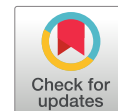


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Comparative Cross-Species Evaluation of the Phytochemical Composition and Functional Properties of the Main Fruit and Berry Crops Leaves of the Rosaceae Family



Altıngül Maksotova¹ , Railya Issagat¹ , Dana Nurzhanova¹  & Nikolay Akatyev¹  

¹ Makhambet Utemisov West Kazakhstan University, Faculty of Natural and Geographical Sciences, Uralsk, Kazakhstan

Abstract

Objective: The main objective of this work was to determine the phytochemical composition and potential of aqueous extracts of leaves of the main fruit and berry crops of the Rosaceae family.

Materials and Methods: The phytochemical compositions and antioxidant properties of the extracts were evaluated using the spectrophotometric method. The phytochemical profiles of the extracts and their capacity to protect human skin from solar radiation were estimated using UV-Vis spectroscopy.

Results: The highest total flavonoid content was observed in the leaves of *Rosaceae canina* (75.97 ± 3.51 mg GAE/g), and the highest total phenolic content was observed in the leaves of *Aronia melanocarpa* (74.86 ± 3.73 mg QE/g). The extract from the leaves of *Prunus armeniaca* was found to have the best sun protection properties (22.55 ± 0.43 at 1.0 g/L). The highest total antioxidant capacity, total reducing power, and hydroxyl radical-scavenging activity values were determined for extracts obtained from the leaves of *R. canina* (3.45 ± 0.08 mmolAA/g, 3.71 ± 0.11 mmolAA/g, and 64.86 ± 1.66 %, respectively). The highest DPPH and nitric oxide-radical scavenging activity values were determined for the extracts of *P. armeniaca* (88.11 ± 1.26 and 85.19 ± 1.24 %, respectively), and the highest hydrogen peroxide scavenging activity values were determined for *Prunus domestica* (87.73 ± 1.38 %). The correlations highlighted the crucial role of phenolic compounds in exhibiting antioxidant properties and the similarity of their action mechanisms.

Conclusion: The main fruit and berry crops of the Rosaceae family offer significant potential for the development of pharmaceuticals with antioxidant and sun protection properties to protect human health.

Keywords

Antioxidant activity · Correlation analysis · Fruit and berry crops · Phytochemistry · Plant extracts · Sun protection factor



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Corresponding author: Nikolay Akatyev nikolay.akatyev@wku.edu.kz



INTRODUCTION

The role of medicinal plants in human health is clearly enormous. Since ancient times, medicinal plants have been integral to traditional medicine systems worldwide, where they have been used to treat various chronic diseases and infections.¹ The use of medicinal plants and their extracts has led to the widespread use of herbal medicine and its rapidly increasing economic significance. According to the World Health Organization (WHO), traditional medicine, which primarily uses plant extracts and their active ingredients, is currently used by 95% of the world's population.²

The Rosaceae family is important to humans because of its diverse range of economically valuable crops, including fruits, nuts, and ornamental plants. This family comprises over 3,000 species, with notable members such as apples, peaches, strawberries, and cherries, which are major sources of human nutrition and economic drivers in many regions worldwide.³ The main areas of use of plants of the Rosaceae family in medicine are well known and have been broadly reviewed.⁴ Consuming fruits from the Rosaceae family offers several specific health benefits due to their rich phytochemical and nutrient profiles. These fruits, such as loquats, apples, peaches, and apricots, are high in phenolic compounds, flavonoids, and antioxidants, which contribute to their health-promoting properties.⁵ In Kazakhstan, the Rosaceae family is represented by 89 species, making it one of the leading families in terms of species diversity within the country's medicinal flora. This diversity includes various genera and species that are significant both ecologically and economically.⁶ The diversity in Kazakhstan is indicative of the family's adaptability and ecological significance, similar to other regions where Rosaceae species are integral to both natural ecosystems and agricultural systems. This diversity supports various ecological functions and provides numerous resources for human use, including food, medicine, and ornamental plants. This diversity is comparable to that of other regions with similar temperate climates, where the Rosaceae family is also well-represented due to its adaptability to various environmental conditions. For instance, in the Caucasus region, the Rosaceae family is similarly diverse, contributing to the region's rich medicinal plant heritage.⁷

In pharmacology and medicine, various parts of fruit and berry crops are of interest due to their rich content of bioactive compounds. The leaves of fruit and berry crops of the Rosaceae family, among other plant parts, are rich in flavonoids and phenolic compounds, which have antibacterial, anti-inflammatory, and antioxidant properties that make them useful for medicinal application. Moreover,

leaves usually contain higher concentrations of active phytochemicals with several practically useful properties. In addition, the extraction of active components from leaves is generally easier and more efficient, and harvesting the leaves is significantly less harmful to the plant when harvesting roots or stems, allowing continuous use of the plant without killing it, making the process more environmentally friendly.⁸ Berry leaves contain the same primary bioactive substances as berry fruits. Moreover, leaves are also a byproduct of berry cultivation, making them an easily accessible resource for study and application.⁹

Representatives of the same plant family often share similarities in their pharmacological properties and phytochemical compositions because of their genetic relatedness. However, the degree of similarity can vary significantly depending on the specific family and the environmental conditions under which the plants grow. For instance, in the Rosaceae family, many species contain similar classes of compounds such as flavonoids, phenolic acids, and tannins, which contribute to shared pharmacological properties like antioxidant and anti-inflammatory effects.¹⁰ However, the concentration and specific types of these compounds may vary between species, which leads to differences in their efficacy and use.¹¹ Environmental factors, such as soil type, climate, and altitude, can also influence the phytochemical profiles of plants, even within the same family, resulting in differences in their pharmacological properties.¹² Therefore, although there is a baseline of similarity due to genetic factors, ecological and environmental influences can lead to significant diversity in the pharmacological and phytochemical characteristics of plants within the same family.

In this regard, a key aspect of our investigation is the comparative cross-species evaluation of the phytochemical composition and functional properties of leaf extracts from fruit and berry crops belonging to the Rosaceae family that are locally grown in Western Kazakhstan. This research systematically examines key functional characteristics such as antioxidant and sun protection activity in relation to phytochemical composition, including total phenolic and flavonoid content. By establishing a correlation between the phytochemical composition and functional characteristics, this study provides valuable insights into the pharmaceutical potential of these plant extracts and fills gaps in existing research on their antioxidant and photoprotective properties.



MATERIALS AND METHODS

Reagents and Solvents

All analytical purity reagents used for qualitative and quantitative analyses were purchased from commercial vendors. Double-distilled water (DDW) with an electric conductivity of 1.17 ± 0.22 mg/L (NaCl) was used to obtain the extracts and prepare all the reagents and aqueous solutions.

Harvesting and Preparing Plant Samples

The fresh leaves of the plants were collected in their natural habitat in the summer of 2024, away from highways and industrial facilities. The plant material was thoroughly washed with tap water and DDW and then stored at room temperature in a shaded atmosphere to completely eliminate moisture. The dried samples were ground in a stainless-steel mill, sieved through a 1.0-mm sieve, and stored at 4°C.

Extract Preparation

10 g of dried and ground plant material was extracted three times with 100 mL portions of DDW in a 250 mL Erlenmeyer flask at 60°C for 6 h. After mixing and evaporating the resulting extracts, the solid residue was dried at 50°C to a constant weight. The extracts obtained were utilized for analysis and kept in glass vials at 4°C.

Qualitative Phytochemical Analysis

The qualitative determination of phytochemicals in the plant extracts was carried out using standard methods.^{13,14}

Carbohydrates (Molish's Test)

To perform the test, 2 drops of a 20% ethanolic solution of naphth-1-ol were added to 1 mL of the extract solution and thoroughly mixed. Then, 1 mL of concentrated H_2SO_4 was carefully added along the side of the test tube until two layers were formed. An intensely colored purple ring appears on the border of the two layers, confirming the positive results for carbohydrates.

