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Comparison of Biochemical and Color Parameters in Fresh Flower Stalks of Wild Rhubarb (*Rheum ribes* L.) from Diverse Locations

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HIGHLIGHTS

- *R. ribes* genotypes showed high variability in biochemical and color traits.
- Succinic acid was the dominant organic acid across all rhubarb accessions.
- Erçek genotypes had the highest antioxidant capacity and sugar contents.
- PCA and clustering revealed strong trait separation by location and genotype

Abstract

This study aimed to evaluate the biochemical and color parameters of fresh flower stalks of *Rheum ribes* L. collected from four distinct locations in Eastern Anatolia, Türkiye. Significant variation was observed among genotypes in terms of organic acids, sugars, ascorbic acid, antioxidant capacity, and color traits. Succinic acid was the predominant organic acid, peaking in Bahçesaray (14718.47 µg g^{-1} FW). Erçek accessions had the highest levels of fructose (1346.72 µg g^{-1} FW), glucose (970.88 µg g^{-1} FW), and total antioxidant capacity (73.97 µmol TE g^{-1} FW). Muradiye samples contained the highest ascorbic acid (63.71 µg g^{-1} FW), while Mount Erek showed maximum oxalic acid and chroma values. Color measurements also indicated greater vividness in Mount Erek and Muradiye accessions.

Principal Component Analysis (PCA) explained 76.46% of the total variance in the first two components, effectively separating genotypes based on trait profiles. Cluster analysis confirmed these differences, grouping accessions by location with 100% bootstrap support. These results demonstrate the considerable biochemical and visual diversity of *R. ribes* populations across different environments, supporting their potential in food and nutraceutical applications.

Keywords: Biochemical; Color parameters; *Rheum ribes* L.; Wild rhubarb

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1. Introduction

Rhubarb is a perennial vegetable belonging to the family *Polygonaceae*, which includes nearly 60 species within the *Rheum* genus (Zheng et al. 2013; Ekincialp et al. 2019). While some species, such as *R. officinale* and *R. palmatum* are cultivated as medicinal rhubarb, others, like *R. rhabarbarum* (syn. *R. undulatum*) are consumed as vegetables. Additionally, species including *R. rhaponticum* and *R. ribes* are used both for culinary and traditional medicinal purposes due to their edible petioles, roots, and flower stalks (Chin and Youngken 1947; Will and Dietrich 2013; Niziol et al. 2017; Ekincialp et al. 2019).

Among these, *R. ribes* L. is the only wild rhubarb species naturally growing in Türkiye and is predominantly distributed in the eastern provinces, especially in the Lake Van Basin (Cullen 1967; Tuzlacı and Meriçli 1992; Munzuroğlu et al. 2000; Öztürk et al. 2007). This species, which thrives in high-altitude and rocky terrains, is a widely consumed endemic plant in the region (Türkmen et al. 2005; Ekincialp et al. 2019). It is a perennial herb with a wind-pollinated flowering structure and a rhizomatous underground system, while its aerial parts are annual (Aziz 2013; Tartik et al. 2015). Among its edible parts, the flower stalks are particularly appreciated and commonly used in raw or cooked forms.

Due to its bioactive compounds, particularly anthraquinones concentrated in the rhizomes *R. ribes* also plays an important role in traditional medicine across the Middle East (Andiç et al. 2009). Extracts of this plant have been reported to be effective against hemorrhoids, measles, smallpox, and gallstones (Baytop 1999), while its fresh shoots and petioles are traditionally used for gastrointestinal ailments (Tabata et al. 1994; Abu-Irmaileh and Afifi 2003).

Previous studies have focused on the morphological traits and general nutrient content of *R. ribes* accessions in the Lake Van Basin (Türkmen et al. 2005; Andiç et al. 2009; Meral 2017; Ekincialp et al. 2019). However, to date, no comprehensive study has evaluated the detailed biochemical composition, including organic acids, sugars, ascorbic acid, antioxidant capacity, and colorimetric parameters of *R. ribes* genotypes from this region.

The aim of this study is to determine the variation in organic acid content, sugar profile, ascorbic acid levels, total antioxidant capacity, and color parameters of *Rheum ribes* L. genotypes collected from locations with different altitudes and environmental conditions in Eastern Anatolia, and to reveal the relationship of this variation with ecological differences.

2. Materials and Methods

2.1 Plant materials

The plant material (flower stalks, Figure 1) was collected from the naturally growing areas of wild rhubarb (*R. ribes* L.) in Van province and its districts in Lake Van Basin, located in the Eastern Anatolia Region of Turkey. The collection was carried out in the spring season, during the morning hours of May and June, when the flower stalks were at optimal physiological maturity. After the samples were taken in the field, they were kept with dry ice and their vitality was ensured to be preserved until they were transported to the laboratory. Then, the samples were kept at -20 ºC until the biochemical analyses were carried out in the laboratory. The passport data of the collected wild rhubarb genotypes is presented in Table 1.

