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Evaluation of antioxidant, antimicrobial, and α-amylase enzyme inhibition properties of *Rosa canina* seed extracts prepared with different solvents using maceration and ultrasound-assisted extraction methods

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Abstract

The efficient extraction of bioactive molecules from medicinal plants is strongly related to the solvents and methods used. This study evaluated the impact of solvent selection and extraction methods on the bioactive potential of Rosa canina seed extracts, focusing on antioxidant, antimicrobial, and α-amylase inhibitory activities. Four solvents —dimethyl sulfoxide (DMSO), ethanol-water (80:20), ethyl acetate, and acetonitrile— were paired with maceration and ultrasonic techniques to generate eight extracts (DMM, DMU, ESM, ESU, EAM, EAU, ACM, ACS). Antioxidant activity was assessed via total phenolic (TPC) and flavonoid (TFC) content, FRAP, DPPH, and ABTS assays. Antimicrobial efficacy was tested against seven microorganisms (Gram+, Gram−, and yeast) using well diffusion and MIC methods, while α-amylase inhibition was quantified via IC50.

The ethyl acetate ultrasonic extract (EAU) exhibited the highest TPC (63.86 mg GAE/g) and TFC (210.23 mg QE/g), along with superior antioxidant activity across all assays ($P < 0.05$). Ethyl acetate maceration (EAM) ranked second, underscoring ethyl acetate's efficacy. Acetonitrile maceration (ACM) demonstrated the strongest α-amylase inhibition (IC_{50} = 20.46 mg/mL). All extracts showed notable antimicrobial activity against *Aeromonas hydrophila* and *Klebsiella pneumoniae*, though efficacy varied for other strains. DMSO-based extracts (DMM/DMU) consistently underperformed. These findings highlight ethyl acetate (EAU/EAM) as optimal for antioxidant-rich extracts and ACM for enzyme inhibition, positioning *Rosa canina* seeds as a promising source of natural bioactive compounds for functional food and therapeutic applications.

Keywords: Antioxidant, antimicrobial, maceration, rosa canina seeds, solvent, ultrasonication

1. Introduction

There is an increasing interest in medicinal plant products today, and according to the World Health Organization (WHO), although there are differences between developed, underdeveloped, and developing countries, it has been reported that the global trust and usage rate of herbal products for therapeutic purposes has reached up to 80% [1]. During metabolic processes in plants, chemical reactions result in the formation of compounds known as primary and secondary metabolites, which are commonly used in various industrial fields, including agriculture, medicine, and pharmaceuticals, to produce a wide range of products [2].

The genus *Rosa*, which belongs to the Rosaceae family, includes more than 100 species found in North America, Europe, Western Asia, and the Middle East [3, 4], and approximately 25% of these species are found in Türkiye [5]. Rosa species are widely used in traditional medicine. *Rosa canina*, commonly known as Rosehip, Dog Rose, Wild Rose, or Hip Rose, is a shrub that can grow at various altitudes [3]. With cultural diversity, rosehip is consumed in various forms such as herbal tea,

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marmalade, juice, jam, sweets, cakes, and even soup. Its maturation process lasts from the end of September until the beginning of November, and it is harvested during this period [6].

Rosa species are commonly used in traditional treatments for digestive disorders, colds, constipation, and some inflammatory diseases. The fruits of *Rosa canina* are especially consumed in the form of tea during the winter months. Various studies have demonstrated that the fleshy fruit, seeds, roots, flowers, and leaves of rosehip possess bioactive properties that can contribute to modern medicine, in addition to their traditional medicinal uses. These properties include anticancer, antimicrobial, anti-inflammatory, antidiabetic effects, and immune system enhancement [7–9]. It is stated that the therapeutic effects of rose hips are based on its high content of elements and compounds with antioxidant activity [9,10].

The main bioactive compounds in rosehip include amino acids, vitamin C, phenolic acids, flavonoids, carotenoids, anthocyanins, α- and β-tocopherols, tannins, pectins, essential and unsaturated oils, as well as magnesium and calcium [6–11]. Antioxidants neutralize free oxygen radicals, which are known to be effective in the development of various acute and chronic inflammatory diseases, cancer, cardiovascular diseases, and arthritis, thereby playing a protective role against these diseases [5,7]. There are several factors that determine the biological activity of phytochemicals. These factors include the plant part (flower, leaf, fruit, seed, root), maturation time, altitude, temperature, humidity, soil quality, climate-related factors, drying, storage, and preservation conditions, and the method of consumption (infusion, grinding, boiling, direct consumption, etc.) [12,13]. For this purpose, various techniques are employed to effectively utilize bioactive compounds in functional foods.

Rosehip seeds have a higher oil content compared to fruit skin and are considered a good source of unsaturated fatty acids, especially omega-3, oleic, linoleic, and linolenic acids [14,15]. Recently, the use of seed extracts, particularly in industries such as cosmetics, has gained attention, which in turn increases the significance of rosehip. This distinguishing feature of rosehip fruit and seeds may require the use of different extraction methods and solvents. Indeed, less polar or nonpolar solvents may be more effective in extracting the seed content compared to water, particularly for water-insoluble seed compounds.

2. Material and methods

2.1. 2.1. Chemicals and equipment

In this study analytical or HPLC grade reagents and chemicals were used. Chemicals used in phenolic and flavonoid contents, (FRAP, DPPH, and ABTS reactives, gallic acid, quercetin, trolox, Na_2CO_3, $AlCl_3$, NaOH, ascorbic acid, Folin-Ciocalteu reagent), and solvents (dimethyl sulfoxide, acetic acid, ethanol, ethyl acetate, and acetonitrile), Nutrient Broth and Mueller-Hinton Agar media used in antimicrobial activity analysis were purchased from Sigma-Aldrich and Merck (St. Louis and Burlington, MA USA). Instruments and other equipment used are mentioned in the text.

