

Bioactive compounds, antioxidant capacities, and mineral contents of wild strawberries (*Fragaria vesca* and *Fragaria viridis*): A comparative evaluation

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Cite this article as:

Altinkaynak, Y., Altinkaynak Akcan, B. (2026). Bioactive compounds, antioxidant capacities, and mineral content of wild strawberries (*Fragaria vesca* and *Fragaria viridis*): A comparative evaluation. *Food and Health*, 12(2), 135-148. <https://doi.org/10.3153/FH26012>

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Submitted: 03.09.2025

Revision requested: 21.12.2025

Last revision received: 23.12.2025

Accepted: 27.12.2025

Published online: 01.03.2026

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ABSTRACT

This study aimed to evaluate and compare the bioactive profiles of two wild strawberry species (*Fragaria vesca* and *Fragaria viridis*) collected from the Ardahan/Turkey region and processed using the freeze-drying method. Vitamin C content and total antioxidant capacities were measured spectrophotometrically, while phenolic compounds were identified and quantified using LC-MS/MS techniques. Mineral analysis was performed using AAS. *F. viridis* exhibited significantly higher vitamin C content (448.91 mg/100g) and superior antioxidant activity compared to *F. vesca* (312.87 mg/100g). Analysis of phenolic profiles revealed species-specific differences, with *F. vesca* showing distinctly higher anthocyanin levels, including Cyanidin-3-O-glucoside (9029.99 ng/mL), Pelargonidin-3-O-glucoside (4259.54 ng/mL), and Pelargonidin-3-glucoside (5827.36 ng/mL). *F. viridis* had higher concentrations of Quinic acid (5827.97 ng/mL) and Gallic acid (282.37 ng/mL), contributing to its antioxidant potential. Mineral analysis showed that *F. vesca* contained higher levels of calcium (145.29 mg/100g), potassium (1294.13 mg/100g), and magnesium (231.07 mg/100g) compared to *F. viridis*. Our findings demonstrate the phytochemical richness and functional diversity of wild strawberry species in the Ardahan region, with *F. viridis* showing superior antioxidant capacity, and *F. vesca* displaying enhanced mineral content and anthocyanin profile. It was concluded that both wild strawberries have higher bioactivity compared to the literature and are a valuable functional food source with protective potential against chronic diseases, requiring further in vivo and in vitro studies in this context.

Keywords: *Fragaria vesca*, *Fragaria viridis*, Freeze-Drying, Phenolic compounds, Antioxidant activity



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Introduction

Fragaria, commonly known as wild strawberry, is a genus of flowering plants in the *Rosaceae* family native to temperate regions of the Northern Hemisphere. (González & Acuña, 2009). The genus includes at least 19 species and numerous hybrids and cultivars, constituting one of the most diverse and economically important fruit products worldwide. (Hancock & Luby, 1993). Among various species, *Fragaria vesca* L. (European wild strawberry, Wood strawberry) and *Fragaria viridis* Duch. (green strawberry, Field strawberry) stand out as valuable for their nutritional and medicinal properties, recognised for centuries in traditional medicine and modern nutraceutical applications (Seeram, 2008).

The Ardahan region of northeastern Türkiye, characterised by high-altitude plateaus and unique climatic conditions, provides an ideal habitat for these wild strawberry species. Located at elevations exceeding 1900 meters above sea level, this region experiences a continental climate with cold winters and mild summers, creating optimal conditions for the development of bioactive compounds in wild fruits. (Akman & Ketenöglü, 1986). Although the local people of Ardahan do not sufficiently appreciate their value, they can traditionally collect these wild strawberries in July-August and consume them fresh or as jam.

Fragaria vesca and *F. viridis* are distinguished from commonly cultivated strawberries (*Fragaria* × *ananassa* Duch.) by their superior bioactive contents and more intense flavour profiles. These wild species contain significantly higher concentrations of vitamins A, B, and C, along with essential minerals such as calcium, iron, and phosphorus. More importantly, they are rich sources of phenolic acids and flavonoid compounds that significantly contribute to their antioxidant properties and potential health benefits (Giampieri et al., 2012).

Scientific research has revealed that wild strawberry species contain significantly higher levels of bioactive compounds compared to their cultivated counterparts. Research on 4 different *Fragaria* species, including *F. vesca* and *F. viridis*, showed that they contain 126 volatile organic compounds, with flavonoids comprising 25% and phenolic acids representing 23% of the total metabolite content. This extraordinary bioactive compound diversity represents nearly half of the dry weight of the fruits, highlighting the exceptional nutritional value of wild strawberry species (Kowalska et al., 2018; Xu et al., 2025).

The major anthocyanins identified in *F. vesca* and *F. viridis* include pelargonidin-3-glucoside, pelargonidin-3-rutinoside,

and cyanidin-3-glucoside, while ellagic acid represents the ellagitannin compound. However, the concentration and composition of these bioactive compounds can vary significantly depending on geographical location, climatic conditions, and genetic factors. (Tulipani et al., 2008).

Studies conducted on *F. viridis* from the Yakutia region of Russia found that a one-week daily intake of the fresh fruits at 250 g/day increased serum total antioxidant capacity to about 544 µM Trolox equivalents per litre (\approx +30 µM), with a higher dose (400 g/day) reaching 557 µM (\approx +45 µM) (Olennikov et al., 2020). These findings demonstrate that both *F. viridis* and *F. vesca* possess superior antioxidant properties compared to the commonly cultivated *F. ananassa*.

The preservation of bioactive compounds in wild strawberries poses a significant challenge for both research and commercial applications. Traditional drying methods often cause significant losses in heat-sensitive compounds, particularly vitamin C and phenolic compounds. Freeze-drying (lyophilisation) has emerged as a superior preservation technique that maintains the structural integrity of bioactive compounds while extending shelf life (Asami et al., 2003).

Despite the growing interest in wild strawberry species and their bioactive properties, comprehensive analytical studies focusing on Turkish populations are very rare. The present study addresses this research gap by providing the first comprehensive analysis of bioactive compounds and mineral content in *F. vesca* and *F. viridis* species collected from the Ardahan region. Particularly, the *F. viridis* species has never been analysed throughout Turkey.

