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Research Article

Investigating the Content and Bioaccessibility of Phenolic Compounds In Roots of *Rosa canina* L. and *Rosa pimpinellifolia* L.

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Abstract: Rosehip is among the most important plants with high economic value, mainly used in foods and beverages from ancient times to the present. In this study, *Rosa canina* L. and *Rosa pimpinellifolia* L. roots, consumed as tea in Aktoprak Village of Erzurum province, were collected together with the fruits. The main goal of the study was to investigate the *in vitro* bioaccessibility of phenolic compounds in the roots and fruits of *R. canina* and *R. pimpinellifolia* by a simulated gastrointestinal digestion procedure. Methanolic and aqueous extracts were prepared for the analysis of phenolic compounds in roots, whereas only methanolic extracts were used for the analyses of fruits. Total phenolic and total flavonoid contents were evaluated spectrophotometrically, while four different methods were used for antioxidant capacity measurements. The quantification of individual phenolic acids, flavonoids, and anthocyanins was performed with HPLC-PDA. Results demonstrated that *R. canina* and *R. pimpinellifolia* have high levels of total phenolics, total flavonoids, and antioxidant capacity. The roots of *R. pimpinellifolia* and *R. canina* were observed to contain higher amounts of phenolics compared to the fruits. Epicatechin, 4-hydroxybenzoic acid, gallic acid, syringic acid, p-coumaric acid, naringenin, and ellagic acid were not determined in the fruit extracts of *R. pimpinellifolia* and *R. canina*, while they were detected in aqueous extracts of roots. Bioaccessibility analyses carried out on aqueous root extracts showed total phenolic recovery was 12.73% in *R. canina*, 10.71% in *R. pimpinellifolia*, and total flavonoid recovery was 0% in both species.

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1. Introduction

Türkiye has one of the largest production areas of rosehips (Yıldız and Çelik, 2011). There are a hundred species in the world belonging to the genus *Rosa* of the Rosaceae family, and 26 species out of a hundred are found in Türkiye. Rosehip is a shrub-shaped plant that is usually 0.3-3 m (rarely 6 m) tall. It is widely grown in the Central and Western Black Sea regions such as Corum, Kastamonu, Amasya, Gumushane, Tokat, and in the Eastern Anatolian region, such as Erzincan and Erzurum, Hakkari, Bitlis, and Van, where continental climates are experienced (Dogan and Kazankaya, 2006). Rosa species fruits are generally rich in the bioactive compounds with antioxidant activity, especially in flavonoids, carotenoids, tannins, phenolic acids, mineral compounds and fatty and organic acids (Ayati et al., 2019). The medicinal properties of Rosaceae fruits can be partially attributed to their abundance of phenolics which possess a broad spectrum of biochemical activities; like antioxidant, antimutagenic effects, anticarcinogenic, and the ability to change gene expression (Tapiero et al., 2002; Nakamura et al., 2003, Sevket et al., 2016).

Many different factors including pollution in the environment, UV rays from the sun, industrial wastes, petrochemical products, infection, cosmic rays, chemicals, X-rays, viruses, extreme stress, and exhaust gases from automobiles, etc. which continuously increase the amount of exposure to free radicals in our body (Sies et al., 1991). Phenolic compounds help prevent chronic and oxidative stress-related disorders such as cardiovascular, cancer, and neurodegenerative diseases due to their free radical scavenging abilities (Bhuyan and Basu, 2017). Furthermore, phenolics with antioxidant properties have an imperative function in the human defense system against free radical species, carcinogenesis, the relative inducer of DNA damage, cardiovascular diseases, aging, lipid peroxidation, and diabetes. Besides, phenolics are connected with other biological functions like antimicrobial and antiinflammatory activities (Garavand et al., 2021).

