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# Geographical variation in phytochemical composition and antioxidant activity of supercritical $CO_2$ extracts of *Rheum ribes* L. assessed by HPLC-DAD

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## ABSTRACT

Within the scope of this study, the phytochemical profile and antioxidant capacity of root samples belonging to *Rheum ribes*, collected from five regions of Eastern Türkiye and one region of Iran, were evaluated with respect to geographical variation and extraction method. Extracts were obtained from the root parts using supercritical $CO_2$ extraction (SFE), ethanol-co-solvent supercritical $CO_2$ extraction (SFE+EtOH), and subcritical water extraction (SWE). Total phenolic content (TPC) was determined using the Folin–Ciocalteu method, antioxidant capacity was analyzed by the DPPH radical scavenging assay, and aloe-emodin content was quantitatively assessed using a validated HPLC-DAD method.

SWE extracts exhibited pronounced geographical variation compared to SFE+EtOH extracts, whereas neat SFE resulted in negligible phenolic recovery. The highest phenolic contents were detected in the Sivas and Malatya populations. Antioxidant activity was particularly evident in the Iran population ($IC_{50}$ ≈ 79.2 ppm, SWE) and the Sivas sample ($IC_{50}$ ≈ 255.5 ppm,

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SFE+EtOH), and these findings were interpreted in relation to marked edaphic differences among the collection sites. Aloe-emodin recovery was significantly higher in SFE+EtOH extracts (3.7–7.4 mg $g^{-1}$ d.w.) compared to neat SFE (≤1.2 mg $g^{-1}$ d.w.). Overall, differences in extraction polarity and ecological–edaphic conditions played a decisive role in metabolite recovery. SWE proved superior for phenolic enrichment, whereas ethanol-modified SFE was more efficient for anthraquinone extraction.

**Keywords:** *Rheum ribes*, supercritical extract, antioxidant activity, HPLC-DAD, phytochemical composition

## INTRODUCTION

*Rheum* L. species (Polygonaceae) hold an important place in pharmacological and natural product research due to their rich secondary metabolite content. Specifically, anthraquinones (emodin, rhein, aloe-emodin, chrysophanol, etc.), anthrones, flavonoids, stilbenes, and phenolic compounds are among the main component groups associated with antioxidant, antimicrobial, and anticancer effects[1]. Current reviews emphasize that rhubarb (*Rheum*) species can show promising biological effects in various malignancies at the preclinical level, and that these activities may be largely related to anthraquinone derivatives[2]. Furthermore, the phytochemical diversity and multiple biological effects of rhubarb necessitate consideration of inter-species and sample origin variability[3,4]. Therefore, it is critically important to evaluate not only biological activity but also phytochemical differentiation depending on the extraction method in *Rheum* species[5].

In recent years, environmentally friendly approaches have come to the fore in obtaining bioactive compounds from plant materials; supercritical $CO_2$ extraction SFE ($scCO_2$) in particular has become widespread due to its selectivity, low solvent residue, and sustainability advantages. It has been reported that the $scCO_2$ method offers advantages, particularly in the recovery of more lipophilic/semi-polar components, and that a broader spectrum of compounds can be achieved with the use of co-solvents[6,7]. In contrast, subcritical water extraction (SWE) enables environmentally friendly extraction of many bioactives, particularly phenolic compounds, by utilizing the changing solvent properties of water at high temperature and pressure. The SWE literature demonstrates that the solubility of phenolic compounds and the composition of the extract can be significantly influenced by modifying the temperature and process conditions. This offers a significant advantage for targeted extract design in species rich in phenolics and anthraquinones, such as *Rheum*[8,9].

*R. ribes* is noteworthy not only from a pharmacological perspective but also from an ethnobotanical one. Its fresh stems and leaves are consumed as food in some regions, as well as used for various purposes in traditional medicine[1]. In Türkiye, it is mostly used as a diuretic, while in Iran, it is reported to be used as a sedative; in addition, its traditional uses have been related to stomach disorders, hemorrhoids, hypertension, and some infections mentioned in the sources[10].

