

Morphological and Biochemical diversity among medicinal wild barberry (*Berberis vulgaris* L.) from Isfahan Province

Elmira Ziya Motalebipour

e.ziyamotalebipour@iau.ir

Isf. C, Islamic Azad University

Akbar Pirestani

Isf. C, Islamic Azad University

Article

Keywords: Diversity, Morphological traits, Biochemical, Barberry

Posted Date: September 11th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7451414/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Scientific Reports on November 17th, 2025. See the published version at <https://doi.org/10.1038/s41598-025-23994-w>.

Abstract

Berberis vulgaris L. is a shrub exhibiting significant morphological and biochemical diversity. This study evaluated nine wild genotypes from Isfahan Province, Iran, based on 21 morphological and biochemical traits. Fruit length varied from 0.6 to 1.07 cm, width from 0.47 to 0.72 cm, and weight from 0.14 to 0.26 g, with colors ranging from light yellow to dark red. Pearson correlation analysis revealed key relationships: fruit weight correlated positively with seed length ($r = 0.50$), fruit color had a strong negative correlation with fruit length ($r = -0.68$) and a positive correlation with leaf color ($r = 0.74$). The number of fruits per panicle correlated negatively with seed width ($r = -0.73$), while fresh fruit weight correlated positively with leaf blade length ($r = 0.79$). Biochemical analysis showed notable antioxidant activity linked to phenolic and flavonoid content. Sample 2 had the highest total phenolics (14.34mg/ml), flavonoids (13.83 mg/ml), whereas sample 7 exhibited the highest flavonoid concentration (16.86 mg/ml) and sample 3 showed the strong antioxidant capacity (97.10%). Hierarchical clustering grouped genotypes into three clusters: Cluster I (samples 7 and 8) with distinct biochemical profiles; Cluster II (samples 1 and 2) with darker fruits and higher phenolics; Cluster III with lighter fruits and lower biochemical levels. These results highlight the genetic diversity and medicinal potential of *B. vulgaris*, suggesting fruit color and morphological traits as useful markers for selecting genotypes with high antioxidant capacity. Conservation and breeding efforts should prioritize these valuable wild resources for pharmaceutical and nutraceutical uses.

1. Introduction

The *Berberis* genus, a member of the Berberidaceae family, comprises an extensive array of approximately 650 species distributed across 15 genera. This botanical diversity is predominantly found across regions of Asia, Europe, North Africa, and South America [1]. Among these species, barberry stands out for its small fruit endowed with remarkable medicinal properties. Its natural habitats are particularly prevalent in Asia and Europe, where it has been cultivated and harvested for centuries, playing a significant role in traditional medicine [2].

The historical significance of barberry in folk medicine extends over two millennia, rooted in ancient civilizations [3]. Ancient Egyptian records reveal the ingenious use of barberry combined with fennel seeds to repel pests, showcasing early recognition of its versatile properties. Similarly, Babylonian documents dating back to 650 BCE highlight barberry's esteemed medicinal virtues, underscoring its long-standing reputation as a therapeutic agent [4].

By the 7th century CE, barberry had become a staple of traditional medicine across the Middle East. Renowned for its efficacy in treating infectious fevers and combating typhus, the fruit became an indispensable component of historical therapeutic regimens, attesting to its enduring relevance in medicinal practices [5].

The biochemical composition of *Berberis vulgaris* is a key factor in its widespread use in traditional and modern medicine. The fruit is rich in bioactive compounds, particularly phenolics, flavonoids, and alkaloids such as berberine, which are responsible for its diverse pharmacological properties. These compounds exhibit potent antioxidant, anti-inflammatory, antimicrobial, and cardioprotective effects, making *B. vulgaris* a promising candidate for therapeutic applications [6].

Phenolic compounds in *B. vulgaris* contribute significantly to its antioxidant capacity by neutralizing free radicals and reducing oxidative stress, which is linked to various chronic diseases. Flavonoids, another major group of phytochemicals in the fruit, are known for their ability to modulate cellular signaling pathways, enhancing the body's defense mechanisms. Additionally, berberine, a key alkaloid found in the root and fruit, has demonstrated remarkable efficacy in controlling blood glucose levels, reducing cholesterol, and exhibiting antimicrobial properties [7].

Recent studies have quantified the phenolic and flavonoid content of *B. vulgaris*, highlighting its exceptional antioxidant activity, which varies among wild populations due to genetic and environmental factors. This biochemical diversity underpins the importance of conserving wild genetic resources and exploring the medicinal potential of different ecotypes. By understanding the biochemical profile of *B. vulgaris*, researchers can identify high-value individuals for potential applications in pharmaceutical development and functional foods. [8–10].

This study emphasizes the need to evaluate the biochemical properties of wild barberries to uncover their therapeutic potential and promote their sustainable utilization. Furthermore, it aims to explore significant disparities in morphological and biochemical traits among various wild barberries, commonly referred to as Seedy barberry in Iran. The goal is to evaluate their potential for domestication and to harness their medicinal properties in the future. By examining these traits, we aim to contribute to the sustainable utilization and broader application of barberry as both a cultivated crop and a medicinal resource.

2. Material and method

2.1. Plant material

Totally, 9 wild individuals of *B. vulgaris* were obtained from Zarin Gol Feridunshahr company (Longitude: 49°55'35.8"E, Latitude: 32°56'19.2"N) of Isfahan province from the center area of Iran. The studied areas are near each other and have similar climates (Fig. 1). The collected plant materials were transferred to the Islamic Azad University, Isfahan (Khorasgan) Branch, where the plants were confirmed as wild *B. vulgaris*. Voucher specimens were stored at this center at -70°C for future reference.

2.2. Morphological evaluation

Eighteen morphological variables of 9 wild individuals of *B. vulgaris* were examined (Fig. 2; Table 1). Leaves and fully mature fruits were harvested from each shrub, with random samples collected consisting of 30 fruit bunches from each shrub. Samples of adult fruit bunches were randomly taken from various

parts of the shrubs. Some variables were measured using laboratory equipment. Leaf length, leaf width, leaf tail length, fruit length, fruit width, fruit tail length, seed length, and seed width were measured with a digital caliper. Fresh fruit weight, dry fruit weight, fresh leaf weight, dry leaf weight, and seed weight were recorded using an electronic balance with a precision of 0.01 g. Thirty experimental units were tested for each variable examined. Additionally, the number of leaves and the number of fruits per panicle were recorded. Leaf color and fruit color were assessed according to a scoring and coding system (Table 1).

2.3. Biochemical analysis

Extraction Procedure

One gram of dried fruit samples was ground and immersed in 10 ml of an 70:30 ethanol-water solution for four hour while being shaken. The resulting extracts were filtered and then stored at 4°C in a refrigerator for future use.

Total Phenolics (TP) Analysis

The total phenolic content in the extracts was measured using a spectrophotometric method based on Folin-Ciocalteu's procedure [11]. In summary, 0.5 ml of the diluted extract was mixed with 0.45 ml of water and 2.5 ml of a 1:10 diluted Folin-Ciocalteu phenol reagent, followed by the addition of 2 ml of a 7.5% (w/v) sodium carbonate solution. After allowing the mixture to incubate for 5 minutes at 50°C, the absorbance was recorded at 760 nm. The phenolic content was calculated using a standard curve of gallic acid, with the results expressed as mg of gallic acid equivalents (GAE) per ml of extract. Calibration graphs (Fig. 3) were created for linear regression analysis, plotting peak area against concentrations of 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 µg/ml.