Reducing Sugars (Benedict's Test)

To perform the Benedict's test, 2 mL of Benedict's reagent was added to 1 mL of an aqueous solution of the extract, and the resulting mixture was heated for several minutes in a boiling water bath.¹⁵ The formation of a red precipitate confirmed the presence of reducing sugars in the tested samples.

Proteins (Biuret Test)

The biuret test for the presence of proteins consisted of adding of 2-3 drops of 2% copper (II) sulfate solution to 2 mL of an alcoholic solution of the analyzed extract, followed by

the addition of several pellets of solid KOH. The appearance of a pink coloration confirmed the positive test result.

Amino Acids

The appearance of purple coloration upon adding 0.2% ninhydrin solution in acetone to the extract is a positive qualitative test for the presence of amino acids.

Alkaloids (Dragendorff's Test)

Dragendorff's test involves the formation of a precipitate when alkaloids react with Dragendorff's reagent, a solution containing bismuth nitrate and potassium iodide. The addition of 1 mL of Dragendorff's reagent to 2-3 mL of extract solution led to the appearance of orange or red precipitates, indicating the presence of alkaloids.

Phenolic Compounds

The determination of phenolic compounds in plant extracts using ferric chloride (FeCl_3) is based on the ability of phenols to form colored complexes with ferric ions (Fe^{3+}). When adding a neutral 5% FeCl_3 solution to the plant extract solution, a dark green coloration appears, indicating the presence of phenolic compounds.

Flavonoids (Shinoda's Test)

The Shinoda test for flavonoids is based on the reduction of flavonoid compounds in the presence of magnesium shavings or turnings and concentrated hydrochloric acid (HCl), leading to the formation of a characteristic reddish, pink, or orange coloration, depending on the specific flavonoid present. To perform the test, several magnesium turnings and a few drops of concentrated HCl were added to an alcoholic solution of the extract. The appearance of a characteristic coloration indicated the presence of flavonoids.

Phlobatannins

When a plant extract containing phlobatannins is boiled with an equal volume of 1% HCl, the acid induces oxidation and polymerization of the tannins, leading to the formation of insoluble phlobaphene. The appearance of a red precipitate indicates a positive result, confirming the presence of phlobatannins in tested extract.

Leucoanthocyanins

When 5 mL of $i\text{-C}_5\text{H}_{11}\text{OH}$ was added to an aqueous solution of the tested extract, a red color of the alcohol layer was observed, indicating the presence of leucoanthocyanins.

UV-Vis-Spectroscopy Analysis

For obtaining UV-Vis profiles, the extracts were dissolved in ethanol to achieve a 500 µg/mL concentration. After filtration, UV-Vis spectra of the solutions were recorded on an SF-56 UV-Vis spectrophotometer at wavelengths ranging from 250 to 900 nm in 1.0 nm steps using a 10.0 mm quartz cuvette.

Quantitative Phytochemical Analysis

Determination of Total Phenolic Content (TPC)

To determine TPC the Folin-Ciocalteu reagent was used.¹⁶ Absorption was measured at $\lambda = 760$ nm against blank. TPC of the studied extracts was estimated using the gallic acid calibration curve (0-100 µg/mL; $y = 0.0497x + 0.0382$; $R^2 = 0.9993$) and expressed in milligrams of gallic acid equivalents (GAE) per g of dry weight of the sample (mg GAE/g). TPC was calculated as follows:

$$\text{TPC} = \frac{C \cdot V}{M} \quad (1)$$

where TPC-total phenolic content mg/g dry plant extract in GAE, C-concentration of gallic acid obtained from calibration curve in (mg/mL), V-volume of extract (mL), M-weight of extract in (g).

Determination of Total Flavonoid Content (TFC)

TFC was determined calorimetrically using aluminum chloride.¹⁷ Absorption was measured at $\lambda = 510$ nm against a blank. TFC was calculated using a quercetin calibration curve ($y = 0.0534x + 0.0508$, $R^2 = 0.9994$) and expressed as mg quercetin equivalents (QE) per g extract (mg QE/g).

Determination of Antioxidant Properties

Determination of Total Antioxidant Activity (TAC)

The phosphomolybdate method was used to measure TAC at 765 nm using ascorbic acid as a standard.¹⁸ The results are presented in mmol of ascorbic acid per g of extract (mmolAA/g).

Determination of Total Reducing Power (TRP)

TRP is a measure of the plant extract's ability to reduce Fe(III) to Fe(II). TRP evaluation is based on photometric determination of formed Fe(II) at 700 nm by the formation of Prussian blue.¹⁹ TRP is expressed in mmol of ascorbic acid per g of extract (mmolAA/g).

DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

The DPPH assay evaluates the extract's ability to neutralize free radicals by donating electrons or hydrogen atoms. The

color change from purple (DPPH radical) to yellow (reduced form) reflects the extract's efficiency in neutralizing free radicals.²⁰ The absorption was measured at 517 nm against pure solvent. The following equation was used to calculate the capacity of the extracts to scavenge the DPPH radical:

$$\text{DPPH (\%)} = \frac{A_0 - A_1}{A_0} \cdot 100 \quad (2)$$

where A_0 is the absorption of the blank solution and A_1 is the absorption of the extract.

Hydrogen Peroxide Scavenging Activity (HPSA)

HPSA was determined using 1,10-phenanthroline and Mohr's salt at 510 nm, as described by Mukhopadhyay.²¹ Ascorbic acid was used as a standard. The HPSA was calculated as follows:

$$\text{HPSA (\%)} = \frac{A_{\text{sample}}}{A_0} \cdot 100 \quad (3)$$

where A_{sample} is the absorption of a solution containing an extract or standard, Mohr's salt and hydrogen peroxide; A_0 is the absorption of a solution containing Mohr's salt and 1,10-phenanthroline.

Hydroxyl Radical-Scavenging Activity (HRSA)

HRSA was determined using the sodium salicylate and iron(II) sulfate method with mannitol as the standard.²² The absorption of the salicylate complex was measured at 562 nm. HRSA was calculated using the following equation:

$$\text{HRSA (\%)} = 1 - \frac{A_0 - A_2}{A_0} \cdot 100 \quad (4)$$

where A_0 is the absorption of the control sample (without extract), A_1 is the absorption of the sample containing the extract, A_2 is the absorption of the sample without sodium salicylate.

NO-Radical Scavenging Activity (NO-RSA)

NO-radicals are spontaneously generated at physiological pH in aqueous solution from sodium nitroprusside ($\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]$). The spectrophotometric measurement of nitrite ions (NO_2^-), which are produced as a result of the interaction of NO-radicals with oxygen, can be carried out at 546 nm using Griess reagent.²³ The percentage of inhibition was calculated using the following formula:

$$\text{NO.RSA (\%)} = \frac{A_0 - A_1}{A_0} \cdot 100 \quad (5)$$

where A_0 is the absorption of the control sample and A_1 is the absorption of the sample containing the extract or standard (ascorbic acid). The amount of nitrite formed was estimated using a sodium nitrite calibration curve ($y = 0.211x - 0.045$, $R^2 = 0.9992$).



Sun Protection Factor (SPF) Determination

SPF was determined by measuring the absorbance of the alcoholic solutions of the investigated extracts in SF-56 spectrophotometers at a wavelength range of 290–320 nm at concentrations of 0.1, 0.5, and 1.0 g/L. The measurements were carried out in 10.0 mm quartz cuvettes with 5 nm intervals against pure solvent. The SPF values were calculated using the Mansur equation as follows²⁴:

$$\text{SPF} = \text{CF} \sum_{290}^{320} \text{EE}(\lambda) \cdot \text{I}(\lambda) \cdot \text{abs}(\lambda) \quad (6)$$

where $\text{EE}(\lambda)$ denotes as the erythemal effect spectrum, $\text{I}(\lambda)$ is the solar intensity spectrum, $\text{abs}(\lambda)$ is the absorbance of the solution tested, and CF is the correction factor (10). The standard values of $\text{EE} \times \text{I}$ ²⁵ and listed in Table 1.