Figure 1. Photographs from flower stalk sample of *R. ribes* L. collection from nature (GPS records of the regions were taken)

Table 1. The passport data of the collected wild rhubarb genotypes

Locations	Altitude (m)	Latitude (N)	Longitude (E)	Locations	Altitude (m)	Latitude (N)	Longitude (E)
ERCEK DISTRICT				*BAHCESARAY DISTRICT*			
PLANT-01	1983	38 36' 23,41"	43 44' 12, 28"	PLANT -21	1925	38 0' 29,67"	42 44' 45, 74"
PLANT-02	2019	38 36' 22, 52"	43 44' 10,2"	PLANT -22	1960	38 0' 31, 26"	42 44' 31,17"
PLANT-03	2015	38 36' 23,14"	43 44' 10,2"	PLANT -23	1960	38 0' 31,21"	42 44' 31,18"
PLANT-04	2016	38 36' 23, 23"	43 44' 7,83"	PLANT -24	1960	38 0' 31,32"	42 44' 30,95"
PLANT-05	2018	38 36' 23,26"	43 44' 6,37"	PLANT -25	1960	38 0' 30,92"	42 44' 31,68"
PLANT-06	2064	38 36' 23,21"	43 44' 2,62"	PLANT -26	1970	38 0' 30,52"	42 44' 31,45"
PLANT-07	2066	38 36' 23,46"	43 44' 1,27"	PLANT -27	1980	38 0' 30,08"	42 44' 31,47"
PLANT-08	2081	38 36' 22,62"	43 44' 0,01"	PLANT -28	1980	38 0' 30,08"	42 44' 31,47"
PLANT-09	2076	38 36' 22,02"	43 43' 58,22"	PLANT -29	1985	38 0' 29,71"	42 44' 32,48"
PLANT-10	2083	38 36' 21,76"	43 43' 57,77"	PLANT -30	1985	38 0' 29,48"	42 44' 32,39"
PLANT-11	2083	38 36' 21,54"	43 43' 57,77"	PLANT -31	1990	38 0' 29,33"	42 44' 32,54"
PLANT-12	2082	38 36' 21,53"	43 43' 55,39"	PLANT -32	1985	38 0' 29,62"	42 44' 32,57"
PLANT-13	2126	38 36' 18,25"	43 43' 55,39"	PLANT -33	1985	38 0' 29,6"	42 44' 32,85"
PLANT-14	2128	38 36' 18,12"	43 43' 54,3"	PLANT -34	1980	38 0' 29,64"	42 44' 33,07"
PLANT-15	2147	38 36' 12,69"	43 43' 50,44"	PLANT -35	1975	38 0' 29,85"	42 44' 33,26"
PLANT-16	2138	38 36' 12,24"	43 43' 50,98"	PLANT -36	1970	38 0' 30,17"	42 44' 33,57"
PLANT-17	2122	38 36' 10,01"	43 43' 50,53"	PLANT -37	1965	38 0' 30,01"	42 44' 33,72"
PLANT-18	2117	38 36' 11,01"	43 43' 50,7"	PLANT -38	1960	38 0' 30"	42 44' 33,91"
PLANT-19	2128	38 36' 11,19"	43 43' 50,73"	PLANT -39	1960	38 0' 30"	42 44' 33,91"
PLANT-20	2119	38 36' 11,05"	43 43' 507,3"	PLANT -40	1960	38 0' 30,31"	42 44' 34,03"
MURADIYE DISTRICT				*CENTRAL(EREK MOUNT)*			
PLANT -41	2245	38 45' 28,41"	43 45' 1,25"	PLANT -61	2110	38 29' 50,76"	43 29' 0,76"
PLANT -42	2250	38 45' 27,94"	43 45' 1,18"	PLANT -62	2110	38 29' 50,39"	43 29' 0,76"
PLANT -43	2255	38 45' 27,56"	43 45' 2,15"	PLANT -63	2095	38 29' 49,45"	43 29' 0,45"
PLANT -44	2265	38 45' 25,46"	43 44 59,14"	PLANT -64	2145	38 29' 46,58"	43 28' 55,7"
PLANT -45	2280	38 45' 22,66"	43 44' 54,96"	PLANT -65	2145	38 29' 44,17"	43 28' 54,53"
PLANT -46	2290	38 45' 20,92"	43 44' 54,67"	PLANT -66	2145	38 29' 44,9"	43 28 53,78"
PLANT -47	2335	38 45' 18,58"	43 44' 54,46"	PLANT -67	2150	38 29' 45,54"	43 28' 54,42"
PLANT -48	2340	38 45' 16,69"	43 44' 53,73"	PLANT -68	2165	38 29' 44,31"	43 28 54,365"
PLANT -49	2350	38 45' 15,83"	43 44' 53,92"	PLANT -69	2135	38 29' 39,82"	43 28' 54,46"
PLANT -50	2360	38 45' 15,66"	43 44' 53,24"	PLANT -70	2135	38 29' 39,82"	43 28' 54,46"
PLANT -51	2360	38 45' 15,69"	43 44 53,23"	PLANT -71	2135	38 29'39,82"	43 28' 54,46"
PLANT -52	2370	38 45' 14,38"	43 44' 53,11"	PLANT -72	2135	38 29' 40,62"	43 28' 54,07"
PLANT -53	2370	38 45' 13,74"	43 44' 53,34"	PLANT -73	2145	38 29' 40,16"	43 28' 54,56"
PLANT -54	2395	38 45' 13,01"	43 44 51,63"	PLANT -74	2145	38 29' 39,25"	43 28' 54,36"
PLANT -55	2395	38 45' 12,53"	43 44' 52,42"	PLANT -75	2145	38 39' 39,45"	43 28' 54,33"
PLANT -56	2395	38 45 12,71"	43 44' 52,32"	PLANT -76	2155	38 29' 39,09"	43 28' 54,56"
PLANT -57	2395	38 45' 12,93"	43 44' 52,64"	PLANT -77	2155	38 29' 39,09"	43 28' 54,56"
PLANT -58	2395	38 45' 12,46"	43 44' 53,11"	PLANT -78	2165	38 29' 38,13"	43 28' 54,41"
PLANT -59	2395	38 45' 12,32"	43 44' 53,83"	PLANT -79	2165	38 29' 38,43"	43 28' 54,23"
PLANT -60	2420	38 45 10,82"	43 44' 53,12"	PLANT -80	2165	38 29' 37,98"	43 28' 54,33"