Total Flavonoids (TF) Analysis

The flavonoid content was measured using a spectrophotometric method that relies on the formation of an aluminum-flavonoid complex [12]. All analyses were performed in triplicate. To begin, 0.5 mL of a 2% (w/v) AlCl₃ solution was added to 1 mL of the test solution (either standard or sample). Following this, 0.5 mL of water, HCl, CH₃COONa, or CH₃COONH₄ (each at a concentration of 1 M) was added. The standard solutions of flavonoids used had a concentration of 100 µM. The mixture was then shaken vigorously and left to incubate at room temperature for 10 minutes before undergoing spectral analysis at 425 nm. In the blank, the AlCl₃ solution was replaced with an equal volume of water. The flavonoid content was determined using a standard curve based on quercetin, with results expressed as mg of quercetin equivalents (Q) per mL of extract. Calibration graphs (Fig. 4) were subsequently plotted for linear regression analysis, correlating peak area with concentrations of 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 µg/mL.

Antioxidant Capacity by DPPH Assay

The DPPH radical scavenging activity of the extract was assessed using the stable free radical DPPH, as described previously [13]. In this method, 1 mL of each sample (at a concentration of 1 mg/mL) was incubated with a methanolic solution of DPPH (1 mM). After 30 minutes of incubation at room temperature, the absorbance at 517 nm was measured using a spectrophotometer. The percentage of radical inhibition (%I) was calculated using the following formula:

$$\%I = \left(\frac{A_{\text{DPPH}} - A_{\text{P}}}{A_{\text{DPPH}}} \right) \times 100$$

where A_{DPPH} is the absorbance of the DPPH solution containing ethanol, and A_P is the absorbance of the DPPH solution with the plant extract.

3.3. Statistical analysis

Data analysis was performed using the XLSTAT software v5.03 (2016). Statistical analysis was carried out using the ANOVA procedure and the means were compared with a Least Significant Difference (LSD) test at p ≤ 0.05. The p-values of less than 0.05 were considered statistically significant. Simple correlation (Pearson) between traits were performed using Microsoft Excel (version 2013).

Hierarchical Cluster Analysis (HCA) was conducted using the Ward linkage method and Euclidean distance matrix to assess the genetic diversity and grouping patterns among the samples. Clustering and dendrograms were generated using Python (libraries: pandas, scipy, sklearn, matplotlib). All data were first normalized using Z-score standardization to ensure comparability across different units and scales [14–15].

Pearson correlation coefficients were calculated to assess the strength and direction of relationships between morphological and biochemical traits. The correlation matrix was visualized using a heatmap for interpretability [16].

3. Result and discussion

Morphological evaluation

The results indicated a statistically significant difference (p < 0.01) among the studied genotypes in terms of morphological traits. This diversity highlights the existence of considerable genetic variation, which can be utilized in breeding programs. Genotype 1 showed the highest number of fruits per panicle (17), along with dark red fruit color, suggesting its superiority in fruit production potential. Although Genotype 8 also had a high fruit count, it lacked in other key traits, making Genotype 1 a better overall performer.

In terms of fruit characteristics, Genotype 5 exhibited the greatest fruit width (0.72 cm), fresh fruit weight (0.41 g), seed length (0.57 cm), and seed width (0.27 cm). These traits suggest Genotype 5 could be ideal for breeding focused on fruit and seed quality. Moreover, Genotype 2 had the darkest fruit color,

followed closely by Genotype 1, which could be desirable for markets that prefer deeply pigmented fruits.

Regarding vegetative traits, Genotype 7 had the highest leaf length (3.15 cm), while Genotype 2 recorded the greatest leaf width (1.72 cm). Fresh and dry leaf weights were also highest in Genotype 5, further supporting its overall robustness. Seed weight varied among genotypes, with Genotypes 5 and 4 showing the most promising results.

Overall, performance ranking based on yield-related traits was as follows: Genotype 1 > Genotype 3 > Genotype 2 > Genotype 7 > Genotype 5 > Genotype 6 > Genotype 4. The variation in fruit color ranged from dark red to yellow, with the following trend: 2 > 1 > 3 & 4 > 5 & 6 > 7. This indicates that Genotype 2 is preferable for color-related traits, while Genotype 1 is superior for fruit count and general productivity, and Genotype 5 excels in fruit and seed size and weight. Such diversity makes these genotypes suitable candidates for future improvement and selection efforts. Trait correlation analysis is used to investigate and establish meaningful relationships between traits (Table 2). Establishing this relationship between traits leads to studying traits that may be difficult to measure [17].

Simple correlation coefficients between traits showed that some measured traits have a significant positive or negative correlation. Barberry fruit weight has a positive and significant correlation with seed length ($r = 0.5$). Barberry color showed negative correlation with fruit length ($r = -0.68$) and positive correlation with leave color ($r = 0.74$). Furthermore, Number of fruits per panicle showed negative correlation with seed width ($r = -0.73$). Also fresh fruit weight has positive correlation ($r = 0.79$) with blade length. Increasing number of fruits per panicle will be decreased the seed weight. Many studies investigated and evaluated significant difference between morphological traits which depends on studied region [18–19]. Wild germplasm of barberry plants were assessed on their morphological parameters.

Table 1
Mean comparison of morphological of wild barberry (*Berberis vulgaris* L.) fruits genotype.

Sample	leaf length	leaf width	leaf tail length	fruit length	fruit width	fruit tail length	Number of fruit per of panicle	Number of leave per panicle	Fruit color	leaves color	fresh fruit weight	fresh leaf weight	Blade length	dry leaf weight	dry leaves weight
1	2.30 ^a	1.30 ^{ab}	0.75 ^{bcd}	0.75 ^c	0.65 ^{ab}	0.62 ^{cd}	17.00 ^b	16.00 ^a	5.00 ^b	3.00 ^b	0.25 ^{bc}	0.037 ^{bc}	1.97 ^{bc}	0.06 ^b	0.02 ^a
2	2.50 ^a	1.72 ^a	0.70 ^{bcd}	0.60 ^d	0.47 ^c	0.42 ^e	13.00 ^{cd}	9.00 ^c	6.00 ^a	4.00 ^a	0.14 ^d	0.060 ^{abc}	1.85 ^c	0.03 ^{de}	0.02 ^a
3	2.20 ^a	1.47 ^{ab}	0.67 ^{bcd}	1.07 ^a	0.57 ^{abc}	0.72 ^c	14.50 ^{bc}	10.50 ^b	3.00 ^d	2.00 ^c	0.20 ^{cd}	0.068 ^{abc}	2.25 ^b	0.09 ^a	0.02 ^a
4	3.12 ^a	1.15 ^{bc}	0.97 ^{bc}	1.07 ^a	0.55 ^{bc}	0.70 ^{cd}	9.50 ^e	8.75 ^c	3.00 ^d	3.00 ^b	0.19 ^d	0.050 ^{abc}	2.10 ^{bc}	0.06 ^b	0.02 ^a
5	2.97 ^a	1.55 ^{ab}	1.72 ^a	0.95 ^b	0.72 ^a	0.60 ^{cde}	11.00 ^{de}	8.00 ^{cd}	4.00 ^c	3.00 ^b	0.41 ^a	0.093 ^a	2.70 ^a	0.09 ^a	0.02 ^a
6	2.25 ^a	1.05 ^{bc}	0.50 ^d	1.02 ^{ab}	0.65 ^{ab}	0.60 ^{cde}	10.20 ^{de}	7.00 ^d	4.00 ^c	2.00 ^c	0.26 ^b	0.033 ^{bc}	2.10 ^{bc}	0.05 ^c	0.02 ^a
7	3.15 ^a	0.70 ^c	0.95 ^{bcd}	0.95 ^b	0.57 ^{abc}	0.95 ^b	14.75 ^{bc}	4.75 ^e	2.00 ^e	2.00 ^c	0.18 ^d	0.086 ^{ab}	1.97 ^{bc}	0.04 ^{cd}	0.01 ^a
8	2.80 ^a	1.20 ^b	1.12 ^b	1.05 ^{ab}	0.72 ^a	1.20 ^a	25.75 ^a	7.25 ^d	3.00 ^d	3.00 ^b	0.15 ^d	0.034 ^{bc}	2.20 ^b	0.06 ^b	0.02 ^a
9	3.15 ^a	1.45 ^{ab}	0.60 ^{cd}	0.95 ^b	0.52 ^{bc}	0.52 ^{de}	12.25 ^{cde}	7.50 ^d	1.00 ^f	2.00 ^c	0.20 ^d	0.022 ^c	2.05 ^{bc}	0.03 ^e	0.02 ^a

Table 2
Correlation coefficients between all measured morphological traits in the wild barberry genotype.

