 ± 0.5	12.7 ± 0.2	n.d.	0.955 ± 0.009
RDN2	1.99 ± 0.02	n.d.	3.55 ± 0.04	6.01 ± 0.08	6.72 ± 0.08	23.9 ± 0.5	10.5 ± 0.2	n.d.	1.24 ± 0.02
RDN3	1.89 ± 0.02	n.d.	3.64 ± 0.04	5.77 ± 0.07	6.41 ± 0.08	17.4 ± 0.3	13.3 ± 0.3	n.d.	0.529 ± 0.004
RDN4	2.07 ± 0.03	n.d.	3.88 ± 0.05	6.80 ± 0.08	10.1 ± 0.2	27.5 ± 0.6	16.9 ± 0.4	n.d.	0.344 ± 0.005
RCC1	1.97 ± 0.02	42 ± 1	3.63 ± 0.04	n.d.	2.52 ± 0.03	21.1 ± 0.5	9.6 ± 0.2	3.96 ± 0.04	n.d.
RCC2	1.82 ± 0.02	38 ± 1	3.22 ± 0.04	n.d.	3.51 ± 0.04	27.7 ± 0.5	10.2 ± 0.2	2.83 ± 0.03	n.d.
RCC3	2.01 ± 0.02	48 ± 1	3.73 ± 0.05	n.d.	2.61 ± 0.03	21.8 ± 0.5	10.0 ± 0.2	3.83 ± 0.04	n.d.
RCC4	1.97 ± 0.02	34 ± 1	3.44 ± 0.04	n.d.	3.97 ± 0.04	26.5 ± 0.6	11.5 ± 0.2	4.96 ± 0.05	n.d.
RA1	1.92 ± 0.02	23.9 ± 0.6	3.22 ± 0.04	5.21 ± 0.07	4.35 ± 0.05	23.4 ± 0.5	22.9 ± 0.5	5.22 ± 0.06	1.29 ± 0.02
RA2	1.83 ± 0.02	24.0 ± 0.6	3.19 ± 0.04	5.21 ± 0.07	4.07 ± 0.05	28.3 ± 0.6	16.2 ± 0.4	7.42 ± 0.09	1.03 ± 0.02
RA3	1.89 ± 0.02	21.3 ± 0.6	3.23 ± 0.04	6.10 ± 0.08	5.75 ± 0.07	31.9 ± 0.8	12.5 ± 0.2	5.21 ± 0.06	1.50 ± 0.02
RA4	1.96 ± 0.02	26.6 ± 0.6	3.58 ± 0.04	6.49 ± 0.08	6.53 ± 0.08	26.2 ± 0.6	14.2 ± 0.3	6.01 ± 0.08	1.02 ± 0.02
RCV1	1.54 ± 0.02	25.9 ± 0.6	n.d.	5.43 ± 0.07	3.34 ± 0.04	17.0 ± 0.3	23.7 ± 0.5	5.96 ± 0.02	1.69 ± 0.02
RCV2	2.05 ± 0.03	27.2 ± 0.6	n.d.	5.97 ± 0.07	5.05 ± 0.06	22.5 ± 0.5	16.1 ± 0.4	4.86 ± 0.02	2.41 ± 0.03
RCV3	1.65 ± 0.02	19.4 ± 0.3	n.d.	6.29 ± 0.08	4.95 ± 0.05	24.9 ± 0.6	15.1 ± 0.4	5.20 ± 0.02	1.35 ± 0.02
RCV4	2.11 ± 0.03	30.7 ± 0.9	n.d.	6.97 ± 0.08	5.33 ± 0.06	24.2 ± 0.6	25.2 ± 0.6	4.28 ± 0.02	1.93 ± 0.02
RDV1	1.75 ± 0.02	21.6 ± 0.6	3.26 ± 0.04	5.15 ± 0.07	4.10 ± 0.05	32.8 ± 0.8	21.6 ± 0.5	1.26 ± 0.01	1.75 ± 0.02
RDV2	1.59 ± 0.02	23.5 ± 0.6	3.49 ± 0.04	5.69 ± 0.07	7.96 ± 0.03	30.8 ± 0.8	15.7 ± 0.3	1.73 ± 0.01	1.11 ± 0.02
RDV3	1.88 ± 0.02	18.6 ± 0.3	3.53 ± 0.04	5.77 ± 0.07	5.96 ± 0.06	27.7 ± 0.6	17.9 ± 0.4	1.12 ± 0.01	1.58 ± 0.02
RDV4	1.98 ± 0.02	26.1 ± 0.6	3.99 ± 0.05	6.02 ± 0.08	4.57 ± 0.02	31.9 ± 0.8	16.1 ± 0.4	1.99 ± 0.01	1.01 ± 0.02
RS1	3.81 ± 0.04	42 ± 1	n.d.	6.96 ± 0.08	7.57 ± 0.09	24.7 ± 0.5	24.2 ± 0.6	3.37 ± 0.02	8.00 ± 0.09
RS2	4.69 ± 0.05	47 ± 1	n.d.	6.51 ± 0.03	6.67 ± 0.08	27.3 ± 0.6	21.0 ± 0.5	8.55 ± 0.03	9.52 ± 0.09
RS3	2.73 ± 0.03	44 ± 1	n.d.	6.05 ± 0.08	6.99 ± 0.08	15.2 ± 0.3	30.1 ± 0.8	3.05 ± 0.02	8.30 ± 0.09
RS4	3.06 ± 0.04	41 ± 1	n.d.	5.94 ± 0.07	5.94 ± 0.06	30.2 ± 0.8	20.0 ± 0.5	4.38 ± 0.02	6.88 ± 0.08