2.2. Plant material

Rosehip fruits were collected in early October 2024 from the Arzular Kabaköy district of Gümüşhane province (40°26'44.4" N - 39°44'40.4" E). The fruits, each weighing approximately 250 grams, were placed in airtight refrigerator bags and transported to the laboratory. The fresh fruit samples were dried at room temperature, away from direct sunlight. After washing, the fruits were further dried in an oven at 37 °C . The seeds were separated from their peels with a scalpel and the remaining fibers were removed with an air sprayer. Seeds were ground into a powder using a grinder (IKA A-10, Germany). The seed powders were aliquoted into 15 mL Falcon tubes and stored at -80 °C in a deep freezer (Thermo Scientific, USA) until further analysis.

2.3. Extraction of rosehip seeds

The extraction methods were partially modified from the method of Oz et al. [16]. Rosehip seed powders were weighed to 2 grams using a precision balance (KERN, Germany) and transferred into 100 mL capped glass bottles. To each seed powder sample, 40 mL of one of the following solvents was added: acidified dimethyl sulfoxide (DMSO, 1% acetic acid), ethanol-distilled water (80:20, v/v), ethyl acetate, or acetonitrile. After the bottles were thoroughly shaken, they were mixed or 30 minutes at room temperature on a magnetic stirrer at 250 rpm.

2.3.1. Maceration method

After magnetic stirring, the samples were incubated for 24 hours in a shaking incubator (Shel Lab, UK) at 37 °C and 150 rpm under dark conditions. Following incubation, the samples were sequentially filtered through coarse filter paper and a 0.45 µm membrane filter. The filtrates were then concentrated using a rotary evaporator (Heidolph, Germany) under reduced pressure (10 mbar) at 60 °C. Any remaining solvent was completely evaporated in a fanned and air-flow oven at 60 °C until dryness was achieved. The dried residues were subsequently dissolved in dimethyl sulfoxide (DMSO), and the final concentrations of the extracts were determined for further analysis (Table 1).

2.3.2. Ultrasonic-assisted extraction (UAE) method

For the ultrasonic-assisted extraction, the extraction procedure was identical to the maceration method up to

the shaking incubator stage. However, prior to the shaking incubator, the samples were treated in an ultrasonic water bath (cleaner ultrasonic bath Daihan Industries South Korea) for 45 minutes at 40 °C and 50% frequency intensity. Following this treatment, the samples were incubated in a shaking incubator, as described in the maceration method, at 37 °C and 150 rpm for 24 hours in a dark environment. After incubation, the samples were filtered as described above, evaporated in a rotary evaporator, and the residues were dissolved in DMSO, with final concentrations determined (Table 1). Samples obtained from both extraction methods were coded and aliquoted into small volumes, then stored at -20 °C until further analysis. Extract methods are shown in Fig. 1.

Extract methods were coded and grouped as follow: **DMM:** Dimethyl sulfoxide –maceration; **DMU:** Dimethyl sulfoxide-ultrasonic; **ESM:** Ethanol/water 80:20–maceration; **ESU:** Ethanol/water 80:20–ultrasonic; **EAM:** Ethyl acetate-maceration; **EAU:** Ethyl acetate-ultrasonic; **ACM:** Acetonitrile-maceration; **ACU:** Acetonitrile-ultrasonic.

Table 1. Final concantrations of *Rosa canina* seed extracts

Extracts	Concentration (mg/mL)
DMM.	38,48
DMU	32,14
ESM	28,42
ESU	30,68
EAM	24,02
EAU	22,97
ACM	20,48
ACU	25,02

2.4. Determination of antioxidant content and activities of seed powder extracts

In this study, the antioxidant content of rosehip seed extracts was evaluated using various solvents and extraction methods, including maceration and ultrasonic-assisted extraction. As antioxidant content and activity parameters, total phenolic content (TPC) total flavonoid content (TFC), ferric reducing antioxidant power (FRAP) assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, and 2,2'-azino-bis 3-ethylbenzothiazolin-6-sulfonic acid scavenging activity (ABTS) assay, which are widely used in the literature of similar studies, were analyzed. In analyses, each sample and standard were run in 3 replicates.