	<i>leaf length</i>	<i>leaf width</i>	<i>leaf tail length</i>	<i>fruit length</i>	<i>fruit width</i>	<i>fruit tail length</i>	<i>Number of fruits per panicle</i>	<i>Number of leave per panicle</i>	<i>Fruit color</i>	<i>leaves color</i>	<i>fresh fruit weight</i>	<i>fresh leaf weight</i>	<i>Blade length</i>	<i>dry leaf weight</i>	<i>dry leaves weight</i>
leaf length	1.00														
leaf width	-0.27	1.00													
leaf tail length	0.47	0.08	1.00												
fruit length	0.23	-0.45	0.15	1.00											
fruit width	-0.09	-0.18	0.59	0.37	1.00										
fruit tail length	0.23	-0.59	0.28	0.52	0.50	1.00									
Number of fruits per panicle	-0.10	-0.11	0.11	0.03	0.43	0.76	1.00								
Number of leave per panicle	-0.56	0.39	-0.17	-0.39	0.05	-0.31	0.10	1.00							
Fruit color	-0.61	0.44	0.03	-0.68	0.05	-0.39	-0.03	0.51	1.00						
leaves color	-0.04	0.53	0.31	-0.65	-0.07	-0.19	0.14	0.31	0.74	1.00					
fresh fruit weight	0.00	0.15	0.59	0.12	0.60	-0.28	-0.37	0.14	0.13	-0.08	1.00				
fresh leaf weight	0.19	-0.02	0.61	-0.04	0.06	0.03	-0.23	-0.23	0.11	0.08	0.36	1.00			
Blade length	0.15	0.22	0.78	0.46	0.67	0.10	-0.08	-0.12	-0.10	-0.09	0.79	0.44	1.00		
dry leaf weight	-0.23	0.17	0.54	0.45	0.55	0.19	0.05	0.29	0.09	-0.05	0.55	0.46	0.77	1.00	
dry leaves weight	-0.49	0.67	0.04	-0.17	-0.03	-0.41	-0.22	0.05	0.62	0.46	0.13	0.11	0.26	0.27	1.00
Seed length	-0.34	0.53	-0.10	-0.07	-0.12	-0.76	-0.63	0.60	0.28	0.05	0.50	-0.07	0.25	0.37	0.37
seed width	-0.23	0.52	-0.01	-0.10	-0.20	-0.76	-0.73	0.44	0.39	0.25	0.44	0.02	0.22	0.31	0.51
seed weight	-0.28	0.34	-0.08	-0.33	-0.03	-0.71	-0.53	0.67	0.49	0.30	0.46	-0.22	0.01	0.09	0.20

Biochemical analysis

The data collected from the analysis of nine samples of *Berberis vulgaris* L. reveal notable concentrations of total phenolics and flavonoids, expressed in mg/ml, along with their respective antioxidant percentages (Table 3). The results indicate that sample 2 showcased the highest total phenolic content of 14.34 mg/ml and flavonoid concentration of 13.83 mg/ml, and sample 3 achieving an impressive antioxidant percentage of 97.10%. Similarly, sample 7 exhibited a remarkable flavonoid concentration of 16.86 mg/ml, with a corresponding antioxidant activity of 96.45% (Table 3). The findings on *Berberis vulgaris* L. align with the research indicating high phenolic and flavonoid concentrations, showcasing significant antioxidant activity. This supports the notion that even lower concentrations can effectively scavenge free radicals, reinforcing its potential as a natural antioxidant resource [20]. The study on Iranian *Berberis* species found varying total phenolic contents, with Black berberis bakhtiarica showing the highest levels and antioxidant activity, supporting the notion that phenolic and flavonoid compounds in *Berberis* contribute significantly to their antioxidant properties [21–22].

Conversely, samples with lower concentrations, such as sample 8 (5.60 mg/ml for total phenolics) and sample 9 (5.18 mg/ml for total phenolics), still exhibited considerable antioxidant properties, with percentages of 93.12% and 89.20%, respectively. This suggests that even at lower concentrations, the phenolic and flavonoid compounds in *Berberis vulgaris* L. are effective in scavenging free radicals, reinforcing the herb's potential as an antioxidant resource.

concentrations of total phenolics in *Berberis vulgaris* L. (5.60 mg/ml and 5.18 mg/ml) demonstrated significant antioxidant activity (93.12% and 89.20%), highlighting the effectiveness of its phenolic and flavonoid compounds in free radical scavenging. concentrations of total phenolics in *Berberis vulgaris* L. (5.60 mg/ml and 5.18 mg/ml) demonstrated significant antioxidant activity (93.12% and 89.20%), highlighting the effectiveness of its phenolic and flavonoid compounds in free radical scavenging. [23]. The paper confirms that *Berberis vulgaris* L. exhibits significant antioxidant activity, particularly in acetone extracts, with the highest total phenolic content at 92.75 mg GAE per g, supporting the findings of notable antioxidant percentages in various samples [24].

The overall antioxidant percentages across all samples consistently exceed 89%, underscoring the robust antioxidant activity associated with *Berberis vulgaris* L. In the present study, the antioxidant activity of the barberry fruit extract was found to be significantly high, reaching levels comparable to or potentially exceeding those of ascorbic acid. While previous studies such as Hosseinihashemi et al. [25] reported an antioxidant activity of 98.61% in inner peel extracts of barberry, our findings show that the fruit itself—without isolating the peel—also possesses remarkable antioxidant properties. This suggests that antioxidant compounds are not limited to the peel, but rather are distributed throughout the fruit tissue. The high antioxidant capacity observed in our samples may be attributed to the presence of bioactive phytochemicals such as anthocyanins, polyphenols, and flavonoids, naturally abundant in barberry. These findings align with previous literature that highlights the rich phytochemical profile of this plant, attributing its protective effects largely to its complex array of flavonoids and phenolic acids (22, 26). Altogether, these results suggest that whole barberry fruit could serve as a potent natural antioxidant source for use in food, nutraceutical, and pharmaceutical applications.

Table 3
Biochemical composition of wild barberry (*Berberis vulgaris* L.) fruit genotypes

Sample	Total Phenol mg/ml	Total Flavonoid mg/ml	Antioxidant %
1	11.15	12.55	93.84
2	14.34	13.83	91.30
3	11.93	10.07	97.10
4	7.75	6.22	95.58
5	7.71	8.24	93.99
6	6.53	7.05	96.01
7	8.65	16.86	96.45
8	5.60	10.26	93.12
9	5.18	5.30	89.20

Cluster analysis

To explore the diversity patterns among the studied samples, a hierarchical clustering analysis was conducted using 21 morphological and biochemical traits. This analysis was implemented using Python software, employing the Ward linkage method to group the samples based on their similarity. The resulting dendrogram provided a visual representation of the relationships among the samples, effectively categorizing them into three distinct clusters (Fig. 5).