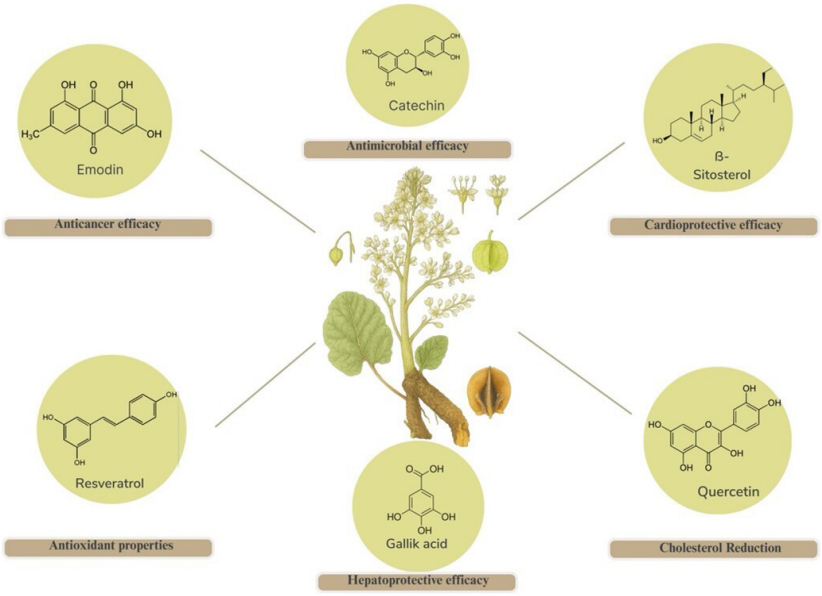


**Figure 1.** Major phytochemical constituents of *Rheum ribes* and their reported biological activities

In this study, six *R. ribes* samples collected from different regions of Türkiye and 1 Iranian *R. ribes* sample were evaluated. The samples were freeze-dried (<14% moisture), ground (<1 mm), and subjected to a three-step extraction: (i) supercritical $CO_2$, (ii) SFE with EtOH co-solvent, and (iii) subcritical water extraction. Subsequently, moisture, total phenolic content (TPC), DPPH radical scavenging activity/$IC_{50}$, and anthraquinone–flavonoid–phenolic profile analyses were performed on the extracts using HPLC-DAD. This approach provides a robust experimental design for comparatively demonstrating how the same plant matrix can be fractionated into different chemical fractions using different extraction technologies.

## METHODOLOGY

### Plant material and herbarium authentication

Roots of *R. ribes* were collected from different geographical regions of Türkiye and Iran, including Bahçesaray, Erzurum, Erzincan, Sivas, Malatya, and Iran (Figure 2).

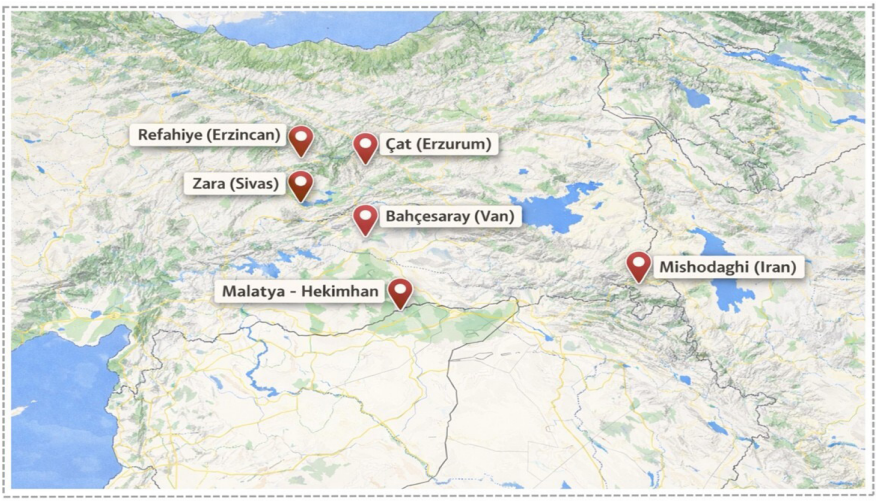


**Figure 2.** Geographic distribution of analyzed *Rheum ribes* populations from Eastern Türkiye and Iran

Plant materials were harvested during the vegetation period from natural populations in their native habitats (Figures 3, 4, 5, 6, 7, 8).