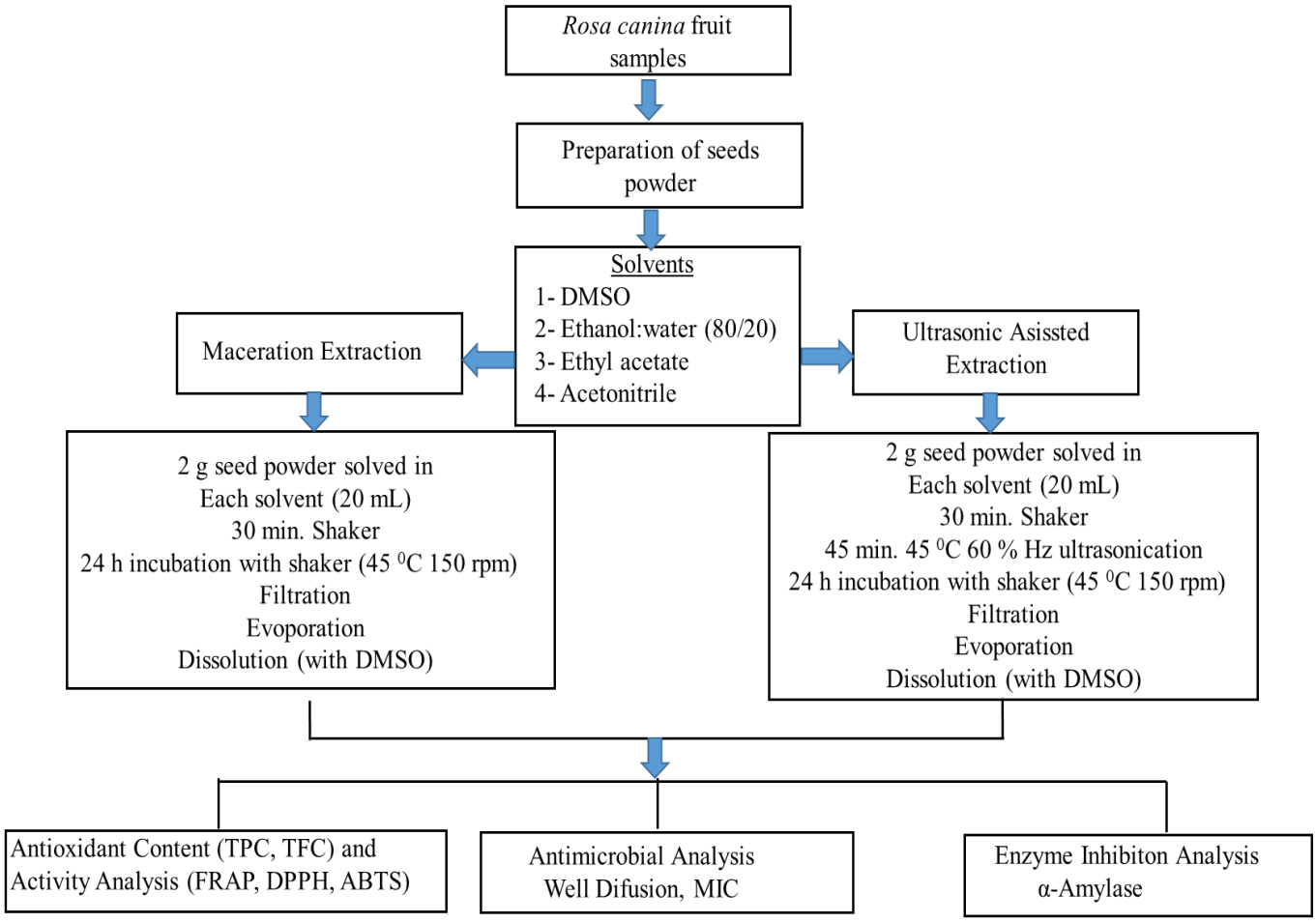

Figure 1. Figurative summary of extract methods and analyses parameters

2.4.1. Determination of total phenolic content (TPC)

Total phenolic content analysis was performed by Johnson et al. modified from the Folin-Ciocalteu procedure of Slinkard and Singleton adapted to microplate [17,18]. The method is based on the reduction of phosphotungstic acid to phosphotungstic blue in an alkaline environment. Gallic acid prepared in different concentrations was used as the standard in the study. Standard graphic equation is determined as y = 0.00723461x + 0.0512804 R^2=0.999. The extracts/standards were diluted as necessary and pipetted into the microplate in a volume of 25 µL, followed by the addition of 10% (v/v) Folin-Ciocalteu reagent (125 µL). 10% sodium carbonate (Na_2CO_3) (100 µL) solution was added to the mixture, and the microplate was thoroughly shaken. The final volumes in the wells were 250 µL. The plate was incubated at room temperature for 2 hours in the dark and read spectrophotometrically at 760 nm using a microplate reader (Thermo Fisher Sci. MultiScan Go Walham, Massachusetts USA). The results were expressed as mg/g of gallic acid equivalent (mg GAE /g extract).

2.4.2. Determination of total flavonoid content (TFC)

The total flavonoid assay will be performed according to the method adapted to a 96-well microplate, as described by Kamış et al. [19] and based on the aluminum chloride colorimetric method by Beara et al. The procedure was carried out as follows: 50 µL of sample/standard was pipetted into the microplate, followed by the addition of 30% methanol-water (120 µL), 0.5 M 15 µL sodium nitrite ($NaNO_2$), and 0.3 M 15 µL aluminium chloride ($AlCl_3$) in sequence. After a 5-minute incubation, 1 M 100 µL sodium hydroxide (NaOH) was added to the mixtures, and the final well volume was adjusted to 300 µL. The mixtures were incubated in the dark at room temperature for 40 minutes. Absorbance values were measured spectrophotometrically at 406 nm against reagent and sample blanks using a microplate reader. Quercetin, prepared in five different concentrations, was used as the standard. The standard curve equation was determined as y=0.000581872x+0.0183755 with R^2 = 0.998. The total flavonoid content (TFC) of the extracts was expressed as quercetin equivalents (mg QE/g extract).

2.4.3. DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging method

The DPPH radical scavenging method was adapted from the procedure used by Soler-Rivas et al. [20] for a 96-well microplate. In this method, 25 µL of diluted extract/standard was pipetted into the microplate. Then, 100 µM DPPH 185µL (1,1-diphenyl-2-picrylhydrazyl) and 90 µL methanol were added in sequence. The final volume was adjusted to 300 µL. After incubation for 30 minutes in the dark at room temperature, the absorbance was measured spectrophotometrically at 517 nm against blanks using a microplate reader. Trolox and ascorbic acid were used as standards. The results were expressed as trolox equivalent antioxidant capacity (TEAC) and ascorbic acid equivalent (AAE). The standard curve equations were determined as y = -0.00433359x + 1.6969 with R^2 = 0.9946 for Trolox and y = -0.00368613x + 1.29333 with R^2 = 0.990 for ascorbic acid.

2.4.4. Ferric reducing antioxidant power (FRAP) method

This method was modified from Benzie and Strainin's method to be applied to a 96-well microplate [21]. The principle of the method is based on the reduction of Fe^{3+} to Fe^{2+} in the presence of antioxidants. Standard solutions of Trolox and ascorbic acid were prepared in five different concentrations, and standard curves were generated. The standard curve equations were y = 0.0110624x + 0.0412819 with R^2 = 0.999 for Trolox and y = 0.013392x + 0.127341 with R^2 = 0.995 for ascorbic acid. In short, 25 µL of extract/standard was pipetted into the microplate, and 275 µL of FRAP reagent [300 mM acetate buffer + 10 mM 2,4,6-Tris(pyridyl)-S-triazine (TPTZ) solution + 20 mM $FeCl_3$ solution (10:1:1)] was added. The final volume was adjusted to 300 µL. Absorbance values were measured spectrophotometrically at 593 nm against blanks using a microplate reader. Results were expressed as mg TEAC/g and mg AAE/g.