n.d.: not detected.

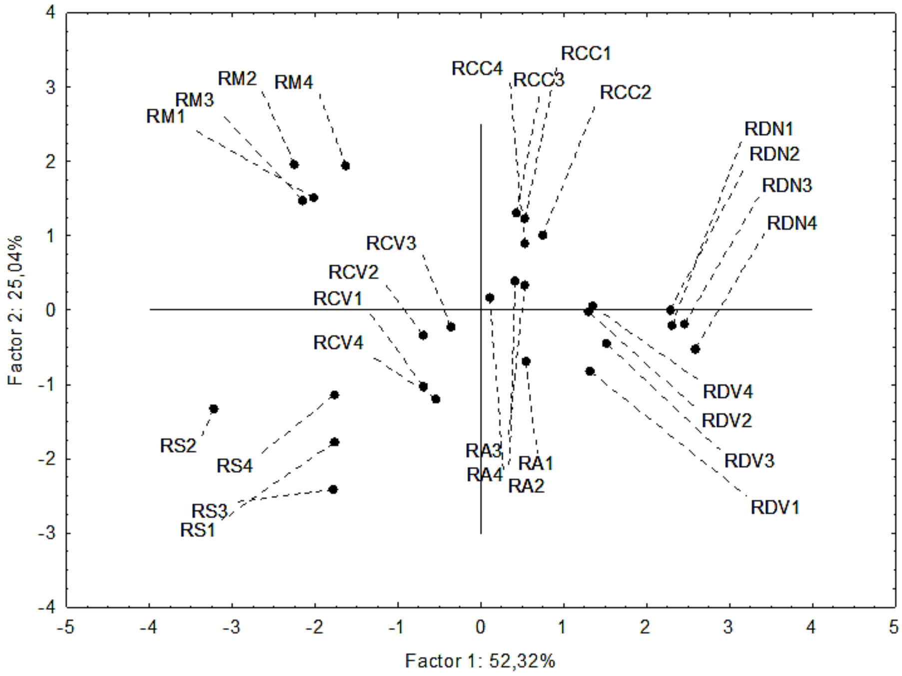

Figure 2. Scatter plots of first two principal components (PC1 *vs*. PC2) for rosehip samples. Classification of 28 samples based on individual phenolic data.

CV, TP, TF, and antioxidant assays emphasised the reliability of the obtained results. In accordance with the calculated ACI values, the antioxidant capacity of the samples was estimated. Vitamin C did not contribute significantly to the antioxidant capacity of rosehips. Based on the pronounced anodic peak appearing between 0.951 and 1.056 V in cyclic voltammograms and the HPLC analysis, quercetin was identified as one of the most abundant compounds in the analysed samples.

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