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Determination of antioxidant activities of rosehip (*Rosa canina* L.) fruits grown in Sivas province

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ABSTRACT

Rosa canina L., belonging to the Rosaceae family, is the most common *Rosa* species found in T*ürkiye*. Known commonly as rosehip, it is widely used in various traditional medicines. This study investigates the antioxidant activities of ethanol and water extracts of rosehip (*Rosa canina* L.) fruit samples naturally grown in Sivas. Using DPPH and ABTS assays, we measured the antioxidant capacities of the samples and compared results based on the solvents. Findings show that the ethanol extract exhibited a higher antioxidant capacity, with an IC_{50} value of 13.28 ± 1.3 µg/mL in the DPPH assay and 24.98 ± 5.3 µg/mL in the ABTS assay, compared to the water extract with IC_{50} values of 18.099 ± 2.4 µg/mL and 38.47 ± 6.1 µg/mL, respectively. These results suggest that rosehip fruits could serve as effective sources for antioxidant-rich pharmaceutical products, with ethanol extracts demonstrating a stronger antioxidant effect than water extracts.

Keywords: *Rosa canina*, antioxidant activity, rosehip, DPPH, ABTS

INTRODUCTION

Rosa canina, belonging to the Rosaceae family, refers to the fruits of wild rose species that can grow in almost all regions of Türkiye. *Rosa canina*, which also

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grows in Northeast Africa and Eurasia, is widely distributed in the Anatolian region. It grows wild along the edges of forests, ponds, shrublands, and grasslands[1,2]. Among the over 30 *Rosa* species in Türkiye, *Rosa canina* is the most widespread and is especially suited for processing due to its distribution and fruit characteristics. It is often found along forest edges, fields, and roadsides, especially in Türkiye's colder, more mountainous regions[3,4]. Regionally, rosehip is also known by various names: wild rose, dog rose; Askil, Civil, Gül burnu, Gül elması, İp burması, İp burnu, İt burnu, Kuşburnu, Kuşburni, Asker gülü, İt gülü[5,6]. Rosehip is a highly beneficial food source with a rich nutritional value, commonly preferred by the public for various health purposes. Traditionally, rosehip fruits have been used for generations to treat kidney stones, gastroenteritis, hypertension, and respiratory infections[7,8]. Rosehip fruits contain vitamins C, P, A, B1, B2, E, and K and are used to produce products such as jam, marmalade, juice, and tea. Besides its anti-inflammatory properties, rosehip is an excellent natural source of vitamin C and lycopene. Due to its nutritional composition, rosehip supplementation has shown beneficial effects in managing chronic conditions such as osteoarthritis, rheumatoid arthritis, and cancer[9,10]. Due to its nutritional content, rosehip supplements have positive effects on certain chronic diseases such as osteoarthritis, rheumatoid arthritis and cancer[10].

The fruits are rich in phenolic compounds such as apigenin, phloroglucinol, quercetin, gallic acid, and caffeic acid, with smaller amounts of catechin, resveratrol, and chlorogenic acid. The high antioxidant activity of rosehip is attributed to its ascorbic acid, beta-carotene, tocopherol, anthocyanin, and other phenolic compounds. Studies report the phenolic compounds anti-inflammatory, antioxidant, anticarcinogenic, antimicrobial, and antimutagenic properties. Additionally, rosehip fruits contain high amounts of Ca, Mg, Fe, Ag, Cu, Mn, Na, P, Sr, Zn, and pectin[3,8,11-14].

Oxidative stress occurs due to an imbalance between free radicals and antioxidants in the human body, a factor known to play a significant role in human health. Accumulation of free radicals in the body, reaching high levels, can damage cellular components such as lipids, proteins, and DNA, leading to neuronal dysfunction, chronic diseases, and even death. Experimental studies show that oxidative stress plays a critical role in the progression of diseases such as cancer, cardiovascular diseases, neurodegenerative disorders, aging, and age-related diseases. The damage caused by oxidative stress can be prevented by endogenous (superoxide dismutase, catalase, and glutathione) and exogenous (phenolic acids, flavonoids, and vitamins) antioxidant systems[15-17]. While antioxidants are naturally produced by some plants and animals, this process differs

in humans, making a balanced and regular diet crucial for sufficient antioxidant levels[18]. In this study, the antioxidant activities of rosehip fruits obtained from the *Rosa canina* plant naturally growing in the Zara district of Sivas province and prepared with ethanol and water solvents were investigated, with results compared and interpreted according to the solvents used.

