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Research Article

Phenolic Compound Content and Antioxidant Activity of *Rheum ribes* Shells

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The antioxidative and therapeutic properties of *Rheum ribes*, a plant indigenous to Turkey, have been extensively researched. However, little attention has been paid to compounds extracted from *R. ribes* shells. This study focused on assessing the phenolic compound content and antioxidative capabilities of *R. ribes* shells. We identified 44 out of 88 phytochemical compounds present in these shells using liquid chromatography-high-resolution mass spectrometry. Among these compounds, rutin hydrate M-OH_2, kuromanine, and procyanidin B2 emerged as the most abundant, whereas sinapic acid had the lowest concentration. Furthermore, antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl assay, with the *R. ribes* shell extract exhibiting an activity level of 1.06 ± 0.3 mg Trolox equivalent/g of sample. In summary, this research explored the potential health advantages of *R. ribes* shells, thereby offering valuable insights for discovering novel bioactive compounds in natural resources for future drug development.

1. Introduction

Phytochemicals are plant-derived chemicals primarily composed of polyphenols. In addition to essential nutrients, vitamins, and minerals, polyphenols are important for human health. The spectrum of phytochemicals within the human diet is estimated to encompass a wide range, from 5,000 to 25,000 phytochemicals, found abundantly in various food sources, including fruits, vegetables, seeds, and other plant parts. The bioactivity of these phytochemicals extends to a myriad of health benefits, contributing to the mitigation of health risks, such as Alzheimer's disease, heart diseases, cancers, and diabetes [1–4]. Nonetheless, among approximately 250,000 flowering plants cultivated globally, only a scant fraction has been studied for active substances. Consequently, numerous unexplored phytochemical compounds exist within plant sources, awaiting discovery and characterization [5–8].

Rheum ribes L., also known as Uşgun, belongs to the Polygonaceae family. Uşgun grows in rocky and hilly areas at altitudes of 2100–2800 m. Exclusive to Turkey's eastern Anatolia region, this plant is the only species within the *Rheum* genus.

The stems of this plant have thick rhizomes and can grow up to a height of 50 cm. The leaves are characterized by their green and red coloration, wide kidney-shaped appearance, and the presence of stems. The flowers are small and yellow. Petioles and fresh stems can be consumed raw or after being cooked [9].

Free radicals are composed of atoms and molecules with unshared electrons. Free radicals are either generated endogenously (via mitochondrial propagation, respiration, enzymes, and auto-oxidation reactions) or exogenously (in response to drugs, UV rays, alcohol, ionizing radiation, xenobiotics, environmental pollution, and cigarette smoke). In living systems, oxygen usage generates free radicals, which scavenge electrons from vital cellular components and disrupt membrane structures [10, 11]. Therefore, to prevent the damage caused by toxic free radicals, organisms have developed defense systems based on antioxidants that can neutralize free radicals, which can contribute to a variety of health issues, including premature aging, cardiovascular diseases, diabetes, and cancer. Antioxidants prolong food shelf life, preserve sensory qualities, and enhance overall quality by preventing rancidity in oily foods. They play

a crucial role in improving nutritional value and meeting consumer preferences in the food sector [12–14]. Phenolic compounds are prevalent in plant- and animal-derived foods, and this is valuable in the context of both human and animal health. They exert antioxidant, anti-inflammatory, antibacterial, and antiviral effects, thereby contributing to disease prevention and bolstering overall health [15]. Due to cost considerations, synthetic antioxidants are generally preferred over their natural counterparts. However, some studies have revealed that synthetic antioxidants can be toxic and carcinogenic. Previous studies have investigated the applicability of natural substances as antioxidants in food, reporting them to be more effective than synthetic antioxidants [16–18]. According to the World Health Organization (WHO), approximately 20,000 plants are utilized for their medicinal properties. Research conducted by the Food and Agricultural Organization revealed 21,000 medicinal plant varieties, 5000 of which are commercially traded. In Turkey, only 500 of the 9000 available medicinal plants are used for therapeutic purposes. Between 2001 and 2005, the WHO initiated a program to support and improve traditional treatment methods in developing countries [19–22]. Hence, organic sources, such as plants abundant in antioxidants, hold significant value for human health and scientific research [23].