Materials and Methods

Plant Material Collection and Preparation

Fragaria vesca L. (FV) and *Fragaria viridis* subsp. *viridis* (FVi) wild strawberry fruits were collected in August 2024 from around the Ardahan University Yenisey Campus located in Ardahan province, northeastern Turkey (GPS coordinates: 41.1345°N, 42.7776°E; altitude: 1928 m). A total of 3 kg of *F. vesca* and 1 kg of *F. viridis* samples were obtained at the peak of the ripening period to ensure optimal bioactive compound content. Fresh samples of about 1 kg were also collected from the same regions for total phenolic matter and vitamin C analysis only.

Prof. Dr. Yusuf Kaya performed plant material identification at the Plant Systematics Laboratory of the Biology Depart-

ment, Faculty of Science, Atatürk University. Voucher specimens were deposited in the herbarium collection with the following registration numbers: 791 for *Fragaria vesca* L. and 793 for *Fragaria viridis* subsp. *viridis*.

To preserve biochemical stability and prevent degradation of phenolic compounds, the collected fruit samples were immediately processed using freeze-drying (lyophilisation) technology at the Eastern Anatolia High Technology Application and Research Centre (DAYTAM) of Atatürk University.

Freeze-Drying Process

Freeze-drying is a dehydration technique that enables the removal of water through direct sublimation under vacuum conditions at low temperatures. The collected strawberry samples were first frozen at -80°C for 24 hours to ensure complete ice crystal formation throughout the fruit tissue. Subsequently, the frozen samples were transferred to a laboratory-scale freeze-dryer equipped with a vacuum system capable of maintaining pressures below 0.1 mbar (Karam et al., 2016). After harvesting, strawberry samples were immediately freeze-dried to remove moisture and inhibit microbial and enzymatic activity. The lyophilised samples were subsequently stored in airtight, light-protected containers at room temperature until analysis. Due to the very low water activity of freeze-dried materials, chemical stability was preserved during storage, minimising degradation processes.

Chemicals and Standards

High-purity analytical standards ($\geq 95\%$) used for LC-MS/MS phenolic compound identification and quantification, including phenolic acids (gallic acid, ellagic acid, quinic acid), flavan-3-ols (catechin, epicatechin), and anthocyanins (cyanidin-3-O-glucoside, peonidin-3-O-glucoside, pelargonidin-3-glucoside, and pelargonidin-3-rutinoside), were obtained from Sigma-Aldrich (Germany). LC-MS grade solvents (methanol, acetonitrile, and formic acid) used for UHPLC-ESI-MS/MS analyses were purchased from Merck (Germany).

Experimental Design and Extraction Strategy

In this study, different extraction procedures were deliberately applied depending on the analytical purpose. Fresh and freeze-dried samples were used for vitamin C and total phenolic content analyses to evaluate the effect of freeze-drying on these parameters. All antioxidant activity assays and LC-MS/MS phenolic profiling were conducted exclusively using freeze-dried samples to ensure analytical consistency and stability of bioactive compounds.

For antioxidant activity assays, freeze-dried samples were extracted using three different solvent systems (water, ethanol–water, and methanol) at defined concentrations (1, 5, and 10 mg/mL) to assess solvent-dependent extraction efficiency and antioxidant performance. In contrast, LC-MS/MS analysis was performed using a methanol–water (70:30, v/v) solvent system optimised for comprehensive phenolic compound identification and quantification.

Sample Extraction

Freeze-dried fruit samples were thawed at room temperature and homogenised using an IKA® ULTRA-TURRAX T25 high-speed homogeniser to ensure uniform particle size distribution. Extraction for UHPLC-MS/MS analysis was performed using a methanol: water (70:30, v/v) solvent system at a concentration of 10 mg/mL (Robbins, 2003).

Vitamin C Analysis by HPLC

Vitamin C (L-ascorbic acid) content was determined by high-performance liquid chromatography (HPLC) using a Shimadzu LC20AD system equipped with a column oven (CTO-10AS) and a Photo-diode Array (PDA) detector (SPD-M20A). For vitamin C analysis, fresh and freeze-dried strawberry samples were extracted using a metaphosphoric acid solution (3%, w/v) to stabilise ascorbic acid and prevent oxidation. Samples (1 g) were homogenised with 10 mL of extraction solution, centrifuged at $10,000 \times g$ for 10 min at 4°C , and the supernatant was filtered. Chromatographic separation was performed using an Ultra Aqueous C18 column (250 mm \times 4.6 mm \times 5 μm) maintained at 30°C .

Total Phenolic Content Determination

Total phenolic content (TPC) was determined using the Folin–Ciocalteu colourimetric method with minor modifications based on Slinkard and Singleton (1977). Briefly, 0.5 mL of appropriately diluted extract was mixed with 2.5 mL of 10-fold diluted Folin–Ciocalteu reagent. After 5 min, 2.0 mL of sodium carbonate solution (7.5%, w/v) was added, and the mixture was incubated at room temperature in the dark for 30 min. Absorbance was measured at 765 nm using a UV–Vis spectrophotometer. Gallic acid was used as the calibration standard, and results were expressed as mg gallic acid equivalents (GAE).

UHPLC-ESI-MS/MS Analysis

Phenolic compound identification and quantification were performed using an Agilent 1290 Infinity UHPLC system coupled with an Agilent 6460 Triple Quadrupole mass spectrometer equipped with an electrospray ionisation (ESI) source.

Chromatographic separation was performed using a Zorbax SB-C18 column (4.6 × 100 mm, 3.5 µm particle size, Agilent, USA) maintained at 30°C. The mobile phase consisted of two components: (A) 0.1% formic acid in ultra-pure water and (B) 0.1% formic acid in acetonitrile.

The mass spectrometer was operated in negative electrospray ionisation mode, which provides optimal sensitivity for most phenolic compounds. The Multiple Reaction Monitoring (MRM) mode was used for selective and sensitive detection of target analytes. (Gruz et al., 2011).

Mineral Content Analysis

Mineral analysis was performed using a Perkin Elmer PinAAcle 900H model instrument. Standard calibrations were performed in the range of 0.5 ppm to 5 ppm. Merck brand 1000 ppm standards were used for each standard. Samples were weighed at 0.5 g each into Erlenmeyer flasks, and 4 ml of 65% HNO₃ and 2 ml of 30% H₂O₂ were added. Wet digestion was applied on a magnetic stirrer heater until nitrogen oxide fumes were no longer visible and the solution became clear. After the wet digestion process, the solution was cooled to room temperature, filtered through a 0.45 µm filter, diluted to 25 ml with dilute nitric acid, and submitted to the instrument.