2.2 Reagents and chemicals

Analytical-grade chemicals were used in this study. Phenolic reagent standards, including gallic acid, chlorogenic acid, Q-coumaric acid, ferulic acid, syringic acid, vanillic acid, phloridzin, rutin, and protocatechuic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Organic acid standards such as fumaric acid, citric acid, oxalic acid, malic acid, and succinic acid were also obtained from Sigma-Aldrich. Other chemicals were procured from Merck (Darmstadt, Germany).

2.3 Analysis of phenolic compounds and extraction procedure

Phenolic compound concentrations were determined using standards on an HPLC device (modified method from Rodriguez-Delgado et al., 2001). Peak adjustment was conducted with different concentrations (5-70 ppm) of chlorogenic acid, ferulic acid, gallic acid, Q-coumaric acid, phloridzin, protocatechuic acid, rutin, syringic acid, and vanillic acid. Rhubarb flower stalk samples (1 g) were homogenized with 4 ml of methanol using a Heidolph Silent Crusher. After centrifugation and filtration through a 0.45 µm filter, the supernatant was injected into an Agilent 1100 HPLC system equipped with a DAD detector and an ODS column. Chromatographic separation utilized a methanol, acetic acid, and water (28:2:70) mobile phase with gradient elution. Detection occurred at 254 nm and 280 nm, with a flow rate of 1 ml/min and an injection volume of 20 µL.

2.4 Extraction and determination of organic acid

Organic acid analysis modified from Bevilacqua and Califano (1989) utilized a 0.009 N H_2SO_4 mobile phase passed through a 0.45 µm membrane filter. Concentrations were determined using externally provided standards on an HPLC device. To quantify organic acids (citric acid, malic acid, oxalic acid, and succinic acid), different concentrations (50-800 ppm) were prepared. Extraction involved homogenizing 1 g of sliced rhubarb flower stalks with 20 ml of 0.009 N H_2SO_4 at room temperature (Heidolph Silent Crusher M, Germany). The homogenate was chilled and mixed for 1 hour, followed by centrifugation and filtration through coarse filter paper, a 0.45 µm membrane filter, and a SEP-PAK C18 cartridge. HPLC analysis with an Aminex column (HPX-87H, 300mm×7.8mm, Bio-Rad) connected to an Agilent 1100 series HPLC G 1322 A (Germany) was used for measuring organic acid concentrations at 214 nm and 280 nm wavelengths.

2.5 Sugar Content Analysis

The modified method of Melgarejo et al. (2000) was used for sugar analysis. After adding 10 ml of acetonitrile, 1 g of rhubarb flower stalks was crushed using a homogenizer and centrifuged for 2 min at 12,000 rpm. The supernatants were passed through a SEP-PAK C-18 cartridge, and the filtrate was stored at -20 °C until analysis. HPLC readings were performed using a µbondapak-NH_2 column with 85% acetonitrile as the liquid phase and a refractive index detector. Concentrations were calculated based on external standards.

2.6 Vitamin C Content Analysis

For ascorbic acid (AsA) analysis, 3 g of pureed *R. ribes* samples were extracted with 5 ml of 3% metaphosphoric acid and centrifuged at 6500 g for 10 min at +4 °C. The supernatant (0.5 ml) was diluted to 10 ml with 2.5% metaphosphoric acid, filtered through a 0.45 µm membrane, and analyzed using a C18 column (Phenomenex Luna, 250 × 4.60 mm, 5 µm) with a DAD detector at 254 nm. L-ascorbic acid standards (50–2000 ppm) were used for quantification (Cemeroğlu 2010).

2.7 Total Antioxidant Activity (TE)

Antioxidant activity was determined using the Ferric Reducing Antioxidant Power (FRAP) analysis method according to Benzie and Strain (1996). The absorbance was measured spectrophotometrically at 593 nm, and the antioxidant activity values were expressed as µmol Trolox equivalents (TE) per gram of fresh weight (FW).

2.8 Total Phenolic Contents (TP)

The total phenolic contents were measured using a modified version of the method described by Jang et al. (2007). 1 g of the sample was extracted with 4 ml of methanol. Vacuum filtration was used to separate the solid material from the extract, and 0.15 ml of each extract was mixed with 0.15 ml of Folin-Ciocalteu reagent (1:10). After resting for 3 min at 25°C, a saturated sodium carbonate solution (0.30 ml) was added, and the mixture was incubated for 30 min at room temperature. Absorbance readings were then taken at 725 nm using a UV-Vis spectrophotometer. The results were reported as gallic acid equivalents in milligrams per 100 g of fresh weight (FW).

2.9 Analysis of color parameters

Variations in color values of wild rhubarb (*R. ribes* L.) were determined using a Minolta CR-400 colorimeter. The results were expressed as L, a, b, Chroma, and Hue angle values. L represents brightness, a represents the degree of redness or greenness (+red, -green), b represents the degree of yellowness or blueness (+yellow, -blue), Chroma represents color intensity, and Hue angle represents the dominant color.

