



Antioxidant and Bioactive Potential of *Rheum ribes* L. Flowers: A Comprehensive Study on Secondary Metabolites and Volatile Compounds

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ARTICLE INFO

Research Article

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Received: 06 October 2024 / Revised: 22 December 2024 / Accepted: 14 January 2025 / Online: 29 July 2025

Cite this article

Binici H İ (2025). Antioxidant and Bioactive Potential of *Rheum ribes* Flowers: A Comprehensive Study on Secondary Metabolites and Volatile Compounds. *Journal of Agricultural Sciences (Tarım Bilimleri Dergisi)*, 31(3):690-695. DOI: 10.15832/ankutbd.1558025

ABSTRACT

Many antioxidants and therapeutic properties of *Rheum ribes* L., a rich medicinal plant in Türkiye, have been studied. However, little attention has been given to the compounds present in the flowers of *Rheum ribes* L.. The aim of this research was to comprehensively assess the antioxidant activity, secondary metabolites, and volatile compounds present in the flowers of *Rheum ribes* L.. The total phenolic content, total flavonols, total flavonoids, DPPH and ABTS values of the *Rheum ribes* L. flowers were 19.94 mg GAE/g, 1.09 mg QE/g, 7.31 mg QE/g, 15.89 mM TEAC/g

and 5.05 mM TEAC/g, respectively. Very high amounts of rutin and gallic acid were found as secondary metabolites. High levels of heptacosane (31.81%), 17-pentatriacontene (15.05%), octatriacontyl pentafluoropropionate (8.33%), and oleic acid (7.75%) were identified as volatile components. Briefly, this study investigated the identification of key secondary metabolites, such as rutin and gallic acid, as well as volatile components, highlighting the potential for discovering new bioactive compounds that could contribute to the development of natural herbal medicines.

Keywords: *Rheum ribes* L. flowers, Bioactive compounds, Secondary metabolites and volatile compounds

1. Introduction

Fruits and vegetables play important roles in the human diet due to their low energy content and high levels of minerals and vitamins. Additionally, they contain bioactive compounds that are beneficial for human health (Shashirekha et al. 2015). Plants with valuable bioactive components are often preferred for their pharmacological properties in the treatment of disorders such as cancer, respiratory and heart-vascular diseases, and blood pressure (Lubbe & Verpoorte 2011; Prasathkumar et al. 2021). Recently, with the onset of the coronavirus pandemic, there has been an increased interest in herbal remedies as an alternative medicine approach (Kadioğlu & Kadioğlu 2021). The use of plants for the treatment of various diseases has a long history. Various parts of plants, such as leaves, stems and roots, have been used to prevent symptoms and normalize abnormalities. Despite the limited number of scientific studies on the antioxidant activities of some plants, important findings are available (Özyurt et al. 2021). Functional foods are food substances of plant, animal or mineral origin formulated to maintain a healthy life or to support the treatment of certain diseases. Phytotherapeutic products are the best-known group of plant-derived functional foods and are generally herbal formulations used regionally for different purposes. Medicinal plants have various beneficial pharmacological activities and especially contain anticarcinogenic phytochemicals (Singh & Rawat 2016). *Rheum ribes* L., commonly known as 'Işkın', which is one of Türkiye's rich medicinal plant flora, belongs to the family of *Sorrelaceae* (*Polygonaceae*). Several studies on the rhubarb plant have shown that its edible fruit has antioxidant effects and therefore can be evaluated as a nutraceutical. The obtained evidence has shown that rhubarb has a high phenolic content and therefore has antioxidant effects through the binding of free radicals at a high rate. Recent studies have reported that *Rheum ribes* L. exhibits positive effects on diseases such as cancer, as well as antioxidant, antidiabetic, blood pressure-regulating, antibacterial, anti-ulcer, anti-obesity, and anti-diarrheal properties. Furthermore, it has been found that the expression of genes associated with obesity and obesity-related complications is downregulated following treatment with *Rheum ribes* L. methanolic root extract, suggesting its potential as a therapeutic agent for obesity and related complications (Meral 2017; Kaya et al. 2023).

In this demonstrated that rhubarb flowers are used both as food and in the treatment of various diseases. Currently, the raw materials of many drugs are derived from compounds synthesized from medicinal and aromatic plants. While the literature includes numerous studies on the rhubarb plant, no in vitro studies have been found on the antioxidant activity, secondary metabolites, organic acids, or volatile compounds in the flower part of this plant. Therefore, this study investigated the antioxidant activity, secondary metabolite, organic acid and volatile compound contents of rhubarb plants.