Cluster I included Samples 7 and 8, which demonstrated clearly distinct biochemical profiles. Notably, Sample 7 exhibited a remarkably high flavonoid content, setting it apart from the rest of the dataset. This elevated level of flavonoids suggests a strong potential for antioxidant activity, making Sample 7 a candidate of interest for further phytochemical or pharmacological studies (Fig. 5).

Cluster II comprised Samples 1 and 2. Among these, Sample 2 stood out due to its exceptionally dark fruit color (color code 6, the darkest in the range). It also exhibited the highest concentrations of total phenolic compounds (14.34 mg/ml) and total flavonoids (13.83 mg/ml). These values were significantly higher than those observed in the other samples, indicating a possible correlation between darker fruit pigmentation and greater accumulation of antioxidant compounds. Such a relationship aligns with the widely reported role of anthocyanins—natural pigments responsible for red, purple, and blue hues—in contributing to the antioxidant properties of plant-based materials (Fig. 5).

Cluster III consisted of Samples 3, 4, 5, 6, and 9. These samples displayed relatively lower levels of biochemical compounds, particularly phenolics and flavonoids. The fruit color codes in this cluster ranged from 1 to 4, suggesting lighter pigmentation compared to the samples in Cluster II. This pattern supports the hypothesis that fruit color may serve as an indirect indicator of phytochemical content, especially antioxidant-related compounds (Fig. 5).

The findings of this study are consistent with previous research indicating that the intensity of natural pigmentation in plant tissues, especially dark coloration due to anthocyanin accumulation, is positively associated with antioxidant capacity. Given the ease of observing fruit color in the field, this trait could serve as a practical and rapid screening tool for selecting genotypes with enhanced medicinal and nutraceutical potential.

These insights have several important implications for future plant breeding and natural product development initiatives: Genotypes exhibiting darker fruit coloration should be prioritized in breeding programs aimed at developing cultivars with high levels of bioactive compounds, particularly those with antioxidant properties. Also, The data provide a scientific basis for the development of natural antioxidant supplements, functional plant extracts, and herbal medicinal products derived from high-value genotypes [27, 28].

hierarchical clustering based on integrated morphological and biochemical data has proven to be a powerful method for identifying diversity and selecting elite genotypes. These findings offers a simple yet effective strategy for the preliminary assessment of medicinal potential in plant breeding and conservation programs.

Correlation Between Morphological and Biochemical Traits

To further investigate the relationships between morphological characteristics and biochemical composition in the studied plant samples, Pearson correlation analysis was performed. The results revealed several statistically significant associations, indicating that morphological features may act as reliable predictors of the plant's biochemical and antioxidant potential (Fig. 6).

Fruit Color (coded on a scale from 1 = light to 6 = dark) exhibited a strong positive correlation with total phenol content ($r = 0.60$, $p < 0.01$), as well as moderate positive correlations with total flavonoid content ($r = 0.42$, $p < 0.05$) and fresh fruit weight ($r = 0.51$, $p < 0.01$). These results suggest that darker-colored fruits tend to accumulate higher concentrations of bioactive compounds. For instance, Sample 2, which had the darkest fruit color (code 6), showed the highest levels of phenolic and flavonoid content among all samples, supporting the hypothesis that fruit pigmentation is closely tied to antioxidant potential [29].

Fruit Tail Length was positively correlated with antioxidant activity ($r = 0.49$, $p < 0.05$), suggesting a potential link between fruit structural traits and secondary metabolite biosynthesis. This relationship may indicate that longer fruit tails are associated with physiological or metabolic pathways that favor the accumulation of antioxidant compounds [27, 28].

Leaf Width showed a moderate negative correlation with total phenol content ($r = -0.53$, $p < 0.05$), implying that narrower leaves may be more efficient in accumulating phenolic compounds. This could be due to differences in light interception, transpiration, or metabolic partitioning in narrower versus broader leaves (Fig. 3).

Similarly, Dry Leaf Weight demonstrated a negative correlation with total flavonoid content ($r = -0.52$, $p < 0.05$). This suggests that an increase in dry matter may be associated with either the dilution of flavonoid compounds or their oxidative degradation during the drying process [30–31].

Taken together, these findings reinforce the hypothesis that morphological traits can serve as practical, indirect indicators of biochemical capacity in plant genotypes. This correlation between observable physical features and underlying phytochemical richness provides a valuable tool for rapid, non-destructive assessment in both field and laboratory settings. Research shows that specific morphological traits, such as seed dimensions and weight, are correlated with biochemical properties like dry matter and sugar content, suggesting these traits as indicators of nutritional value [32]. In pumpkin genotypes, significant variations in morphological traits like fruit yield and vine length were associated with biochemical traits such as ascorbic acid and carotene levels, highlighting the link between physical characteristics and biochemical potential [33].

The observed correlations between morphological and biochemical traits have important implications for both applied plant breeding and the development of natural pharmaceutical products. Easily observable phenotypic traits—such as fruit color, tail length, and leaf shape—can serve as preliminary screening markers in breeding programs. These morphological indicators can help identify high-value genotypes that are rich in antioxidants and other medicinally important compounds without the immediate need for time-consuming and costly biochemical assays. In this context, genotypes exhibiting dark fruit pigmentation and elevated levels of total phenolics and flavonoids can be strategically selected as parent lines in hybridization programs aimed at developing improved cultivars with enhanced nutraceutical and therapeutic properties [34]. Furthermore, the strong antioxidant capacity demonstrated by these dark-colored genotypes supports their potential use as raw material for the production of functional foods, dietary supplements, and natural therapeutic agents [35]. They may also serve as important sources for the extraction of bioactive compounds in the development of herbal medicines and antioxidant-enriched formulations. In brief, the integration of morphological assessments with biochemical profiling provides a powerful framework for targeted selection in both agricultural and pharmaceutical contexts. Such an approach enhances the efficiency of genotype evaluation and paves the way for the development of high-performance cultivars with tangible health benefits [36].

4. Conclusion

In this study, wild genotypes of *Berberis vulgaris* L. were collected from different regions of Isfahan Province, Iran, and thoroughly evaluated for their morphological and biochemical diversity. The results revealed considerable variability in morphological traits such as fruit color, fruit weight, leaf shape, and tail length, emphasizing the genetic richness of this species and its potential for use in breeding programs and conservation efforts. This morphological diversity offers valuable opportunities for the selection and improvement of genotypes with desirable agronomic or medicinal characteristics.

While barberry fruit is known as a source of essential nutrients including carbohydrates, proteins, fats, fiber, and minerals, its high seed content and wild nature limit its direct application in large-scale dietary consumption. Nevertheless, the species' strong ecological adaptability and resilience to harsh conditions underscore its importance as a strategic genetic resource, particularly in the face of climate change and biodiversity loss. Biochemical analyses further demonstrated that *Berberis vulgaris* L. possesses notable antioxidant potential, largely attributable to its rich content of phenolic and flavonoid compounds. These bioactive substances, although not widely utilized in culinary applications, show promising therapeutic properties. Strong positive correlations between darker fruit pigmentation and elevated levels of phenolic and flavonoid compounds support the idea that fruit color can serve as a practical, field-level indicator of medicinal potential.

The findings suggest that darker-fruited genotypes should be prioritized for breeding and industrial exploitation aimed at the development of functional foods, nutraceuticals, and natural pharmaceutical products. Moreover, the identified correlations between morphological and biochemical traits provide a foundation for more efficient screening and selection of elite genotypes in future breeding initiatives.