Bioaccessibility is described as the amount of food product accessible for intestinal absorption after digestion (Hedren et al., 2002). Moreover, the bioaccessibility of a compound indicates the amount released from the food matrix and which is assumed to be available for absorption (Minekus et al., 2014). Bioaccessibility of a compound deal with various processes, such as the stages of release, absorption, distribution, metabolism, and elimination from a food matrix (Rain et al., 2013; Cosme et al., 2020). From a nutritional point of view, bioavailability accepts the part of a particular food that the body can use; therefore, it is considered as a nutritional activity (Benito and Miller, 1998; Fernàndez-Garcìa et al., 2009; Cosme et al., 2020). The current literature focuses mainly on the phenolics in the fruits of rosehip. Moreover, there are only a few studies about the *in vitro* bioaccessibility of *Rosa canina* L. and *Rosa pimpinellifolia* L. roots teas, in which the phenolic and flavonoid contents as well as antioxidant capacity were investigated before and after digestion.

This study aimed to determine the total phenolic and total flavonoid contents and antioxidant capacity of the roots and fruits of *R. canina* and *R. pimpinellifolia* rosehip species and to investigate the bioaccessibility of phenolic compounds in rosehip roots.

2. Material and Methods

2.1. Plant material

Rosa canina L. and *Rosa pimpinellifolia* L. roots and fruits were collected as three separate samples from the Aktoprak village of Erzurum in October 2016, which is the harvest time of the fruits.

2.2. Extraction procedure

According to Capanoglu et al. (2008), the methanolic extract was prepared in triplicate for roots and fruits belonging to two species. First, 1.00 ± 0.01 g from each sample was mixed with 5 mL of 80% methanol and sonicated in an ultrasonic bath (Azakli, Türkiye) for 15 min. Afterward, the extracts were centrifuged (Hettich Zentrifugen Universal 32R, UK) at 2700 × *g* for 10 min at +4 °C, and the supernatant was separated. Then 5 mL of 80% methanol was added to the residue to repeat the procedure. At the end of the extraction process, supernatants were kept at -20 °C until further analyses.

The method of Perk et al. (2016) was used to prepare aqueous extraction from root samples. Briefly, 2 g of root samples were boiled in 50 mL of distilled water. Then, 5 min after the starting point

of the boiling process, 50 mL of distilled water was added. This process was repeated four times until the total volume reached 200 mL, and the total mixture was allowed to cool down to room temperature. After cooling, the root parts were removed, and the mixture was filtered through filter paper and kept at -20 °C.

2.3. *In vitro* bioaccessibility

The methods of McDougall et al. (2005) and Minekus et al. (2014) were adapted for the determination of *in vitro* bioaccessibility. First, salivary fluid, gastric fluid, and intestinal fluid were prepared according to the protocol. For oral digestion, 2.5 mL of sample was taken and 3.5 mL of saliva, 0.5 mL of amylase solution, and 25 µL of 0.3 M $CaCl_2$ were added. After adjusting the pH to 7.0, the mixture was incubated in a shaker water bath for 2 min at 37°C. At the end of salivary digestion, 2 mL of sample was collected, and the residue was mixed with 6 mL of gastric fluid, 1.28 mL of pepsin solution, 4 µL of $CaCl_2$, and 1.6 µL of HCl, and the pH was adjusted to 3.0, and shaken at 37 °C for 2 h for gastric digestion. At the end of digestion, a 2 mL stomach sample was collected. Then, 7.7 mL intestinal fluid, 3.5 mL pancreatin solution, 1.75 mL bile solution, 28 µL of $CaCl_2$, and 972 µL of 1 M NaOH were added to the remaining part. Dialysis bags were prepared with 20 mL $NaHCO_3$ stock solution (10.5 g $NaHCO_3$ was dissolved in 250 mL distilled water). Prepared bags were placed in intestinal fluids media, then all beakers were kept in the shaker at 37 °C for 2 h, and the part remaining in the dialysis bag was separated as 'IN' and the rest as 'OUT'. These fractions were transferred into Eppendorf tubes and centrifuged at 23000 ×*g*, +4°C. The bioaccessibility (%) was calculated as follows;

Bioaccessibility (%) = Intestinal internal value / Initial value * 100

2.4. Total phenolic content

The total phenolic content of the samples was measured using the Folin-Ciocalteu method, according to the method of Turkmen et al., 2006. First, in the analysis tube, 100 µL of the sample and 900 µL of distilled water were added. After the addition of 1.5 mL of 0.2 N Folin-Ciocalteu reagent mixture was held for 5 min. Following the addition of 1.2 mL of saturated Na_2CO_3 solvent, the mixture was left for 90 min. Absorbance measurement of samples was performed at 765 nm using a UV–Vis spectrophotometer (Shimadzu UV-1700; Shimadzu Corporation, Kyoto, Japan) against blank. For the generation of the standard calibration curve, gallic acid solution in 80% MeOH was used.

