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ELEMENTAL ANALYSIS AND DETERMINATION OF TOTAL PHENOLIC AND FLAVONOID CONTENT OF *RHEUM RIBES* L. BARK

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Abstract

Original scientific paper

Increasing evidence shows that *Rheum ribes* bark exhibits therapeutic and pharmacological effects. Therefore, we determined the total phenolic and flavonoid content and performed elemental analysis of *R. ribes* bark using inductively coupled plasma mass spectrometry. The results indicated a phenolic content of 0,61 ± 0,001 mg gallic acid-equivalent/g sample and a flavonoid content of 0,327 ± 0,026 mg catechin-equivalent/100 g sample. Elemental analysis of *R. ribes* bark revealed the presence of numerous essential elements, including Fe, K, Ca, Na, Al, and Mg. Our results show that *R. ribes* bark contains high levels of phenolic and flavonoid compounds and is a source of various important elements. Consequently, it may offer health benefits and might prove to be a valuable resource for the pharmaceutical and food industries.

Keywords: *Antioxidant; flavonoid; phenolic; trace element; Rheum ribes.*

1 Introduction

Plant oils and extracts possess several therapeutic properties and are extensively used in pharmaceuticals and alternative medicine. Antioxidants present in plants protect cells against harmful substances, prevent oxidation caused by free radicals, and can reduce the deleterious effects of disease such as chronic inflammation, obesity, diabetes, cardiovascular diseases, and cancer [1], [2]. Naturally produced antioxidants include vitamins A, C, and E, polyphenols, carotenoids, and flavonoids [3], [4]. Antioxidants often contain phenolic compounds [5], which are important bioactive compounds, and play key roles in defense responses, such as anti-aging, anti-inflammatory, antioxidant, and anti-proliferative activities [6]. Flavonoids are secondary plant metabolites that play important roles in regulating plant growth and inhibiting low-density lipoprotein oxidation [7], [8]. Additionally, plants contain various biologically important macro-elements, such as K, Ca, Mg, and Na, and trace elements, such as Fe, Zn, Cu, Mn, Mo, Cr, and Co, which are necessary for enzyme activity, growth, metabolism, and tissue health. Deficiencies in these elements lead to health problems, whereas excessive intake may result in toxic effects [9]. Although medicinal plants have not exhibited detrimental effects at concentrations evaluated to date, excessive intake of heavy metals negatively impacts human health [2], [10], [11]. Therefore, an accurate evaluation of the elemental composition of plants is pertinent [2], [12].

R. ribes, which belongs to the *Polygonaceae* family, has been an important food source and medicinal plant in Turkey and other parts of the world since ancient times [13]. Annually, tons of *R. ribes* plant bark are discarded. Given its medicinal applications, we aimed to investigate its total phenolic and flavonoid contents, as well as the elemental composition of the bark.

2 Materials and Methods

2.1 Collection of Plant Materials

Wild-growing *R. ribes* was collected from the Pervari district of Siirt province, Turkey, in early May 2023 (Figure 1). The bark of the collected plant samples was dried at 24 °C in a dark room for 1 month. Subsequently, the dried *R. ribes* bark was ground into powder under identical conditions and preserved for further analysis.

Figure 1. Photograph of *Rheum ribes* bark.

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2.2 Extract Preparation

Five milliliters of 75% methanol containing 0.1% phosphoric acid were added to a 0,2 g powdered plant sample, mixed well, and homogenized using an ULTRA-TURRAX homogenizer (MS3-MaxiHomo35, Osaka, Japan) at 600 rpm for 30 s. The sample was centrifuged at 2500 rpm (Archer LC-05A, Istanbul, Türkiye) for 10 min at 24°C to obtain the supernatant, which was then incubated for 15 min in an ultrasonic water bath at 25°C. The extraction process was repeated twice, and the extracts were pooled. The final extract volume was adjusted to 10 mL with methanol, and the extract was transferred to 100 µL tubes.