2. Material and Methods

2.1. Collection of plant materials

Rheum ribes L. was collected in early May 2023 from the Adilcevaz district of Bitlis Province, Türkiye, where it grows naturally. *R. ribes* is a well-known plant in the region, similar to other vegetables and fruits that are commonly consumed there. Approximately 100 ± 0.1 g of flowers were dried in an oven at 40°C for 24 hours after collection. After drying, the flowers were powdered and stored in a refrigerated chamber at -20°C until analysis.

2.2. *Rheum ribes* L. flowers extraction method

Approximately 100 ± 0.001 mg of sample was added to 10 mL of methanol and kept in an ultrasonic water bath for 30 minutes. The mixture was then centrifuged ($4200 \times g$, 15 min) and filtered through (Whatman No:1) filter paper. The filtered sample was subsequently evaporated from the methanol solution using a rotary evaporator at $45 \pm 1.0^\circ\text{C}$, and the extracts were prepared for analysis at a concentration 1 mg/mL.

2.3. Determination of total phenolic content

Briefly, 0.1 mL of the prepared extracts was added to a glass tube, and 0.8 mL of distilled water, Na_2CO_3 (10%), or Folin–Ciocalteu (0.5 N) was added. Measurements were then made at 760 nm and values were given as mg GAE/g (Gülçin et al. 2012).

2.4. Determination of total flavonol content

From these extracts, 0.5 mL of sample extract, 0.5 mL each of AlCl_3 (2%) and sodium acetate (5%) solutions were mixed in a glass tube (2.5 mL with distilled water), and absorbance values were measured at 425 nm (Almaraz-Abarca et al. 2007).

2.5. Determination of total flavonoid content

From these extracts, 0.5 mL of sample extract was added to 0.15 mL NaNO_2 (5%), 0.15 mL 10% AlCl_3 (after 6 minutes) and 0.5 mL 4% NaOH solution (after 6 minutes) in a glass tube (2.5 mL with distilled water) and the absorbance was measured at 510 nm 14 minutes after homogenization (Youssef & Mokhtar 2014).

2.6. DPPH assay

From these extracts, 0.1 mL of sample extract was added to a glass tube, 0.5 mL of DPPH (39 mg/100 g methanol) was added (2.5 mL by volume of methanol), kept in the dark for 30 minutes and measured against the blank at 517 nm. The amount of DPPH was calculated in mM TEAC/g using the Trolox standard curve prepared daily (Spada et al. 2008).

2.7. ABTS assay

The ABTS- $^+$ radical scavenging activities were determined according to previous methods (Köksal et al. 2009). From these prepared extracts, 0.1 mL of sample extract was added to 0.5 mL of ABTS (2 mM, potassium persulfate (2.45 mM)), and the final volume was adjusted with purified water to 2.5 mL in a glass tube. It was then given in mM TEAC/100 g by Trolox standard curve at 734 nm.

2.8. Color analysis

Color analysis was performed according to Şat et al. (2023).

2.9. Determination of secondary metabolites by HPLC

The secondary metabolites of the rhubarb flowers were determined according to the methods of Alwazeer et al. (2023).

2.10. Organic acid determination

Organic acid analysis was performed according to Castellari et al. (2000).

2.11. Determination of volatile compounds by GC–MS

The volatile compound concentrations were determined according to Binici et al. (2024).

2.12. Statistical analysis

The data obtained from the analysis of *Rheum ribes* L. flower were statistically analysed. Total phenolic content, total flavonol, total flavonoid, DPPH and ABTS analyses were conducted in triplicate (n=3) and the results were expressed as mean $\pm$ standard deviation (SD).