2.5. Total flavonoid content

The total flavonoid content was examined spectrophotometrically according to the method of Dewanto et al. (2002). After 250 µL of the sample was taken into the analysis tube, 1.25 mL of distilled water was added. Then, 75 µL of 5% $NaNO_2$ solvent was added, and the mixture was allowed to stand for 6 min. Afterward, 150 µL of 10% $AlCl_3.6H_20$ solution was mixed and held for 5 min, then 0.5 mL of 1 M NaOH solution was mixed, and the total volume was adjusted to 2.5 mL with distilled water. The absorbance was measured against a blank at a wavelength of 510 nm utilizing a UV–Vis spectrophotometer. For the preparation of the calibration curve catechin solution in 80% MeOH was used.

2.6. Antioxidant capacity

2.6.1. ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate)) radical scavenging method

The method was applied using the protocol of Miller and Rice-Evans (1997), 220 mg of ABTS radical was dissolved in 200 mL of distilled water, and 38 mg $K_2S_2O_8$ was dissolved in 2 mL of distilled water. These solutions were mixed and kept in the dark overnight to complete radicalization and obtain the ABTS+ solution. Before starting the analysis, until its absorbance achieved 0.9 ± 0.2 ABTS+ solution was diluted with 0.05 M KPi buffer (pH = 8). Then, approximately 100 µL of the sample was transferred into an analysis tube, and 1 mL of ABTS+ solution was added. The mixture was vortexed

(IKA Vortex Genius 3) for 25 sec. Using a UV–Vis spectrophotometer, the absorbance against the blank was measured at a wavelength of 734 nm.

2.6.2. CUPRAC (Cupric Reducing Antioxidant Capacity) method

According to Apak (2004), 0.4262 g of $CuCl_2H_2O_2$ was dissolved in 250 mL of distilled water, 0.039 g of Neocuproin was dissolved in 96% EtOH and diluted to 25 mL, and 19.27 g of NH_4Ac was diluted in 250 mL of distilled water. Nearly 100 µL of the sample was transferred into an analysis tube, and 1 mL of $CuCl_22H_20$ solution, 1 mL of NH_4Ac buffer, 1 mL of Neocuproin solution, and successively 1 mL of distilled water were added. Afterwards the mixture was waited for 30 minutes, and the absorbance using a UV–Vis spectrophotometer was evaluated at 450 nm against water.

2.6.3. DPPH (2,2-diphenyl-1-picrylhydrazyl) method

Shortly, 2 mL of 0.1 mM DPPH was mixed with 100 µL of sample in a test tube. Samples were stored in the dark at room temperature for 30 minutes. Using a UV–Vis spectrophotometer absorbance was measured against methanol at a wavelength of 517 nm. (Kumaran et al., 2006).

2.6.4. FRAP (Ferric Reducing Antioxidant Power) method

The method Benzie and Strain (1996) was used with slight modifications. In short, 3.1 g of $CH_3COONa·3H2O$ was dissolved in distilled water, 16 mL of 99.85% acetic acid was added and the total volume was put into 1 L with distilled water. 0.504 g of FeCl3·6H2O was dissolved in distilled water and mixed with 1 M HCl. The total volume of the mixture was put into 100 mL with distilled water. 0.156 g of tripyridyl triazine (TPTZ) was dissolved in 50 mL ethanol. FRAP reagent was made with a 10:1:1 volume rate with these solution sequences. Then, 100 µL of the sample was taken into a test tube and 900 µL of FRAP reagent was added. After keeping the mixture for 4 min at room temperature, absorbance was measured at 593 nm against distilled water.