Table 1. Normalized product function for SPF calculation.

Wavelength (λ), [nm]	EE x I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1.0000

*EE: erythemal effect spectrum. I: solar intensity spectrum.

The experimental results are presented as the mean of three independent determinations with a standard deviation ($\pm$ SD) at a confidence probability (α) of 0.95.

RESULTS

There are a lot of studies about the main species of fruit and berry crops within the Rosaceae family in Kazakhstan's

flora.^{26–29} The main fruit and berry crops of the Rosaceae family chosen for our investigation is given in Figure 1.

Phytochemical Composition

Qualitative Phytochemical Analysis

The qualitative phytochemical screening data of the aqueous extracts of leaves of the main species of the Rosaceae family are presented in Table 2. Simple methods based on qualitative reactions and visual observation allowed assessment of the relative presence of primary and secondary metabolites in the studied extracts. It was found that the investigated extracts are rich in phenols, flavonoids, and carbohydrates. Alkaloids were absent only in *Prunus domestica* and *Rubus idaeus*. Phlobatannins were present in *Rosa canina*, *P. domestica*, *Prunus armeniaca* and *Prunus cérasus*. Proteins and amino acids were detected in several species, notably *P. domestica*, *Aronia melanocarpa*, and *R. idaeus*. Leucoanthocyanins were found in *Ribes nigrum*, *Crataegus laevigata*, *P. domestica*, and *P. cérasus*.

UV-Vis Absorption Analysis

UV-Vis spectroscopy provides valuable preliminary information on the composition and structural characteristics of plant metabolites, which exhibit characteristic absorption spectra. The ethanolic solutions of the investigated extracts were subjected to scanning at wavelengths ranging from 280 to 750 nm to estimate the UV-Vis profile. The UV-Vis spectra of aqueous extracts of leaves of fruits and berry crops of the Rosaceae family are shown in Figure 2. Peak values (λ_{max} , nm) and their attributions are presented in Table 3. The UV-Vis spectra showed the most intense absorption at 285–420 nm, suggesting the presence of phenolic compounds. Peaks near 310 and 350 nm are characteristic of flavonoids. The absorption maximum at 325 nm and nearby shoulders further support the presence of polyphenolic constituents. Minor

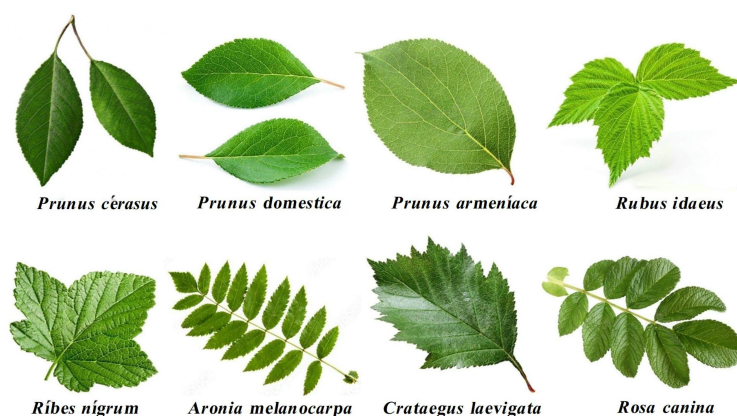


Figure 1. Leaves of the main fruit and berry crops of the Rosaceae family.

Table 2. Qualitative analysis of aqueous extracts of leaves of the main species of the Rosaceae family.

Phytocomponents	<i>R. canina</i>	<i>R. nigrum</i>	<i>C. laevigata</i>	<i>P. domestica</i>	<i>A. melanocarpa</i>	<i>R. idaeus</i>	<i>P. armeniaca</i>	<i>P. cérasus</i>
Carbohydrates	+	++	++	+	++	++	+++	+
Reducing sugars	-	+	-	+	+	+	-	+
Phenolic compounds	+	+	++	+	+++	+	+	+
Flavonoids	+++	+	++	+	++	+	+	+
Alkaloids	+	+	++	-	++	-	+	++
Phlobataninns	+	-	-	+	-	-	+	+
Proteins	+	-	+	-	-	+	+	+
Amino acids	-	-	-	+	+	+	-	-
Leucoanthocyanins	-	+	+	+	-	-	-	+

(+++)- highly present, (++)- moderately present, (+)- low present, (-)- absence

bands at 400–420 nm likely arise from natural pigments. A noticeable absorption is also observed in the range of 600–700 nm, which corresponds to flavonoids and chlorophylls.

Table 3. UV-Vis peak values of aqueous extracts of leaves of the main species of the Rosaceae family (solution in ethanol 0.5 mg/mL).

Plant species	λ_{max} nm	Abs	Phytocomponent
<i>R. canina</i>	288	1.4483	Phenolic compounds
	295	1.3562	Phenolic compounds
	364	0.5067	Polyphenolics
	412	0.1457	Pigments
	435	0.0608	Flavonoids
	606	0.0075	Chlorophyll-porphyrins
<i>R. nigrum</i>	664	0.0386	Chlorophyll A
	292	0.7268	Phenolic compounds
	350	0.2931	Polyphenolics
	611	0.0014	Chlorophyll-porphyrins
<i>C. laevigata</i>	664	0.0072	Chlorophyll A
	289	0.7632	Phenolic compounds
	292	0.7264	Phenolic compounds
	313	0.6415	Flavonoids
	412	0.1081	Pigments
	605	0.0074	Chlorophyll-porphyrins
<i>P. domestica</i>	664	0.0358	Chlorophyll A
	297	1.1674	Phenolic compounds
	325	1.2212	Phenolic compounds
	606	0.0011	Chlorophyll-porphyrins
<i>A. melanocarpa</i>	664	0.0077	Chlorophyll A
	289	0.4323	Phenolic compounds
	298	0.4192	Phenolic compounds
	314	0.3924	Flavonoids
<i>R. idaeus</i>	664	0.0031	Chlorophyll A
	289	1.0017	Phenolic compounds
	365	0.6075	Polyphenolics
	664	0.0053	Chlorophyll A
<i>P. armeniaca</i>	295	1.9295	Phenolic compounds
	325	1.8322	Phenolic compounds
	606	0.0029	Chlorophyll-porphyrins
	664	0.0174	Chlorophyll A
<i>P. cérasus</i>	295	0.6861	Phenolic compounds
	305	0.6532	Flavonoids
	610	0.0024	Chlorophyll-porphyrins
	664	0.0068	Chlorophyll A

Qualitative Phytochemical Analysis

Phenols and flavonoids are essential secondary metabolites in plants and play vital roles in their growth, development, and defense mechanisms. These compounds contribute to various physiological and biochemical processes that enhance survival and adaptation to environmental changes. Figure 3 shows the TPC and TFC as well as their total content (TPC+TFC) and ratio (TPC/TFC) in the examined extracts. The results indicate that the content of phenols and flavonoids directly depends on the plant species. *P. cérasus* had the lowest TPC (17.51 ± 0.85 mg GAE/g), while *R. canina* had the highest (75.97 ± 3.51 mg GAE/g). TFC was highest in *A. melanocarpa* (74.86 ± 3.73 mg QE/g) and lowest in *P. cérasus* (16.17 ± 0.81 mg QE/g). In all cases, TPC exceeded TFC, reflecting broader phenolic diversity. *R. canina* showed the highest relative phenolic levels, with TPC 2.85 times greater than TFC. In addition, *R. canina*, *P. domestica*, and *A. melanocarpa* were richest in both compounds; *P. armeniaca* and *P. cérasus* contained the least. Therefore, Rosaceae leaves from western Kazakhstan are notable sources of phenolics and flavonoids.