3. Results and Discussion

Antioxidants are chemical compounds that block or inhibit the formation of free radicals and usually contain phenolic compounds in their structure. In addition, the reaction of an antioxidant chemical mixture with a radical has been defined as the antioxidant capacity (Özli et al. 2024). Flavonoids are classified as secondary metabolites in plants (do Rosário Bronze et al. 2012). The total phenolic content, total flavonol, total flavonoid, DPPH and ABTS values of the flowers of *Rheum ribes* L. are shown in Table 1. The total phenolic content, total flavonol, total flavonoid, DPPH and ABTS values of the *Rheum ribes* L. flowers were determined to be 19.94 mg GAE/g, 1.09 mg QE/g, 7.31 mg QE/g, 15.89 mM TEAC/g and 5.05 mM TEAC/g, respectively. In a study, the DPPH value of the bark of *Rheum ribes* L. was determined to be 1.06 mg TEAC/g (Yolbaş, 2024). Keser et al. (2020), in their study on the antioxidant, antimicrobial, and cytotoxic properties of different *Rheum ribes* extracts, reported the ABTS values of water, ethanol, and methanol extracts as 99.27%, 99.91%, and 99.88%, respectively. Additionally, the DPPH values of water, ethanol, and methanol extracts were found to be 93.49%, 94.21%, and 95.86%, respectively. Öztürk et al. (2007), in their study, determined the total phenolic content of the stem and root parts of *Rheum ribes* to be between 22.68 and 48.66 μ g PE (pyrocatechol equivalent)/mg extract. They also reported the total flavonoid content of the stem and root parts to range from 13.66 to 145.59 μ g QE/mg extract. Taşkın & Bulut (2019) reported the total phenolic content, DPPH IC₅₀ and ABTS content as 58.00-167.00 μ g GAE/g extract, 5.00-144.00 μ g/mL and 12.70-51.90 mM TEAC/mg extract, respectively, in an in vitro study on different parts of *Rheum ribes* L. plants. It was determined that the rhubarb plant was different from that in the literature. This difference may be attributed to differences in growth conditions, harvesting methods, extraction methods and solvents used (Mısır et al. 2023; Şat et al. 2023).

Table 1- Total phenolic content, total flavonol content, total flavonoid content, and antioxidant (DPPH and ABTS) content of *Rheum ribes* L. flowers

Analyzes/Sample	<i>Rheum ribes</i> L. Flowers
Total phenolic content (mg GAE/g)	19.94 $\pm$ 0.17
Total flavonol (mg QE/g)	1.09 $\pm$ 0.00
Total flavonoid (mg QE/g)	7.31 $\pm$ 0.21
DPPH (mM TEAC/g)	15.89 $\pm$ 0.01
ABTS (mM TEAC/g)	5.05 $\pm$ 0.00

The color values of *Rheum ribes* L. flowers are shown in Table 2. The L*, a*, b*, C* and H° values were 60.25, 0.94, 21.26, 21.29 and 87.47, respectively. As shown by the L* values, the flowers of *Rheum ribes* L. were lighter in color. When the b* value was examined, it was determined that the flowers of the rhododendron were dark yellow in color. Additionally, when the chroma and hue values of the rhubarb flowers were examined, it was determined that the plants were brighter yellow in color. Şat et al. (2023) determined L*, a* and b* values of 45.69, -7.13 and 18.41, respectively, in fresh rhubarb samples. The difference in color among the different plant species in these studies may be due to differences in the specific parts of the plant species.

Table 2- Color (L*, a*, b*, C* and h°) values of *Rheum ribes* L. flowers

Color	<i>Rheum ribes</i> L. Flowers
L*	60.25 $\pm$ 2.52
a*	0.94 $\pm$ 0.27
b*	21.26 $\pm$ 0.56
C*	21.29 $\pm$ 0.55
h°	87.47 $\pm$ 0.75

The phenolic profile and organic acid values of the *Rheum ribes* L. flowers are shown in Table 3. Chlorogenic acid, catechin, epicatechin and rosmarinic acid were not detected in the phenolic profile. The most phenolic compounds in the *Rheum ribes* L. flowers were rutin (352.90 μ g/mL) and gallic acid (11.47 μ g/mL), and the least phenolic compound was caffeic acid (0.88 μ g/mL). Can et al. (2024) examined the phenolic profiles of four different medicinal plants. According to the results, *Rheum ribes* was found to have a higher dominance of gallic acid.

It has been reported that quercetin in flowers inhibits the growth of cancer cells (Zhang et al. 2015). It is also known that phenolic acids and flavonoids are widely present in plants. It has been reported that these compounds have biological and pharmacological properties and may benefit human health. Phenolic compounds have been reported to have antioxidant, anti-inflammatory, antimutagenic, anticarcinogenic and antiobesity properties (Hsu et al. 2006; Hsu & Yen 2008; Weng & Yen 2012;

Yen et al. 2011; Sinh et al. 2017). In a study, it was reported that resveratrol, gallic acid, o-coumaric acid and rutin could significantly increase the mitochondrial DNA content compared to that of other compounds (Chan et al. 2021). The highest organic acid content of *Rheum ribes* L. flowers was found in succinic acid (1959.92 µg/mL), and the lowest organic acid content was found in fumaric acid (0.50 µg/mL). Tartaric acid, formic acid, barbutyric acid and ascorbic acid were not detected. A study reported that succinic acid and its derivatives have insulinotropic and antioxidant properties. It was also found to help lower blood sugar in a mouse model of type 1 diabetes (Pari & Saravanan 2007; Saravanan & Pari 2006). Fumaric acid is reportedly used as a food acidifier, especially in beverages and baking powders. Fumaric acid is also known to be a pharmaceutical and antimicrobial agent used to treat psoriasis and multiple sclerosis (Yadav et al. 2022).