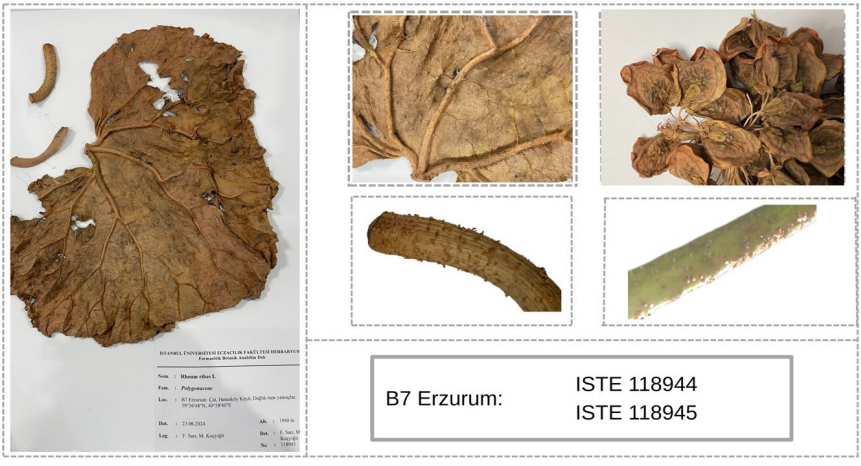


**Figure 3.** Herbarium specimen and detailed morphological characteristics of *Rheum ribes* from B7 Erzurum (ISTE 118944–118945)

**Figure 4.** Herbarium specimen and reproductive morphological characteristics of *Rheum ribes* from B8 Van (Bahçesaray) (ISTE 118932–118936)

**Figure 5.** Herbarium specimen and reproductive morphological characteristics of *Rheum ribes* from B6 Erzincan (ISTE 118940–118943)

**Figure 6.** Herbarium specimen and reproductive morphological characteristics of *Rheum ribes* from B6 Sivas (ISTE 118939)

**Figure 8.** Herbarium specimen and reproductive morphological characteristics of *Rheum ribes* from Iran (ISTE 118913)

All collected plant materials were taxonomically identified with reference to the

Polygonaceae family treatment in Volume 2 of *Flora of Türkiye and the East Aegean Islands*. Taxonomic identification was carried out by Mine Koçyiğit Avcı and Fatma Sarı. After drying, the collected *R. ribes* specimens were mounted on herbarium sheets, and the relevant information was recorded on labels, which were subsequently affixed to the corresponding specimens.

In addition to the freshly collected specimens, historical herbarium records housed in the leading herbaria of Türkiye ISTE (Herbarium of Istanbul University, Faculty of Pharmacy), HUB (Hacettepe University Herbarium), and ANK (Herbarium Turcicum, Ankara University) were examined to confirm the geographical distribution of *R. ribes* in Eastern Türkiye. Voucher numbers and detailed locality information are presented in Table 1. Voucher specimens were deposited in the Herbarium of Istanbul University, Faculty of Pharmacy (ISTE). Voucher numbers were assigned as follows: Sivas (ISTE 118939), Erzurum (ISTE 118944, 118945), and Erzincan (ISTE 118940, 118941, 118942, 118943), Bahçesaray (ISTE 118932, 118933, 118934, 118935, 118936), Malatya (ISTE 118914, 118915), and Iran (ISTE 118913) (Table 1).

**Table 1.** Locality information and voucher numbers of *Rheum ribes* specimens examined

| Locality Information | ISTE Code |
|---|---|
| **B7 Erzurum:** Çat, Hatunköy Village, slopes of mountainous hills, 39°36'48"N, 40°58'40"E, 1940m, F. Sarı, M. Koçyiğit | ISTE 118944<br>ISTE 118945 |
| **B6 Erzincan:** Refahiye, Sivas–Erzincan Highway, mountainous hill slopes, 39°57'41"N, 38°40'37"E, 2010m, F. Sarı, M. Koçyiğit | ISTE 118940<br>ISTE 118941<br>ISTE 118942<br>ISTE 118943 |
| **B6 Sivas:** Zara, 10 km beyond the Kızılırmak River, mountainous areas, 39°54'59"N, 37°44'24"E, 1528m, F. Sarı, M. Koçyiğit | ISTE 118939 |
| **B8 Van:** Bahçesaray, Karabet Plateau, mountainous area, south-facing slopes, m38.1261°N, 42.8006°E, 2750m, F. Sarı, M. Koçyiğit | ISTE 118932<br>ISTE 118933<br>ISTE 118934<br>ISTE 118935<br>ISTE 118936 |
| **Iran:** East Azerbaijan Province, Mishodaghi Mountain (Misho Mountain), rocky slopes, high-altitude habitat, 38.311°N, 45.553°E, 2847 m. M. Rahro | ISTE 118913 |
| **B7 Malatya:** Malatya – Arapgir / Hekimhan road, foothills of Yama Mountain (stony–rocky slopes, plateau margins), 38.9115°N, 38.1320°E, 1500 m, F. Sarı, M. Koçyiğit | ISTE 118914<br>ISTE 118915 |