To fully harness the medicinal potential of *Berberis vulgaris*, future research should focus on: Elucidating the mechanisms of action of its bioactive compounds, Assessing their bioavailability and pharmacokinetics and Evaluating clinical efficacy through in vivo and clinical studies. Such studies will advance the application of this native species in modern phytotherapy and integrative medicine and support its sustainable utilization and conservation.

Declarations

Conflict of Interest

The authors declare no conflicts of interest.

Funding statement

The authors received no financial support for the research, authorship, and/or publication of this article.

Author Contribution

E.ZM and A.P contributed equally to this work. E.ZM was responsible for [mention specific contributions, e.g., data collection, analysis, or writing the first draft], while A.P contributed to [mention specific contributions, e.g., data interpretation, reviewing the manuscript, or conducting experiments]. Both authors reviewed and approved the final manuscript.

Acknowledgement

The authors are thankful to the Director of the 3Medicinal Planr Rsearch Center, Isf. C., Islamic Azad University, Isfahan, Iran for providing all the research facilities during this study. We are also thankful for the cooperation Zarin Gol Feridunshahr company for preparing samples of this study.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Ahrendt, L. W. A. *Berberis* and *Mahonia*: a taxonomic revision. *Bot. J. Linn. Soc.* **57** (369), 1–410 (1961).
2. Chauhan, N. S. *Medicinal and aromatic plants of Himachal Pradesh* (Indus Publishing Co., 1999).
3. Sun, W., Shahrajabian, M. H. & Cheng, Q. Barberry (*Berberis vulgaris*), a medicinal fruit and food with traditional and modern pharmaceutical uses. *Isr. J. Plant. Sci.* **68** (1–2), 61–71. <https://doi.org/10.1163/22238980-bja10019> (2021).
4. Arayne, S. M., Sultana, N. & Bahadur, S. S. The *Berberis* story: *Berberis vulgaris* in therapeutics. *Pak J. Pharm. Sci.* **20**, 83–92 (2007).
5. Ebrahimi-Mamaghani, M., Arefhosseini, S. R., Golzarand, M., Aliasgarzadeh, A. & Vahed-Jabbary, M. Long-term effects of processed *Berberis vulgaris* on some metabolic syndrome components. *Iran. J. Endocrinol. Metab.* **11** (1), 41–47 (2009).
6. Hashmi, S. S. et al. Genus *Berberis*: A comprehensive and updated review on ethnobotanical uses, phytochemistry and pharmacological activities. *Chem. Biodivers.* <https://doi.org/10.1002/cbdv.202400911> (2024).
7. Crozier, A., Jaganath, I. B. & Clifford, M. N. Dietary phenolics: Chemistry, bioavailability and effects on health. *ChemInform* **40** (46). <https://doi.org/10.1002/chin.200946244> (2009).
8. Gundogdu, M. Determination of antioxidant capacities and biochemical compounds of *Berberis vulgaris* L. fruits. *Adv. Environ. Biol.* **7** (Special Issue), 344–349 (2013).
9. Yildiz, H. et al. Some physicochemical characteristics, bioactive content and antioxidant characteristics of non-sprayed barberry (*Berberis vulgaris* L.) fruits from Turkey. *Erwerbs-Obstbau* **56** (4), 123–129. <https://doi.org/10.1007/S10341-014-0216-4> (2014).
10. Garazhian, M., Gharaghani, A. & Eshghi, S. Genetic diversity and inter-relationships of fruit bio-chemicals and antioxidant activity in Iranian wild blackberry species. *Sci. Rep.* **10**, 18983. <https://doi.org/10.1038/s41598-020-75849-1> (2020).
11. Pantelidis, G. E., Vasilakakis, M., Manganaris, G. A. & Diamantidis, G. R. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and Cornelian cherries. *Food Chem.* **102**, 777–783. <https://doi.org/10.1016/j.foodchem.2006.06.021> (2007).
12. Pękal, A. & Pyrzynska, K. Evaluation of aluminium complexation reaction for flavonoid content assay. *Food Anal. Methods.* **7**, 1776–1782. <https://doi.org/10.1007/s12161-014-9814-x> (2014).
13. Brand-Williams, W., Cuvelier, M. E. & Berset, C. L. W. T. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci. Technol.* **28**, 25–30 (1995).
14. Köhn, H. F. & Hubert, L. Hierarchical cluster analysis. In: Wiley StatsRef: Statistics Reference Online. (2014). <https://doi.org/10.1002/9781118445112.stat02449.pub2>

15. Rocha, D. Hierarchical clustering analysis data. figshare. (2022). <https://doi.org/10.6084/m9.figshare.20738449.v1>
16. Perveen, N. et al. Correlation and path coefficient analysis for morphological and biochemical traits in diverse pomegranate (*Punica granatum* L.) genotypes. *Curr. J. Appl. Sci. Technol.* **35** (3), 1–9. <https://doi.org/10.9734/cjast/2019/v35i330186> (2019).
17. Rezaei, M., Sarkhosh, A. & Balandari, A. Characterization of valuable indigenous barberry (*Berberis* sp.) germplasm by using multivariate analysis. *Int. J. Fruit Sci.* **20** (1), 1–19. <https://doi.org/10.1080/15538362.2018.1555508> (2020).
18. Khodabandeh, M., Azizi, M., Balandary, A. & Arouiee, H. Evaluation of some physical properties of sixteen Iranian indigenous barberry genotypes. *J. Hortic. Sci.* **36** (3), 549–562. <https://doi.org/10.22067/jhs.2021.58409.0> (2022).
19. Khoshandam, L., Farokhzad, A. & Rezaei, M. Evaluation of some morphological and biochemical characteristics of wild seeded barberry genotypes (*Berberis* L.) in Shahrood region. *Iran. J. Hortic. Sci.* **53** (4), 2423–2930. <https://doi.org/10.22059/ijhs.2022.330341.1967> (2023).
20. El-Zahar, K. M. et al. Antioxidant, antibacterial, and antifungal activities of the ethanolic extract obtained from *Berberis vulgaris* roots and leaves. *Molecules* **27** (18), 6114. <https://doi.org/10.3390/molecules27186114> (2022).
21. Sharifi, F. & Poorakbar, L. The survey of antioxidant properties of phenolic compounds in fresh and dry hybrid barberry fruits (*Berberis integerrima* × *vulgaris*). *Cumhuriyet Sci. J.* **36** (3), 1609–1617. <https://doi.org/10.17776/cs.69976> (2015).
22. Rahimi-Madiseh, M. et al. Evaluation of minerals, phenolics and anti-radical activity of three species of Iranian *Berberis* fruit. *Der Pharma Chem.* **8** (2), 191–197 (2016).
23. Och, A. et al. Evaluation of the antioxidant and anti-lipoxygenase activity of *Berberis vulgaris* L. leaves, fruits, and stem and their LC–MS/MS polyphenolic profile. *Antioxidants* **12** (7), 1467. <https://doi.org/10.3390/antiox12071467> (2023).
24. Aliakbarlu, J., Ghiasi, S. & Bazargani-Gilani, B. Effect of extraction conditions on antioxidant activity of barberry (*Berberis vulgaris* L.) fruit extracts. *Vet. Res. Forum.* **9** (4), 361. <https://doi.org/10.30466/vrf.2018.33090> (2018).
25. Hosseinihashemi, S. K., Anooshei, H., Aghajani, H. & Salem, M. Z. Chemical composition and antioxidant activity of extracts from the inner bark of *Berberis vulgaris* stem. *BioResources* **10** (4), 7958–7969 (2015).
26. Iskender, H. et al. Determination of essential oil and phenolic compounds of *Berberis vulgaris* grown in Şavşat, Artvin; revealing its antioxidant and antimicrobial activities. *Z. Naturforsch. C.* **79** (1), 1–10. <https://doi.org/10.1515/znc-2024-0163> (2024).
27. Wang, S. Y. Maximizing antioxidants in fruits. *Acta Hort.* **877**, 81–93. <https://doi.org/10.17660/ACTAHORTIC.2010.877.5> (2010).
28. Hu, W., Guan, Y. & Feng, K. Biosynthesis of phenolic compounds and antioxidant activity in fresh-cut fruits and vegetables. *Front. Microbiol.* **13**, 906069. <https://doi.org/10.3389/fmicb.2022.906069> (2022).
29. Wang, R., Dong, P., Shuai, X. & Chen, M-S. Evaluation of different black mulberry fruits (*Morus nigra* L.) based on phenolic compounds and antioxidant activity. *Foods* **11** (9), 1252. <https://doi.org/10.3390/foods11091252> (2022).
30. Uthairat, C., Srisook, E. & Srisook, K. Effects of drying methods and extraction conditions on total phenolic and flavonoid content and antioxidant activities of *Helicteres isora* L. fruit extracts. *Burapha Sci. J.* **22** (1), 151–165 (2017). <http://science.buu.ac.th/ojs246/index.php/sci/article/download/1184/1259>
31. Sarjadi, M. S., Majid, M. H. A., Laurence, Y. M. & Khan, A. Total flavonoid content and antioxidant activity by different drying and extraction methods of *Clinacanthus nutans* leaves. *Borneo Sci.* **39** (2). <https://doi.org/10.51200/bsj.v39i2.4424> (2023).
32. Rakhmetov, D., Bondarchuk, O., Rakhmetova, S. O., Rashydov, N. & Kutsokon, N. Biological, morphological and biochemical features of seeds of introduced genotypes of *Cicer arietinum* L. Plant Production and Soil Science (Рослинництво Та Ґрунтознавство). (2023). <https://doi.org/10.31548/plant3.2023.97>
33. Kumar, V., Mishra, D., Yadav, G. & Dwivedi, D. K. Genetic diversity assessment for morphological, yield and biochemical traits in genotypes of pumpkin. *J. Pharmacogn. Phytochem.* **6** (3), 14–18 (2017). <https://www.phytojournal.com/archives/2017.v6.i3.1187>
34. Blando, F. et al. Nutraceutical characterization of anthocyanin-rich fruits produced by Sun Black tomato line. *Front. Nutr.* **6**, 133. <https://doi.org/10.3389/fnut.2019.00133> (2019).
35. Irakli, M., Samanidou, V. F., Katsantonis, D., Biliaderis, C. G. & Papadoyannis, I. N. Phytochemical profiles and antioxidant capacity of pigmented and non-pigmented genotypes of rice (*Oryza sativa* L.). *Cereal Res. Commun.* **44** (1), 98–110. <https://doi.org/10.1556/0806.43.2015.033> (2016).
36. Rezvani Moghaddam, P., Fallahi, J., Aghavani Shajari, M. & Nassiri Mahallati, M. Effects of harvest date, harvest time, and postharvest management on quantitative and qualitative traits in seedless barberry (*Berberis vulgaris* L.). *Ind. Crops Prod.* **42**, 30–36. <https://doi.org/10.1016/j.indcrop.2012.05.007> (2013).