2.7. HPLC analysis

HPLC analysis was performed based on the method described by Capanoglu et al. (2008). Standard calibration curves were created using solutions of catechin, gallic acid, *p*-hydroxy benzoic acid (PHBA), protocatechuic acid, vanillic acid, caffeic acid, p-coumaric acid, kaempferol, fumaric acid, naringenin, syringic acid, quercetin, quercetin di-hydrate, ellagic acid, cyaniding and rutin. These samples and their stock solutions mixture were filtered through a 0.45 µm membrane filter, and 1 mL of the filtered sample was accommodated in vials and analyzed in a Waters W600 HPLC system with a PDA (Waters 996) detector. Luna C18 column (Phenomenex, Utrecht, The Netherlands), was utilized as the stationary phase. The mobile phase consisted of solvent A, 0.1% (v / v) trifluoric acid (TFA) with distilled water, and solvent B, 0.1% (v / v) TFA with acetonitrile. A linear gradient was used: 95% solvent A and 5% solvent B at 0 min; At 45 minutes, 65% solvent A and 35% solvent B; At 47 minutes, 25% solvent A and 75% solvent B; Returns to primary conditions in 54 minutes. The flow rate was 1 mL/min. Detections were performed at wavelengths 280, 312, 360, and 520 nm.

2.8. Statistical analysis

The results were analyzed by Tukey's New Multiple Comparison post hoc test ($p<0.05$) using the One-Way Analysis of Variance (One–way ANOVA) with SPSS (21st version, IBM, New York, NY, USA) program. Each analysis was made in triplicate, and the results were reported as the mean ± standard deviation.

3. Results and Discussion

3.1. Total phenolic content and total flavonoid content of *Rosa canina* L. and *Rosa pimpinellifolia* L. roots and fruits

The total phenolics and flavonoid contents of the aqueous and methanolic extracts are presented in Table 1. Total phenolic content was measured as gallic acid equivalent (GAE). Total flavonoid

content measured as catechin equivalent (CA). When *R. canina* and *R. pimpinellifolia* are compared in terms of total phenolic content (TPC), it is observed that the *R. pimpinellifolia* root aqueous extracts exhibited the highest value with 52.81±6.81 mg GAE/g. Similarly, in terms of total flavonoid content (TFC), the highest value was observed in *R. pimpinellifolia* root aqueous extractions with 25.34±4.00 mg CA g^{-1}. The TFC of fruit methanolic extracts constituted the lowest level, while the lowest TFC was observed to be 3.43±1.44 mg CA g^{-1} in the fruit methanolic extracts of *R. canina*. The TFC of *R. pimpinellifolia* root aqueous extracts was observed to be higher than that of *R. canina*. Previous studies indicated that the TPC was 929.27 mg GAE 100 g^{-1} in the aqueous extract, higher than the value observed in the methanol extract of *R. pimpinellifolia* fruit (Çakır and Ergen, 2021). In another study, the total phenolic content levels of *R. canina* fruits were determined as 247.60 ± 0.95 µg EAG mg^{-1} and total flavonoid content as quercetin equivalents 187.66 ± 5.25 µg EQ mg^{-1}. (Fetni et al., 2020). Ozdogan and Coruh (2015) studied *R. heckeliana* roots by comparing methanolic, petroleum ether, ethyl acetate, chloroform, and n-butanol extracts. Accordingly, the TFC was determined as 0.184±0.0047 µg CA mg^{-1} in the methanolic extract. TPC of the six *R. pimpinellifolia* genotypes ranged from 1018-1407 mg GAE per 100 g fresh weight (Karatas, 2021). Encapsulation extract into lipid vesicular systems and biological activity of *Rosa canina* L. bioactive compounds in dermocosmetic use, TPC value was found to be 88.71 ± 0.95 µg GAE mg^{-1} and TFC value was 25.38 ± 0.49 µg QE mg^{-1} (Sallustio et al., 2022). In particular, *R. pimpinellifolia* root aqueous extracts were observed to have a significantly higher TFC compared to the other samples analyzed within the scope of this study. Compared with the values reported in the previous studies, the values observed for *R. pimpinellifolia* aqueous extractions were remarkably higher.