2.3 Total Phenolic Content Determination

Folin–Ciocalteu reagent (FCR) is used to measure the total phenolic content of a substance. Phenolic compounds are antioxidants and can react with free radicals, reducing cellular damage. The FCR reacts with phenolic compounds, causing a color change that is proportional to the amount of phenolic compounds present. This test is commonly used in the food, beverage, and natural product industries to assess antioxidant capacity and is usually reported in terms of gallic acid equivalents. Briefly, 900 µL pure water (18,2 MΩ, Arium Pro Ultrapure Water System, Sartorius, Göttingen, Germany) and 5 mL 0,2 M FCR were added to 100 µL *R. ribes* extract. The mixture was shaken vigorously and allowed to rest for 8 min. Next, 5 mL 7.5% sodium carbonate was added to the mixture and vortexed for 20 s. The mixture was then incubated in the dark at 22–24°C for 2 h, after which the absorbance was measured at 765 nm using a spectrophotometer (Biochrom Libra S70 Double Beam Spectrophotometer, Cambridge, England).

2.4 Total Flavonoid Content Determination

The *R. ribes* extract (0,4 mL) or catechin standard was transferred to a 10-mL measuring cup, and 4 mL pure water was added. Then, 0,3 mL 5% $NaNO_2$ was added to the mixture and allowed to rest for 5 min. Next, 0.3 mL 10% $AlCl_3$ was added to the mixture and allowed to rest for 6 min. Finally, 2 mL 1 M NaOH and 3 mL distilled water were added to the mixture, shaken, and the absorbance measured at 510 nm using a spectrophotometer (Biochrom Libra S70 Double Beam Spectrophotometer, Cambridge, England). Pure water was used as a blank.

2.5 Inductively Coupled Plasma Mass Spectrometry Elemental Analysis

For inductively coupled plasma mass spectrometry (ICP–MS), a 1 g powdered *R. ribes* bark sample was transferred to a Teflon container. Next, 10 mL concentrated 65% nitric acid (Merck, Darmstadt, Germany) was added to each sample; 10 mL 65% nitric acid was added to an empty Teflon container and used as a blank. The Teflon containers were placed in a MARS 6 One Touch microwave digestion system (CEM, Matthews, NC, USA). The maximum temperature was increased to 210°C over 25 min and maintained for 15 min. Dissolution was performed in a closed system for 40 min. After the microwave temperature returned to 22–24°C, the solutions and Teflon container lids were thoroughly washed with ultrapure water and transferred to 50-mL volumetric flasks.

Calibration solutions for ICP–MS (Table 1) were prepared by diluting commercially available multi-element standards with 1% Suprapur nitric acid (Millipore Sigma, Burlington, MA, USA) in ultra-pure water and used to construct a calibration curve.

Table 1. Calibration standards.

Analytes	Std1 (ppb)	Std2 (ppb)	Std3 (ppb)	Std4 (ppb)	Std5 (ppb)	Std6 (ppb)	Internal standard
Li, B, Al, Ti, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Nb, Mo, Ru, Pd, Ag, Cd, Sn, Ba, Hf, Ta, W, Au, Pb, U	0,5	1	5	25	50	100	^{89}Y
Na, Mg, K, Ca, Fe	25	50	2,50	1250	2500	5000	

Appropriate sample dilutions were prepared, and ICP–MS analyses were performed on a NexION 2000B ICP mass spectrometer (PerkinElmer, Waltham, MA, USA) fitted with a quartz nebulizer, cyclonic spray chamber, and integrated auto-sampler under the operating conditions summarized in Table 2.

A washing solution containing 1% Suprapur nitric acid–ultrapure water was prepared at the concentrations specified in Table 1, and 25 ppb 89Y (Yttrium) was used as the internal standard. Samples were injected into the cyclonic spray chamber with argon gas flow via a peristaltic pump. In addition to argon, a high helium percentage was used to prevent interference. Syngistix for ICP–MS software version 2.2 (PerkinElmer) was used for instrument setup, data acquisition, and data analysis.