2.10 Statistical analysis

The mean values of the samples from each location were calculated and presented as mean ± standard deviation (SD). For each wild rhubarb genotype, three biological replicates were collected and analyzed. Analysis of the biochemical compounds was performed using analysis of variance (ANOVA) followed by Duncan's multiple comparison test to determine the differences among the locations. Principal Component Analysis (PCA) based on the studied traits and correlation analysis were conducted using XLSTAT software to identify variations among the wild rhubarb genotypes (Vidal et al. 2020).

3. Results and Discussion

3.1 Phenolic Compounds

The role of phenolic compounds in plant physiology and their effects on human health have been emphasized in numerous studies (Cemeroğlu et al. 2004; Gundogdu et al. 2011). In the present study, the concentrations of nine identified phenolic compounds, particularly in samples where rutin was dominant, varied significantly depending on genotype and location (Table 2). This finding is consistent with the study by Clapa et al. (2020) on *R. rhabarbarum*, which reported a wide range of variation in similar phenolic profiles. Clapa et al. (2020) and Kolodziejczyk-Czepas and Liudvytska (2021) reported that rutin was present in the leaves of *Rheum* species at levels ranging between 0.61–0.70%. In our study, rutin content in the fresh flower stalk tissue ranged from 441.48 to 840.32 μg g^{-1} FW. These values may differ significantly depending on plant organ and environmental conditions. Similarly, Neupane and Lamichhane (2020) demonstrated that *R. australe* from Nepal had high total phenolic and flavonoid contents. This suggests that *Rheum* species growing in mountainous regions tend to have rich phenolic profiles, which aligns with our findings in the high-altitude regions of the Lake Van Basin. Jahan et al. (2013) reported the presence of gallic acid (90.9 μg g^{-1} DW) and ferulic acid (102.8 μg g^{-1} DW) in the rhizomes of *R. emodi*. Compared with our results for gallic acid (32.31–55.45 μg g^{-1} FW) and ferulic acid (1.62–5.96 μg g^{-1} FW), this reflects interspecies and organ-specific differences. Keser et al. (2020) reported rutin (15.90 mg g^{-1} DW) and catechin (24.85 mg g^{-1} DW) as dominant flavonoids in *R. ribes* samples from Elazığ, along with high levels of phenolic acids such as ferulic and gallic acids. These findings are consistent with our phenolic profile, though the plant parts analyzed differ. Meral (2017) reported that rutin, ferulic acid, and gallic acid contents in *R. ribes* may vary depending on temperature treatments. In our study, the highest levels of rutin and ferulic acid were observed in samples from Mount Erek, suggesting that microclimatic factors such as sunlight exposure and temperature may influence the synthesis of these compounds. In a study by Kosikowska et al. (2010) on the roots and rhizomes of three *Rheum* species (*R. palmatum, R. undulatum, and R. rhaponticum*) grown in Poland, total polyphenol contents ranged from 46.11 to 76.45 mg g^{-1}. When compared to the values determined in our study from fresh flower stalk tissue (76.10–95.55 mg GAE 100 g^{-1} FW), these levels are relatively lower. This indicates that factors such as plant organ (root vs. stalk), extraction method (ethanol vs. methanol), and moisture content (DW vs. FW) may cause substantial

variation. Furthermore, genetic differences among species appear to have a significant influence on phenolic synthesis. Ceylan et al. (2019) reported that among traditional medicinal plants in Erzurum, *R. ribes* exhibited the highest antioxidant capacity. Similarly, in our findings, the Erçek samples exhibited the highest antioxidant activity with 73.97 μmol TE g^{-1} FW. The antiradical and antimicrobial activities reported by Keser et al. (2020) also support these findings.

Table 2. Phenolic contents (μg g^{-1} FW) of wild rhubarb (*Rheum ribes* L.) genotypes collected from different locations in the Lake Van Basin (mean ± standard deviation).

Phenolics	Ercek Town	Bahçesaray Town	Muradiye Town	Central (Mount Erek)
Protocatechuic	13.04±3.32	16.38±5.34	14.93±4.10	14.64±4.56
Vanillic acid	0.91±0.40	1.48±0.54	1.97±1.34	1.57±1.15
Rutin	518.34±459.91^{ab}	441.48±222.59^{b}	537.93±285.20^{ab}	840.32±445.09^{a}
Gallic Acid	32.31±25.57	47.06±33.03	35.25±33.27	55.45±37.81
Chlorogenic	24.48±8.83	21.13±13.01	9.97±3.97	26.82±24.38
Syringic acid	50.67±31.96	55.50±42.86	29.07±19.26	58.55±60.97
Ferulic acid	2.53±1.85^{b}	3.16±2.22^{b}	1.62±0.83^{b}	5.96±3.56^{a}
Q-Coumaric acid	0.57±0.32	0.99±1.04	0.41±0.32	0.52±0.32
Phloridzin	2.38±0.50	2.50±0.77	2.28±0.40	2.62±0.58
Total Phenolic (mg GAE 100 g^{-1} FW)	95.55±17.87	88.36±13.58	76.10±23.47	87.35±21.28

Values in the same row followed by different letters are significantly different at $p \leq 0.05$