Figures



Figure 1

Natural habitat of wild *Berberis vulgaris* collected from Zarin Gol, Feridunshahr, Isfahan province, Iran (49°55'35.8"E, 32°56'19.2"N). The sites are nearby with similar climates.

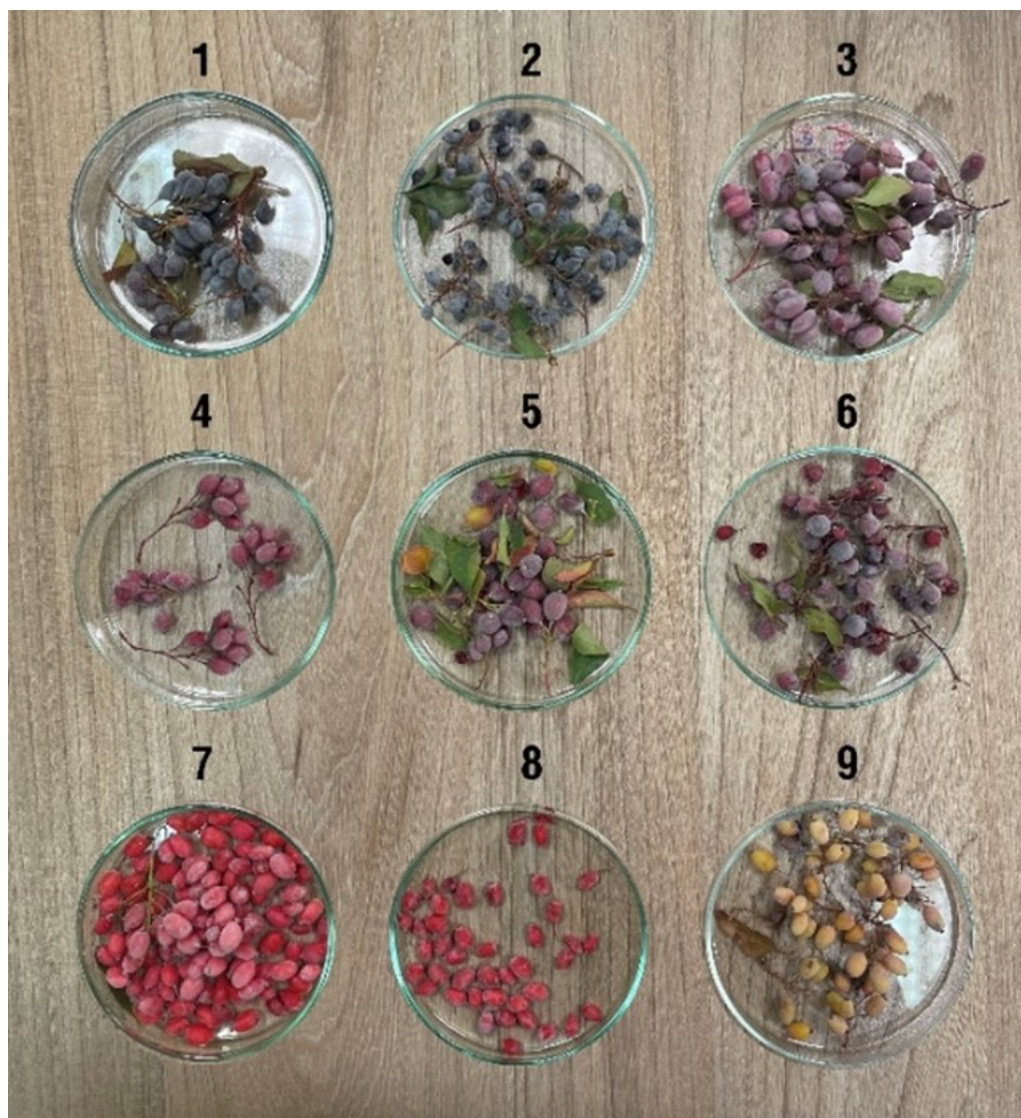


Figure 2

Visual representation of the 9 wild individuals of *Berberis vulgaris* examined in this study