2.4.5. ABTS radical scavenging capacity

This method was modified from the procedure used by Silva et al. [22], which was adapted for a 96-well microplate from Re et al. [23]. Briefly, 25 µL of sample/standard was pipetted into the microplate. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical solution, adjusted to an optical density of 0.7 with 7 mM ABTS + 2.45 mM potassium persulfate) was added to achieve a final volume of 200 µL. After incubating at room temperature for 5-6 minutes, absorbance was measured spectrophotometrically at 734 nm using a microplate reader. Trolox and ascorbic acid solutions at five different concentrations were used as standards. The standard curve equations were determined as y=-0.00766426x+2.0314 with R^2=0.999 for Trolox and y=-0.010095x+2.03325 with R^2=0.999 for ascorbic acid. The analysis was performed in triplicates, and the results were expressed as mg TEAC/g and mg AAE/g.

2.5. Determination of antimicrobial activities

2.5.1. Agar well diffusion method

The antimicrobial activities of the 8 different rosehip extracts were determined using the well diffusion method with some modifications based on the CLSI (Clinical & Laboratory Standards Institute: CLSI

Guidelines [24]. The test organisms used were Gram-positive bacteria *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 9634; Gram-negative bacteria *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *Proteus vulgaris* ATCC 13315, *Aeromonas hydrophila* ATCC 35654 and fungi *Candida albicans* ATCC 18804. Fresh cultures of the microorganisms were prepared and diluted to a turbidity of 0.5 McFarland standard (1.5×10^8 microorganisms/mL) using sterile distilled water. The microorganisms were inoculated onto Müller-Hinton Agar petri dishes with the help of a sterile swab. Wells were created on the inoculated Petri dishes, and then 50 µL of the extract solutions were pipetted into the wells. The plates were incubated at 4 °C for 2 hours to allow for diffusion of the extracts into the agar. Chloramphenicol was used as a positive control, and DMSO was used as a negative control. After incubation, the dishes were incubated at 37 °C for 24 hours for bacteria and at 28 °C for 48 hours for yeast. The inhibition zone diameters surrounding the wells were measured using a digital caliper. Analyses were run in 3 replicates.

2.5.2. Minimal inhibitory concentrations analysis of extracts

The minimum inhibitory concentration (MIC) values of the extracts were analyzed following the CLSI [24]. guidelines with some modifications. The method involved applying the broth dilution technique to 96-well sterile microplates with lids. Mueller-Hinton Broth was used as the culture medium. Unlike the other wells, the first wells received 100 µL of the culture medium prepared at twice the concentration, while the other wells were inoculated with 100 µL of the standard concentration medium. Then, 100 µL of rosehip extract was pipetted into the first wells. Starting from an initial concentration of 2750.0 µg/mL, a twofold serial dilution was performed to prepare 10 different concentrations, ranging down to 5.0 µg/mL. Similarly, chloramphenicol was used at an initial concentration of 256 µg/mL. Suspensions of standard microorganisms (at a 0.5 McFarland turbidity) were inoculated into the microplates. After incubation in an incubator at 37 °C for 24 hours for bacteria and 28 °C for 48 hours for yeast, microbial growth was observed using a microplate reader. The lowest concentrations of rosehip extracts and antibiotics that inhibited the growth of microorganisms were given as µg/mL as MIC values.

2.6. Determination of α-amylase enzyme inhibitory effect of seeds extracts

The α-Amylase inhibitory activity of rosehip extracts was determined using the iodine/potassium iodide method, adapted from the method of Yang et al. [25]. Extract solutions were prepared at five different concentrations and pipetted into the wells of a microplate in 25 µL volumes. Then, a 0.5 mg/ml α-amylase enzyme solution was prepared using phosphate buffer (pH 6.9, 6 mM sodium chloride) and 50 µL of this solution was added to the wells. The samples in the microplate were incubated at 37 °C for 10 minutes. After the pre-incubation, 50 µL of 0.1% starch solution was added to each well to initiate the reaction. The samples were incubated again at 37 °C for 10 minutes, after which 25 µL of 1 M HCl was automatically added to stop the reaction. To observe the color change, 100 µL of iodine-potassium iodide solution was added to the wells. The absorbances of the extracts and the control were spectrophotometrically measured in a microplate reader set to 630 nm wavelength. Acarbose was used as a standard at concentrations of 10, 25, 50, 100, and 200 µg/ml. The α-amylase inhibitory activity results of the extracts and acarbose were presented as IC_{50} (mg/mL) values.

2.7. Statistics

To compare the extracts with each other, ANOVA test was applied. ANOVA, Analysis of Variance, was conducted using Microsoft Excel with the help of XLSTAT (Addinsoft, 2024, XLSTAT statistical and data analysis solution, New York, USA). Results are given as mean ± standard deviation. P <0.05 was considered a statistically significant difference.

3. Results and discussion

3.1. Results of TPC TFC, and antioxidant activity

In this study, a total of 8 different extracts were obtained with 4 different solvents and 2 different extraction methods. TPC and TFC antioxidant activity results are shown in Table 2.

Total phenolic content (TPC) is an indicator of the antioxidant potential of medicinal plants because phenolic compounds are known for their ability to neutralize free radicals. Rosehip is known as an important source of antioxidants. As with many plants, the bioactive properties of rosehip fruits can vary depending on several factors such as ripening and harvesting time, storage, altitude, temperature, humidity, and climate [6]. Moreover, the effective extraction of the total phenolic content (TPC) and total flavonoid content (TFC) from rosehip fruits and seeds is influenced not only by the aforementioned conditions but also by solvents and extraction methods [11]. The polarity of the solvent is a significant parameter that affects the release of phytochemicals responsible for bioactive properties during the extraction process [26,27].