3.2 Organic Acids

Organic acids play an important role in fruit and vegetable flavor formation, intracellular pH regulation, and metabolic processes. They also act as intermediate compounds in the respiratory chain and contribute to energy metabolism (Cemeroğlu et al. 2004). Furthermore, these compounds can form complexes with heavy metal ions, thereby reducing toxicity and limiting oxidation (Savran 1999). In this study, five organic acids—fumaric, malic, oxalic, citric, and succinic acid—were identified in *Rheum ribes* L. genotypes. Among them, succinic acid was the predominant compound, ranging from 10431.30 to 14718.47 μg g^{-1} FW, with the highest value detected in Bahçesaray accessions. This result presents a unique biochemical profile for *R. ribes*, contrasting with previous reports that identified malic and oxalic acids as dominant in related species (Will and Dietrich, 2013; Stoleru et al. 2019). Citric acid ranged from 4770.85 to 5873.01 μg g^{-1} FW, with the highest average found in Mount Erek samples. Malic acid was highest in the Erçek region (1319.91 μg g^{-1} FW), while oxalic acid peaked in Mount Erek samples (768.93 μg g^{-1} FW). Fumaric acid was found at the lowest levels across all locations, with a statistically significant minimum value recorded in Mount Erek samples (0.52 μg g^{-1} FW, $p < 0.05$).

When compared to studies on *Rheum rhabarbarum*, these findings highlight notable interspecific differences in organic acid composition. For example, Stoleru et al. (2019) reported oxalic acid at 256–377 mg 100 g^{-1} FW, malic acid at 670–687 mg 100 g^{-1} FW, and citric acid at 441–534 mg 100 g^{-1} FW in *R. rhabarbarum*. Will and Dietrich (2013) identified malic acid as the dominant compound, reporting concentrations between 16.2 and 17.2 g L^{-1}. These differences are likely due to species, cultivation conditions, extraction methods, and analytical protocols.

Regarding total antioxidant capacity, values ranged from 46.76 to 73.97 μmol TE g^{-1} FW, with the highest value recorded in Erçek samples. This suggests a strong correlation between antioxidant capacity and phenolic compound richness. Compared with previous studies, Alkaya et al. (2019) reported antioxidant values between 8.88 and 62.70 μmol TE g^{-1} in *R. ribes* roots using the FRAP method, while Samancioglu et al. (2015) reported a value of 32.66 using the DPPH method. Kalisz et al. (2020) reported antioxidant capacities ranging from 3.25 to 18.26 mmol Trolox 100 g^{-1} dry matter in different *R. rhabarbarum* cultivars. These findings support the high antioxidant potential of the flower stalk tissues analyzed in this study.

Concerning sugar content, two major monosaccharides glucose and fructose were identified. The highest fructose concentration was observed in Erçek genotypes (1,346.72 μg g^{-1} FW), while glucose followed with an average of 901.83 μg g^{-1} FW, also peaking in Erçek (970.88 μg g^{-1} FW). Mezeyova et al. (2021) reported fructose

and glucose contents in *R. rhabarbarum* as 32.80–38.46 g L^{-1} and 4.74–11.74 g L^{-1}, respectively, confirming the significant compositional differences among species.

Ascorbic acid (vitamin C) content ranged from 24.46 to 63.71 μg g^{-1} FW, with the highest average detected in Muradiye samples (63.71 μg g^{-1} FW), and the lowest in Mount Erek (24.46 μg g^{-1} FW). This variation in vitamin C levels is likely influenced by environmental factors such as sunlight exposure, altitude, and temperature. Stoleru et al. (2019) reported ascorbic acid contents of 339–438 mg 100 g^{-1} FW in *R. rhabarbarum*. The lower values observed in this study may be attributed to differences in the plant part analyzed (flower stalks) and interspecies variation.

In conclusion, this study demonstrated that the flower stalks of *Rheum ribes* L. exhibit high variability in organic acid, sugar, vitamin C, and antioxidant content, influenced not only by genetic diversity but also by environmental conditions. Specifically, Erçek genotypes stood out for their antioxidant capacity and sugar content, Bahçesaray accessions for succinic acid levels, and Muradiye genotypes for ascorbic acid concentration. These biochemical profiles may serve as valuable indicators for the functional food potential and medicinal use of *R. ribes*.

Table 3. Organic acids (μg,gr^{-1} FW), sugars (μg,gr^{-1} FW), and total antioxidant (μmol (TE) g^{-1}) contents of *Rheum ribes* L. genotypes collected from different locations in the Lake Van Basin (mean ± standard deviation).

Organics	Ercek Town	Bahçesaray Town	Muradiye Town	Central (Mount Erek)
Fumaric acid	2.36±1.26^{a}	1.91±0.93^{a}	1.82±1.29^{a}	0.52±0.30^{b}
Malic acid	1319.91±596.79^{a}	976.33±553.14^{b}	957.77±547.24^{b}	866.66±412.07^{b}
Oxalic	462.92±193.70^{b}	675.64±201.31^{a}	616.18±253.03^{ab}	768.93±410.87^{a}
Citric acid	5621.31±1587.75	5713.52±1748.53	4770.85±2106.15	5873.01±2327.58
Succinic acid	10431.30±17245.02	14718.47±7855.67	13034.26±12068.57	11331.00±6873.76
Glucose	970.88±148.29^{a}	802.88±149.07^{b}	948.70±204.26^{a}	888.33±131.66^{ab}
Fructose	1346.72±177.01^{a}	1157.77±168.61^{b}	1312.27±243.17^{a}	1317.71±171.58^{a}
Ascorbic acid	58.84±36.09^{a}	57.82±18.05^{a}	63.71±27.02^{a}	24.46±13.97^{b}
Total antioxidant	73.97±42.81^{a}	52.97±35.89^{ab}	46.76±29.05^{b}	48.47±24.12^{b}

Values in the same row followed by different letters are significantly different at $p \leq 0.05$

3.3 Color parameters

The optical characteristics of wild rhubarb (*R. ribes* L.) flower stalks showed significant variation among genotypes from different collection sites (Table 4). These traits, including L (lightness), a (red-green axis), b (yellow-blue axis), chroma (color intensity), and hue angle, are commonly used in colorimetric evaluations of plant materials, particularly in assessing market quality, maturity, and phytochemical content.