Antioxidant Activity Determination

The values of TAC and TRP of aqueous extracts of leaves of the main Rosaceae family species are presented in Figure 4. TAC and TRP often demonstrate similar trends for members of the same botanical family, owing to shared phytochemical profiles. TAC and TRP values were generally below 70% relative to pure ascorbic acid, showing consistent trends across samples. The highest TAC and TRP were observed in *R. canina* and *R. idaeus* leaf extracts, while *P. armeniaca* and *P. cérasus* showed the lowest values. Figure 5 presents the results of determining the antioxidant and antiradical activity of leaf extracts of the main species of the Rosaceae family. DPPH radical scavenging activity exceeded 80% for most extracts, with *P. armeniaca* and *R. nigrum* exhibiting the strongest activity; *P. cérasus* was the least effective. HPSA was high in



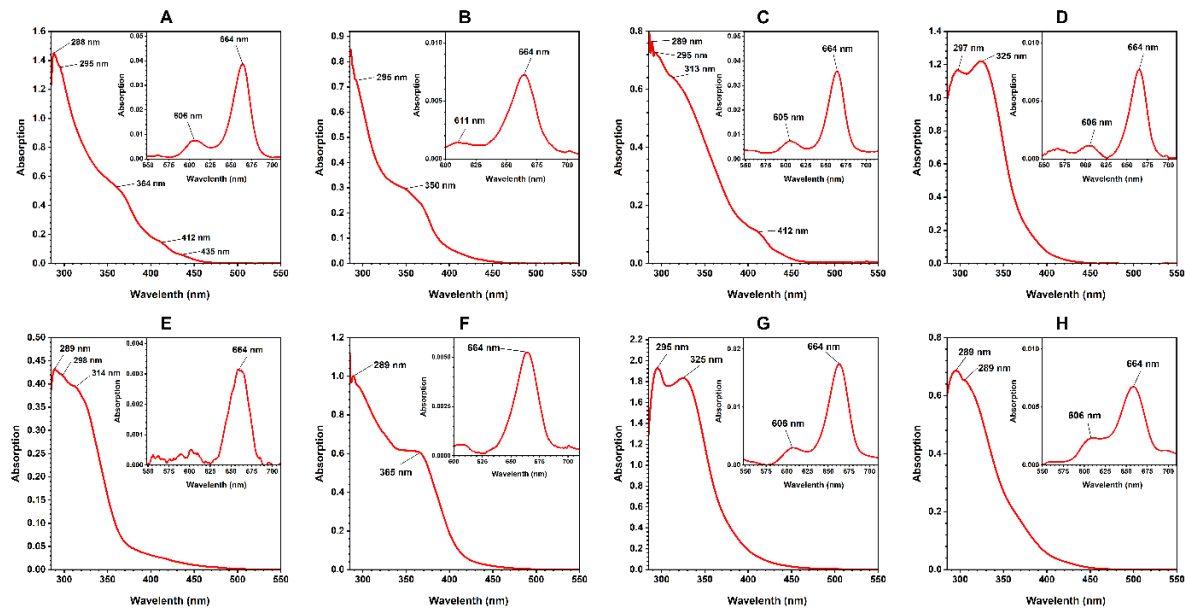


Figure 2. UV-Vis profile of aqueous extracts of leaves of the main species of fruit and berry crops of the Rosaceae family. (A) *R. canina*. (B) *R. nigrum*; (C) *C. laevigata*. (D) *P. domestica*. (E) *A. melanocarpa*. (F) *R. idaeus*. (G) *P. armeniaca*; (H) *P. cerasus*.

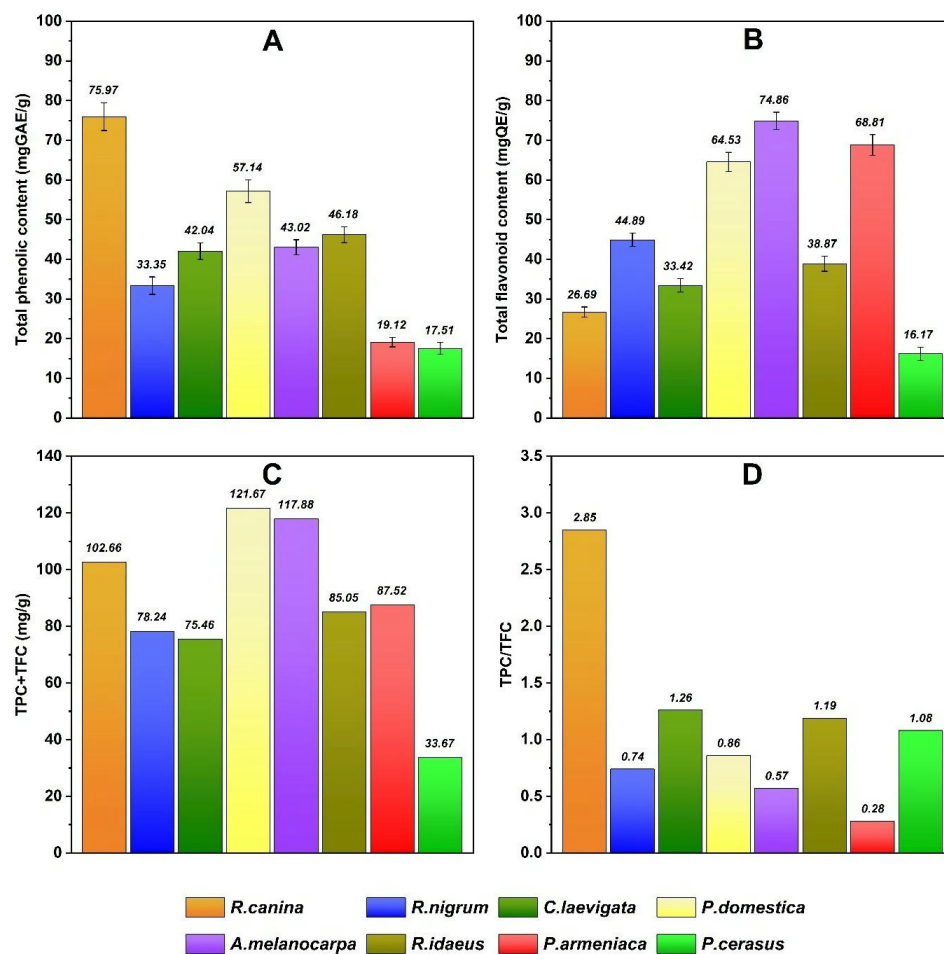


Figure 3. Phytochemical composition of leaf extracts of the main species of the Rosaceae family. (A) Total phenolic content (TPC). (B) Total flavonoid content (TFC). (C) Total content of phenols and flavonoids. (D) TPC/TFC ratio.

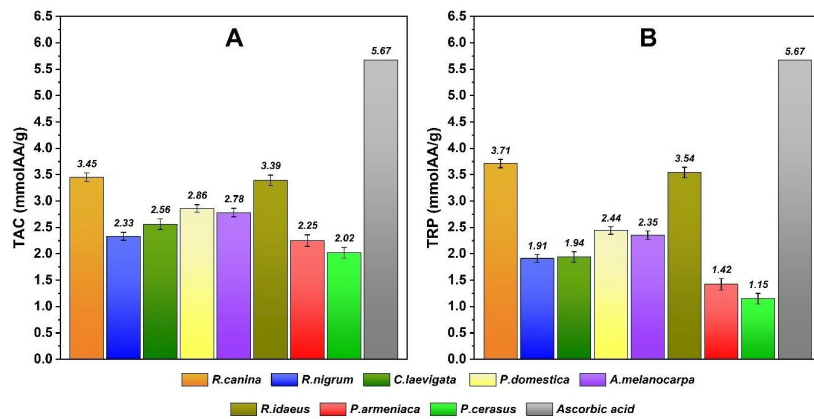


Figure 4. Total antioxidant activity (TAC) (A) and total reducing power (TRP) (B) of aqueous extracts of leaves of the main species of the Rosaceae family.