METHODOLOGY

Plant material

In this study, the fruits obtained from the *Rosa canina* L. plant were collected fresh from the Esenler Village region of Zara, Sivas, in September 2022. These fruits were then dried under suitable conditions and prepared for experimental use. Species identification was conducted by Assoc. Prof. Dr. Mustafa Sevindik.

Chemicals

All chemicals and reference standards utilized in the experimental protocols were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The chemicals used were of analytical grade.

Extraction

The dried fruits were ground into coarse powder. A sample of 5g of plant material was weighed and placed into an Erlenmeyer flask, to which 50 mL of ethanol was added. The flask was then sealed and left to macerate at room temperature, with occasional shaking to ensure thorough maceration. After 24 hours, the macerate was filtered, new solvent (50 mL) was added to the plant material, and the maceration was continued for three days. The collected macerates were then concentrated using a rotary evaporator at a low temperature (40°C) under vacuum[19]. The extracts were combined in dark-colored, capped glass containers and stored at -20°C in a refrigerator until use in experimental studies, with percentage yield calculations recorded.

For the water extract, an infusion process was used. A sample of 5g of plant material was weighed and placed into Erlenmeyer flasks, to which 50 mL of hot distilled water was added. The flask was sealed and left to stand at room temperature for 10–15 minutes with occasional shaking. After this period, the extract was filtered, and another 50 mL of hot distilled water was added to the plant material. After three repetitions, the collected extracts were placed in a lyophilizer (freeze-dryer) to ensure complete removal of water. After four days, the remaining extract in the freeze-dryer was transferred to a dark-colored, capped glass container, and the percentage yield was calculated. Extracts were stored at -20°C in a refrigerator until used in experimental studies.

For biological activity, stock solutions at a concentration of 1 mg/mL were prepared from each extract. The stock solution for the ethanol extract was prepared in DMSO (dimethyl sulfoxide), and for the water extract, it was prepared in distilled water.

Determination of antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity

The DPPH free radical method[20] is a practical and highly reliable technique for determining the free radical scavenging capacity of antioxidants. DPPH is a stable nitrogen radical that is commercially available; its ethanol solution appears purple and is measured at an absorbance of 517 nm. When antioxidants are introduced into the DPPH solution, they reduce DPPH, changing the solution from purple to yellow. This reaction is monitored using a spectrophotometer[21,22].

For this test, the stock solution of each sample was prepared in methanol (MeOH) at a concentration of 1 mg/mL. After filtration, 200 µL of the clear stock solutions were transferred to the first column of a 96-well microtitration plate. Using a multi-channel pipette, eight serial dilutions were made in equal amounts of MeOH, and the mixtures were vortexed for 5 minutes. The DPPH stock solution was prepared by dissolving 2 mg of DPPH in 25 mL of MeOH, yielding a final concentration of 80 µg/mL. To each well, 100 µL of the DPPH solution was added to initiate the reaction, which was then incubated in the dark at room temperature for 30 minutes[23,24]. Ascorbic acid at the same concentration was used as a positive control, DPPH + MeOH as the negative control, and MeOH alone as the blank. The UV absorbance was read at 517 nm using a microplate spectrophotometer (Epoch) at room temperature[24].

The % inhibition value of DPPH was calculated using the following formula[25,26]:

$$\% \text{ Inhibition} = [(A \text{ Control} - A \text{ Sample}) / A \text{ Control}] \times 100$$

ABTS (2,2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radical-scavenging activity

The ABTS radical-scavenging activity, which follows the standard TEAC (Trolox Equivalent Antioxidant Capacity) method, was performed by optimizing the procedures established by Papandreou et al. (2006), Re et al. (1999), and Ardağ (2008)[27-29].