Table 1. Total phenolic content and total flavonoid content of *R. canina* and *R. pimpinellifolia* roots and fruits

***R. canina* and *R. pimpinellifolia* Extracts**	**Total Phenolic Content (mg GAE g^{-1})**	**Total Flavonoid Content (mg CA g^{-1})**
R. canina root methanolic extract	27.22±0.71^{c}	11.16±4.29^{b}
R. canina root aqueous extract	48.01±7.69^{b}	24.06±1.63^{a}
R. canina fruit methanolic extract	10.74±3.09^{d}	3.43±1.44^{c}
R. pimpinellifolia root methanolic extract	28.22±5.10^{c}	12.88±4.99^{b}
R. pimpinellifolia root aqueous extract	52.81±6.81^{a}	25.34±4.00^{a}
R. pimpinellifolia fruit methanolic extract	14.35±2.62^{d}	7.32±2.48^{c}

3.2. Phenolic compounds in *R. canina* and *R. pimpinellifolia* root and fruit extracts

The phenolic compounds and their amounts in *R. canina* and *R. pimpinellifolia* root and fruit extracts are presented in Table 2. Mainly 4- hydroxybenzoic acid was found in *R. canina* root methanolic extract and *also in both R. pimpinellifolia* root and fruit methanolic extracts. Cyanidin, an anthocyanin, was detected only in *R. pimpinellifolia* fruit methanolic extract. Rutin was also present in fruit extracts of both species; however, its amount was observed to be higher in *R. canina*. On the other hand, *p*-coumaric acid was not found in *R. canina* fruit methanolic extracts. The extract with the highest value among the catechin derivatives was observed to be *R. pimpinellifolia* root aqueous extract. Moreover, catechin and its derivatives were detected in large amounts in both species. Among the phenolic components in *R. pimpinellifolia* fruits, the most dominant ones are chlorogenic acid, gallic acid, and vanillic acid (Çakır and Ergen, 2021). In a previous study, phenolic compounds including procyanidin B1, epicatechin, chlorogenic acid (trans-5-O-caffeoylquinic acid) procyanidin B2, salicylic acid, gallic acid, catechin, etc. were found in rosehip (Ghendov-Mosanu et al., 2020). On the other hand, the root of *R. canina* was reported to show the highest content of epigallocatechin (almost 3% of the total catechins) among the determined catechins (Oproshanska et al., 2021). Catechin and catechin derivatives observed in the present study were in accordance with the previous reports. The highest amount of catechin was determined in *R. canina* fruit methanolic extract. The diversity of phenolic substances in *R. pimpinellifolia* fruit methanolic extraction, especially in terms of catechin derivatives, is variable compared to other studies.

Table 2. Phenolic compounds in *R. canina* and *R. pimpinellifolia* root and fruit extracts

Phenolic Substance	*R. canina* Root Ext.		*R. canina* Fruit Ext.	*R. pimpinellifolia* Root Ext.		*R. pimpinellifolia* Fruit Ext.
	Aqueous ext. (mg g^{-1})	Methanolic ext. (mg g^{-1})	Methanolic ext. (mg g^{-1})	Aqueous ext. (mg g^{-1})	Methanolic ext. (mg g^{-1})	Methanolic ext. (mg g^{-1})
4-Hydroxybenzoic acid	-	0.94±0.02	-	-	0.90±0.3	0.13±0.01
Catechin	4.58±0.07	3.3±0.14	8.40±0.96	2.08±0.14	2.21±0.08	4.27±0.23
Epicatechin	0.69±0.08	0.44±0.04	-	2.30±0.10	1.26±0.11	-
Gallic acid	0.20±0.04	0.12±0.02	-	0.40±0.02	2.21±0.08	-
Syringic acid	0.56±0.04	0.49±0.03	-	-	1.26±0.11	-
P-coumaric acid	0.30±0.05	0.11±0.02	-	0.23±0.02	0.06±0.01	0.01±0.0
Naringenin	-	0.11±0.01	-	-	-	-
Ellagic acid	0.39±0.06	0.55±0.07	-	1.53±0.15	0.34±0.02	0.21±0.03
Quercetin	1.33±0.07	0.14±0.02	0.03±0.00	0.60±0.16	0.18±0.01	0.10±0.01
Rutin	-	-	0.13±0.01	-	-	0.06±0.01
Quercetin di hydrate	0.12±0.03	0.05±0.01	-	0.15±0.01	0.09±0.01	-
Catechin derivative 3 minutes	-	-	2.56±0.15	-	-	1.86±0.14
Catechin derivative 5 minutes	-	-	-	-	-	1.01±0.06
Catechin derivative 7 minutes	-	-	-	-	-	0.48±0.10
Catechin derivative 10 minutes	3.50±0.36	2.69±0.15	-	5.86±0.41	2.75±0.13	-
Catechin derivative 11 minutes	-	-	-	3.45±0.18	1.51±0.08	-
Catechin derivative 14 minutes	-	-	-	0.95±0.14	1.31±0.06	-
Anthocyanin cyanidin	-	-	-	-	-	0.48±0.05