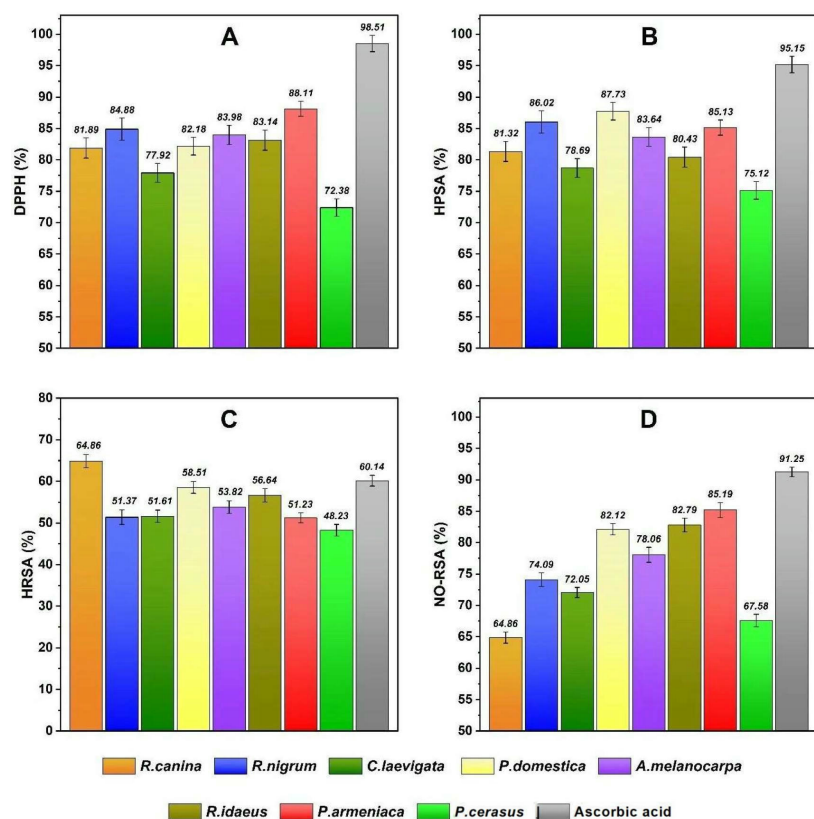


Figure 5. Antioxidant activity of aqueous extracts of leaves of the main species of the Rosaceae family at a concentration of 1.0 mg/mL. (A) DPPH scavenging activity. (B) HPSA. (C) HRSA. (D) NO-RSA.

all samples, notably in *P. domestica*, while *C. laevigata* and *P. cerasus* had the lowest activity. HRSA was significant across all extracts, with *R. canina* and *P. domestica* being the most active. In contrast, *P. armeniaca* and *P. cerasus* again demonstrated the lowest HRSA. NO-RSA was slightly lower compared to other, with *P. armeniaca* and *R. idaeus* showing the highest activity, and *P. cerasus* and *R. canina* the lowest. As can be seen, the extracts demonstrated strong antioxidant potential against key reactive oxygen and nitrogen species.

Sun Protection Properties

Figure 6 shows the results of the evaluation of the sun protection ability of the studied extracts. Investigating the sun protection properties of plant extracts is crucial for developing eco-friendly skin care products as natural alternatives to synthetic sunscreens, which may contain chemicals that can cause skin irritation or environmental damage.^{30,31} The sun protection factor was evaluated using UV-Vis spectroscopy for freshly prepared alcoholic solutions of the studied extracts. The study found that SPF values of all

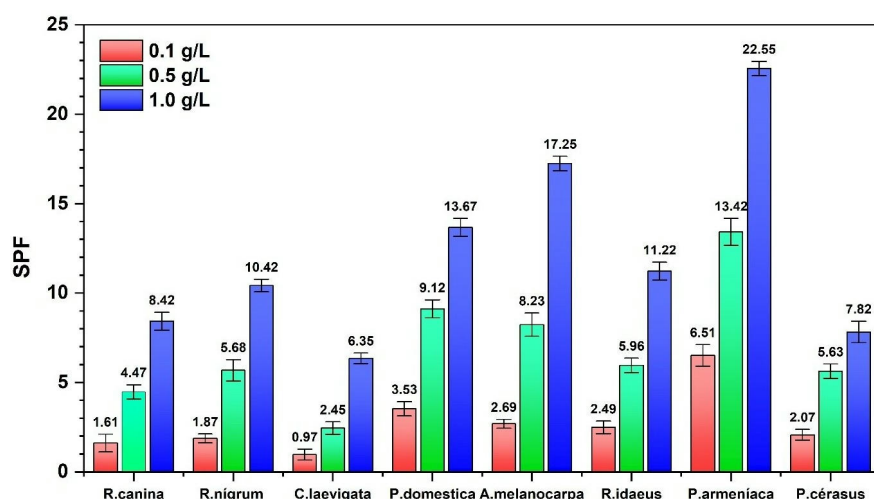


Figure 6. Sun protection factor (SPF) of freshly prepared ethanolic solutions of aqueous extracts of leaves of the main fruit and berry crops of the Rosaceae family.

tested extracts increased with concentration and ranging from 22.55 ± 0.43 (*P. armeniaca*, 1.0 g/L) to 0.97 ± 0.22 (*C. laevigata*, 0.1 g/L). Aqueous extracts from the leaves of Rosaceae family members showed significant UVB protection.

Correlations of the Functional Properties of Aqueous Extracts of Leaves of the Main Species of the Rosaceae Family with Phytochemical Composition

Phenolic compounds and flavonoids primarily influence the correlation between phytochemical composition and antioxidant activity in plants due to their chemical structure and biological properties.³² The presence of multiple hydroxyl groups in the structure of these compounds allows them to interact with various reactive oxygen species (ROS) and reactive nitrogen species (RNS), providing a robust defense mechanism for living cells.³³ In the present work, correlations of the antioxidant and sun protection properties of aqueous extracts of leaves of the main species of the Rosaceae family with the content of phenolic compounds, flavonoids, and their ratios were revealed using a linear regression coefficient (R^2) and Pearson's correlation analysis. Results, given in Figure 7, highlight the critical role of phenolic compounds and flavonoids in the functional properties of leaves from fruit and berry crops of the Rosaceae family. Strong positive correlations ($p < 0.05$) were found between TPC and TAC, TPC and TRP, and TPC with HRSA. Moderate correlations were observed for TFC with DPPH, HPSA, NO-RSA, and SPF, indicating that phenolic compounds and flavonoids contribute distinct functions due to their qualitative composition. Therefore, higher TPC improves TAC, TRP, and DPPH-scavenging activity, while increased TFC leads to increased NO-RSA, HPSA, and sun protection properties.