Among all locations, the L values (which range from 0 = black to 100 = white) varied between 57.42 and 62.86, with Ercek Town samples showing the highest brightness (62.86 ± 4.49) and Central (Mount Erek) samples the lowest (57.42 ± 12.26). This suggests that samples from Mount Erek had a slightly darker surface color, which could be linked to higher concentrations of pigments such as flavonoids and anthocyanins.

The "a^{*}" parameter, which represents the red-green scale (positive values toward red, negative toward green), ranged from -3.86 to 1.06. The most negative value was recorded in Muradiye Town (-3.86 ± 5.81), indicating a pronounced greenish hue in these samples. Conversely, Bahçesaray Town showed a slight tendency toward redness with a positive "a" value (1.06 ± 6.32). Such differences may reflect local environmental factors or genotypic variability affecting pigment biosynthesis, including chlorophyll and anthocyanins.

The "b^{*}" values (yellow-blue scale) revealed more substantial differentiation, ranging from 13.70 to 22.95. Notably, Central (Mount Erek) and Muradiye Town exhibited significantly higher "b" values (22.95 ± 6.00 and 22.91 ± 8.01, respectively), while Ercek Town showed the lowest (13.70 ± 5.87). These findings indicate that samples from higher altitudes like Mount Erek tend to have a more yellowish appearance, which may be due to increased carotenoid accumulation under higher solar radiation.

The chroma values, expressing color saturation or vividness, showed a similar trend. Mount Erek samples displayed the highest chroma (25.78 ± 4.14), followed by Muradiye and Bahçesaray, while Ercek samples

exhibited the lowest (14.27 ± 5.57). Since chroma is closely associated with pigment content, these differences could reflect both environmental stress factors and metabolite richness, supporting previous findings that stress conditions can intensify coloration through enhanced secondary metabolism.

The hue angle, which describes the dominant perceived color (0° = red, 90° = yellow, 180° = green, 270° = blue), ranged from 84.09° (Bahçesaray) to 94.20° (Muradiye), with all samples clustering within the yellow-green quadrant. Although hue values did not differ significantly across locations, subtle shifts might still be biologically relevant, especially in connection with specific phenolic profiles.

In general, higher chroma and "b" values in Mount Erek and Muradiye Town align with the elevated levels of phenolic compounds—particularly rutin, syringic acid, and gallic acid—observed in these samples. This supports earlier reports suggesting that color intensity in plant tissues can be correlated with antioxidant content (Oktay et al. 2007; Meral 2017). Furthermore, the slight darkening and greener hue in Muradiye samples (low L and low a values) may reflect elevated chlorophyll retention or lower degradation due to microclimatic factors.

Table 4. Color parameters *(L, a, b*,* Chroma, and Hue angle) of *Rheum ribes* L. genotypes collected from different locations in the Lake Van Basin (mean ± standard deviation)

	Ercek Town	Bahçesaray Town	Muradiye Town	Central (Mount Erek)
L^*	62.86±4.49	62.66±5.96	61.13±5.16	57.42±12.26
a^*	-0.85±3.28	1.06±6.32	-3.86±5.81	-0.07±11.05
b^*	13.70±5.87^{b}	18.24±4.25^{ab}	22.91±8.01^{a}	22.95±6.00^{a}
Chroma	14.27±5.57^{c}	19.72±2.80^{b}	24.14±7.82^{ab}	25.78±4.14^{a}
Hue	91.69±17.36	84.09±21.73	94.20±18.16	87.28±28.48

Values in the same row followed by different letters are significantly different at $p \leq 0.05$

3.4 Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was conducted to identify the underlying structure among the biochemical and colorimetric traits of wild rhubarb genotypes. The eigenvalues and explained variance ratios of the first three components are summarized in Table 5. These three axes cumulatively accounted for 100% of the total variation, with PC1 explaining 43.66%, PC2 explaining 32.80%, and PC3 23.54% of the total variability. This high cumulative variance strongly supports the robustness of the multivariate structure (Ozdamar 2004). According to Seymen et al. (2019), the first two components explaining over 25% of the variance provide a sound basis for interpretation. Here, PC1 and PC2 together account for 76.46%, indicating a reliable dimensionality reduction.