Traditional uses of *Rheum ribes* include the utilization of fresh shoots and stems in the amelioration of diverse medical conditions, such as hemorrhoids, measles, smallpox, gastrointestinal ailments, and diarrhea. Moreover, the plant exhibits therapeutic efficacy against disorders, such as diabetes, ulcers, hypertension, obesity, and digestive disorders. In countries other than Turkey, this plant is cultivated from young shoots. Notably, the roots and shoots of *R. ribes* exhibit high antioxidant activity [24–27]. In Turkey, where *R. ribes* proliferates abundantly in its natural habitat, it is a staple in the local diet, leading to the routine disposal of considerable quantities of its shells on an annual basis. The discarded shells of *R. ribes* carry components with promising applications in health, cosmetics, food, and pharmaceutical sectors. Surprisingly, despite their potential, there is little scientific evidence regarding the properties of compounds derived from these shells. This unexplored territory presents an opportunity for further scientific investigation that may lead to potential innovations in various industries.

In this study, our main goal was to analyze the phenolic composition and antioxidant capacity of the *R. ribes* shells. We evaluated the antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and determined the phenolic composition of the *R. ribes* shells using liquid chromatography-high-resolution mass spectrometry (LC-HRMS). This investigation aimed to identify new nutritional resources that could be instrumental in the prevention of various diseases. This study can serve as a guide for the production of herbal medicines and offers a valuable resource, namely, the shell of *R. ribes*, for future research in this area.

2. Materials and Methods

2.1. Collection of Plant Materials. *R. ribes* was collected in the Pervari district of Siirt province, Turkey, where it grows naturally, at the beginning of May 2023. *R. ribes* plant is a plant known to everyone in the region, like other vegetables and fruits widely consumed in the region. After collection, the shells were left to dry in a dark room at 24°C for 20 d. Once dried, the shells were ground into a powder and utilized for subsequent experiments.

2.2. Antioxidant (DPPH) Activities

2.2.1. DPPH Radical-Scavenging Capacity. The extract was prepared by combining 0.2 g powdered shell samples with 5 mL methanol solution (75%, with 0.1% phosphoric acid). Phosphoric acid is used in the DPPH method to provide acidic conditions, increase solubility, control reaction rate, and ensure stability. The mixture was thoroughly homogenized using an Ultra-Turrax (MS3-MaxiHomo35, Staufen, Germany) device at 600 rpm for 30 s. Next, the mixture was centrifuged at $3600 \times g$, 5000 rpm (Archer LC-05A; Archer, San Jose, CA, USA) for 10 min at 24°C, followed by incubation of the resulting supernatant in an ultrasonic water bath at 25°C for 15 min. After incubation, the supernatant was extracted twice, and the combined extracts were adjusted to a final volume of 10 mL using methanol. Subsequently, the prepared extract was transferred to a 100 μL tube.

2.2.2. Antioxidant Activity Analysis. The assessment of the DPPH radical-scavenging activity of the shell samples was conducted by adapting a method described earlier [28]. Briefly, 0.1 mL extract was placed into a 10 mL tube, and then, 3.9 mL of a 60 μM DPPH solution, prepared with methanol as the solvent, was added. The mixture was incubated at 21°C for 30 min. After incubation, the absorbance was measured at 517 nm using a Libra S70 double-beam spectrophotometer (Biochrom, Cambridge, England). In this study, Trolox was used as the blank reference, and the DPPH solution was regarded as the control. The results were then compared with the standard antioxidant compound, Trolox. The content of the plant extract was measured in milligrams of Trolox equivalents per gram (mg Trolox equivalent/g).