3.3. Total antioxidant capacities of *R. canina* and *R. pimpinellifolia* root and fruit

The total antioxidant capacities of *R. canina* and *R. pimpinellifolia* root and fruit are presented in Table 3. Antioxidant capacity methods DPPH, CUPRAC, FRAP, and ABTS were applied to the root and fruit extracts of *R. canina* and *R. pimpinellifolia*. The highest values among fruit and root parts were observed in aqueous root extractions. The highest values were determined to be 191.86 ± 17.95 mg TEAC g^{-1} and 171.47 ±25.32 mg TEAC g^{-1} in aqueous root extractions of *R. canina* and *R. pimpinellifolia* according to the CUPRAC method, respectively. The aqueous extracts of these two Rosa species and especially *R. canina* and *R. pimpinellifolia* roots were observed to show a much stronger antioxidant effect compared to the fruit (Table 3). In a study conducted on *R. canina* fruit powders, the result obtained with the DPPH method was 1793 mg TE 100 g^{-1} (Igual et al., 2022). Genome size, iPBS profiles, antimicrobial and antioxidant activities, characterization of *Rosa canina* L. fruits in a study that examined collected in urban areas of Slovakia, results on the total antioxidant activity was detected with DPPH method ranged from 6.99 to 7.73 mg TEAC g^{-1} (Rovna et al., 2020). In another recent study examining the functional compounds and antioxidant activities of rosa species cultivated in Türkiye, the antioxidant capacity of *R. canina* species was determined as 1932.50 ± 47.31 mg Trolox/100 g according to the CUPRAC method (Kayahan et al., 2022).

Table 3. *R. canina* and *R. pimpinellifolia* root and fruit total antioxidant capacities

***R. canina* and *R. pimpinellifolia* Extraction**	**DPPH (mg TEAC g^{-1})**	**CUPRAC (mg TEAC g^{-1})**	**ABTS (mg TEAC g^{-1})**	**FRAP (mg TEAC g^{-1})**
R. canina root methanolic extract	69.19±3.75^{b}	77.33±6.64^{b}	48.22±5.00^{cd}	49.05±1.40^{b}
R. canina root aqueous extract	157.68±4.81^{a}	191.86±17.95^{a}	66.56±13.63^{b}	84.44±8.69^{a}
R. canina fruit methanolic extract	25.03±4.91^{c}	64.93±12.41^{b}	35.93±3.23^{d}	13.14±1.82^{c}
R. pimpinellifolia root methanolic extract	67.31±7.62^{b}	77.39±15.26^{b}	59.84±11.50^{bc}	47.62±4.89^{b}
R. pimpinellifolia root aqueous extract	148.15±18.78^{a}	171.47±25.32^{a}	81.64±13.07^{a}	66.41±9.64^{a}
R. pimpinellifolia fruit methanolic extract	30.11±2.26^{c}	71.81±7.66^{b}	39.15±5.44^{d}	15.21±2.31^{bc}

3.4. Total phenolic and total flavonoid content in *in vitro* bioaccessibility analysis of *R. canina* and *R. pimpinellifolia* roots