Table 2. Comparison of antioxidant content and activites of rosehip seed extracts prepared by maceration and ultrasonic assisted methods using different solvents

Extract	TPC mg GAE/g	TFC mg QE/g	DPPH mg TEAC/g	DPPH mg AAE/g	ABTS mg TEAC/g	ABTS mg AAE/g	FRAP mg TEAC/g	FRAP mg AAE/g
DMM	5.66 ±0.36 [a]	22.53 ±0.78 [a]	5.98 ±0.49 [a]	4.74 ±0.28 [a]	19.44 ±0.09 [a]	17.82 ±0.06 [a]	4.63 ±0.09 [a]	2.15 ±0.08 [a]
DMU	7.26 ±0.70 [a]	25.88 ±3.21 [a]	10.79 ±0.85 [b]	9.47 ±0.77 [b]	24.72 ±0.52 [b]	22.33 ±0.36 [b]	6.93 ±0.26 [b]	±3.73 ±0.21 [a]
ESM	47.31 ±3.29 [d]	133.04 ±3.25 [e]	57.37 ±2.85 [d]	39.13 ±2.17 [d]	59.26 ±2.50 [e]	46.90 ±1.73 [e]	50.63 ±2.48 [f]	39.56 ±2.05 [e]
ESU	41.10 ±0.77 [c]	107.22 ±2.81 [d]	63.51 ±1.30 [e]	38.79 ±1.85 [d]	54.74 ±2.97 [d]	43.34 ±2.05 [d]	46.79 ±0.72 [e]	36.55 ±0.59 [d]
EAM	60.53 ±3.14[e]	189.90 ±3.12 [f]	115.34 ±2.53 [f]	107.57 ±1.93 [f]	84.07 ±1.83 [f]	64.61 ±1.97 [f]	63.88 ±1.07 [h]	49.18 ±2.30 [g]
EAU	63.86 ±3.11[e]	210.23 ±3.61[g]	117.43 ±2.49 [f]	91.08 ±2.39 [e]	97.52 ±2.16 [g]	73.65 ±2.67 [g]	59.40 ±1.09 [g]	45.56 ±1.94 [f]
ACM	16.00 ±1.13 [b]	31.09 ±2.13 [b]	17.40 ±1.53 [c]	14.37 ±0.33 [c]	26.59 ±2.01 [b]	26.60 ±1.39 [c]	±17.45 ±0.66 [c]	10.25 ±0.84 [b]
ACU	16.62 ±2.69 [b]	44.22 ±1.56 [c]	18.61 ±0.90 [c]	16.69 ±0.62 [c]	31.25 ±1.42 [c]	28.34 ±0.99 [c]	19.63 ±0.68 [d]	13.71 ±0.52 [c]

DMM: Dimethyl sulfoxide –maceration; **DMU:** Dimthyl sulfoxide-ultrasonic; **ESM:** Ethanol/water 80:20 –maceration; **ESU:** Ethanol/water 80:20–ultrasonic; **EAM:** Ethyl acetate-maceration; **EAU:** Ethyl acetate-ultrasonic; **ACM:** Acetonitrile-maceration; **ACU:** Acetonitrile-ultrasonic. Different letters (a-h) in the same column are significantly different (p <0.05).

In our study, combinations of 4 solvents with varying polarities and extraction methods were employed.

Among the 4 solvents, EAU (Ethyl Acetate Ultrasonic) exhibited the highest TPC amounts (63.86 mg GAE/g) and significant differences from all other extracts (except EAM) demonstrating that ultrasonic-assisted extraction of the rosehip seeds with ethyl acetate was highly efficient for TPC ($p<0.05$). EAM (Ethyl Acetate Maceration) had the second highest content (60.53 mg GAE/g) after EAU. However, there was no significant difference between these two extracts. It was observed that DMM (Dimethyl sulfoxide –Maceration) and DMU (Dimethyl sulfoxide –Ultrasonic) extracts had the lowest TPC values (5.66; 7.26 respectively). When the extracts are compared in terms of TPC content, the order EAU>EAM>ESM>ESU> ACU>ACM>DMU>DMM is seen. When TFC values were examined, it was observed that, similar to TPC, the highest level was in the EAU (210 mg QE/g) extract, followed by EUM (189.9 mg QE/g) and DMM (22.5 mg QE/g), and DMU (25.8 mg QE/g) had the lowest values, respectively. In TFC content comparison, extracts are listed as EAU>EAM>ESM>ESU>ACU>ACM>DMU>DMM. In this study, these findings suggest that ultrasonic-assisted extraction using ethyl acetate was again the most effective method for extracting phenolics and flavonoids from rose hip seeds.

The DPPH and ABTS assays are commonly used to assess the free radical scavenging ability of plant extracts. The FRAP assay evaluates the ability of extracts to reduce ferric ions, which is another important measure of antioxidant activity. In this study, these 3 commonly used antioxidant activity determination methods were preferred (Table 2).

It was determined that the ethyl acetate extracts (EAU and EAM) exhibited the highest values in all antioxidant activity analysis methods. Additionally, these values showed a significant difference compared to the values of other solvent extracts. However, when considering TEAC and AAE standards, the highest antioxidant activity values varied between EAU and EAM. For instance, in the DPPH assay, EAU had 117.43 mg TEAC/g, while EAM had 115.34 mg TEAC/g, and in terms of ascorbic acid standard, EAM was at 107.57 mg AAE/g, while EAU was at 91.08 mg AAE/g. Similarly, in the FRAP analysis, EUM was found to be significantly higher than EUA and all other extracts. Among the extracts, DMM and DMU showed the lowest values in terms of all three antioxidant activity methods.

In the literature, there are more studies on the antioxidant content and activity of rosehip fruit samples extracted with different methods and solvents compared to studies involving rosehip seeds. This could be due to the higher usage of the fruit compared to the seed. Montazari et al. used six different solvents (*n*-hexane, ethyl acetate, chloroform, acetone, water and methanol) to extract Iranian *Rosa canina* fruits using the maceration method and dissolved the residues in DMSO after evaporation. The researchers found that the four extracts with the highest TPC contents were ranked as methanol, acetone, water, and ethyl acetate (424.4, 295.8, 220.2, 173.3 mg GAE/g extract respectively). In terms of TFC, ethyl acetate performed better than water in the same ranking [28].