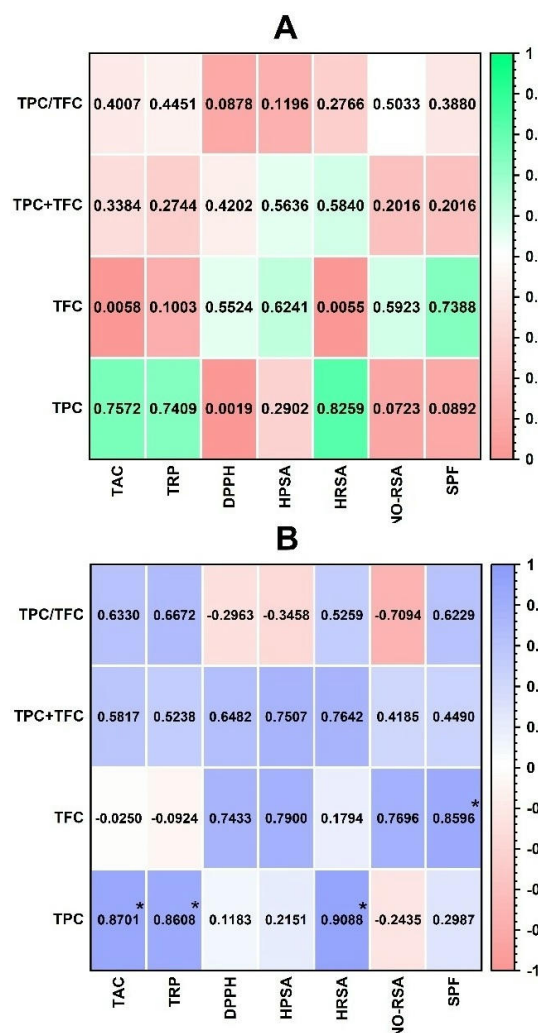


Figure 7. Relationships between the content and ratio of phenolic compounds versus the antioxidant and sun protection capacity of aqueous extracts of leaves of the main species of the Rosaceae family. (A) Linear regression coefficient (R^2). (B) Pearson's correlation coefficient (r) (*significant at $p < 0.05$ probability level).

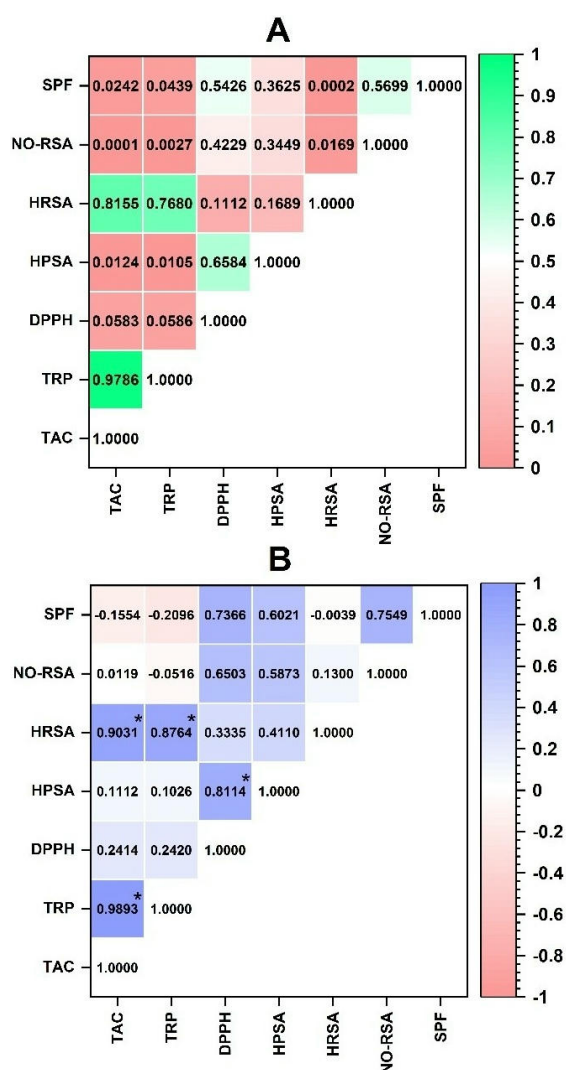


Figure 8. Relationship between antioxidant properties and sun protection capacity of aqueous extracts of leaves of the main species of the Rosaceae family. (A) Linear regression coefficient (R^2). (B) Pearson's correlation coefficient (r) (*significant at $p < 0.05$ probability level).

The chemical structure and functional groups of phytochemicals influence the correlation between different types of antioxidant properties. The correlation between different antioxidant properties reflects the multifunctional nature of antioxidant compounds. Such correlations typically indicate shared mechanisms, complementary activities, or synergistic effects. This indicates that different functional groups in the same molecule or extract contribute to distinct antioxidant mechanisms. The corresponding correlations between antioxidant activity and SPF of the studied extracts are shown in Figure 8. Significant positive correlations ($p < 0.05$) were observed between TAC-TRP, TAC-HRSA, TRP-HRSA, and DPPH-RSA, and moderate correlations between DPPH-SPF and NO-RSA-SPF. The correlations between TAC, TRP, and HRSA are attributed to antioxidants' ability to donate electrons or hydrogen atoms. The DPPH-HRSA correlation

reflects the ability of antioxidants to neutralize free radicals, despite differences in reactivity between DPPH and hydroxyl radicals. The DPPH-SPF and NO-RSA-SPF correlations suggest that antioxidants also help reduce UV-induced radicals and enhance SPF. The correlations found highlight the multifunctional role of Rosaceae species leaf extracts.

DISCUSSION

The results of the qualitative analysis showed that the extracts of leaves of the main species of the Rosaceae family were a rich source of various phytochemicals, predominantly phenols and flavonoids. Carbohydrates are present in all extracts at significant amounts. Reducing sugars were detected in *R. nigrum*, *P. domestica*, *A. melanocarpa*, *R. idaeus*, and *P. cerasus* extracts. Alkaloids were absent in only *P. domestica* and *R. idaeus*. A positive test of phlobatannins was observed for *R. canina*, *P. domestica*, *P. armeniaca* and *P. cerasus* extracts. Extracts from *R. canina*, *C. laevigata*, *R. idaeus*, *P. armeniaca* and *P. cerasus* demonstrated a moderate presence of proteins. Amino acids were found only in extracts obtained from *P. domestica*, *A. melanocarpa*, and *R. idaeus*. In turn, the presence of leucoanthocyanins was detected in *R. nigrum*, *C. laevigata*, *P. domestica*, and *P. cerasus* leaf extracts.

The UV-Vis profile obtained for ethanolic solutions of investigated extracts revealed the presence of a large baseline with peaks at 288-350 nm, corresponding to aromatic rings of phenolic compounds, which absorb UV radiation in this region. Peaks around 310 and 350 nm can also be attributed to polyphenolic compounds, including flavonoids.^{34,35} The maximum absorption at 325 nm and the shoulders in the same region are the result of π - π -transitions because the tested extracts contain polyphenolic compounds. Peaks and shoulders at 360-370 nm range also correspond to polyphenolic components.³⁶ This absorption is often associated with π - π^* - or n - π^* -transitions in aromatic and heterocyclic compounds. Absorption peaks around 400-420 nm can be explained by the presence of natural pigments and can be associated with charge transfer and π - π^* interactions, depending on the molecular structure.³⁷ The absorption wavelength of 435 nm corresponds to flavonoids.³⁸ The insignificant absorption at approximately 610 nm can be attributed to chlorophyll-porphyrins.³⁹ The absorption at 664 nm corresponds to chlorophyll A.⁴⁰ The UV-Vis analysis clearly indicates the presence of diverse phytocomponents, including polyphenolic compounds, flavonoids, natural pigments, and chlorophyll derivatives. These components exhibit strong UV absorption, suggesting their potential for photoprotection and antioxidant activity. The results highlight the complex

molecular composition of the extracts, which contributes to their functional properties.