**Table 2.** Historical herbarium records of *Rheum ribes* examined from ISTE, ANK, HUB, ISTF and MARE collections

| Collector | Herbarium (Code) | Collection Date | Altitude (m) | Locality Information | Grid |
|---|---|---|---|---|---|
| E. Tuzlacı | ISTE | 13.08.1984 | 1950 | Van: Gürpınar, road to Güzelsu (24 km from Gürpınar) | B9 |
| A. & T. Baytop | ISTE | 19.06.1981 | 2200 | Van: Erek Mountain, road to Gölardı Village | B9 |
| A. & T. Baytop, A. Atilla | ISTE | 19.06.1981 | 2200 | Van: road to Bahçesaray, Karabel Pass | B9 |
| A. & T. Baytop, A. Atilla | ISTE | 21.06.1981 | 2600 | Van: road to Bahçesaray, Karabel Pass | B9 |
| T. Baytop | ISTE | 03–04.06.1971 | — | Bitlis: between Ahlat and Adilcevaz | B9 |
| T. Baytop | ISTE | 03.06.1971 | 2200 | Ağrı: Tahir Pass–Eleşkirt, Kavurman Mountain | B9 |
| B. Yıldız, Z. Bahçecioğlu | ISTE | 08.07.2004 | 1700–1800 | Kahramanmaraş: between Göksun and Geben, Kayranlı Mountain | C6 |
| N. & M. Tanker | ISTE | 28.04.1957 20.08.1961 | 1400 | Elazığ: Pekinik Village, Çakıllı Çukur Hill | B7 |
| Davis & O. Polunin | ISTE | 18.07.1954 | 2300–2600 | Van: Erek Mountain, volcanic rocky slopes | B9 |
| V. Akman | ISTE | 13.06.1967 | 1250 | Kahramanmaraş: Andırın, Sarım Plateau (Amanos Mountains) | C6 |
| Davis & O. Polunin | ANK | 18.07.1954 | 2300–2600 | Van: Erek Mountain, volcanic rocky slopes | B9 |
| V. Akman | ANK | 13.06.1967 | 1250 | Kahramanmaraş: Andırın, Sarım Plateau, Amanos Mountains | C6 |
| Adil Güner | HUB | 24.05.1978 | 1800 | Bitlis: Yumurtatepe Village, Soğanlıdere, volcanic sandy terrain | B9 |
| Bayram Yıldız | HUB | 13.05.1978 | 2000 | Kahramanmaraş: between Süleymanlı and Bertiz Mountain, volcanic terrain | C6 |
| Adil Güner | HUB | 1978 | 1750–1850 | Hakkari: Yüksekova, Esendere, Keçili Locality | C10 |

| Collector | Herbarium (Code) | Collection Date | Altitude (m) | Locality Information | Grid |
|---|---|---|---|---|---|
| Bayram Yıldız | HUB | 13.05.1978 | 2000 | Kahramanmaraş: between Süleymanlı and Bertiz Mountain | C6 |
| Adil Güner | HUB | 1978 | 1800 | Bitlis: Yumurtatepe Village | B9 |
| Fatma Nujin Ertaş | MARE: 18578 | 25.06.2016 | — | Van, Gürpınar, foothills of Dibekli Mountains | — |
| Ertan Tuzlacı | MARE: 450 | 11.07.1986 | 2000 | Sivas, Şerefiye, Karabayır Pass | A6 |
| Muharrem Biçer | MARE: 15295 | 12.07.2012 | — | Hakkâri, Yüksekova, Tarlacık Village | C9 |
| Ertan Tuzlacı | MARE: 874 | 16.VII.1987 | 1700 | Erzincan, between Kuruçay and Refahiye, 32 km from Kuruçay | B7 |
| M. Nuri Gültepe | MARE: 8959 | 05.05.2002 | — | Van | — |
| Bülent Kişi, Fuat Yakupoğlu | MARE: 22847 | 27.05.2023 | — | Erzurum, Pasinler, Otlukkapı (39°58’58”N, 41°46’00”E) | A8 |
| Fatma Nujin Ertaş | MARE: 18049 | 03.05.2015 | — | Van, Gürpınar, Dibekli Mountain | — |
| Ertan Tuzlacı | MARE: 1017 | 17.07.1987 | 2100 | Erzincan, road from Erzincan to Demirkent, 15 km from Erzincan | B7 |
| Reyhan Nur Sönmez | MARE: 23801 | 27.06.2024 | — | Elazığ, Merkez, Koruk Village | B7 |
| Cem Işık | MARE: 17337 | 21.06.2016 | — | Siirt, Merkez | C8 |
| Ahmet Doğan | MARE: 10807 | 16.06.2007 | 1520 | Tunceli, Ovacık, Yenikonak Village (39°17’31.96”N, 39°58’30.99”E) | B7 |
| Serdar Öztürk | MARE: 23482 | 02.VI.2024 | — | Ağrı, local market (purchased material) | B10 |
| Musa Türker | MARE | 15.09.19.. | — | Bitlis (Bg.), Van, Çatak | — |
| A. Atilla | ISTF: 11040 | 09.05.1951 | — | Eğim Mountain–Elazığ | B7 |