Antioxidant Activity Tests

Multiple complementary assays were employed to evaluate the antioxidant properties of strawberry extracts comprehensively. Total antioxidant capacity (TAC) was determined using the thiocyanate method, which measures the inhibition of lipid peroxidation in a linoleic acid emulsion system. At the same time, radical scavenging activity was assessed using the DPPH assay and expressed as per cent inhibition (Mitsuda, Yasumoto, & Iwami, 1966).

Freeze-dried strawberry samples were extracted using distilled water, ethanol–water (1:1, v/v), and methanol to evaluate solvent-dependent antioxidant performance. Extracts were prepared at final concentrations of 1, 5, and 10 mg/mL and used consistently across all antioxidant activity assays. Fresh strawberry samples were not initially included in the antioxidant activity assays, as the primary experimental design focused on the comprehensive evaluation of freeze-dried materials. Fresh samples were analysed only for a limited number of parameters, including vitamin C and total phenolic content, as a preliminary and cost-effective comparison to assess the impact of freeze-drying.

All prepared extracts at concentrations of 1, 5, and 10 mg/mL were used for both DPPH radical scavenging activity and total antioxidant capacity (thiocyanate) assays.

Statistical Analysis

All experiments were performed in triplicate, and results are presented as mean values ± standard deviation. Statistical analyses were performed using SPSS 20.0 software (IBM Corporation, USA). One-way analysis of variance (ANOVA) was used to determine significant differences between groups, followed by Duncan's multiple range test for post-hoc comparisons. Statistical significance was set at $p < 0.05$.

Results and Discussion

Vitamin C Content Analysis

Vitamin C analysis revealed significant differences between the two wild strawberry species and demonstrated the effect of freeze-drying on ascorbic acid preservation (Figure 1, Table 1). *Fragaria viridis* exhibited significantly higher vitamin C content with an average concentration of 448.91 ± 1.07 mg/100g in freeze-dried samples, while *F. vesca* contained 312.87 ± 13.83 mg/100g. These values represent high ascorbic acid concentrations that significantly exceed those typically found in cultivated strawberry varieties and most other fruits.

The comparison between fresh and freeze-dried samples showed that freeze-dried samples consistently contained higher vitamin C levels than their fresh counterparts. Fresh *F. viridis* samples contained 171.59 ± 7.26 mg/100g vitamin C, while fresh *F. vesca* samples contained only 34.10 ± 1.22 mg/100g.

This apparent increase in vitamin C content in freeze-dried samples can be attributed to the concentration effect resulting from water removal during the lyophilisation process and the prevention of enzymatic degradation that typically occurs during traditional storage and processing.

The ascorbic acid levels obtained in this study were notably higher than many values reported in the literature and results reported from the Gümüşhane region. The freeze-drying method significantly contributed to the preservation of the thermolabile structure of vitamin C. Thus, the measured concentrations were found to be approximately 10-20 times higher than samples analysed by traditional methods. Particularly, the ascorbic acid content of 448.91 mg/100 g determined in *F. viridis* species was not only significantly higher than *F. vesca* (312.87 mg/100 g) but also obtained a value far above the 50-60 mg/100 g range reported in cultivated strawberries. (Fidan et al., 2023).

This situation reveals that interspecies genetic differences, climatic conditions, and the processing techniques used (freeze-drying) play a critical role in the preservation of bioactive

compounds. Furthermore, these high vitamin C levels underline the potential nutritional value of wild strawberry species in the Ardahan region and support their functional food development potential.

Total Phenolic Content and Antioxidant Properties

Total phenolic content analysis revealed significant differences between species and processing methods (Figure 2, Table 2). Freeze-dried *F. viridis* samples contained 1050.958 ± 20.794 mg GAE/L, while freeze-dried *F. vesca* samples contained 696.047 ± 2.179 mg GAE/L.

Comparative analysis showed that *F. viridis* extracts exhibited superior antioxidant performance across multiple parameters. The inhibition percentage of *F. viridis* extracts was consistently higher than that of *F. vesca* extracts in all extract types (water, ethanol-water, and methanol) and concentrations,

showing stronger radical scavenging activity. Phenolic content was also consistently higher in *F. viridis* extracts compared to *F. vesca*, indicating higher phenolic contents in *F. viridis* extracts compared to *F. vesca*. Additionally, the antioxidant capacities of *F. viridis* and *F. vesca* extracts were evaluated in different solvent systems. *F. viridis* extracts reached the highest inhibition percentage (80.11%, 10 mg/mL) and phenolic compound content (20.39 mg GAE/g), especially in an ethanol-water mixture. *F. vesca* extracts, despite showing lower phenolic content, exhibited strong antioxidant activity (78.56%), especially in ethanol-water extracts. While methanol extracts exhibited moderate activity in both species, the inhibition rate and phenolic content were lower in water extracts. In comparison with trolox, extracts of both species showed strong free radical scavenging activity (Figure 3, Table 3).