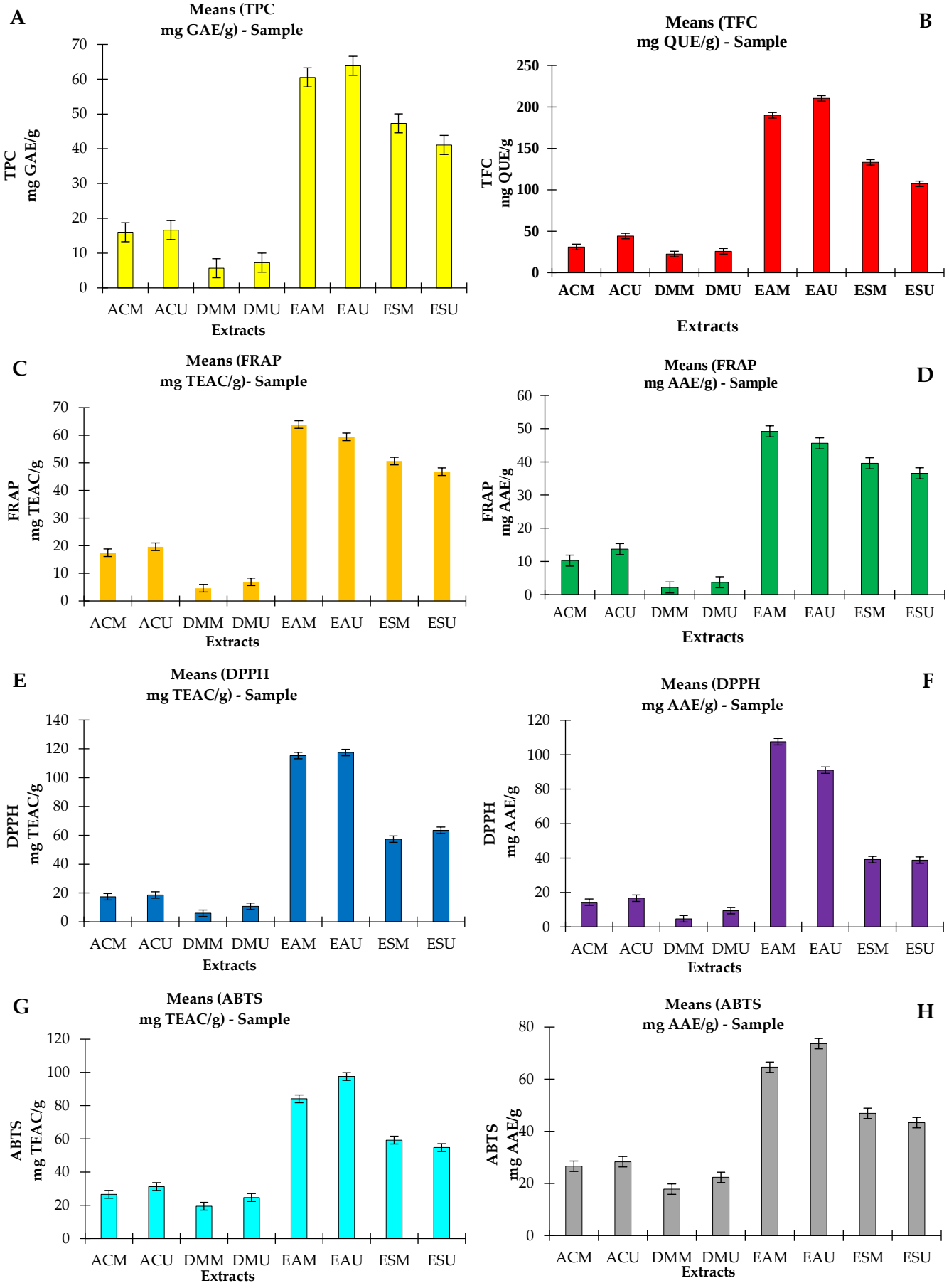

Figure 2. Graphical representations of solvent and methodological comparisons of phenolic and flavonoid contents and antioxidant activities of seed extracts. **A:** TPC (mg GAE/g); **B:**TFC mg QUE/g; **C:** FRAP (mg TEAC/g); **D:** FRAP mg AAE/g); **E:** DPPH (mg TEAC/g); **F:** DPPH (mg AAE/g): **G:**ABTS mg (TEAC/g); **H:** ABTS (mg AAE/g)

Graphical representations of solvent and methodological comparisons of phenolic and flavonoid contents and antioxidant activities of seed extracts are given in Fig 2.

In another study conducted in Turkey, the whole fruit (WF), pulp (P), and seeds (S) of *Rosa pimpinellifolia* (black rosehip) were analyzed for TPC, DPPH, ABTS, and CUPRAC (cupric reducing antioxidant capacity). It was determined that the seed TPC values were lower compared to those of WF and P; however, the seeds exhibited higher values in all three antioxidant activity assays than the other parts. The researchers, using an acidified ethanol-water mixture (50:50, v/v) as the solvent, employed the ultrasound-assisted extraction (UAE) method with three repetitions (30 minutes at temperatures ranging from 30 to 40 °C) for the extraction process [29].

TPC and TCF values in rosehip seeds may vary according to regional and methodological differences. In some studies conducted on Rosa canina seeds collected from Gümüşhane-Erzurum regions, TPC values were reported to be between 2.55 mg GAE/g and 52 mg GAE/g [30]. In a study using 80% methanol, 70% acetone and 60% ethanol as solvents by maceration method, ABTS values in rosehip seed powders were determined as approximately 2.5 mg TEAC/g for methanol and acetone, while ethanol was found to be lower, and TPC was determined as 2.55 mg GAE/g in the same study [31]. Çürük et al. determined DPPH value as 16.2 mg TEAC/g in rosehip seed powder obtained by supercritical carbon dioxide extraction method [32]. In another study, the DPPH value of 75% methanolic and 0.1% formic acid extract of dried and frozen rosehip seed powder in liquid nitrogen was found to be 5.38 mg TEAC/g. [30].

In recent years, the use of different extraction methods other than the traditional maceration-solvent extraction method, such as ultrasonic assisted extraction (UAE), microwave assisted extraction (MAE), and supercritical fluid extraction (SFE) has increased. UAE uses a combination of thermal and mechanical effects to elicit bioactive components and is generally effective at low frequencies of 20–100 kHz. Higher frequencies can do more harm than good [33,34].