2.2.3. Calibration Curve. The DPPH calibration analysis included concentrations of 20, 40, 60, 80, and 100 μg/mL. Using these concentrations, a calibration curve for DPPH was constructed, and the IC_{50} value was calculated from the curve. A Trolox calibration curve was constructed using Trolox standard solutions of different concentrations. The IC_{50} value for DPPH was calculated as the Trolox equivalent based on the Trolox and DPPH curves. The calculation of percentage inhibition was performed using the following formula:

$$\% \text{ Inhibition} = \left[\frac{(\text{Ak} - \text{A0})}{\text{Ak}}\right] \times 100, \quad (1)$$

where *Ak* signifies the absorbance value of the control sample (lacking antioxidants) and *A*0 denotes the absorbance value of the sample containing antioxidants. The IC_{50} values of the samples (50% scavenging concentration of DPPH radicals) were obtained by calculating the percentage inhibition values from the plotted graph.

2.3. LC-HRMS Analysis of R. ribes Shells

2.3.1. Sample Preparation. At the beginning of the procedure, a 10 mg powdered shell sample was dissolved in a solution consisting of equal parts (10 mL each) of methanol and water. Subsequently, the solution was filtered using a 0.22 μm polytetrafluoroethylene syringe filter. Finally, the solution was transferred into a 1.5 mL flask for analysis.

2.3.2. Chromatography and High-Resolution Mass Spectrometry Conditions. The samples were analyzed using a Phenomenex Gemini 3 μm NX-C18 110 Å (100 m × 2 mm) column (Phenomenex, Torrance, CA, USA) maintained at 30°C. The elution process involved mobile phase A, composed of 2% (v/v) glacial acetic acid in ultrapure water obtained from an ultrapure water system (GFL 2004/human power 1, company name, city, state, country), and mobile phase B, consisting of 99.9% pure LC-MS-grade methanol (Sigma-Aldrich, St. Louis, MO, USA). The elution parameters included a flow rate of 0.3 mL/min, a sample injection volume of 20.0 µL, and a total analysis time of 20 min. Analysis was carried out using a heated electrospray ionization Orbitrap HRMS instrument (Exactive Plus™; Thermo Fisher Scientific, Waltham, MA, USA) operating in both positive (full MS/all-ion fragmentation [AIF]) and negative (full MS/AIF) modes. Device settings included an automatic gain control target of 3e6, spray voltage of 3.5 kV, S-lens RF level of 50, maximum ion deposition time per scan event of 2 ms, ionization interface sheath gas flow rate of 35 mL/min, auxiliary gas temperature of 350°C, auxiliary gas flow rate of 7, MS scan range of 60–800 m/z, capillary temperature of 350°C, resolution of 17500×, and collision energy/step below 25 V.

2.3.3. LC-HRMS. For LC-HRMS analysis, the DIONEX UltiMate 3000RS autosampler, pump, and column furnace (Thermo Fisher Scientific, Waltham, MA, USA) were used. The analysis was conducted using the Exactive Plus Orbitrap version 2.1.0.I140 (Thermo Fisher Scientific). The calibration of the Orbitrap LC-MS instrument was performed using the Pierce™ Negative Ion Calibration Solution and the Pierce™ LTQ Velos ESI Positive Ion Calibration Solution. Both LC and MS processes were conducted simultaneously using TraceFinder 3.2 (Thermo Fisher Scientific) during the LC-HRMS analyses. Data acquisition and recording were executed using Xcalibur version 2.1.0.I140 (Thermo Fisher Scientific).

3. Results and Discussion

3.1. Chemical Composition Analysis. A comprehensive analysis of the *R.* ribes shell using LC-HRMS led to the identification of 44 phytochemical compounds (Table 1). The compounds present in the highest concentrations were rutin hydrate M-OH_2 (8917.999 mg/kg), kuromanine (5243.637 mg/kg), and procyanidin B2 (2930.503 mg/kg), whereas sinapic acid (0.409 mg/kg) was the compound with the lowest detection limit.

Catechin, one of the compounds present in the shells, offers various benefits in preventing lung and cardiovascular diseases including cancer [29]. Quercetin also inhibits cancer cell growth [30]. Moreover, a diet enriched with cyanidin may be effective in preventing diabetic arterial damage and diabetic nephropathy in obese [31]. Additionally, the intake of cyanidin-3-O-glycoside can reduce body fat accumulation in mice fed a high-fat diet, regulate blood sugar, and increase insulin sensitivity [32].