Investigating the antioxidant properties of plant extracts is crucial because of their potential to reduce oxidative stress, which has been implicated in aging and various chronic diseases. Plant-based antioxidants provide a natural, sustainable, and often safer alternative to synthetic compounds, which aligns with the growing demand for eco-friendly and health-conscious solutions. Scientific interest in the main species of fruit and berry crops of the Rosaceae family has increased significantly in recent decades. *R. nigrum*, *R. idaeus*, and *P. cerasus* are of significant interest for researchers due to their rich phytochemical profile and potential health advantages. *R. nigrum* is particularly noted for its high anthocyanin content, which contributes to its antioxidant properties. The anti-inflammatory and antimicrobial properties of these compounds are associated with many health benefits, and they may help treat conditions involving inflammation and blood glucose control. The potential use of *R. nigrum* in the development of natural food additives and its economic importance in the food industry also drive interest in the plant.²⁶ *R. idaeus* is valued for its high antioxidant content, which contributes to its health-promoting effects, including potential anticancer and anti-inflammatory activities. The plant is also a rich source of phenolic compounds and vitamins, making it a popular ingredient in food supplements and cosmetics intended to enhance skin hydration and health.²⁷ *P. cerasus* is also of great interest to researchers because of its rich phytochemical profile. It is particularly notable for its high anthocyanin content, which contributes to its strong antioxidant properties. These antioxidants have various health benefits, including anti-inflammatory effects and potential improvements in fertility outcomes.²⁸ Additionally, *P. cerasus* leaf extracts have been used in the synthesis of silver nanoparticles, which exhibit significant antibacterial activity against multidrug-resistant bacteria, offering a promising eco-friendly and non-toxic method for producing antimicrobial agents.²⁹ TAC and TRP are measures used to characterize the antioxidant properties of plant extracts. These properties are crucial for evaluating the potential health benefits of plant-derived medicines, particularly their ability to reduce oxidative stress. From the results of the quantitative assessment of the absolute and relative content of phenolic compounds, it follows that the lowest TPC was observed in leaves of *P. cerasus* (17.51 ± 0.85 mg GAE/g) and the highest TPC was observed in leaves of *R. canina* (75.97 ± 3.51 mg GAE/g). The determination of TFCs confirms that their content in plants also varies depending on the species. The highest flavonoid

content was observed in the leaves of *A. melanocarpa* (74.86 ± 3.73 mg QE/g). The lowest flavonoid content was noted in the leaves of *P. cerasus* (16.17 ± 0.81 mg QE/g). This indicates that the levels of these phytochemicals vary depending on the plant species, even within the same family. TPC in plants is generally higher than TFC because phenolic compounds present a broader category of chemical structures. *R. canina* had the highest relative content of phenolic compounds. TPC values were 2.85 times higher than TFC values in aqueous extracts of its leaves. The total phenol and flavonoid content did not exceed 122 mg/g dry weight. The results of the quantitative analysis show that *R. canina*, *P. domestica*, and *A. melanocarpa* were the richest in phenolic compounds and flavonoids. *P. armeniaca* and *P. cerasus*, in turn, had the lowest content of both types of phytochemicals. The leaves of the main Rosaceae family plants growing in the Western region of Kazakhstan are rich in phenolics and flavonoids. It is known that plant extracts rich in biologically active compounds have significant antioxidant properties and can effectively neutralize free radicals and other highly reactive particles by exhibiting redox properties. The results of the evaluation of the antioxidant activity show that the TAC and TRP values of the studied aqueous extracts did not exceed 70% compared to pure ascorbic acid, and both parameters are in the same trend. Plants within the same family typically possess similar types of bioactive compounds that are responsible for both the antioxidant capacity and the reducing power of plant extracts.⁴¹ The highest TAC and TRP values were determined for extracts obtained from leaves of *R. canina* (3.45 ± 0.08 and 3.71 ± 0.11 mmolAA/g, respectively) and *R. idaeus* (3.39 ± 0.12 and 3.54 ± 0.09 mmolAA/g, respectively). The lowest TAC and TRP values were obtained for the extracts of leaves of *P. armeniaca* (2.25 ± 0.13 and 1.42 ± 0.07 mmolAA/g, respectively) and *P. cerasus* (2.02 ± 0.06 and 1.15 ± 0.09 mmolAA/g, respectively).

DPPH scavenging activity also characterizes the antioxidant properties of plant extracts. It measures an extract's ability to donate hydrogen atoms or electrons to neutralize the DPPH radical. This property indicates the extract's potential to reduce cell damage caused by free radicals. Figure 5A shows that, for most of the extracts studied, the DPPH scavenging activity was very high, exceeding 80% compared to pure ascorbic acid. The ability to scavenge DPPH radicals was the highest in the extracts of *P. armeniaca* and *R. nigrum* (88.11 ± 1.26 and 84.88 ± 1.12 %, respectively). The extract obtained from *P. cerasus* leaves was the least active for scavenging DPPH radicals (72.38 ± 1.62 %).

HPSA, in turn, characterizes antioxidant properties by measuring the ability of plant extracts to neutralize hydrogen peroxide, which is one of the most ROS. Hydrogen peroxide



is a byproduct of various metabolic processes and can contribute to oxidative stress if not adequately managed by the antioxidant systems of living organisms. Excess hydrogen peroxide formation can lead to cell damage and inflammation and contribute to the development of chronic diseases. The experimental results, presented in Figure 5B, demonstrated that the ability of the investigated extracts to scavenge hydrogen peroxide was very high. Only extracts of *C. laevigata* and *P. cerasus* demonstrated relatively low HPSA values of about 78.69 ± 1.53 and 75.12 ± 1.45 %, respectively. The highest HPSA value was determined for *P. domestica* (87.73 ± 1.38 %). The effectiveness of plant extracts in supporting antioxidant defense mechanisms can be assessed by evaluating HPSA, which is crucial for their potential therapeutic applications in the prevention and treatment of diseases related to oxidative stress.⁴²

HRSA specifies the properties of plant extracts that neutralize hydroxyl radicals. Hydroxyl radicals (OH-radicals) are considered the most active and dangerous ROS due to their high reactivity and non-selective nature. Unlike other ROS, the high reactivity of OH-radicals is attributed to their unpaired electrons, which makes them extremely unstable and prone to react with almost any molecule they encounter, including DNA, proteins, lipids, and carbohydrates, resulting in widespread cell damage. These reactivities allow them to initiate chain reactions that spread oxidative damage throughout cells and tissues. Therefore, determining HRSA is a major focus of studies and developments in pharmacology because it helps evaluate the antioxidant potential of compounds or plant extracts.⁴³ Figure 5C demonstrates that the activity of all the extracts studied in the scavenging of OH radicals was significant and comparable to that of mannitol as a standard. *R. canina* and *P. domestica* demonstrated the highest ability to scavenge hydroxyl radicals (64.86 ± 1.66 and 58.51 ± 1.59 %, respectively). In contrast, *P. armeniaca* and *P. cerasus* had the lowest HRSA values (51.23 ± 1.25 and 48.23 ± 1.39 %, respectively).

NO-RSA describes the properties of plant extracts to neutralize nitric oxide radicals (NO $\cdot$), which are RNS involved in various physiological and pathological processes. Excessive production of NO radicals can lead to inflammation and tissue damage and is involved in the pathogenesis of several diseases.⁴⁴ The NO radical-scavenging activity of the plant extract indicates its potential to mitigate these harmful effects by reducing the concentration of NO radicals. The results shown in Figure 5D indicate that the activity of the extracts in scavenging NO radicals was insignificantly lower than that of HPRS and DPPH scavenging activities. The highest NO-RSA values were determined for *P. armeniaca* (85.19 ± 1.24 %) and

R. idaeus (82.79 ± 1.21 %) and the lowest for *P. cerasus* and *R. canina* (67.58 ± 1.06 and 64.86 ± 0.94 %, respectively). The results show that the study extracts have significant antioxidant activity in scavenging the most dangerous ROS and RNS.