The starting point of the study is that the local people have consumed the roots of rose hips since ancient times, as well as the fruits. In particular, the fact that black rose hips do not have a widespread distribution and show high values in terms of content enriched our study. Since the benefits of rosehip fruits are widely known, content analyzes of the fruits were also applied in order to make a comparison. According to the *in vitro* bioaccessibility result, the TPC of *R. canina* was 6.11±1.49 mg GAE/g, and the recovery was 12.73%. The TPC and TFC obtained from the mouth, stomach, and intestinal fractions and recovery values are presented in Table 4. In *R. canina,* the highest TPC was observed in the stomach fraction whereas the highest TFC was in the mouth fraction. The lowest phenolic and flavonoid content was found in the intestinal IN fraction. The recovery rate of the TPC was 12.73%, while the recovery of the TFC was 0%. In *R. pimpinellifolia*, the highest phenolic and flavonoid contents were observed in the gastric fractions (Gibis et al., 2012; Toktas et al., 2018). *R. pimpinellifolia* root aqueous extract the recovery rate of the TPC was 10.71%, while the recovery of the TFC was 0%. Reduction of TFC is attributed to the possible interaction between flavonoids, anthocyanins, phenolic compounds, and dissolved proteins, as well as the polymerization of phenolics which may result in the deterioration of their structure. The results of the bioaccessibility analysis show that there was no recovery in the flavonoid content of both *Rosa* species and the highest recovery rate in terms of TPC (12.73%) was found in *R. canina*. In the *in vitro* bioaccessibility study on sour cherry, the initial value of 147.6% was obtained as 10.1% in the inner part (Oksuz et al., 2019). In another recent study examining the *in vitro* bioaccessibility after thermal treatment applied to rosehip fruits, the TPC was determined as 74.9 ± 4.5%, and the TFC was 30.4 ± 0.4% (Ozkan et al., 2022). In the present study, significantly lower bioaccessibility was observed for the bioactives in the root parts compared to the fruits. Since there is limited data on the bioaccessibility of bioactives in rosehip roots, our findings were found to be consistent with the bioaccessibility studies with other plant species belonging to the Rosaceae family and rosehip fruits.

Table 4. Total phenolic and total flavonoid content in *in vitro* bioaccessibility of *R. canina* and *R. pimpinellifolia* plants root aqueous extracts

***R. canina* and *R. pimpinellifolia* *in vitro* bioaccessibility tested environments**	**Total Flavonoid Content (mg CA g^{-1})**		**Total Phenolic Content (mg GAE g^{-1})**	
	R. canina	***R. pimpinellifolia***	***R. canina***	***R. pimpinellifolia***
Initial	22.42±1.26^{bc}	26.43±5.76^{bc}	48.01±7.69^{a}	52.81±6.81^{a}
Mouth	41.38±5.36^{a}	21.98±4.49^{bc}	20.84±1.37^{de}	22.24±5.47^{cd}
Stomach	31.85±3.86^{bc}	32.98±6.19^{ab}	30.85±3.12^{b}	27.98±5.33^{bc}
Intestinal external	-	-	15.61±3.69^{de}	13.93±2.21^{ef}
Intestinal internal	2.16±1.01^{c}	7.75±13.93^{bc}	6.11±1.49^{fg}	5.66±1.1^{g}
Recovery %	0	0	12.73	10.71

3.5. Antioxidant capacity of *R. canina* and *R. pimpinellifolia* root aqueous extracts after *in vitro* digestion

Results of DPPH, CUPRAC, ABTS, and FRAP antioxidant capacity methods in aqueous extractions of *R. canina* and *R. pimpinellifolia* root after *in vitro* digestion are presented in Tables 5 and 6. The highest antioxidant capacity was found in *R. pimpinellifolia* in the gastric fraction according to the CUPRAC method. The highest recovery rate (52.16%) was observed in *R. pimpinellifolia* with the CUPRAC method. Antioxidant capacity values measured with CUPRAC and ABTS tests, after *in vitro* digestion, of *R. canina* and *R. pimpinellifolia* root aqueous extractions were observed to be higher than the values measured with DPPH and FRAP methods. The highest antioxidant capacity was found in *R. pimpinellifolia* with the gastric fraction according to the CUPRAC method, and the recovery rate was 52.16%. In the *in vitro* bioaccessibility study with sour cherry (*Prunus cerasus* L.), the gain was determined as 6.5% as a result of the DPPH method, which is one of the antioxidant capacity determination methods (Oksuz et al., 2019).