The collected roots were air-dried at room temperature in a shaded environment and stored in airtight containers until extraction.

### Supercritical fluid extraction

Subsequent to drying, the samples were pulverized and sieved through a 600 μm mesh using a Retsch® sieve system to standardize particle size for extraction. The powdered root material was subsequently stored at 4°C until extraction. This pre-treatment step ensured chemical integrity and homogeneity of the samples prior to further analyses (Figure 9).

Supercritical fluid extraction was carried out using a SuperEx SC-1000 system equipped with a 1 L extraction vessel (maximum operating conditions: 500 bar and 200°C). Prior to extraction, dried roots were comminuted using a high-speed bladed cutter (28000 rpm) to obtain particles smaller than 1 mm. The powdered material was loaded into a stainless-steel extraction basket and placed inside the extraction vessel.

Extraction was performed in dynamic mode with continuous solvent flow. Initially, extraction was conducted using pure $CO_2$ at 400 bar and 40°C for 180 min. This was followed by a second extraction step at 300 bar using $CO_2$ modified with 15% (v/v) food-grade ethanol for 180 min. $CO_2$ flow rate was maintained at 100 g $min^{-1}$ throughout the experiments. Extracts were collected in a separator vessel, and ethanol was removed under reduced pressure using rotary evaporation.

**Figure 9.** Drying, grinding, and sieving of *Rheum ribes* root materials from different populations prior to extraction

### Subcritical water extraction

Following supercritical fluid extraction, subcritical water extraction was performed in the same system by introducing pressurized water at 140°C for 30 min. The water flow rate was maintained at 30 mL $min^{-1}$. Following extraction, aqueous extracts were collected and freeze-dried to remove residual moisture.

### Total phenolic content (TPC)

Total phenolic content was determined using the Folin–Ciocalteu colorimetric method. Briefly, appropriately diluted extract solutions were mixed with diluted Folin–Ciocalteu reagent and sodium carbonate solution, followed by incubation at 40°C for 30 min. Absorbance was measured at 760–765 nm using a UV–Vis spectrophotometer (Shimadzu i1900 model). Gallic acid was used as the reference compound and results were expressed as mg gallic acid equivalents (GAE) per g extract[11].

### DPPH scavenging activity

DPPH radical scavenging activity was determined using the method described in the literature[2]. A 0.1 mM DPPH solution was prepared in methanol and mixed with extract solutions at different concentrations (20–400 ppm). The reaction mixtures were incubated in the dark at room temperature for 20 min and absorbance was recorded at 517 nm using a UV–Vis spectrophotometer (Shimadzu i1900 model). Radical scavenging activity (%) was calculated relative to the control and $IC_{50}$ values were determined from concentration–response curves[11].

### HPLC conditions

Aloe-emodin analysis was performed using a Shimadzu i-Series 2050-3D HPLC system equipped with a diode array detector (Shimadzu, Kyoto, Japan). Aloe emodin was purchased from Sigma Aldrich (St. Louis, MO, USA). Samples were dissolved in methanol (10 mg $mL^{-1}$) and filtered prior to injection through a 0.45 μm membrane filter. Chromatographic separation was achieved on a reversed-phase C18 column (250 × 4.6 mm, 0.6 μm particle size) maintained at 45°C. The mobile phase consisted of acetonitrile (solvent A) and 0.05 M phosphoric acid in water (solvent B) using a gradient elution program: 8% A to 27% A over 50 min, followed by 80% A for 10 min, and re-equilibration to initial conditions within 10 min (total run time: 80 min). The flow rate was set to 0.8 mL $min^{-1}$ and detection was carried out at 440 nm (Figure 10). Aloe-emodin was identified by comparing retention time and UV spectra with those of an authentic standard.