Standard Curve

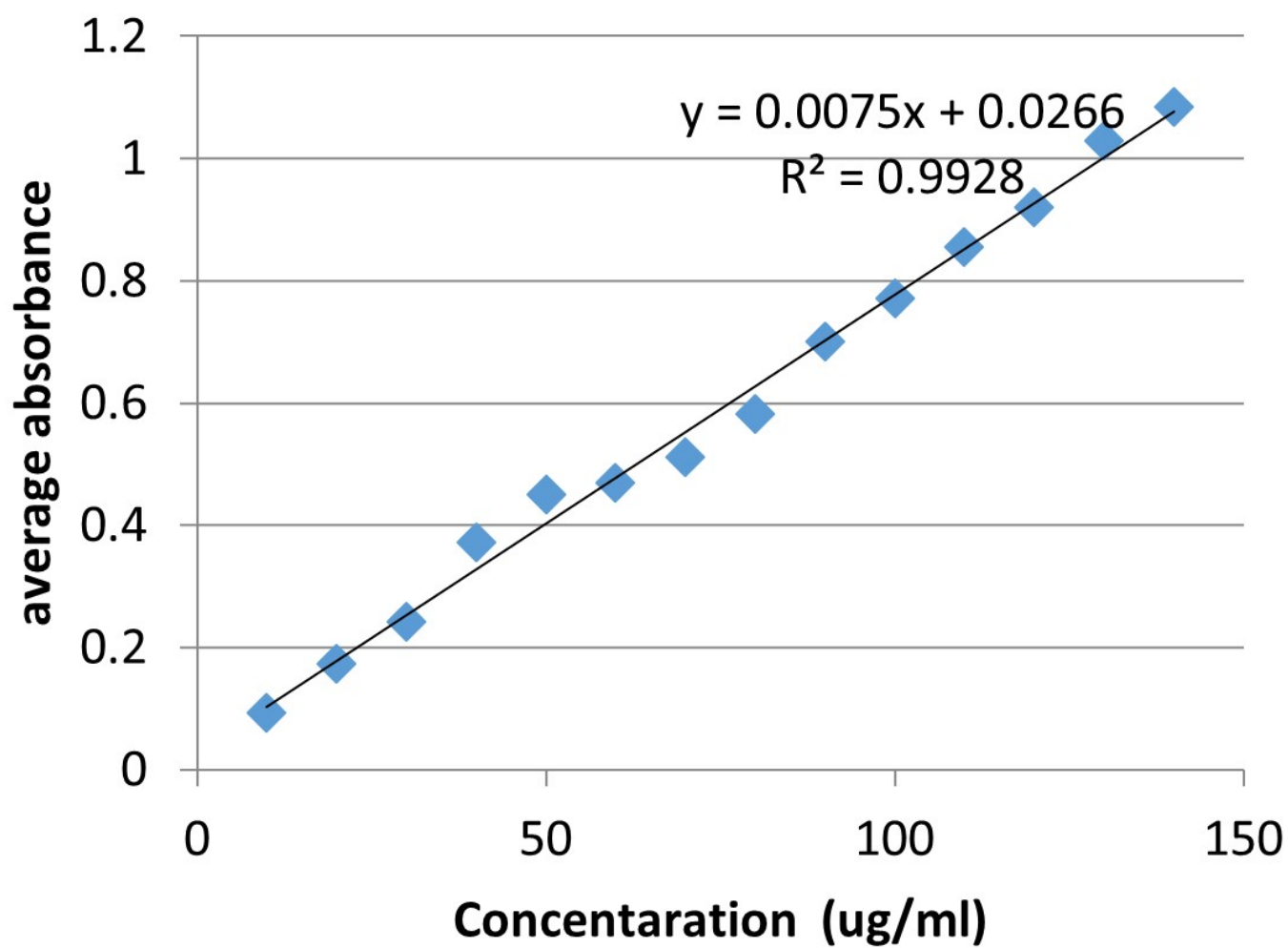


Figure 3

Linear relationship between concentration and absorbance for total phenol determination using the standard curve.

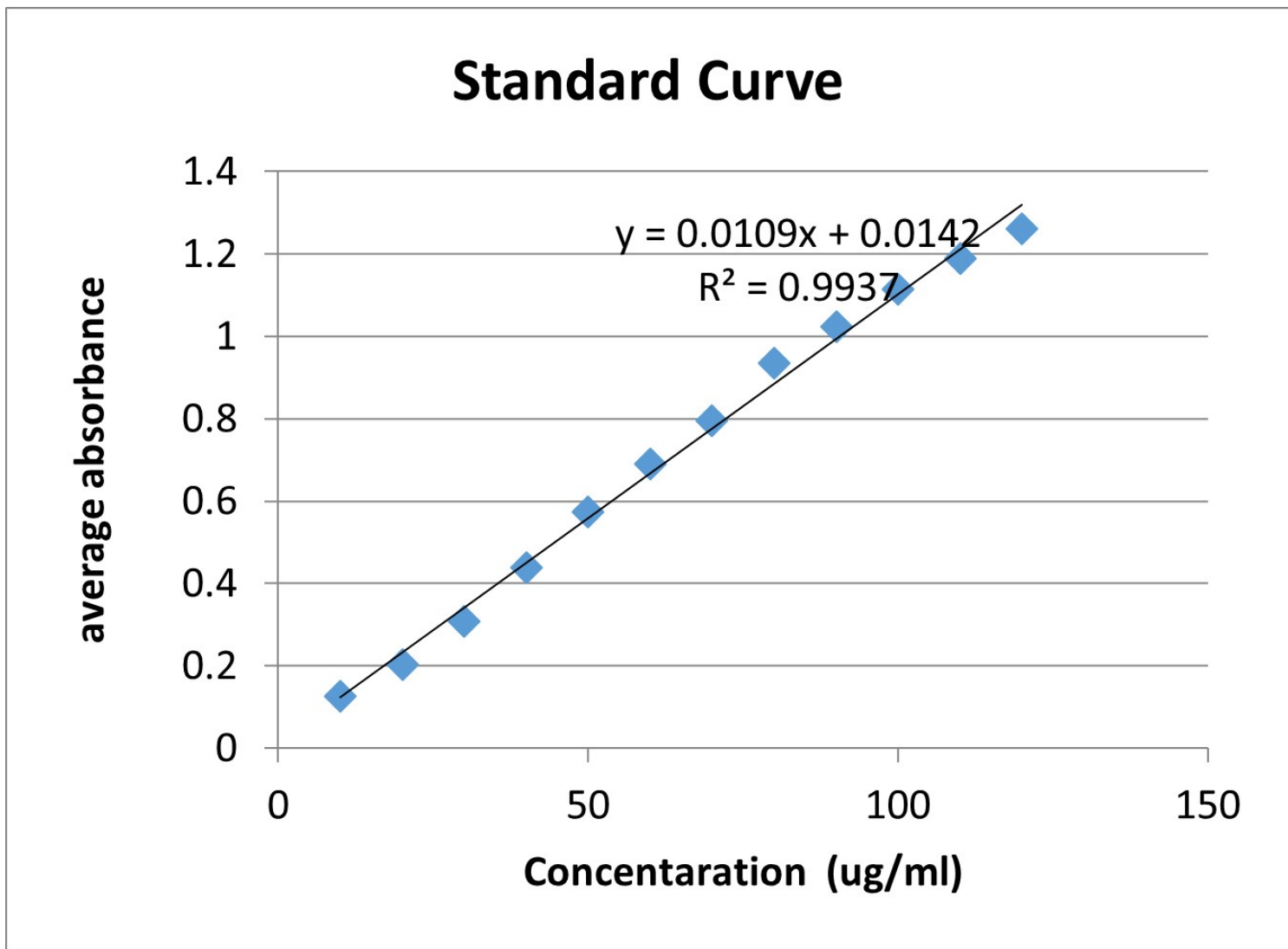


Figure 4

Standard curve showing the linear relationship between flavonoid concentration and average absorbance.