Table 5. *In vitro* bioaccessibility, antioxidant capacity of *R. canina* and *R. pimpinellifolia* root aqueous extracts

***R. canina* and *R. pimpinellifolia* *in vitro* bioaccessibility tested environments**	**DPPH (mg TEAC g^{-1})**		**CUPRAC (mg TEAC g^{-1})**	
	R. canina	***R. pimpinellifolia***	***R. canina***	***R. pimpinellifolia***
Initial	157.68±4.81^{a}	148.15±18.78^{a}	191.86±17.95^{bc}	171.47±25.32^{cd}
Mouth	35.91±3.64^{d}	43.68±8.58^{d}	185.68±20.57^{cd}	144.5±26.76^{d}
Stomach	75.83±5.19^{b}	62.93±11.92^{c}	253.68±36.03^{a}	230.13±38.46^{ab}
Intestinal external	13.79±1.84^{e}	16.12±3.97^{e}	152.28±29.05^{cd}	69.49±13.17^{e}
Intestinal internal	10.92±1.5^{e}	10.51±3.51^{e}	45.63±9.34^{e}	89.43±13.46^{e}
Recovery %	6.93	7.09	23.78	52.16

Table 6. *In vitro* bioaccessibility, antioxidant capacity of *R. canina* and *R. pimpinellifolia* root aqueous extracts

***R. canina* and *R. pimpinellifolia* *in vitro* bioaccessibility tested environments**	**ABTS (mg TEAC g^{-1})**		**FRAP (mg TEAC g^{-1})**	
	R. canina	***R. pimpinellifolia***	***R. canina***	***R. pimpinellifolia***
Initial	66.56±13.63^{b}	81.64±13.07^{a}	84.44±8.69^{a}	66.41±9.64^{b}
Mouth	27.42±0.28^{d}	27.06±0.23^{d}	32.81±5.06^{c}	13.23±2.99^{e}
Stomach	51.82±0.29^{c}	47.01±5.02^{c}	65.54±10.04^{b}	52.46±9.72^{b}
Intestinal external	10.97±1.84^{e}	11.37±2.05^{e}	8.58±2.01^{d}	10.08±1.14^{d}
Intestinal internal	13.65±2.65^{e}	14.89±2.99^{e}	4.75±1.88^{d}	2.73±0.68^{d}
Recovery %	20.51	18.24	5.62	4.1

4. Conclusion

The aim of this study was to investigate the phenolic content, flavonoid content, and antioxidant capacity of the root parts of 2 species of rose hips, which were known to show high values in terms of

phenolic compounds and antioxidant capacity. In addition, the *in vitro* bioaccessibility of root aqueous extractions was investigated. The literature review showed that there were limited studies on rosehip roots. Our findings revealed that the content of rosehip root parts generally showed higher values compared to the previous reports on commonly known fruits. Moreover, the existing studies are mostly framed around the fruit of rosehip. In the black rosehip, *Rosa pimpinellifolia* L. cyanidin was detected, which is one of the valuable findings of this study as an essential component in the anthocyanin class of flavonoids. Unlike the fruit parts, phenolic compounds such as epicatechin, gallic acid, syringic acid, quercetin dihydrate, and naringenin were detected in the roots of *R. canina* and *R. pimpinellifolia*. On the other hand, rutin was observed only in *R. canina* and *R. pimpinellifolia* fruits. Catechin and its derivatives were observed to be intense in both fruit and root parts of both species. In the *in vitro* bioaccessibility analyzes applied to root aqueous extractions of 2 rosehip species, the CUPRAC method showed the highest values in both species in the results of total antioxidant capacity methods DPPH, CUPRAC, ABTS, and FRAP. In addition, in the *in vitro* bioaccessibility of root aqueous extractions of the two species, the total phenolic content was found to be the highest in *R.canina* with 12.73%, while the total flavonoid content was found to be 0% in both species.

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