In ultrasound-assisted extraction, several variables must be considered: sonication time, frequency, and temperature, along with the solvent/solute ratio, which are identified as the main variables [35]. In this study, both maceration and UAE methods were applied using the same solvents. When comparing the UAE and maceration methods, the highest values in terms of antioxidant activity, TPC, and TFC content were found in the EAM and EAU solutions. Among these, EAU exhibited statistically significantly higher TFC and ABTS values (mg TEAC/g and mg AAE/g, respectively) than EAM ($P < 0.05$). Ultrasonic extracts of dimethyl sulfoxide and acetonitrile (DMU and ACU) had higher values for all antioxidant parameters compared to the extracts obtained by maceration (DMM and ACM). These increases were more distinctive in DMU. After ethyl acetate extracts, ESM and ESU extracts exhibited the highest antioxidant parameters, with the exception of DPPH (mg TEAC/g), where ESM showed higher values than ESU.

In general, it is reported that increasing the ultrasonication time will increase the mass transfer to the solvent and accordingly the amount of phenolic and flavonoid substances will increase, but the phenolic content may decrease due to the structural degradation of phenolic compounds as the ultrasonication time increases [36]. In a study, antioxidant activity and penolic content were compared by ultrasonically assisted extraction of rosehip fruits using variable parameters consisting of 3, 6, 9, 12, and 15 minutes sonication time, 25, 50 and 100 % amplitude and 20, 30, and 50 °C temperature. It was shown that ultrasonication time and temperature increased the amount of phenolic matter, but amplitude was not effective [35].

3.2. Results of the antimicrobial activity of different rosehip extracts

3.2.1. Results of agar well diffusion method

Results of the antimicrobial activity of different rosehip extracts against 7 microorganisms were shown in Table 3.

In this study, all rosehip seed powder extracts showed significant antimicrobial activity against *A. hydrophilia and K. pneumonia* strains. It was found that the highest activities were shown by DMU, ESU, and DMM extracts against *A.hydrophila* strain (24.43, 23.23, and 22.23 mm, respectively). No antimicrobial activity of any extract was detected against *E. facialis*. Against *S. aureus*, activity was seen only in EAM and EAU. It was also determined that ACM and ACU only exhibited activity against *A. hydrophila* and *K. pneumoniae* strains. *Rosa canina* is a well-known and extensively studied medicinal plant for its antimicrobial activity. However, most studies on antimicrobial activity have focused primarily on the fruit and flowers. Extraction methods, solvents used, the time of fruit collection, and climatic conditions are influential factors in the expression of the plant's antimicrobial properties [13,37]. In a study with methods similar to ours, *Rosa pimpinellifolia* plant parts, including seeds, were extracted using UAE with a solvent consisting of an ethanol-water (50:50) mixture. In the antimicrobial activity analyses, it was observed that the seed extract was effective against *B. cereus* and *S. aureus*, but exhibited a lower effect compared to the whole fruit [31].

Table 3. Result of antimicrobial activity of different rosehip extracts (zone diameter mm)

Extracts	*S. aureus*	*E. faecialis*	*C. albicans*	*P. vulgaris*	*A.hydrophila*	*B. cereus*	*K. pneumoniae*
DMM	NI	NI	11.43 ±0.31	16.70 ±0.72	22.23 ±0.67	NI	17.53 ±0.67
DMU	NI	NI	NI	15.43 ±0.50	24.43 ±0.91	NI	17.70 ±0.72
ESM	NI	NI	15.50 ±0.66	15.40 ±0.46	23.23 ±0.70	12.57 ±0.68	15.33 ±0.35
ESU	NI	NI	14.63 ±0.40	15.63 ±0.55	20.60 ±0.46	12.10 ±0.36	15.03 ±0.61
EAM	15.70 ±0.46	NI	19.90 ±0.56	NI	19.33 ±0.38	10.70 ±0.44	16.90 ±0.56
EAU	15.60 ±0.36	NI	14.00 ±0.75	NI	20.73 ±0.68	10.67 ±0.25	17.57 ±0.47
ACM	NI	NI	NI	NI	21.40 ±0.89	NI	15.40 ±0.53
ACU	NI	NI	NI	NI	19.87 ±0.60	NI	16.73 ±0.60
CHL	26.63 ±0.31	25.73 ±0.51	30.10 ±0.80	30.63 ±0.86	25.10 ±0.62	22.93 ±0.74	30.40 ±0.85
DMSO	NI	NI	NI	NI	NI	NI	NI

CHL: Chloramphenicol; **DMSO:** as a negative control **NI**: No inhibition. Results were given mean ± Standard deviation (SD)

In a homogenization-based study, evaluating rosehip fruit samples from Samsun, Türkiye, where phosphate buffer was used as the solvent, it was observed that, in contrast to our study, *E. facialis* and *S. aureus* strains were effectively inhibited by the rosehip fruits [37].

Phytochemical components of the extract may show different effects against different microorganisms. For example, citric acid has a pronounced antimicrobial effect against *Staphylococcus aureus* and *Klebsiella aerogenes*, and quinic acid against *S. aureus*. Researchers suggest that solvents may play an important role in antimicrobial activity in this case [28,38].

3.2.2. Results of minimal inhibitory concentrations of extracts

The minimal inhibitory concentration (MIC) values of the extracts obtained by maceration and UAE methods with different solvents against various microbial strains are given in Table 4.

In this context, a lower MIC value indicates a higher level of antimicrobial activity. When the extracts were evaluated in terms of both solvent and method, it was determined that the minimum inhibitory concentrations (MICs) varied according to the microorganisms.

The lowest MIC values were observed against the Gram (-) bacteria *A. hydrophila* and *K. pneumoniae* in the EAU and EAM extracts (0.29 and 0.30 mg/mL, respectively). All other extracts had lower MIC values against these two strains compared to other microorganisms. The highest MIC values were observed against *E. faecialis*. DMM and DMU had the highest MIC values against *S. aureus, E. faecialis*, *A. hydrophila*, and *K. pneumoniae* compared to the other extracts. Extracts obtained by UAE and maceration exhibited similar MIC values for the same solvents, and these values varied depending on the microorganisms.