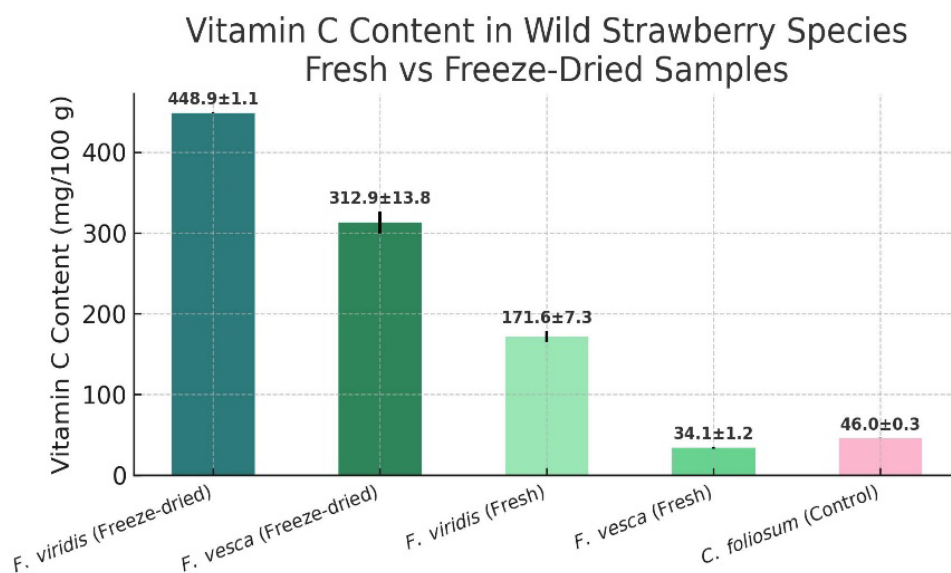
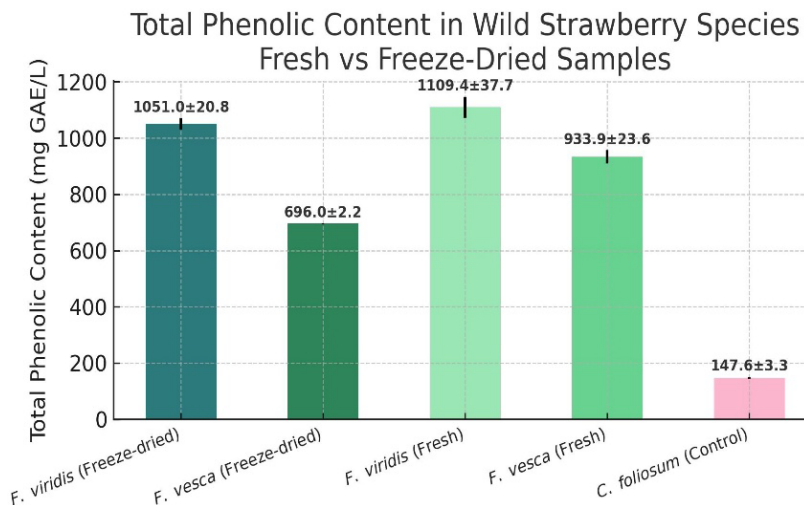


Figure 1. Comparison of vitamin C content of fresh and freeze-dried samples of wild strawberry species (n=3).

Table 1. Vitamin C Content in Fresh and Freeze-Dried Wild Strawberry Species

Species	Processing Method	Vitamin C (mg/100g)	Standard Deviation
<i>F. viridis</i>	Freeze-dried	448.91	±1.07
<i>F. vesca</i>	Freeze-dried	312.87	±13.83
<i>F. viridis</i>	Fresh	171.59	±7.26
<i>F. vesca</i>	Fresh	34.10	±1.22
<i>C. foliosum</i>	Fresh (control)	45.97	±0.30



Results are expressed as mg gallic acid equivalents (GAE) per liter. Error bars represent standard deviation (n=3)

Figure 2. Comparison of total phenolic content of fresh and freeze-dried wild strawberry samples.

Table 2. Total Phenolic Content in Wild Strawberry Species

Species	Processing Method	Total Phenolic Content (mg GAE/L)	Standard Deviation
<i>F. viridis</i>	Freeze-dried	1050.958	±20.794
<i>F. vesca</i>	Freeze-dried	696.047	±2.179
<i>F. viridis</i>	Fresh	1109.445	±37.709
<i>F. vesca</i>	Fresh	933.877	±23.578
<i>C. foliosum</i>	Fresh (control)	147.582	±3.315

Total phenolic content analyses revealed distinct differences between both species and applied processing methods. The values obtained in samples analysed with the freeze-drying technique (*F. viridis*: 1050.96 mg GAE/L; *F. vesca*: 696.05 mg GAE/L) are considerably above the fresh or solvent extract-based values reported in the literature. For example, Yıldız et al. (2014) reported approximately 274-278 mg GAE/100 g phenolic content in *F. vesca* samples collected in Turkey, while Milivojevic et al. (2011) reported values in the range of 300-600 mg GAE/100 g in wild species. In the study conducted in the Gümüşhane region, 165.90 mg GAE/100 g was found for *F. vesca* (Milivojević et al., 2011; Yildiz et al., 2014).

Total antioxidant activity measurements showed lower absorbance values in *F. viridis* extracts compared to *F. vesca*.

Since lower absorbance corresponds to higher antioxidant potential in this test, these results confirm the superior antioxidant activity of *F. viridis*. Overall, *F. viridis* exhibited higher total phenolic content and antioxidant activity compared to *F. vesca*, which contains higher anthocyanin levels.

The findings suggest that *F. viridis* has a superior antioxidant capacity compared to *F. vesca*, with its phenolic density and high inhibition percentages. This result is consistent with literature findings that *F. viridis* has a high ascorbic acid and phenolic acid content (Olennikov et al., 2020). Although *F. vesca* exhibited lower phenolic values, it exhibited significant radical scavenging activity, particularly due to its richness in anthocyanins (Yildiz et al., 2014).

The solvent type also influenced antioxidant activity. Ethanol-water extracts yielded the highest inhibitory and reducing

power values in both species. This may be explained by the more effective extraction of polyphenols in semi-polar solvents. (Aaby et al., 2012).

Comprehensive Phenolic Compound Profiling by LC-MS/MS

Comprehensive phenolic profiling using LC-MS/MS provided detailed information on the phenolic composition of the wild strawberry species (Figure 3, Table 6). The analysis identified and quantified multiple phenolic compound classes, including anthocyanins, phenolic acids, and flavan-3-ols.

Anthocyanin analysis revealed striking differences between the two species, with *F. vesca* showing significantly higher concentrations of these pigmented compounds (Figure 4). Cyanidin-3-O-glucoside, the dominant anthocyanin in both species, was found at concentrations of 9029.99 ± 427.53 ng/mL in *F. vesca* and 3245.68 ± 198.37 ng/mL in *F. viridis* ($p < 0.05$).