Natural phytocomponents have UV-absorbing properties and can act as natural UV filters, providing effective UV protection with potentially fewer side effects. These compounds often exhibit antioxidant and anti-inflammatory activities, providing further protection against UV-induced damage. The results indicated that the SPF values of all tested extracts increased significantly with increasing concentration. The corresponding SPF values ranged from the maximum of 22.55 ± 0.43 (*P. armeniaca*, 1.0 g/L) to the lowest of 0.97 ± 0.22 (*C. laevigata*, 0.1 g/L). In general, aqueous extracts obtained from the leaves of the main representatives of the Rosaceae family exhibited significant UVB protection activity.

The obtained relationships clearly demonstrate the key role of phenolic compounds and flavonoids in the expression of the functional properties of the leaves of fruit and berry crops of the Rosaceae family. Significant positive correlations ($p < 0.05$) were obtained for TPC-TAC ($R^2 = 0.7572$, $r = 0.8701$), TPC-TRP ($R^2 = 0.7409$, $r = 0.8608$), and TPC-HRSA ($R^2 = 0.8259$, $r = 0.9088$). A moderate correlation was established for TFC-DPPH ($R^2 = 0.5524$, $r = 0.7433$), TFC-HPSA ($R^2 = 0.6241$, $r = 0.7900$), TFC-NO-RSA ($R^2 = 0.5923$, $r = 0.7696$), and TFC-SPF ($R^2 = 0.7388$, $r = 0.8596$). The correlations obtained indicate that phenolic compounds and flavonoids in the studied plants have different properties and therefore perform different functions, which is apparently due to their qualitative composition. The correlations obtained for the total (TPC+TFC) and relative (TPC/TFC) contents of phenols and flavonoids confirm this assumption. For instance, the high negative correlation between the TPC/TFC ratio and NO-RSA ($R^2 = 0.5033$, $r = -0.7094$) indicated that the higher the flavonoid content in the leaves and the lower the phenolic compounds content, the higher the NO radical scavenging activity of the extracts. Therefore, the higher the TPC, the higher the TAC and TRP of the extracts, and the DPPH-scavenging activity. The increased TFC contributes to the high activity of the extracts in scavenging NO-radicals, removing hydrogen peroxide, and achieving significant sun protection properties. In addition, there was a significant positive correlation ($p < 0.05$) between TAC-TRP ($R^2 = 0.9786$, $r = 0.9893$), TAC-HRSA ($R^2 = 0.8155$, $r = 0.9031$), TRP-HRSA ($R^2 = 0.7680$, $r = 0.8764$), and DPPH-HRSA ($R^2 = 0.6584$, $r = 0.8114$). Moderate correlations were revealed between DPPH-SPF ($R^2 = 0.5426$, $r = 0.7366$) and NO-RSA – SPF ($R^2 = 0.5699$, $r = 0.7549$). The correlation between TAC and TRP is explained by their dependence on the electron-donating ability of antioxidant compounds. The high correlation between TAC, TRP and HRSA may be due to the



ability of antioxidants to donate electrons or hydrogen atoms, which is crucial for HRSA. Antioxidants with strong reducing power are effective in neutralizing hydroxyl radicals, leading to a positive correlation.⁴⁵ Extracts rich in phenolics and flavonoids often show strong correlations with TAC and TRP with HRSA because these compounds act through multiple antioxidant mechanisms via similar pathways. The correlation between DPPH and HRSA may be due to both DPPH and HRSA assays measuring the ability of antioxidants to neutralize free radicals by donating electrons or hydrogen atoms. Although DPPH radicals are relatively stable and hydrophobic, hydroxyl radicals are highly reactive and hydrophilic. Despite these differences, natural antioxidants capable of efficiently donating electrons or hydrogen atoms tend to perform well in both scavenging activities, although the correlation may not always be perfect.⁴⁶ Nevertheless, the absence of a significant correlation between DPPH, TAC, and TRP indicates that compounds of similar nature but with different structures are responsible for these properties.

The correlation between DPPH and SPF may be due to the coincident role of antioxidants in protecting against UV rays and neutralizing free radicals. UV light generates ROS and free radicals. Hence, antioxidants with significant DPPH activity can neutralize these radicals, decreasing UV-induced skin damage and enhancing SPF.⁴⁷ The correlation between NO-RSA and SPF can be explained by the fact that UV radiation also induces the production of RNS, including NO radicals. Therefore, antioxidants with strong NO-RSA can indirectly enhance SPF. Thus, the defined correlations reflect the multifunctional role of the aqueous extracts of leaves of the main species of the Rosaceae family in radical scavenging and UV protection. No other significant correlations were found in this study.

In summary, members of the same plant family often share similarities in phytochemical composition owing to evolutionary relationships, but variations can occur depending on environmental factors, plant parts, and specific adaptations. Plants within the same family produce similar classes of secondary metabolites because closely related species often share similar biosynthetic pathways, leading to the production of related compounds. Despite their similarities, phytochemical profiles can vary due to differences in habitat, soil conditions, and genetic diversity. Some species within a family can produce unique compounds with special ecological roles, such as defense and pollinator attraction. Therefore, while representatives of the same plant family often exhibit similar phytochemical profiles, variations can be attributed to genetic, environmental, and ecological factors. Obviously, it is precisely these differences that lead to varying degrees of manifestation of the functional properties

of the investigated extracts. Nevertheless, this study uniquely focused on Rosaceae plant species that were locally grown in Western Kazakhstan, emphasizing their significant antioxidant and photoprotective properties. The study is distinguished by its comprehensive correlation analysis, which not only reinforces established patterns with new reliable data and enhances the understanding of the degree and nature of the influence of phytochemical composition on functionally significant properties. Consistent with similar studies, the findings confirmed the high abundance of phenolic compounds and flavonoids in Rosaceae plants, which is consistent with research conducted in broader geographical regions such as China⁴⁸ and Egypt⁴⁹. This comparison highlights the role of geographical locations and environmental factors in shaping phytochemical profiles and functional attributes. The evaluation of SPF and its correlation with phytochemical composition, along with a broad range of antioxidant activities, represents a novel contribution to research on Rosaceae plants. These insights significantly expand upon previous studies, which have primarily focused on genetic, molecular, and ecological aspects. Together these approaches complement one another, enriching the understanding of the phytochemical diversity and functional potential of this plant family.

CONCLUSION

In this study, the phytochemical composition and practical useful properties of leaf extracts of the main species of the Rosaceae family that grow in the Western region of Kazakhstan were studied. It was found that these plants are rich sources of phenolic compounds and flavonoids that determine their antioxidant and sun protection properties. The revealed correlations indicate that despite belonging to the same family, different species can vary in their functional properties, which is apparently due to environmental and genetic factors. Correlations of antioxidant properties show similar mechanisms of their implementation and consequently similarity in the structure of phytocomponents responsible for these properties. Our research has clearly shown that the main species of the Rosaceae family have a high potential for further research and development of pharmaceutical products with antioxidant and sun protection properties to protect human health and prevent various diseases.



Ethics Committee Approval Ethics committee approval was not required for the study.
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Author Details

Altyngul Maksotova

¹ Makhambet Utemisov West Kazakhstan University, Faculty of Natural and Geographical Sciences, Uralsk, Kazakhstan

 0009-0006-0691-6206

Railya Issagat

¹ Makhambet Utemisov West Kazakhstan University, Faculty of Natural and Geographical Sciences, Uralsk, Kazakhstan

 0009-0008-6599-4276

Dana Nurzhanova

¹ Makhambet Utemisov West Kazakhstan University, Faculty of Natural and Geographical Sciences, Uralsk, Kazakhstan

 0009-0003-8529-0982

Nikolay Akatyev

¹ Makhambet Utemisov West Kazakhstan University, Faculty of Natural and Geographical Sciences, Uralsk, Kazakhstan

 0000-0001-9248-2753 nikolay.akatyev@wku.edu.kz

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