PC1 had strong positive loadings for gallic acid (0.308), oxalic acid (0.294), ferulic acid (0.270), phloridzin (0.275), rutin (0.206), chroma (0.211), and **b* value (0.182)**. These suggest that PC1 is strongly associated with polyphenol biosynthesis and color intensity, particularly yellowish pigments. Notably, negative loadings of fumaric acid (–0.274), malic acid (–0.247), and vitamin C (–0.257) on PC1 indicate an inverse relationship between phenolic accumulation and organic acid/vitamin content, possibly due to resource allocation trade-offs during development or stress response. This axis may thus reflect a phenolic-color saturation gradient, where high-PC1 genotypes have deeper color and richer antioxidant profiles but lower organic acid or vitamin C contents. PC2 showed strong positive loadings for total phenolic content (0.352), chlorogenic acid (0.301), citric acid (0.296), syringic acid (0.289), and a value (0.282)**, and negative loadings for b value (–0.285) and chroma (–0.255). This component captures a contrast between red pigmentation and overall color vividness, with genotypes exhibiting high a* values (more red) tending to have lower yellowness and chroma. The simultaneous positive loading for total phenolics suggests a biochemical shift toward redder phenolic derivatives such as anthocyanins in certain accessions. Interestingly, vanillic acid (–0.331) also showed a strong negative loading, potentially indicating its antagonism with chlorogenic- or syringic-acid-rich pathways. PC3 was marked by positive loadings for fructose (0.392), glucose (0.303), rutin (0.311) and moderate loadings for chlorogenic acid (0.132) and hue angle (0.208). These features suggest that this axis is linked to sweetness perception and pigment hue shifts, possibly during the late stages of maturation or post-harvest storage. Negative contributions from succinic acid (–0.387) and protocatechuic acid (–0.337) indicate that PC3 separates

sugar-rich genotypes from organic-acid-dominant ones, which may reflect a balance between sourness and sweetness in edible quality.

Table 5. Principal Component Analysis (PCA) results based on biochemical and color parameters of *Rheum ribes* L. genotypes

	PC1	PC2	PC3
Eigen values	10.478	7.872	5.650
Explained proportion of variation (%)	43.658	32.799	23.543
Cumulative proportion of variation (%)	43.658	76.457	100.000
Factors (Eigen Vectors)			
Fumaric acid	**-0.274**	0,075	-0,173
Malic acid	**-0.247**	0.207	0.062
Oxalic acid	**0.294**	-0.106	-0.034
Citric acid	0.170	**0.296**	0.031
Succinic acid	0.068	-0.115	**-0.387**
Glucose	-0.207	-0.065	**0.303**
Fructose	-0.105	-0.042	**0.392**
Vitamin-C	**-0.257**	-0.056	-0.224
Total antioxidant	-0.194	**0.268**	0.083
Protocatechuic acid	0.161	-0.105	**-0.337**
Vanillic acid	0.100	**-0.331**	-0.076
Rutin	0.206	-0.031	**0.311**
Gallic acid	**0.308**	0.031	-0.004
Chlorogenic acid	0.134	**0.301**	0.132
Syringic acid	0.181	**0.289**	0.006
Ferulic acid	**0.270**	0.105	0.161
Q-Coumaric acid	0.075	0.184	**-0.346**
Phloridzin	0.275	0.159	0.043
Total phenolic	-0.023	**0.352**	0.056
L^*	-0.234	0.100	**-0.248**
a^*	0.164	**0.282**	-0.128
b^*	0.182	**-0.285**	0.051
Chroma	0.211	**-0.255**	0.063
Hue	**-0.220**	-0.178	0.208

3.5 Hierarchical Cluster Analysis (HCA) and Observation-Based PCA Interpretation

The PCA observation plot (Figure 2) reinforced these results. Genotypes from Ercek were positioned in the upper-left quadrant (high F2, low F1), suggesting elevated antioxidant capacity and vitamin C but relatively lower pigment intensity. In contrast. Central samples were positioned far along the positive PC1 axis, associated with high rutin, gallic acid, and chromatic richness. Muradiye genotypes appeared in the lower-left quadrant (low PC1 and PC2), reflecting reduced metabolite accumulation and duller coloration. Bahçesaray, centrally located in the positive PC2 quadrant, displayed moderate phenolic and color traits. As visualized in Figure 2. most genotypes clustered centrally, while three genotypes from YYU-MER and one from YYU-MUR showed clear separation. These outliers likely represent distinct metabolic profiles with extreme loadings in PC1 or PC2.

To support and complement the PCA-based trait discrimination. Hierarchical Cluster Analysis (HCA) was conducted using the same dataset. The resulting dendrogram (Figure 3) grouped the four wild rhubarb populations into two distinct clusters with high bootstrap support: Central (Mount Erek) and Ercek genotypes clustered together with 66% similarity, most likely due to their shared high values in phenolic compounds such as rutin and gallic acid, as well as elevated chroma and *b* values (i.e.. stronger yellow pigmentation). Bahçesaray and Muradiye formed a second distinct cluster at 72% similarity, reflecting a separate biochemical and optical profile, with Muradiye particularly showing low brightness (L*) and high greenness (negative *a*) values. The branching of these two major clusters was confirmed with 100% reliability, indicating highly distinct trait expression between the two genotype groups.

The loading plot (Figure 4) further illustrates trait interactions: vectors with angles <90° denote positive correlations (e.g. chlorogenic acid & hue). while those >90° (e.g.. a* & b*) indicate antagonistic relations. These

findings corroborate the correlation matrix in Table 5, which revealed both strong positive (up to r = 0.902) and negative (r = –0.966) relationships. The negative correlation between *a and chroma/hue angle** along with syringic acid and L. suggests tissue reddening is inversely related to brightness and saturation, which aligns with visual observations and metabolite distributions.

The PCA loading plot (Figure 4) visually confirmed these trait relationships. Strong positive correlations (acute angles) were observed between gallic acid, ferulic acid, and chroma. In contrast, antagonistic interactions (obtuse angles) were evident between vitamin C and rutin or between *a* and *b* values. These directional loadings align with correlation matrix findings and underscore the reliability of PCA as a discriminant tool.

