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Research Article

Determination of phenolic content of *Berberis vulgaris* L. fruits harvested at different times by multivariate analysis

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Abstract: This study focused on the levels of phenolic compounds present in the fruits of *Berberis vulgaris* genotypes harvested at different times and determined the relationship between the studied traits by multivariate analysis. Harvest time significantly affected the amount of phenolic content. There were significant increases in gallic, catechin, chlorogenic, rutin, and *q*-coumaric acid contents, while caffeic and syringic acid contents decreased significantly as the harvest time was delayed. In PCA analysis, PC1 and PC2 explained 78.3% of the data. It was found that 'Harvest 1' was notable for its high content of caffeic and syringic acids, while 'Harvest 4' excelled in catechin, gallic acid, and chlorogenic acid content. It was also determined that 'Genotype 1' stood out in terms of myricetin, quercetin, *p*-coumaric, *q*-coumaric, and rutin content. This study highlights the importance of phenolic acid content in determining the optimal harvest time. The findings indicate that 'Harvest 4' (December) is the most suitable period for harvesting, particularly in terms of gallic acid, catechin, chlorogenic acid, *p*-coumaric acid, rutin, and *q*-coumaric acid.

1. INTRODUCTION

Plants synthesize many organic compounds such as tocopherol, flavonoids, phenolic compounds, alkaloids, chlorophyll, polyfunctional organic acids, and carotene during their vital activities (Larson, 1988). Phenolic compounds, which are defined as secondary metabolites, undertake vital activities such as growth and reproduction in plants as well as pest control and defense against external influences. The aroma and odor properties of plants are also due to phenolic compounds in the form of essential oils (Ercan, 2024). Additionally, these compounds are fundamental to maintaining oxidative stability and are critical in assessing the plant's antioxidant capacity.

Currently, there is a growing demand in the food and pharmaceutical industries for wild fruits that are rich in nutrients and antioxidants. Scientific research on the medicinal benefits and nutritional content of various wild edible fruits from different regions worldwide has garnered significant interest due to their numerous beneficial properties. The Berberidaceae family, which produces pink-red-black colored fruits, constitutes the most important natural wild fruits (Ağaoğlu & Gerçekçioğlu, 2013). For more than 2,500 years, humans have relied on *B. vulgaris* L., a species within the Berberis genus, as a valuable resource for herbal treatments. This long history of use highlights its significance in traditional medicine practices across various cultures (Končić *et al*., 2010; Mokhber-Dezfuli *et al*., 2014) because the isoquinoline alkaloid in the bark of the root and stem contains berberine (Ağaoğlu & Gerçekçioğlu, 2013). In addition to

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its root and bark, its leaves and fruit are also utilized (Imenshahidi & Hosseinzadeh, 2008; Imenshahidi & Hosseinzadeh, 2016). *B. vulgaris* L. is employed in treating a variety of conditions, such as kidney stones, urinary tract disorders, gastrointestinal issues, liver and gallbladder diseases. It also serves as a circulatory system stimulant and is used in the prevention of heart cardiac hypertrophy, failure, and arrhythmia, as well as in managing high cholesterol and diabetes (Kalmarzi *et al*., 2019). Its tall shrub-like species grow naturally in many countries including Türkiye (Gundogdu, 2013). It is popularly known by different names such 'kızamık', 'karamuk', 'kadıntuzluğu', 'garamık', 'ekşimen', 'sarı çalı', 'çoban tuzluğu', 'zibike', 'diken üzümü'. The yellow flower clusters bloom in April or May, comprising 15-25 blossoms. The fruits, which are 8-12 mm long and elliptical, turn a striking red when they ripen. In both traditional and modern medicine, fruit is the part of the plant most frequently utilized (Karadeniz, 2009). The fruits of *B. vulgaris*, rich in vitamin C, are sour (Kalmarzi *et al*., 2019; Gundogdu, 2013). The fruits are utilized as fresh dried, jam, and jelly. It has also been reported to be used as an additive in food product formulations (Alavi & Mazloumzadeh, 2012). On the other hand, various studies focusing on the fruits, leaves, shoots, and roots of *B. vulgaris* have demonstrated that extracts from these plant parts exhibit substantial anti-carcinogenic and antioxidant properties (Tomosaka *et al*., 2008; Özgen *et al*., 2012; Končić *et al*., 2010).

Various factors, including ecological conditions, genetic influences, and market demands, play a crucial role in determining the timing of fruit harvest. Harvesting fruits too early or too late can adversely impact their quality. Therefore, harvesting at the optimal time, in line with demand, is essential for achieving high yield and, most importantly, quality (Özcan, 2019). The harvest timing and methods also influence the quantity and quality of Berberis fruits (Moghaddam *et al*., 2013). Phenolic compound content in plants may vary according to genetic characteristics, environmental factors, harvest time, and storage conditions (Kırca *et al*., 2023).

This research concentrated on two main objectives: (1) analyzing the phenolic compound content in fruits of *B. vulgaris* genotypes harvested at various times, and (2) using multivariate analysis to uncover the relationships between the traits studied.

2. MATERIAL and METHODS

2.1. Plant Material

The study was carried out on large-fruited, highly attractive 3 *B. vulgaris* genotypes located in the Central Campus of Bolu Abant İzzet Baysal University (Türkiye) and whose pomological analyses were previously conducted (Bak *et al*., 2021). Genotypes were harvested at 4 different times in September, October, November, and December. At each harvest time, 20 cluster fruit samples were collected from each genotype. To determine the phenolic compounds in the genotypes examined, the juice was extracted from the fruit samples and placed in falcon tubes, then stored at -20°C until analysis.