In the experimental protocol, a solution of 7 mM ABTS in distilled water (50 mL) was mixed with a 2.45 mM potassium persulfate solution (25 mL) and left

in the dark for 12–16 hours to form the radical cation. This blue-green ABTS radical solution was diluted with ethanol to a 1:80 ratio until an absorbance of 0.8–0.7 was achieved at 734 nm. Standard Trolox solutions were prepared at concentrations of 3, 2, 1, 0.5, 0.25, and 0.125 mM. Then, 10 µL of each sample was mixed with 990 µL of the prepared ABTS solution, and absorbance was measured at 734 nm. The absorbance results were used to create a linear regression equation for Trolox[24,26,30].

To perform the assay, 1 mL of the ABTS radical solution was added to numbered Eppendorf tubes, followed by 10 µL of each sample solution (100 µg/mL extract). Absorbance readings were taken at 734 nm at both 1 and 6 minutes. The difference in absorbance values between the first (A1) and sixth minute (A6) was calculated to yield ΔA values. Using these values, the percentage of inhibition was calculated using the formula below, and inhibition was plotted against sample concentration.

$$\% \text{ inhibition} = [(A6-A1)/A1] \times 100$$

$$\% \text{ inhibition} = (\Delta A/A1) \times 100$$

The TEAC method, initially developed by Miller et al., measures the decrease in absorbance of the ABTS radical solution in the presence of antioxidants[24,27,29-32].

Statistics

The results were expressed as mean ± standard deviation, and the statistical evaluation and calculations were performed using the GraphPad Data Analysis program. Data were analyzed at a 95% confidence level, and a p-value less than 0.05 was considered statistically significant.

RESULTS and DISCUSSION

This study initially involved the preparation of ethanol and water extracts of *Rosa canina* fruits, followed by the determination of extraction yields. By measuring the amounts used initially and the final amounts obtained, the extraction yields were calculated as percentages. It was found that the yield of the ethanol extract was higher at 10.41%, while the yield of the water extract was comparatively lower at 6.54% (Table 1).

Table 1. Yield values of *Rosa canina* fruit extracts

Extract	Yield %
Ethanol extract	10.41%
Water extract	6.54%

Antioxidant activity determination results

The antioxidant capacities of ethanol and water extracts from *Rosa canina* fruits were assessed using DPPH and ABTS radical-scavenging manual methods, with ascorbic acid, BHT (Butylated Hydroxytoluene), and Trolox as standard substances.

DPPH method activity results

The ability of the extracts to decolorize the DPPH solution was measured, with absorbance values obtained from the Elisa spectrophotometer indicating the extracts capacity to reduce DPPH radicals and overall antioxidant activity. Results showed that the antioxidant capacity of the ethanol extract was higher than that of the water extract, although the water extract still exhibited moderate to high antioxidant capacity. Specifically, the DPPH radical-scavenging activity of the ethanol extract had an IC_{50} value of 13.28 ± 1.3 µg/mL, while the water extract had an IC_{50} value of 18.099 ± 2.4 µg/mL. Table 2 shows the DPPH radical-scavenging activities of the extracts and standard solutions.

Table 2. DPPH radical-scavenging activity values of standard antioxidants and *Rosa canina* fruit extracts

Extracts/Standard Substances	IC_{50} (µg/mL)
Ethanol	13.28 ± 1.3
Water	18.099 ± 2.4
Ascorbic acid	5.35 ± 0.9
BHT	7.65 ± 1.8
Trolox	5.77 ± 1.6

ABTS method activity results

The ability of the extracts to scavenge ABTS radicals was measured spectrophotometrically and compared to standard solutions. Findings indicated that the antioxidant capacity of the ethanol extract was higher than that of the water extract. Specifically, the ABTS radical-scavenging activity of the ethanol extract had an IC_{50} value of 24.98 ± 5.3 µg/mL, while the water extract exhibited an IC_{50} value of 38.47 ± 6.1 µg/mL. Table 3 details the ABTS radical-scavenging activities of the extracts and standard solutions.

Table 3. ABTS radical-scavenging activity values for standard antioxidants and *Rosa canina* fruit extracts

Extracts/Standard Substances	IC_{50} (µg/mL)
Ethanol	24.98 ± 5.3
Water	38.47 ± 6.1
Ascorbic acid	7.48 ± 2.6
BHT	6.94 ± 1.1
Trolox	5.22 ± 0.9

In this study, the antioxidant activities of ethanol and water extracts of *Rosa canina* fruits were evaluated using the DPPH and ABTS methods, revealing that the ethanol extract demonstrated a stronger free radical inhibition capacity compared to the water extract. This result likely stems from the higher concentration of phenolic compounds in the ethanol extract. It is well-documented that the antioxidant activities of plant extracts are largely influenced by the diversity and quantity of phenolic and flavonoid compounds they contain. Secondary metabolites, through functional groups such as phenols and hydroxyls, donate hydrogen and electrons to radicals, playing a role in the reduction of oxidative compounds and consequently exhibiting antioxidant activity[33].