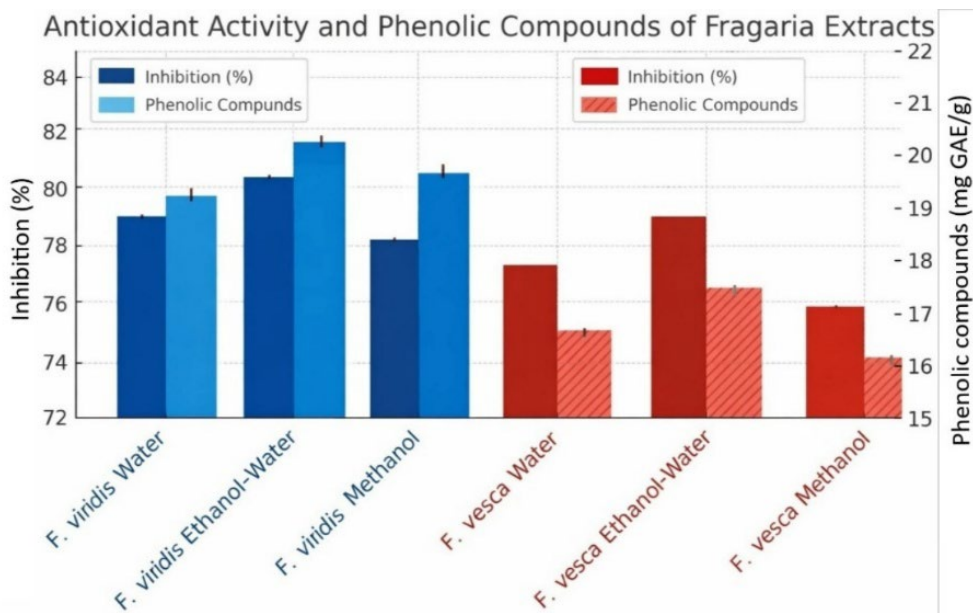
Peonidin-3-O-glucoside showed significant differences, with *F. vesca* containing 4259.54 ± 312.46 ng/mL while *F. viridis* contained 1876.29 ± 145.82 ng/mL ($p < 0.05$). Pelargonidin-3-glucoside was detected at 5827.36 ± 389.42 ng/mL in *F.*

vesca and only 2134.58 ± 176.93 ng/mL in *F. viridis* ($p < 0.05$). Similarly, Pelargonidin-3-rutinoside was found at 782.37 ± 58.29 ng/mL in *F. vesca* and 394.65 ± 32.18 ng/mL in *F. viridis* ($p < 0.05$).

Phenolic acid composition was analysed comparatively between the two species (Figure 6, Table 3). Quinic acid was found significantly higher in *F. viridis* (5827.97 ± 412.36 ng/mL) compared to *F. vesca* (2134.58 ± 187.42 ng/mL) ($p < 0.05$). Gallic acid was found at significantly higher concentrations in *F. viridis* (282.37 ± 21.54 ng/mL) compared to *F. vesca* (145.82 ± 12.37 ng/mL) ($p < 0.05$).

Ellagic acid was found at comparable levels in both species, with *F. vesca* containing 3245.68 ± 245.37 ng/mL and *F. viridis* containing 3128.94 ± 237.65 ng/mL, with no significant difference.

Flavan-3-ol analysis revealed differences in compound distribution between species. Catechin was found more abundantly in *F. viridis* (2134.58 ± 165.29 ng/mL) compared to *F. vesca* (1876.29 ± 143.58 ng/mL), but this difference is not statistically significant. Epicatechin showed similar patterns, with *F. viridis* containing 2876.29 ± 213.76 ng/mL while *F. vesca* contained 3245.68 ± 254.37 ng/mL.



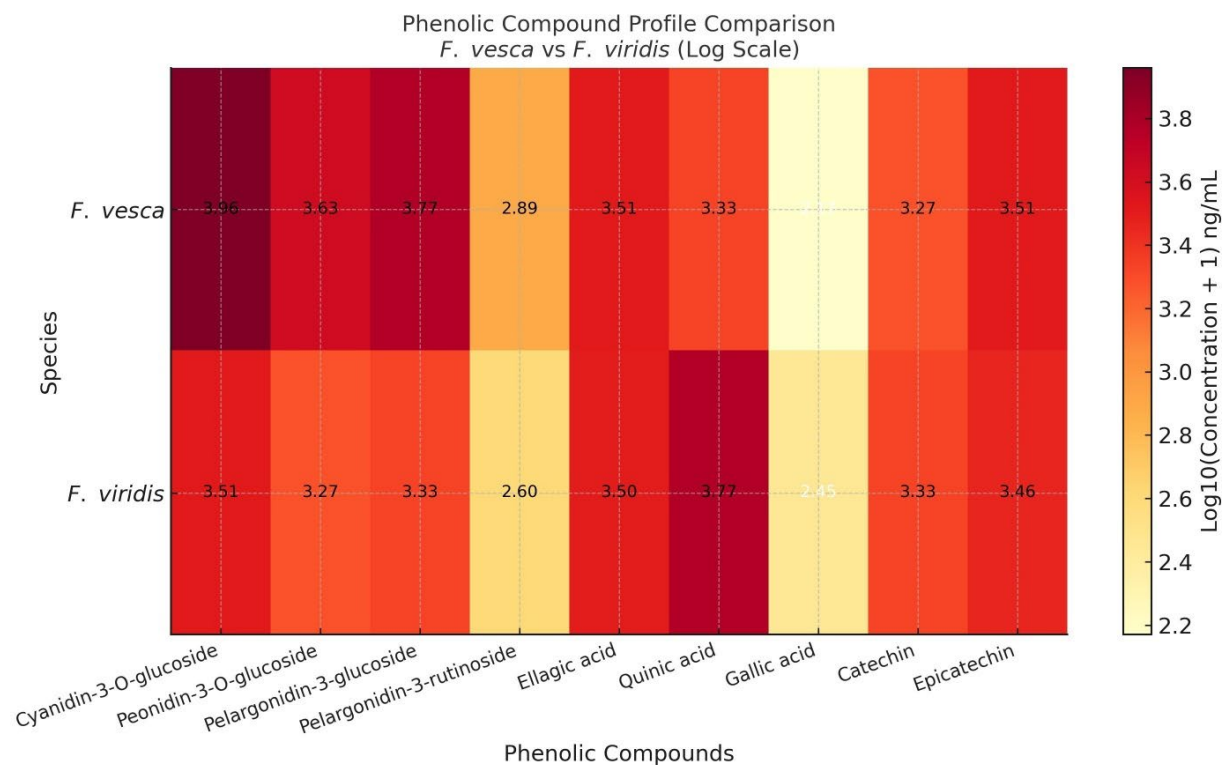
The highest values were obtained in ethanol–water extracts for both species. Error bars indicate standard deviations ($n=3$).