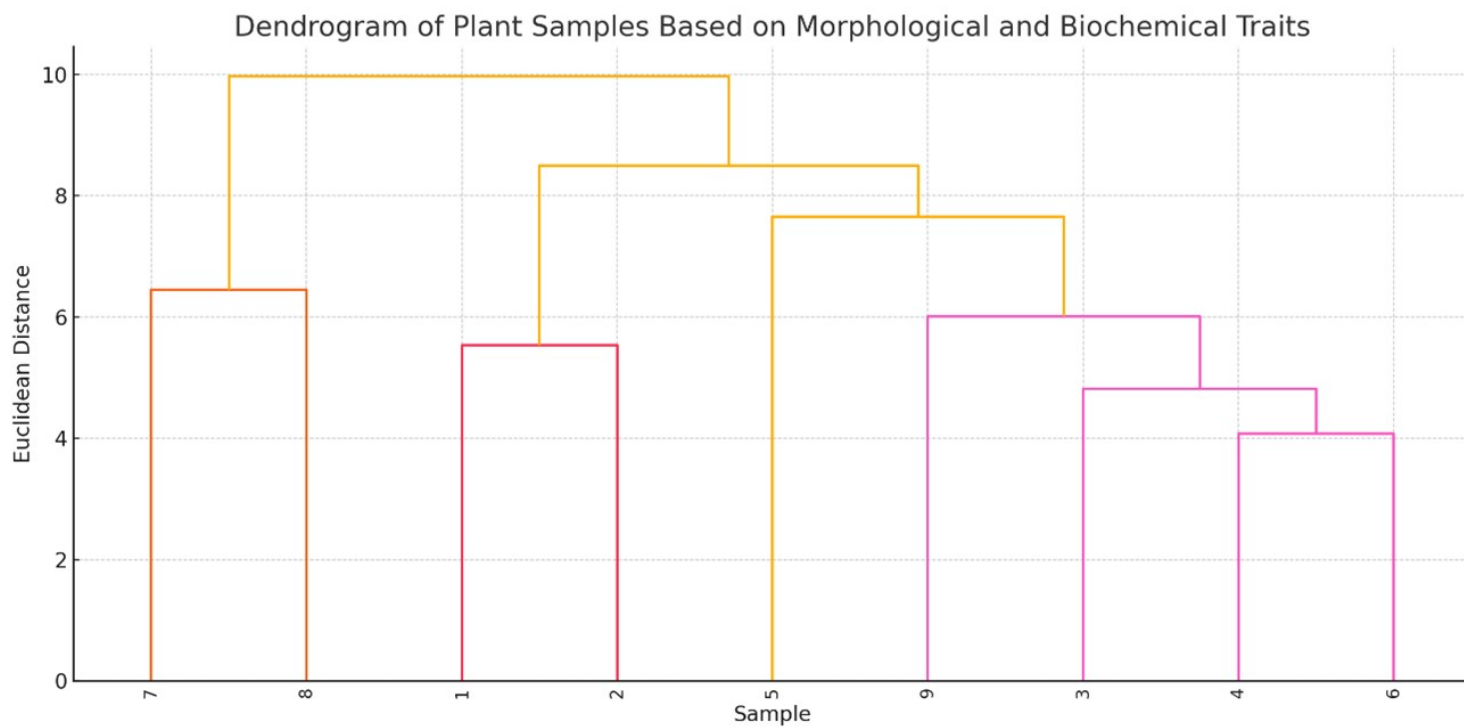


Figure 5

Dendrogram of wild barberry (*Berberis vulgaris* L.) samples based on morphological and biochemical traits.

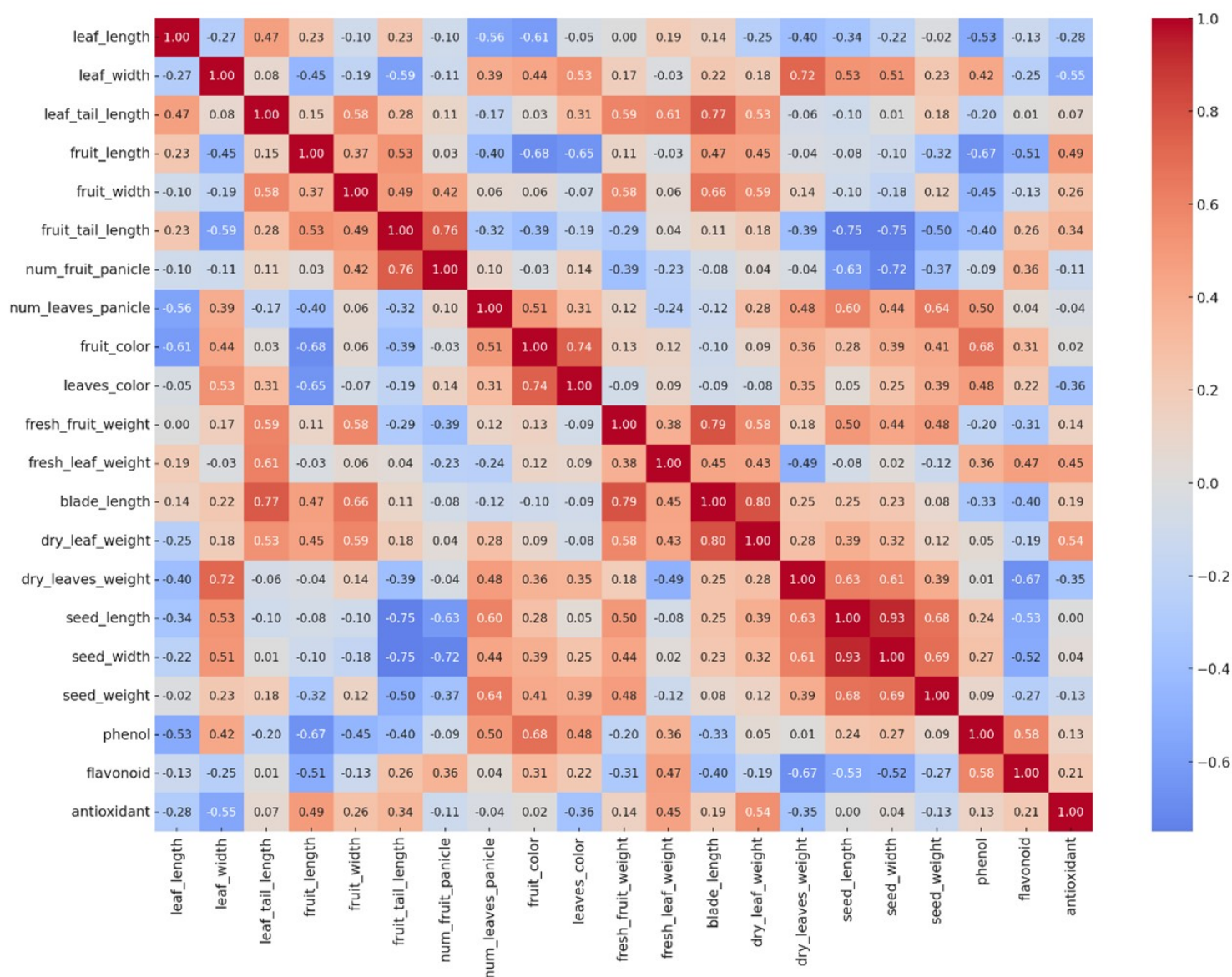


Figure 6

Pearson correlation heatmap showing the relationships between morphological traits and biochemical/antioxidant parameters. Red shades indicate positive correlations, while blue shades represent negative correlations. The numerical values within the cells correspond to the Pearson correlation coefficients. Several statistically significant associations suggest that specific morphological characteristics may serve as reliable predictors of the plant's biochemical composition and antioxidant potential.