Consumption of polyphenol-rich foods not only leads to enhanced antioxidant effects but also aids in mitigating oxidative stress, reducing the likelihood of strokes and coronary cardiovascular diseases. Notably, the anticancer properties of phenolic substances are well-known [33]. Furthermore, salicylic acid, gallic acid, 3,4-dihydroxybenzoic acid, and ferulic acid are noteworthy components [34]. Moreover, epigallocatechin gallate [35], luteolin [36], galangin [37], procyanidin B2 [38], genistein [39], formononetin, apigenin [40], and emodin [41] are components found in ribose shells that exhibit anticarcinogenic and antiproliferative effects in human cancer cell lines.

The HPLC analysis of *R. ribes* root extracts in both aqueous and ethanol solvents revealed the presence of 10 distinct phenolic compounds. The identified compounds were aloe-emodin, gallic acid, emodin, rhein, tannic acid, physcion, chlorogenic acid, kaempferol, chrysophanol, and rutin [42]. Naemi et al. used gas chromatography-mass spectrometry (GC-MS) to analyze the essential oil content of *R. ribes* plants and identified 19 compounds [43]. The major components were n-eicosane (9.9%), palmitic acid (27.08%), linoleic acid (6.56%), n-tetracosane (7.34%), and ethyl linoleate (4.76%) [44] initially analyzed *R. ribes* flowers using gas chromatography-flame ionization detection and GC-MS and identified 23 compounds, accounting for 97.5% of the total detected components. In our study, we analyzed the hydrates of *R. ribes* shells using LC-HRMS and identified 44 compounds, including rutin hydrate M-OH_2, kuromanine, procyanidin B2, and gallic acid (Figure 1). These findings suggest that phenolic compounds are more prevalent in the shells of *R. ribes* than in the flowers and roots.

Phenolic compounds are potent chain-breaking antioxidants [45], and their concentrations show strong linear correlations with their effectiveness with respect to neutralizing free radicals, particularly the DPPH radical [26]. Therefore, the abundance of phenolic compounds in the shells of *R. ribes* offers clear health benefits.

Table 1: Phenolic compounds identified using LC-HRMS.

No	Phenolic compound profile	Compound types	Result (mg/kg)
1	Luteolin-7-O-β-D-glucuronide (luteolin-7-O-glucuronide)	Flavonoid	N/F
2	2,4-Dihydroxybenzoic acid (beta-resorcylic acid)	Dihydroxybenzoic acid and derivative	15.238
3	3,4-Dihydroxybenzaldehyde (protocatechuic aldehyde)	Dihydroxybenzoic acid and derivative	33.948
4	3,4-Dihydroxyphenylacetic acid (DOPAC; homoprotocatechuic acid)	Dihydroxybenzoic acid and derivative	75.782
5	3-Hydroxybenzoic acid (3-HBA)	Dihydroxybenzoic acid and derivative	N/F
6	3-Hydroxyphenylacetic acid (3-HPA)	Dihydroxybenzoic acid and derivative	221.558
7	4-Hydroxybenzoic acid	Dihydroxybenzoic acid and derivative	45.349
8	5,7-Dihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one (acacetin)	Flavon	N/F
9	Afzelin (kaempferol 3-rhamnoside)	Flavon	0.493
10	Apigenin (5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one)	Flavon	N/F
11	Apigenin 7-glucoside	Flavonoid and flavonol glycosides	N/F
12	Apigenin 7-glucuronide	Glycoside and phenolic glycoside	N/F
13	Apiin (apigenin-7-(2-O-apiosylglucoside))	Glycoside and phenolic glycoside	N/F
14	Arbutin	Glycoside and phenolic glycoside	261.139
15	Astragalin (kaempferol 3-glucoside)	Flavonoid and flavonol glycoside	1.134
16	Benzoic acid	Benzoic acid and derivative	144.028
17	Caffeic acid	Phenolic acid	37.866
18	Caffeic acid phenethyl ester (CAPE)	Phenolic acid	N/F
19	Catechin (cianidanol)-p	Flavonoid and flavonol derivative	736.564
20	Chlorogenic acid	Phenolic acid	N/F
21	5,7-dihydroxy-2-phenyl-4H-chromen-4-one (chrysin)	Flavonoid and flavonol derivative	N/F
22	Coumaric acid (*trans*-3-hydroxycinnamic acid)	Cinnamic acid derivative	13.568
23	Daidzin	Isoflavonoid	3.071
24	Diosmetin (luteolin 4′-methyl ether)	Flavonoid metabolite	N/F
25	Doxorubicin hydrochloride	Antineoplastic drug	N/F
26	Ellagic acid	Gallic acid and derivative	5.243.637
27	Emodin	Anthraquinone derivative	4.639
28	Epigallocatechin	Catechin	N/F
29	Epigallocatechin gallate	Catechin	22.173
30	Eriocitrin	Flavanone derivative	N/F
31	Eriodictyol (3,4,5,7-tetrahydroxyflavanone)	Flavanone derivative	N/F
32	Esculin hydrate	Coumarin derivative	N/F
33	Ethylgallate	Gallic acid and derivative	7.788
34	Etoposide	Alkaloid and derivative	N/F
35	Ferulic acid	Phenolic acid	39.199
36	Fisetin hydrate	Flavonol	2,304
37	Formononetin (neochanin)	Isoflavone	N/F
38	3,5,7-trihydroxy-2-phenyl-4H-chromen-4-one (galangin)	Flavan	N/F
39	3,4,5-trihydroxybenzoic acid (gallic acid)	Phenolic acid	1.713.508
40	5,7-dihydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one (genistein)	Phenolic acid	N/F
41	Genkwanin(4′,5-dihydroxy-7-metthoxyflavone, apigenin 7-O-methyl ether)	Flavonol	N/F
42	Gentisic acid	Isoflavone	10,62
43	Glabridin	Flavonol	N/F
44	Hesperidin	Flavanone	N/F