One study highlighted the antioxidant effects of extracts from *Rosa canina*, *Rosa sempervirens*, and *Pyrocantha coccinea*. When assessing the ability to protect against DNA damage, *Rosa canina* exhibited the highest level of protection, followed by *Rosa sempervirens* and *Pyrocantha coccinea*. Furthermore, among these species, the extract of *Rosa canina* was found to significantly reduce reactive oxygen species (ROS) in endothelial cells. Such findings suggest that *Rosa canina* extract could potentially serve as a dietary supplement to prevent pathological conditions arising from oxidative stress[34].

Another study found that the vitamin C content of *Rosa canina* is considerably higher than that found in citrus fruits, with some sources indicating that it has the highest vitamin C content among fruits and vegetables, ranging from 30 to 1300 mg per 100 g[35,36]. In a study aimed at confirming the antioxidant effects of *Rosa canina*, the vitamin C content, which partially contributes to its antioxidant properties, was determined. Extracts were prepared separately from the peel, seeds, and entire fruit, with the antioxidant capacity measured using the DPPH method. The results revealed significant vitamin C content and potent antioxidant properties in *Rosa canina*. Notably, the peel extract showed the highest antioxidant capacity (IC_{50} = 2.05 μg/mL), followed by the whole fruit extract (IC_{50} = 2.59 μg/mL)[37].

Further research involving dried wild *Rosa canina* fruits with three extracts (water, 50% ethanol, and 70% ethanol, all (v/v)). The content of ascorbic acid, tannins, and total phenolics was determined, and antioxidant strength was assessed using DPPH, ABTS, FRAP, and CUPRAC methods. The highest ascorbic acid content was found in the 70% ethanol (v/v) extract, the highest tannin content in the water extract, and the highest phenolic content in the 50% ethanol (v/v) extract. Overall, *Rosa canina* was recognized as a potent source of antioxidants[38]. Using total antioxidant status (TAS) kits, the TAS value of ethanol extracts obtained from the fruits of *Rosa canina* L. collected from Türkiye was determined to be 4.602 mmol/L. As a result, *R. canina* showed high antioxidant activities[39].

A similar study analyzed the antioxidant composition of ethanol extracts of *Rosa canina* fruits using HPLC-UV-MS and investigated their cytotoxic effects on HepG2 and SH-SY5Y cells. The results indicated that *Rosa canina* provides substantial protection against oxidative damage. Additionally, the antioxidant effect was attributed to compounds such as flavonoids, tannins, terpenoids, xanthonoids, and glycerol glucosides. Given its abundance of antioxidant components, *Rosa canina* has been suggested as a potential additive in the food industry and as a dietary component to help control certain cancer types[13]. In a study, it was observed that the highest ascorbic acid content was in the 70% ethanol extract, the highest tannin content was in the water extract, and the highest phenolic content was in the 50% ethanol extract. The results showed that *Rosa canina* L. can be considered as a rich source of antioxidants and has a serious potential as food and herbal cosmetic preparations[38]. A study conducted in Russia revealed that pelargonidin-3,5-diglucoside, an anthocyanin derivative prepared from *Rosa canina*, has a significant radioprotective effect[40].

Rosa canina fruits, which is widely used among the public, should be considered for further in-depth studies to isolate and produce active compounds effective in mitigating damage caused by free radicals. This study is expected to contribute to the existing literature by determining the antioxidant activities of *Rosa canina* fruits, which hold an important position in both domestic and international markets. The findings demonstrate the potential for using the studied extracts as natural sources of antioxidants with health benefits. Additionally, the inclusion of *Rosa canina* extracts in the development of innovative products may contribute significantly to the formulation of value-added food and cosmetic products.

STATEMENT OF ETHICS

Not applicable.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed equally to the article. All authors have read and approved the final published version of the manuscript.

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