In antimicrobial activity analyses of *Rosa canina* fruit extracts prepared using the maceration method and six different solvents, it was observed that methanolic extracts showed the lowest MIC values against *C. albicans, B. cereus, and S. aureus* strains, followed by aqueous extracts. In this study, chloroform and n-hexane exhibited no activity, while ethyl acetate showed activity only against *S. aureus*, and acetone demonstrated activity against all strains except *B. cereus* [28].

Table 4. Results of minimal inhibitory concentrations (MIC) of seed extracts (mg/mL)

Extracts	*S. aureus*	*E. faecialis*	*C. albicans*	*P. vulgaris*	*A.hydrophila*	*B. cereus*	*K. pneumoniae*
DMM	3.85	3.85	1.92	0.96	0.48	3.85	0.48
DMU	3.21	3.21	3.21	0.80	0.40	1.61	0.40
ESM	2.84	2.84	0.71	0.71	0.36	0.71	0.36
ESU	3.07	3.07	0.77	0.77	0.38	0.77	0.38
EAM	0.60	2.40	0.60	2.40	0.30	1.20	0.30
EAU	0.57	2.30	0.57	2.30	0.29	1.15	0.29
ACM	2.05	2.05	2.05	2.05	0.26	2.05	0.26
ACU	2.50	2.50	2.50	2.50	0.31	2.50	0.31
CHL	0.02	0.01	0.001	0.001	0.02	0.01	0.01
DMSO	0.00	0.00	0.00	0.00	0.00	0.00	0.00

CHL: Chloramphenicol as antibiotic; **DMSO:** Dimethyl sulfoxide as negative control. The results are presented in mg/mL (mean ± Standard deviation (SD).

3.3. Results of α-amylase enzyme inhibitory activity of extracts

Results of α-amylase enzyme inhibitory activity of extracts were given in Table 5. α-Glucosidase hydrolyzes starch and disaccharides in the intestine and ensures the absorption of glucose [39]. α-Amylase breaks down polysaccharides into maltose and glucose. Both of these enzymes have the effect of increasing glucose concentration in the blood, and inhibitors of these enzymes are used in diabetes treatments [40,41].

The IC_{50} values presented in the table reflect the inhibitory activity of various extracts on α-amylase enzyme activity, with lower IC_{50} values indicating greater enzyme inhibition. This analysis is crucial as α-amylase plays a significant role in carbohydrate metabolism, making its inhibition relevant for conditions such as diabetes and obesity [40].

In our study, ACM and EAU extracts had the highest inhibitory effect on α-amylase with the lowest IC_{50} values (20.48 and 22.97 mg/mL, respectively). In contrast, DMM (DMSO Mas) exhibited the least inhibition, with an IC_{50} of 38.48 mg/mL, indicating it is less effective compared to the other extracts. In general, the inhibitory effects of rosehip extracts on α- amylase enzyme were found to be weaker than acarbose, which is an α-amylase inhibitor. The effects of solvents on the bioactivity of extracts via phytochemicals may also apply to enzyme inhibition. Indeed, in a study conducted to determine the α-glucosidase enzyme inhibitory effect of rosehip fruits and using acetone, methanol, ethyl acetate, and *n*-hexane as solvents, it was reported that acetone extract had the strongest inhibitory effect, followed by methanol and ethyl acetate, while *n*-hexane showed a very low effect [42]. However, in a study showing the α-amylase inhibitory effect of rosehip fruit extract dissolved in liquid nitrogen and extracted with methanol, it was determined that the fruit extract showed 100% inhibitory effect at a concentration of 5.5 mg/ml. [43].

Table 5. Results of α-amylase enzyme inhibitory activity of extracts

Extracts	IC_{50} (mg/mL)
DMM	38.48 ± 1.26^{g}
DMU	32.14 ± 0.89^{f}
ESM	28.42 ± 1.13^{e}
ESU	30.68 ± 0.46^{f}
EAM	24.02 ± 0.58^{cd}
EAU	22.97 ± 1.23^{c}
ACM	20.48 ± 1.47
ACU	25.02 ± 0.56^{d}
Acarbose (μg/mL)	123.750 ± 2.01^{a}

Note: Results are presented as means ± standard deviations. A variety of letters (a-g) in the same column indicate significant differences, attaining statistical significance at the 95% level ($P < 0.05$).

While their efficacy is lower than that of acarbose, their natural origin and potential for fewer side effects make them an attractive area of study. The role of solvents in enhancing bioactivity further underscores the importance of optimizing extraction methods to maximize the therapeutic potential of plant-based extracts.

4. Conclusion

This study demonstrates the critical role of solvent selection and extraction methodology in efficiently unlocking the bioactive potential of *Rosa canina* seeds. Ethyl acetate combined with ultrasound-assisted extraction (EAU) yielded extracts with the highest phenolic (63.86 mg GAE/g) and flavonoid (210.23 mg QE/g) content, alongside superior antioxidant activity in FRAP, DPPH, and ABTS assays. Ethyl acetate maceration (EAM) also showed exceptional performance, confirming the solvent's efficacy in enhancing bioactive compound recovery.

In particular, acetonitrile maceration (ACM) is thought to be used to control postprandial blood glucose levels in diabetes based on its concentration of α-amylase enzyme inhibition (IC_{50} = 20.46 mg/mL). Antimicrobial analysis revealed broad-spectrum efficacy against *Aeromonas hydrophila* and *Klebsiella pneumoniae*, though activity varied against other microbial strains. This finding highlights the potential for developing targeted antimicrobial formulations.

The aim of this study was to present feasible strategies to maximize the bioactivity of Rosa canina seeds by optimizing solvent polarity and extraction dynamics. The results are expected to bring new solutions and approaches in health and other plant-based industrial fields such as functional foods, natural preservatives and herbal therapeutic products.

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