Table 2. Inductively coupled plasma mass spectrometry operating conditions.

Parameter	Description/Value
Nebulizer	MEINHARD plus Classic Type C
Spray chamber	Glass cyclonic (baffled), 4°C
One piece torch	w/2,5 mm Quartz Injector
Injector	2,0 mm i.d.
Nebulizer flow	Optimized for <2% oxides
Radio frequency power	1600 W
Cones	Ni
Replicates	3
Dwell time	50 ms
Aerosol dilution	Set to 2,5×
Sample delivery rate	350 µL/min
Rinse time	45 s
Nebulizer gas flow rate	0,93 L/min
Deflector voltage	Approximately 12 V
Analog stage voltage	Approximately 1750 V
Pulse stage voltage	1100 V
Discriminator threshold	26
Sample tubing (orange-yellow)	Flared PVC pump tubes 0,51 mm/0,89 mm
Internal standard tubing (orange-red)	Flared PVC pump tubes 0,19 mm/0,91 mm
Peristaltic pump speed	35 rpm
Alternating current rod offset	Approximately 4

3 Results and Discussion

3.1 Total Phenolic Content

The *R. ribes* bark had a high phenolic content of 0,61 ± 0,001 mg gallic acid-equivalent/g sample, as determined using the Folin–Ciocalteu method. A previously reported phenolic content for water, ethanol, and methanol extracts of *R. ribes* was 118,76, 125,07, and 136,82 mg, respectively [14]. Another study reported a total phenolic content of 35,71 mg pyrocatechol-equivalent/mg extract [15]. Although the bark of *R. ribes* may have a lower total phenolic content than other plant parts, these discarded portions still hold potential as valuable source material for health and dietary

supplements because of their phenolic components. Phenolic compounds are widely present in plants and have numerous health benefits, such as antioxidant, anticancer, and anti-inflammatory effects [16]. Their anticancer effects, including growth inhibition and cytotoxic effects, are under active investigation. *R. ribes* may therefore exert beneficial health effects, including antioxidant and anticancer activities.

3.2 Total Flavonoids

In addition to the total phenolic content, the presence and amount of other bioactive compounds, such as flavonoids, tannins, and phytosterols [17], can affect the biological activity of *R. ribes* bark extract. We found that the total flavonoid content of the *R. ribes* bark extract was 0,327 ± 0,026 mg catechin-equivalent/100 g sample. *R. ribes* plant extracts obtained using different methods are reportedly rich in flavonoids; water, ethanol, and methanol extracts were previously shown to have a total flavonoid content of 2681,49, 2345,85, and 1239,74 mg catechin-equivalent/g extract, respectively [14]. Another study reported that the chloroform extract of *R. ribes* roots had the highest flavonoid content at 145,59 ± 0,22 mg, whereas the methanol extract of the stems had the lowest content at 13,66 ± 0,75 mg [15].

Flavonoids are plant-derived compounds with antioxidant, anti-inflammatory, anti-tumor, and antimicrobial properties and are therefore of pharmacological and biochemical interest. The current study indicates that *R. ribes* bark is a rich source of flavonoids, and further studies are required to investigate the health benefits of the bark and the bioavailability of the flavonoids.

3.3 Elemental Analysis

The elemental analysis of *R. ribes* bark using ICP–MS revealed the presence of numerous elements (Table 3), with Fe, K, Ca, Na, Al, and Mg present at the highest concentrations. Therefore, *R. ribes* bark contains potentially valuable elements that can be used in various health and food applications.

Minerals and trace elements play important roles in various metabolic processes, and their deficiency or excess can lead to disease. For instance, Cu plays an important role in tissue regeneration, strengthening of bones, protein synthesis, energy production, and red blood cell formation [18]. However, excessive Cu intake can cause health problems. Similarly, Cd is a toxic element that can persist in the body, and high Cd levels in food can cause serious health problems [19]. K regulates water balance and facilitates the conversion of carbohydrates to glycogen [18], and Mg is important for regulating energy

metabolism, the muscle and nervous systems, bone formation, and blood pressure [20]. Se is a crucial element for human health, involved in reproduction, thyroid hormone metabolism, and protection against oxidative damage and infection through its role in the synthesis of enzymes such as selenoproteins [21]. Mn is an essential co-factor of metalloenzymes, especially superoxide dismutase [22]. Co contributes to the composition of vitamin B12, which is essential for the production of red blood cells and the regulation of nervous system functions [23].