Table 3- Secondary metabolites and organic acid of *Rheum ribes* L. flowers

Analyzes/Sample	<i>Rheum ribes</i> L. Flowers
Gallic acid (µg/g)	11.47±0.05
Cholorgenic acid (µg/g)	nd
Catechin (µg/g)	nd
Epicatechin (µg/g)	nd
Caffeic acid (µg/g)	0.88±0.02
Rutin (µg/g)	352.90±1.44
p-coumaric acid (µg/g)	1.84±0.02
Trans-ferulic acid (µg/g)	0.93±0.01
Rosmaric acid (µg/g)	nd
Quercetin (µg/g)	0.95±0.01
Organic acids	
Tartaric acid (µg/g)	nd
Formic acid (µg/g)	nd
Barbutiric acid (µg/g)	nd
Ascorbic acid (µg/g)	nd
Succinic acid (µg/g)	1959.92±8.88
Fumaric acid (µg/g)	0.50±0.01

nd: not determined

The volatile compound contents of the *Rheum ribes* L. flowers are shown in Table 4. Ethyl propanoate (11.35%), ethyl acetate (0.02%), butyl acetate (22.83%), o-xylene (1.51%), heptacosane (31.81%), oleic acid (7.75%), octatriacontyl pentafluoropropionate (8.33%), 17-pentatriacontene (15.05%) and 5-beta-cholestan-3-one (1.36%) volatile compounds were detected. Kaskoos & Ahamad (2022) conducted a study on the chemical composition of *Rheum ribes* stem parts. Using GC/MS analysis of the ethanolic extract, they identified the presence of several chemical compounds, including hexatriacontane (11.2%), heptadecane 2,6,10,15-tetramethyl (5.53%), 7-hexadecenal, (Z)- (4.63%), vitamin E (3.85%), 3,7,11,15-tetramethylhexadec-2-en-1-yl dodecanoate (3.67%), and tetratriacontane (3.65%). Oleic acid is an omega-9 monounsaturated fatty acid in human adipose tissue. In addition, oleic acid, which is an important component of the human diet and includes animal and vegetable oils, is used as an excipient in pharmaceutical products (Abedi et al. 2020). In addition, oleic acid has been reported to have anti-inflammatory properties (Hwong et al. 2022). In one study, octatriacontyl pentafluoropropionate was used as an antiviral drug in the treatment of COVID-19 (Sahu & Singh 2022). In a literature study, 17-pentatriacontene was shown to have anti-inflammatory, anticancer, antibacterial and antiarthritic properties (Kumar et al. 2018).

Table 4- Volatile compounds in *Rheum ribes* L. flowers

Compounds/Example	<i>Rheum riibes</i> L. Flowers
Ethyl propanoate (%)	11.35
Ethyl acetate (%)	0.02
Butyl acetate (%)	22.83
o-Xylene (%)	1.51
Heptacosane (%)	31.81
Oleic acid (%)	7.75
Octatriacontyl pentafluoropropionate (%)	8.33
17-pentatriacontene (%)	15.05
5-beta-cholestan-3-one (%)	1.36

4. Conclusions

In this study, the primary aim was to analyze the antioxidant activity, secondary metabolites, organic acids, and volatile compounds of *Rheum ribes* L. flowers. The results showed that the *Rheum ribes* L. flowers, a delicious edible and medicinal plant widely used in Anatolia and the Middle East, has antioxidant, anticarcinogenic, antimutagenic and anti-inflammatory effects. Consequently, it was concluded that *Rheum ribes* L. flowers may help protect against lipid peroxidation and oxidative damage, and the dried flowers could be used in the development of safe food products and additives. Furthermore, the findings of the present study suggested that the *Rheum ribes* L. flower can serve as a guide for the production of herbal medicines and will be a valuable resource for future research in this field.

Declarations

Funding

No funding was received.

Declarating of Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conflict of interest Statement

The authors declare no conflicts of interest.

Data Availability Statement

The data are available from the corresponding author upon request.

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