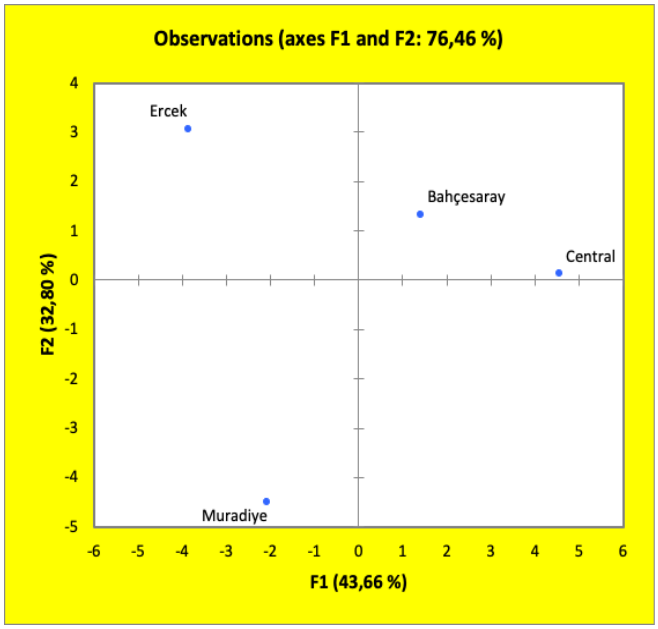

Figure 2. Clustering of the wild rhubarb genotypes collected from different locations based on Principal Component Analysis (PCA)

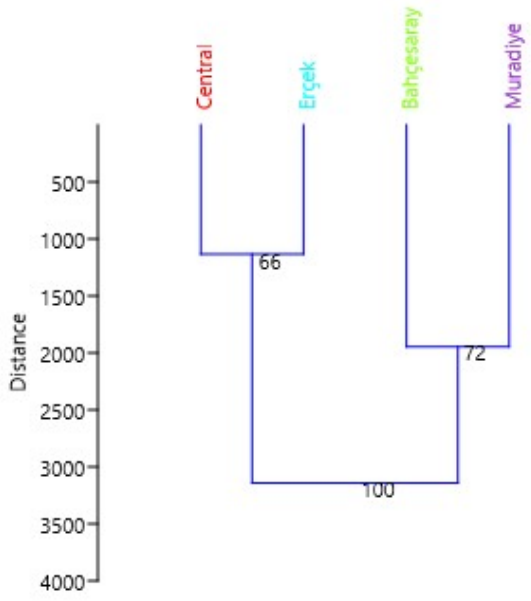

Figure 3. Dendrogram of the wild rhubarb genotypes collected from different locations.

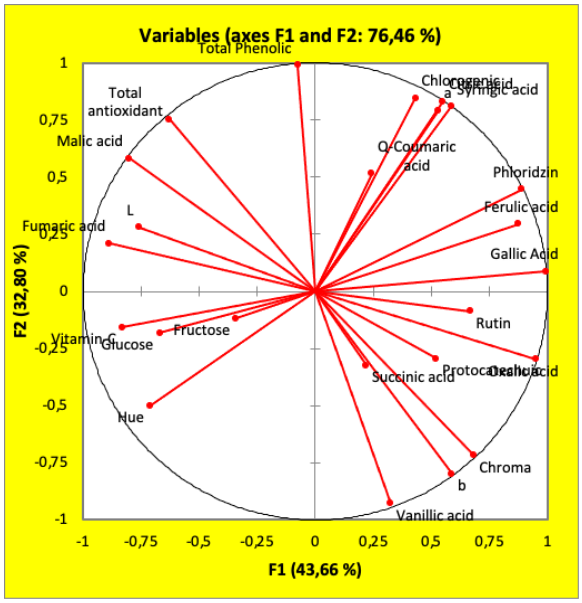

Figure 4. Loading plot graph drawn according to the result of the principal component analysis

4. Conclusions

This study provided a comprehensive evaluation of the biochemical and color properties of *Rheum ribes* L. the only wild rhubarb species naturally growing in Türkiye. A total of 39 genotypes collected from four locations with different altitudes and ecological conditions within the Lake Van Basin were analyzed. The findings revealed substantial variation among the genotypes in terms of organic acids, sugar content, ascorbic acid levels, antioxidant capacity, and colorimetric traits.

Succinic acid was the predominant organic acid, while fructose and glucose were the major sugars identified. Muradiye accessions exhibited the highest ascorbic acid content, whereas Erçek genotypes stood out for their high antioxidant capacity and sugar profile. Color measurements also revealed significant variation among locations, particularly in *b* and chroma values. The most abundant phenolic compound was rutin, followed by syringic acid, gallic acid, chlorogenic acid, and protocatechuic acid.

Multivariate analyses (PCA and HCA) demonstrated that the biochemical and color traits of *R. ribes* genotypes were strongly influenced by geographic origin and altitude. Strong correlations among phenolic compounds and color parameters support the hypothesis that pigmentation intensity may serve as an indirect marker for antioxidant potential.

Given its richness in bioactive compounds, low harvesting cost, and historical use in traditional medicine. *R. ribes* holds considerable promise for pharmacological, Nutraceutical, and functional food applications. The results of this study are expected to fill a significant gap in the literature regarding the biochemical composition of *R. ribes* flower stalks and may serve as a valuable reference for future research and conservation strategies targeting endemic wild plant resources in Türkiye.

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