In summary, *R. ribes* can be used for food products and promoting health. It contains numerous essential minerals and trace elements, which makes it a valuable material for the food industry.

Table 3. Inductively coupled plasma mass spectrometry elemental analysis results.

^{7}Li Helium KED High (ppb)	^{11}B Helium KED High (ppb)	^{23}Na Helium KED High (ppb)	^{24}Mg Helium KED High (ppb)	^{27}Al Helium KED High (ppb)
6756.584	144831.442	5942042.688	3023013.745	851856.794
^{39}K Helium KED High (ppb)	^{43}Ca Helium KED High (ppb)	^{48}Ti Helium KED High (ppb)	^{51}V Helium KED High (ppb)	^{52}Cr Helium KED High (ppb)
37184914.979	8737640.163	17538.989	1587.996	5151.725
^{55}Mn Helium KED High (ppb)	^{57}Fe Helium KED High (ppb)	^{59}Co Helium KED High (ppb)	^{60}Ni Helium KED High (ppb)	^{63}Cu Helium KED High (ppb)
20520.414	369547.785	1156.817	18777.943	11192.606
^{66}Zn Helium KED High (ppb)	^{69}Ga Helium KED High (ppb)	^{75}As Helium KED High (ppb)	^{82}Se Helium KED High (ppb)	^{85}Rb Helium KED High (ppb)
463869.839	3701.328	1331.671	16891.835	43326.721
^{88}Sr Helium KED High (ppb)	^{93}Nb Helium KED High (ppb)	^{98}Mo Helium KED High (ppb)	^{102}Ru Helium KED High (ppb)	^{106}Pd Helium KED High (ppb)
29873.875	1208.499	1570.078	544.923	1384.669
^{107}Ag Helium KED High (ppb)	^{111}Cd Helium KED High (ppb)	^{118}Sn Helium KED High (ppb)	^{138}Ba Helium KED High (ppb)	^{180}Hf Helium KED High (ppb)
1491.508	1261.400	1800.098	15476.094	1315.541
^{181}Ta Helium KED High (ppb)	^{184}W Helium KED High (ppb)	^{197}Au Helium KED High (ppb)	^{208}Pb Helium KED High (ppb)	^{238}U Helium KED High (ppb)
1788.306	2045.404	2297.684	20774.610	5548.620

*KED; kinetic energy discrimination, ppb; parts per billion

4 Conclusion

This study explored the potential use of abundant discarded *R. ribes* bark as a resource for the health and food industries. We analyzed the total phenolic and flavonoid content and determined the elemental composition of *R. ribes* bark and found that it contains a significant amount of phenolic and flavonoid compounds, suggesting that it may be a rich source of antioxidants and anticancer agents. Elemental analysis revealed elevated concentrations of essential minerals, including Fe, Ca, K, and Mg. Our results therefore imply that *R. ribes* bark contains bioactive compounds with potential health benefits.

This study has some limitations. First, the results of this study are limited to specific *R. ribes* bark samples from a particular region, which impacts genetic diversity and growth conditions. Further research involving larger sample sizes is therefore necessary to validate our findings. Second, we used only microwave dissolution and ICP–MS in this study, and different analytical techniques may yield different results. Therefore, our findings should be corroborated by additional, comprehensive studies using diverse analytical methods. Assessing the overall impact of these variables on the results in greater detail will be crucial in future research endeavors. Additionally, in-depth studies are required to investigate the interactions between various bioactive components to elucidate the functional mechanisms involved in the positive impact of *R. ribes* bark on human health.

Declaration

Ethics committee approval is not required.

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