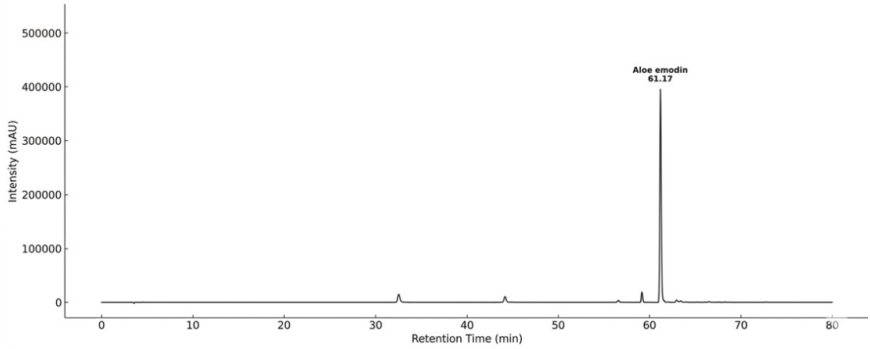


**Figure 10.** HPLC-DAD chromatogram of Aloe-emodin standard recorded at 440 nm

Quantification was performed using an external calibration curve prepared in the concentration range of 10–200 ppm (Table 3).

**Table 3.** HPLC calibration data and quantitative parameters of aloe-emodin

| Compound | Retention Time (min) | Calibration Equation | $r^2$ | Content Range (mg $g^{-1}$ d.w.) |
|---|---|---|---|---|
| Aloe-emodin | 61.17 | y = 24,770x + 5,000 | 0.9994 | 3.7–7.4 |

## RESULTS and DISCUSSION

### Total phenolic content (TPC)

The total phenolic contents of *R. ribes* samples varied depending on both geographical origin and extraction technique (Figure 10). Across all populations, subcritical water extraction (SWE) consistently yielded higher phenolic levels than supercritical fluid extraction performed with ethanol co-solvent (SFE+EtOH).

The highest phenolic contents were obtained from SWE extracts of the Sivas and Malatya populations, while Bahçesaray and Erzurum samples also exhibited relatively high values. In contrast, SFE+EtOH extracts generally produces lower phenolic recoveries, particularly in the Erzincan population. These findings highlight the decisive role of solvent polarity in phenolic extraction.

The enhanced performance of SWE can be attributed to the reduced dielectric constant of water under subcritical conditions, which improves the solubility of moderately polar phenolics and mass transfer efficiency[12,13]. Although ethanol increases the polarity of supercritical $CO_2$, SFE+EtOH extracts still contained

lower phenolic levels compared to SWE in most samples (Figure 11), consistent with previous reports indicating that polar phytochemicals are more efficiently recovered using water-based extraction techniques[14].

Notably, phenolic compounds were not detected in extracts obtained using neat supercritical $CO_2$, indicating negligible recovery under non-modified supercritical conditions. This observation is consistent with the non-polar nature of $CO_2$, which limits the extraction of highly polar phenolics unless a polar co-solvent is employed[14].

Overall, the results emphasize the strong influence of extraction strategy on phenolic yield and support SWE as an efficient green approach for obtaining phenolic-rich *Rheum* extracts.