Figure 3. Antioxidant activity (inhibition, %) and phenolic compound contents (mg GAE/g) of *F. viridis* and *F. vesca* extracts in different solvents

Table 3. Comparison of Different Extracts in Terms of Total Antioxidant Activity, Reducing Power, and Phenolic Compounds

Extract Type	Sample	Dose (mg/ml)	Total Antioxidant Activity	Inhibition (%)	Reducing Power (700 nm)	Phenolic Compounds (mg GAE/g)
<i>Fragaria viridis</i> Extracts						
Water Extract	FviS	1.0	0.501±0.002c	74.05	0.242±0.001f	18.22±0.10
	FviS	5.0	0.473±0.002b	76.02	0.507±0.002g	18.88±0.05
	FviS	10.0	0.430±0.002a	78.04	1.050±0.003h	19.51±0.08
	AA	1.0	0.983±0.001d	49.43		
	Trolox	1.0	0.463±0.001a	76.13		
	Control(water)			1.944±0.002e		
Ethanol-Water Extract	FviES	1.0	0.453±0.001c	77.21	0.374±0.002f	19.07±0.12
	FviES	5.0	0.431±0.001b	78.53	0.692±0.002g	19.71±0.10
	FviES	10.0	0.408±0.002a	80.11	1.456±0.002h	20.39±0.09
	AA	1.0	0.983±0.001d	49.43		
	Trolox	1.0	0.463±0.001b	76.13		
	Control(water)			1.944±0.002e		
Methanol Extract	FviM	1.0	0.561±0.002d	72.20	0.142±0.002g	18.71±0.10
	FviM	5.0	0.506±0.002c	74.19	0.404±0.001h	19.26±0.08
	FviM	10.0	0.470±0.002b	76.35	0.541±0.001i	19.78±0.07
	AA	1.0	0.983±0.001e	49.43		
	Trolox	1.0	0.463±0.001a	76.13		
	Control(water)			1.944±0.002f		
<i>Fragaria vesca</i> Extracts						
Water Extract	FvS	1.0	0.545±0.001c	72.09	0.203±0.001f	15.44±0.05i
	FvS	5.0	0.513±0.001b	73.61	0.464±0.002g	15.74±0.01i
	FvS	10.0	0.465±0.002a	76.09	0.963±0.003h	16.39±0.03j
	AA	1.0	0.983±0.001d	49.43		
	Trolox	1.0	0.463±0.001a	76.13		
	Control(water)			1.944±0.002e		
Ethanol-Water Extract	FvES	1.0	0.476±0.001c	75.51	0.326±0.002f	16.01±0.03i
	FvES	5.0	0.465±0.001b	76.08	0.615±0.001g	16.65±0.04i
	FvES	10.0	0.414±0.002a	78.56	1.346±0.001h	17.42±0.02j
	AA	1.0	0.983±0.001d	49.43		
	Trolox	1.0	0.463±0.001b	76.13		
	Control(water)			1.944±0.002e		
Methanol Extract	FvM	1.0	0.583±0.001d	70.01	0.103±0.002g	15.65±0.02i
	FvM	5.0	0.519±0.003c	73.3	0.366±0.001h	15.88±0.01j
	FvM	10.0	0.494±0.002b	74.59	0.505±0.001i	16.34±0.04k
	AA	1.0	0.983±0.001e	49.43		
	Trolox	1.0	0.463±0.001a	76.13		
	Control(water)			1.944±0.002f		

Note: The results are presented as the mean of six parallel measurements (± standard error). Values sharing the same letter are not statistically different according to Duncan's test ($\alpha = 0.05$). AA: Ascorbic Acid



Shown as a heat map with log-transformed concentrations for visualisation

Figure 4. Phenolic compound profile comparison between *F. vesca* and *F. viridis*.

Mineral Content Analysis

Mineral content analysis revealed significant species-specific differences in the mineral composition of wild strawberry species (Figure 5). Our analysis focused on three essential minerals: calcium (Ca), potassium (K), and magnesium (Mg).

F. vesca contained significantly higher calcium levels (145.29 ± 1.757 mg/100g) compared to *F. viridis* (91.75 ± 0.621 mg/100g), corresponding to a 1.6-fold difference. For potassium, *F. vesca* showed higher levels (1294.13 ± 3.827 mg/100g) while *F. viridis* contained (1108.00 ± 10.988 mg/100g). Magnesium analysis revealed that *F. vesca* contained significantly higher levels (231.07 ± 1.050 mg/100g) compared to *F. viridis* (131.73 ± 0.499 mg/100g), corresponding to an approximately 1.8-fold difference (Table 4).

Water-soluble dry matter content analysis revealed species-specific differences. *F. viridis* samples contained $14.19 \pm 0.16\%$ water-soluble dry matter, while *F. vesca* samples contained $11.09 \pm 0.13\%$. The higher water-soluble dry matter content in *F. viridis* indicates higher concentrations of sugars, organic acids, minerals, and other water-soluble compounds.

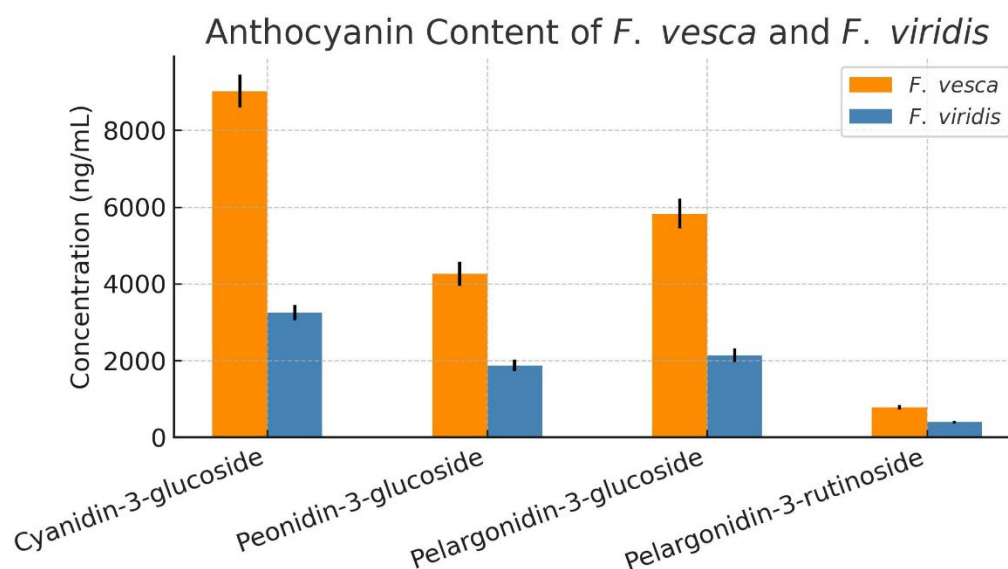
Comparative Analysis and Species-Specific Chemotypes

Comprehensive analysis reveals that *F. vesca* and *F. viridis* represent different chemotypes with complementary bioactive and nutritional profiles. *F. vesca* can be characterised as an anthocyanin- and mineral-rich chemotype, based on its higher concentrations of pigmented compounds and essential minerals. In contrast, *F. viridis* represents a "phenolic acid and vitamin C-rich" chemotype with higher concentrations of gallic acid, quinic acid, vitamin C, and superior overall antioxidant capacity (Tables 1, 2, 3).