Table 1: Continued.

No	Phenolic compound profile	Compound types	Result (mg/kg)
45	Homovanillic acid ((4-hydroxy-3-methoxyphenylacetic acid))	Phenolic acid	14.16
46	Hyperoside (quercetin 3-D-galactoside)	Flavonol glycoside	46.872
47	Isoorientin	Flavonol glycoside	N/F
48	Isorhamnetin (quercetin 3′-methyl ether)	Methoxyflavone	N/F
49	İsoquercitrin (quercetin 3-glucoside)	Flavonol glycoside	15.283
50	Kaempferide	Methoxyflavone	N/F
51	Kaempferitrin	Flavone	N/F
52	Kaempferol	Flavone	N/F
53	Kuromanine (cyanidin 3-glucoside chloride)	Flavone	1.134
54	Leucoside (kaempferol 3-sambubioside)		63.283
55	Liquiritigenin	Flavone	N/F
56	Liquiritin (4′,7-dihydroxyflavanone 4′-glucoside)	Flavonol glycoside	N/F
57	Luteolin	Flavone	11.376
58	Luteolin 7-rutinoside	Flavonol glycoside	2.936
59	Luteoloside (luteolin 7-glucoside)	Flavonol glycoside	N/F
60	Narcissin (narcissoside, isorhamnetin 3-rutinoside)	Flavonol glycoside	N/F
61	Naringenin	Flavonoid	N/F
62	Naringin	Flavonoid	N/F
63	Narirutin (narirutinsa, naringenin rutinoside)	Flavonoid	N/F
64	Neohesperidin	Flavonoid	N/F
65	Kaempferol 3-rutinoside, kaempferol 3-O-β-rutinoside (nicotiflorin)	Flavonol glycoside	316.813
66	Orientin	Flavonol glycoside	N/F
67	Phloridzin	Flavanone glycoside	52.527
68	Procyanidin B2	Procyanidin	2.930.503
69	Protocatechuic acid (3,4-dihydroxybenzoic acid)	Phenolic acid	130.31
70	Ethyl 3,4-dihydroxybenzoate (protocatechuic acid ethyl ester)	Phenolic acid	N/F
71	Quercetin	Phenolic acid	728.899
72	Quercetin 3-rutinoside 7-glucoside	Flavonol glycoside	N/F
73	Quinic acid	Phenolic acid	29.933
74	Rhoifolin (apigenin 7-O-neohesperidoside)	Flavonol glycoside	N/F
75	Rosmarinic acid	Phenolic acid	N/F
76	Rutin hydrate M-OH2	Flavonoid glycoside	8.917.999
77	Sakuranetin (naringenin 7-O-methyl ether)	Flavonoid GlycosideFlavonoid glycoside	N/F
78	Salicylic acid	Phenolic acid	57.63
79	Schaftoside	Flavonoid glycoside	332.969
80	Sinapic acid	Phenolic acid	N/F
81	Syringic acid	Phenolic acid	0.409
82	Tiliroside	Flavonoid glycoside	N/F
83	*trans*-cinnamic acid	Trans-cinnamic acid	N/F
84	Vanillic acid	Phenolic acid	75.782
85	Vanillin	Aromatic acid and aldehyde	12.802
86	Vicenin 2	Flavonoid glycoside	103.119
87	Vitexin (apigenin 8-C-glucoside)	Flavonoid glycoside	25.347
88	α-Cyano-4-hydroxycinnamic acid	Flavonoid	8.684