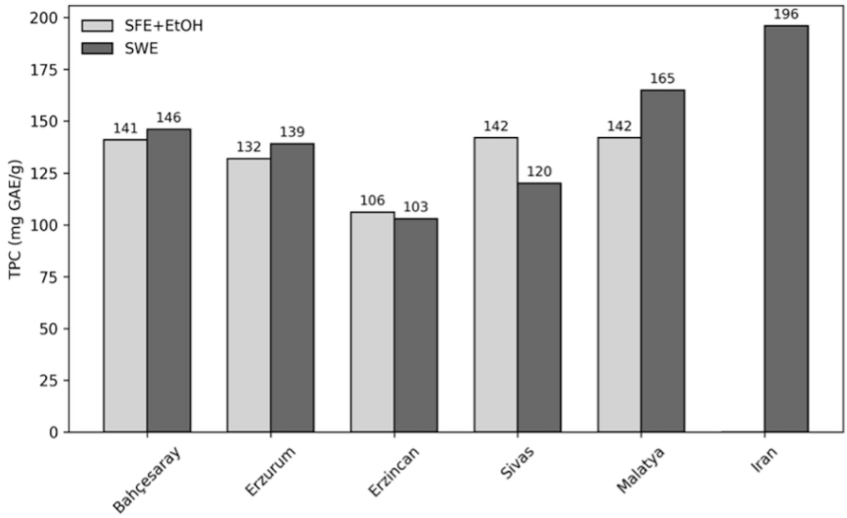


**Figure 11.** Total phenolic content in *R. ribes* extracts

### DPPH radical scavenging activity

The antioxidant capacity of *R. ribes* extracts was evaluated using the DPPH radical scavenging assay.

Among the analyzed samples, measurable radical scavenging activity was primarily observed in the Iran and Sivas populations. The *R. ribes* Iran extract obtained by subcritical water extraction exhibited relatively strong activity ($IC_{50}$ ≈ 79.2 ppm), whereas the *R. ribes* Sivas sample showed detectable activity only in the SFE+EtOH extract and at a markedly higher $IC_{50}$ value (≈255.5 ppm), indicating weaker antioxidant potential. In contrast, several

other populations, including Erzincan, Bahçesaray, Malatya, and Erzurum, exhibited negligible or undetectable activity under the tested conditions.

The selective detection of antioxidant activity suggests that both extraction polarity and population origin play a decisive role in determining radical scavenging efficiency. Although phenolic compounds were detected in multiple samples, the lack of strong DPPH activity in certain populations indicates that antioxidant potential depends not only on total phenolic abundance but also on qualitative differences in phenolic composition and synergistic interactions among secondary metabolites[15,16].

Previous studies on *Rheum* species have reported moderate to strong antioxidant activity, particularly in polar extracts enriched in phenolic compounds. For instance, investigations on rhubarb roots demonstrated significant DPPH scavenging activity associated with phenolic acids and flavonoids[17,18]. Similarly, aqueous extracts of *Rheum officinale* have been shown to exhibit notable radical scavenging capacity, supporting the role of polar phytochemicals in the antioxidant potential of the genus[19]. The variability observed in the present study is therefore consistent with earlier reports emphasizing that antioxidant capacity in wild *Rheum* populations may vary considerably depending on environmental factors and extraction methods.

Overall, the findings underline the different antioxidant profile of geographically distinct *R. ribes* populations and highlight the importance of both extraction strategy and ecological origin in evaluating their bioactive potential.

**Quantitative analysis of phenolic compounds by HPLC**

Quantitative determination of aloe-emodin in *R. ribes* populations was carried out using an external calibration approach based on a validated HPLC-DAD method. The method exhibited excellent linearity within the tested concentration range (10–100 ppm), confirming its suitability for quantitative analysis of anthraquinone derivatives. Aloe-emodin was selected as a target compound due to its well-established role as a characteristic anthraquinone marker of the genus *Rheum*, widely employed for chemotaxonomic evaluation and quality control of rhubarb species[20,21]. Recovery values revealed substantial variability among geographically distinct populations (Table 4).

**Table 4.** Recovery values of aloe-emodin in *Rheum ribes* L. populations determined by HPLC following supercritical extraction

| Locality | SFE Recovery (mg $g^{-1}$ d.w.) | SFE+EtOH Recovery (mg $g^{-1}$ d.w.) |
|---|---|---|
| Bahçesaray | 0.4 | 7.4 |
| Erzurum | 0.3 | 4.2 |
| Malatya | n.d. | 6.3 |
| Sivas | 0.9 | 3.7 |
| Erzincan | 1.2 | 6.8 |
| Iran | n.d. | 4.8 |

Values are expressed as mg $g^{-1}$ dry weight (d.w.). n.d.: not detected.

SFE+EtOH extraction consistently yielded higher aloe-emodin recoveries compared to neat SFE extraction. Across all samples, SFE+EtOH recoveries ranged from 3.7 to 7.4 mg $g^{-1}$ d.w., with the highest levels detected in Bahçesaray (7.4 mg $g^{-1}$) and Erzincan (6.8 mg $g^{-1}$) populations. Malatya and Iran samples exhibited moderate recoveries, whereas Sivas and Erzurum showed comparatively lower levels. In contrast, neat SFE extracts produced only trace amounts of aloe-emodin (≤1.2 mg $g^{-1}$), highlighting the limited extraction efficiency of non-polar supercritical fluids for semi-polar anthraquinones.