The freeze-drying preservation method was effective in maintaining the integrity of bioactive compounds during storage and subsequent analytical characterisation. *F. viridis*'s superior antioxidant performance across multiple test systems, combined with high total phenolic content, may be particularly valuable for applications or research where high antioxidant capacity is desired (Akcicek et al., 2023). Additionally, when compared with *Chenopodium foliosum*, which is reported to have high antioxidant capacity, they have nearly 10 times more effective content in terms of vitamin C and 7 times more effective content in terms of total phenolic matter (Chludil et al., 2008).

Table 4. Phenolic Compound Profile by LC-MS/MS Analysis

Compound	<i>F. vesca</i> (ng/mL)	<i>F. viridis</i> (ng/mL)	Significance
Anthocyanins			
Cyanidin-3-O-glucoside	9029.99 ± 427.53	3245.68 ± 198.37	*
Peonidin-3-O-glucoside	4259.54 ± 312.46	1876.29 ± 145.82	*
Pelargonidin-3-glucoside	5827.36 ± 389.42	2134.58 ± 176.93	*
Pelargonidin-3-rutinoside	782.37 ± 58.29	394.65 ± 32.18	*
Phenolic acids			
Ellagic acid	3245.68 ± 245.37	3128.94 ± 237.65	ns
Quinic acid	2134.58 ± 187.42	5827.97 ± 412.36	*
Gallic acid	145.82 ± 12.37	282.37 ± 21.54	*
Flavan-3-ols			
Catechin	1876.29 ± 143.58	2134.58 ± 165.29	ns
Epicatechin	3245.68 ± 254.37	2876.29 ± 213.76	ns

Significance: * = $p < 0.05$, ns = not significant**Figure 5.** Anthocyanin content comparison between *F. vesca* and *F. viridis*.

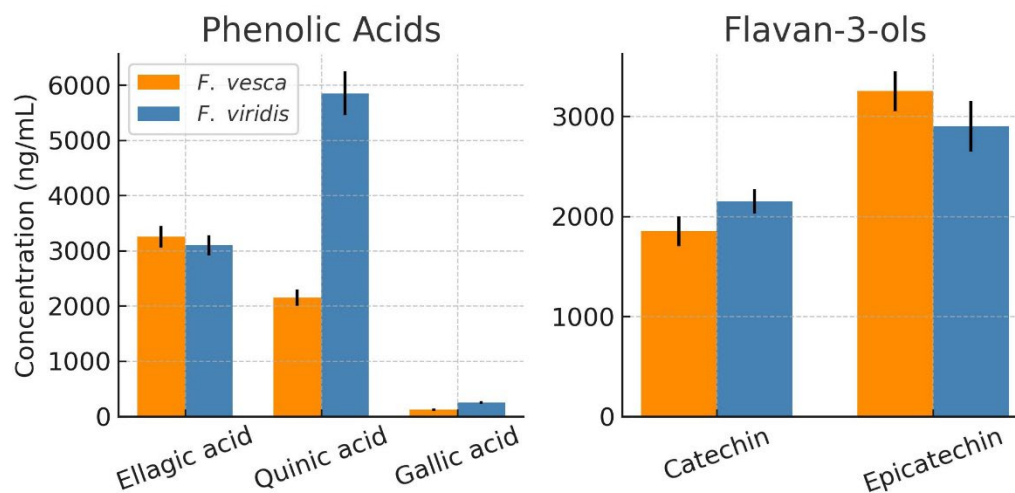
Phenolic Acids and Flavan-3-ols Content of *F. vesca* and *F. viridis*

Figure 6. Comparison of phenolic acids and flavan-3-ols content between *F. vesca* and *F. viridis*.

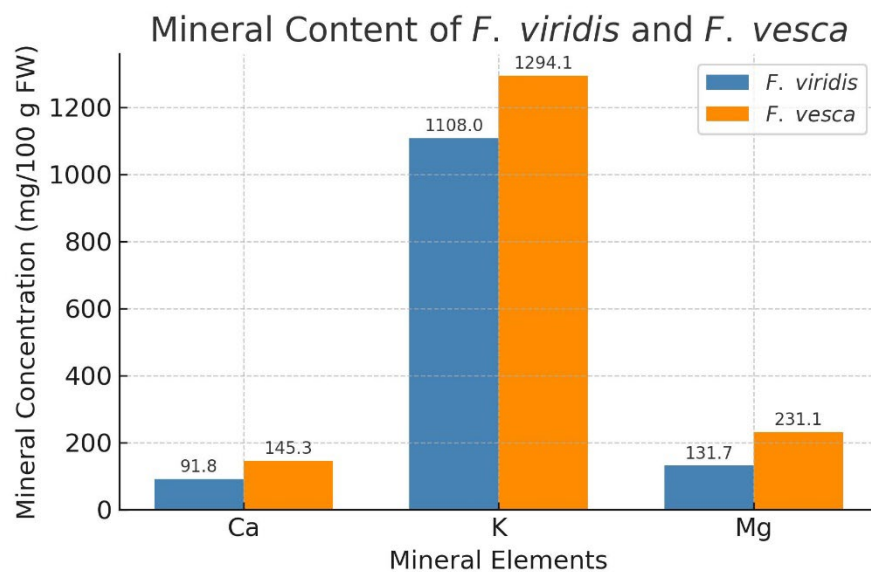


Figure 7. Comparison of calcium, potassium and magnesium concentrations between *F. viridis* and *F. vesca*