Figure 1: Image of *Rheum ribes* plant.

3.2. DPPH Scavenging Capacity. Various components of *R. ribes*, including its roots and stems, demonstrate significant antioxidant properties [21, 26]. In this study, the antioxidant capacity of the plant samples was assessed using the DPPH assay, revealing an antioxidant activity of 1.06 ± 0.3 mg Trolox equivalent/g per sample.

The antioxidant capacities of *R. ribes* root, stem, and aqueous extracts were examined in a previous study. The ethanolic root extract demonstrated the strongest antioxidant activity, with an IC_{50} value of $4.73 \pm 0.21\,\mu$g/mL. However, the aqueous root extract displayed the lowest antioxidant activity, with an IC_{50} value of $25.62 \pm 0.85\,\mu$g/mL [42]. The DPPH radical-scavenging abilities of *R. ribes* stem extracts, prepared using water, ethanol, and methanol solvents, were 93.49%, 94.21%, and 95.86%, respectively [46]. Previous investigations have reported DPPH activities in *R. ribes* stem extracts prepared using aqueous and methanol solvents, yielding values of 83.90% and 87.07%, respectively [47]. Among the diverse extracts obtained from different parts of *R. ribes* in a previous study, including preparations with ether, ethanol, and aqueous extracts, the ethanol extracts of the shell have exhibited the most notable antioxidant activity [48].

Phenolic compounds are typically found in foods such as fruits, vegetables, and grains, and the antioxidant capabilities of these foods are directly linked to their phenolic content [26, 48]. The findings of this study also clearly demonstrate that phenolic compounds in *R. ribes* shells provide antioxidant effects.

4. Conclusion

This study identified 44 compounds phytochemical compounds in the *R. ribes* shell using LC-HRMS. The primary compounds identified in the analysis included rutin hydrate M-OH_2, kuromanine, procyanidin B2, and gallic acid. Notably, this is a pioneering study investigating the composition of R. ribes shells. The existence of these phytochemicals in *R. ribes* shells suggests their potential application in the cosmetic and food industries, as well as in the synthesis of essential fatty acids. Furthermore, our findings indicate that *R. ribes* shells possess substantial antioxidant properties, potentially serving as a natural antioxidant source for the food and pharmaceutical industries. The shells of *R. ribes* contain compounds with notable antioxidant properties, warranting further studies on this topic.

The limitations of this study are the exclusive use of the DPPH antioxidant method and the sole reliance on LC-HRMS for determining chemical compositions. Future research should incorporate a diverse range of analytical methods for a more comprehensive assessment. Additionally, comparing the chemical compositions of *R. ribes* shells from different geographical regions could provide insights into potential variations in the antioxidant and overall chemical profiles of the plant. Taking these steps is crucial for a more profound understanding of the chemical content and antioxidant potential of *R. ribes* shells.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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