The pronounced increase observed in ethanol-assisted extraction can be attributed to the polarity enhancement provided by the co-solvent. Anthraquinones such as aloe-emodin are moderately polar phenolic compounds, and their extraction efficiency has been shown to increase significantly in the presence of polar modifiers. Similar findings have been reported in supercritical and solvent-based extraction studies of rhubarb, where ethanol-modified systems provided higher yields of aloe-emodin and related anthraquinones compared to non-modified supercritical $CO_2$ extraction[14,22].

Geographical variation also appeared to play a critical role in aloe-emodin accumulation. Differences among populations may reflect environmental factors such as altitude, climate, and soil composition, which are known to influence secondary metabolite biosynthesis in medicinal plants[23]. Comparable intra-species variability in anthraquinone profiles has been documented in phytochemical investigations of *Rheum* taxa collected from diverse ecological regions[21]. Such variability supports the use of aloe-emodin as a chemotaxonomic marker for evaluating phytochemical diversity within the genus.

Although anthraquinones are not considered primary contributors to radical scavenging activity, aloe-emodin has been reported to exhibit moderate

antioxidant potential alongside broader pharmacological properties, including antimicrobial and anti-inflammatory effects[19]. The absence of a direct correlation between aloe-emodin abundance and DPPH activity in the present study suggests that other phenolic constituents may play a more dominant role in the overall antioxidant capacity of *R. ribes* extracts.

Overall, the HPLC results confirm aloe-emodin as a consistent phytochemical marker across geographically distinct *R. ribes* populations and demonstrate that both extraction strategy and ecological origin significantly influence its recovery. The enhanced yields obtained using ethanol co-solvent supercritical $CO_2$ emphasize the importance of solvent polarity in the efficient recovery of anthraquinone derivatives from rhubarb species.

Inter-population differences are influenced not only by geographical variability but also by ecological and edaphic stress components that drive metabolic plasticity. It is well established that secondary metabolites are responsive to environmental fluctuations, and factors such as altitude, UV radiation, temperature oscillations, and soil characteristics play a decisive role in regulating the biosynthesis of phenolic compounds and anthraquinones.

The Zara/Sivas locality is characterized by marked topographical and edaphic heterogeneity, microclimatic transition zones, and diverse soil types, collectively creating micro-habitat diversity that may promote enhanced phenolic accumulation. Environmental stress exposure is known to stimulate secondary metabolite production in plants, which is often reflected in increased antioxidant activity.

In contrast, the Bahçesaray–Karabet locality represents a high-altitude zone characterized by prolonged snow cover and increased UV exposure. Elevation-related enhancement of UV-B radiation is known to

activate the phenylpropanoid pathway, thereby stimulating secondary metabolite biosynthesis. Cold and radiative stress, in particular, have been associated with increased accumulation of anthraquinones and other phenolic derivatives. Accordingly, the elevated aloe-emodin recovery detected in the SFE+EtOH extracts of the Bahçesaray population may reflect altitude-driven metabolic adaptation under intensified environmental stress conditions.

The Iranian (Misho/Mishodaghi) locality, situated at approximately 2855 m above sea level, represents a mountainous habitat exposed to high UV radiation, pronounced temperature amplitudes, and potential water stress. The positive effect

of high altitude on phenolic content and antioxidant capacity has been supported by previous studies[24,25]. In line with this ecological framework, the lower $IC_{50}$ values observed in the SWE extract of the Iranian population appear consistent with altitude-related enhancement of bioactive metabolite accumulation.

In conclusion, this study not only provides new data on the chemical profile of *R. ribes*, but also strengthens the scientific basis for selecting appropriate extraction strategies for ravent species, evaluating the chemical diversity of natural populations, and utilizing aloe-emodin as a potential chemotaxonomic marker.

## STATEMENT OF ETHICS

This study does not require any ethical permission.

## CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

## AUTHOR CONTRIBUTIONS

FS: Conceptualization, fieldwork, ethnobotanical data collection, data curation, formal analysis, visualization, writing original draft, writing review & editing, funding acquisition, fieldwork coordination.

MKA: Supervision, methodology, validation, resources, writing review & editing, funding acquisition, fieldwork coordination.

ŞNK: Methodology, validation, resources, writing review & editing, funding acquisition,

AUZ: Funding acquisition, fieldwork coordination.

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