Table 5. Mineral Content and Water-Soluble Dry Matter

Parameter	<i>F. viridis</i>	<i>F. vesca</i>	Unit
Dry Matter Content	14.19 ± 0.16	11.09 ± 0.13	%
Ca	91.75 ± 0.621	145.29 ± 1.757	mg/100g
K	1108.00 ± 10.988	1294.13 ± 3.827	mg/100g
Mg	131.73 ± 0.499	231.07 ± 1.050	mg/100g

In our study, both *F. viridis* and *F. vesca* extracts exhibited a dose-dependent increase in antioxidant capacity. Particularly, ethanol-water extracts stood out with high inhibition percentages (over 80%) and reducing power values. These results are in agreement with previous studies reporting antioxidant activity in methanol extracts. Furthermore, the similarity of the obtained inhibition rates (72-80%) with trolox standard values confirms that these extracts have strong free radical scavenging capacity. This finding is supported by the study of Aaby et al. (2012), which revealed that phenolic compounds play a key role in determining antioxidant capacity in 27 cultivated strawberry varieties (Aaby et al., 2012).

In terms of total phenolic content, *F. viridis* extracts (18.22-20.39 mg GAE/g) and *F. vesca* extracts (15.44-17.42 mg GAE/g) presented high values. Similarly, Milivojević et al. (2011) reported that phenolic compounds in wild *Fragaria* species are higher than in cultivated varieties, while Yıldız et al. (2014) reported a richer bioactive profile in *F. vesca* collected in Turkey compared to cultivated strawberries (Milivojević et al., 2011; Yıldız et al., 2014). In this context, the values obtained in our study (15-20 mg GAE/g) are much higher concentrations than the 300-600 mg GAE/100 g FW range reported in the literature.

Total phenolic contents in *Fragaria* species obtained from the Ardahan region (1500-2000 mg GAE/100 g FW, also *F. viridis*: 1050.96 mg GAE/L; *F. vesca*: 696.05 mg GAE/L) were found to be much higher than the values reported in the literature (for example, 165.9 mg GAE/100 g in Gümüşhane; Yıldız et al., 2014: 274-278 mg GAE/100 g) (Tables 2-5) (Fidan et al., 2023; Milivojević et al., 2011; Yıldız et al., 2014). These observations suggest that geographical conditions, together with freeze-drying and extraction methods, may contribute to the preservation of phenolic compounds. Therefore, the high phenolic density of Ardahan strawberry strongly supports its functional food potential.

F. viridis exhibited higher ascorbic acid and phenolic acid contents, whereas *F. vesca* was characterised by higher anthocyanin levels and mineral contents, including calcium, potassium, and magnesium. This biochemical composition indicates that both species may have nutritional relevance and potential for functional food research. This biochemical composition indicates that both species may have nutritional relevance and potential for functional food research. Similar bioactive profiles in the literature have been reported to provide protective effects in terms of preserving cardiovascular functions, reducing oxidative stress, and supporting the immune system. In this

context, Ardahan strawberries have superior antioxidant capacity compared to typical cultivated varieties and many common fruits. (Giampieri et al., 2012; Yıldız et al., 2014).

From a health perspective, high ascorbic acid levels are of critical importance for strengthening immune function and preserving bone health (Jacob & Sotoudeh, 2002). Anthocyanins and phenolic compounds have been reported to contribute to protective effects against chronic conditions such as type 2 diabetes, obesity, cardiovascular diseases, and neurodegenerative diseases. In addition, mineral content has been associated with supportive roles in metabolic processes and musculoskeletal health (Basu et al., 2010).

Conclusion

This comprehensive study provides the first detailed characterisation of bioactive compounds and mineral content in wild strawberry species (*Fragaria vesca* and *Fragaria viridis*) native to the Ardahan region of northeastern Turkey. The research demonstrates that these wild fruits possess nutritional and functional properties that exceed commercially cultivated strawberry varieties.

F. viridis emerged as a superior source of vitamin C and phenolic acids, exhibiting exceptional antioxidant capacity across multiple test systems. Additionally, having nearly 10 times more effective content in terms of vitamin C and 7 times more effective content in terms of total phenolic matter compared to *Chenopodium foliosum*, which is reported to have high antioxidant capacity, is a remarkable result. *F. vesca* showed superior anthocyanin content, particularly cyanidin-3-O-glucoside and pelargonidin-3-glucoside, along with higher mineral content, including calcium, potassium, and magnesium.

When *F. viridis* and *F. vesca* grown in the Ardahan region are evaluated from a health perspective, this high bioactive profile carries protective potential against various chronic diseases. High ascorbic acid levels are of critical importance for strengthening immune system function, collagen synthesis, and preserving bone health. High levels of phenolic acids and flavonoids in both species play a role in reducing oxidative stress, preserving vascular functions, and reducing the risk of type 2 diabetes, obesity, neurodegenerative diseases, and cardiovascular disease. Mineral richness offers a complementary advantage for musculoskeletal health, electrolyte balance, and metabolic functions. In this framework, advanced in vivo and in vitro research is needed. Additionally, the freeze-drying preservation method for wild strawberries proved to be highly effective in long-term storage and preserving bioactive compound integrity.

These findings highlight the significant untapped potential of wild strawberry species native to the Northeast Anatolia region and indicate that detailed examination of developing high-value functional food components and nutraceuticals may also be important. It was concluded that both wild strawberries have higher bioactivity compared to the literature and are a valuable functional food source with protective potential against chronic diseases, requiring further in vivo and in vitro studies in this context.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare that they have no actual, potential, or perceived conflicts of interest related to this article.

Ethics committee approval: This study involves the collection and analysis of wild plant materials. All plant collection activities were conducted in accordance with local regulations and institutional guidelines. Plant materials were identified, and voucher specimens were deposited in the herbarium collection at Atatürk University with appropriate registration numbers.

Data availability: The data will be made available upon request by the author(s).

Funding: This research was supported by the Scientific Research Support Unit of Ardahan University. The funding organisations had no role in the study design, data collection, analysis and interpretation, or writing of the article.

Acknowledgements: The authors thank Prof. Dr. Yusuf Kaya (Plant Systematics Laboratory, Department of Biology, Faculty of Science, Atatürk University) for plant identification and herbarium services; Dr. Fadime Atalay for her laboratory support; and the Central Research Laboratory of Ardahan University and the Eastern Anatolia High Technology Application and Research Centre (DAYTAM) for providing freeze-drying facilities and technical support.

Disclosure: -

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