2.2. Determination of Phenolic Compound

Phenolic compounds were determined according to the method of Aaby *et al*., (2007) modified by Pehluvan *et al*., (2015). For the extraction of phenolic compounds, 5 mL of previously squeezed fruit juice samples were taken and 10 mL of solvent (50% water and 50% acetonitrile) was added, mixed in a homogenizer and centrifuged at 15.000 rpm for 15 min.

Phenolic extracts were analyzed by Shimadzu CTO-20A HPLC. DGU-20A5 degasser system, LC-20AT model pump, and SPD-M20A model DAD (diode array detector) detector were used. KromoGL Sciences Inc. Inertsil ODS-3V, 5 µm, I.D/L: 4.6 x 250 mm column was used. The injection volume was 20 µL (microliter). Peaks between 273-370 nm were detected at a wavelength of 190-800 nm. The retention times of the standards were determined and then readings were taken by calibration. Chlorogenic acid, caffeic acid, rutin, *q*-coumaric acid, myricetin, *p*-coumaric acid, syringic acid, gallic acid, quercetin, and catechin standards were used in the study (Figure 1).

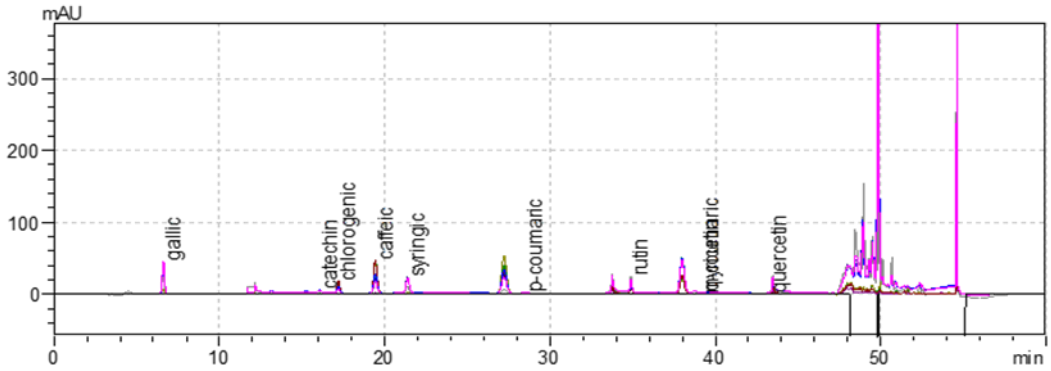

Figure 1. HPLC chromatograms of detected standard phenolic acids according to retention times, 1. Gallic, 2. Catechin, 3. Chlorogenic, 4. Caffeic, 5. Siringic, 6. *p*-coumaric, 7. Rutin, 8. *q*-coumaric, 9. Myricetin, 10. Quercetin

2.3. Statistical Analysis

To explore the relationships between the phenolic compound contents in fruit samples from different *B. vulgaris* genotypes harvested at various times, statistical analyses were conducted. Principal component analysis (PCA) was conducted utilizing JMP® Pro 17 (Copyright © 2022 SAS Institute Inc.). For hierarchical clustering heat maps and correlation analysis, R Studio 2024.04.1 (©2009-2024 RStudio, PBC) was employed to assess the phenolic compound contents in the genotype fruit samples. The analysis in R Studio utilized the open-source data visualization packages 'ggplot2' (Wickham, 2016) and 'corrplot' (Wei *et al*., 2017) (Kırca & Aygün, 2024).

3. FINDINGS

Gallic acid was found to be significantly different among genotypes. In addition, harvest time and Genotype*Harvest time interaction also had a significant effect on gallic acid (p<0.05) (Table 1). The gallic acid content was found to vary among genotypes depending on the harvest time. 'Genotype 1' stood out with the highest gallic acid content, which was recorded in Harvest 4 (H4). (50.24 mg kg-1). This was followed by H3 and H4 of 'Genotype 2' and H2 of 'Genotype 2' which were statistically in the same group (Table 1). Genotype, harvest time, and Genotype*Harvest time interactions were found to be significant in terms of catechin content (p<0.05). The highest amount of catechin was found in 'Genotype 1' and the lowest amount was found in 'Genotype 3'. Again, when Genotype*Harvest time interaction was analyzed, it was determined that there was variation between genotypes and harvest times and the amount of catechin was significantly higher in H4 of 'Genotype 1' (Table 1).

Differences were found between genotypes in terms of chlorogenic acid content (p<0.05) (Table 1). Harvest time was found to be effective on chlorogenic acid. Chlorogenic acid content varied at different harvest times. The highest chlorogenic acid content was recorded in H4 of 'Genotype 1'. It was also determined that Genotype*Harvest time interaction was effective on chlorogenic acid. Genotype, harvest time, and Genotype*Harvest time interaction were found to be effective on caffeic acid content (p<0.05) (Table 1). It was determined that harvest time showed variability among genotypes in terms of caffeic acid content. It was determined that H3 was the best harvest time in terms of caffeic acid content in 'Genotype 1'. In general, it was noted that caffeic acid content decreased at H4 in all genotypes.

Syringic acid content was found to be significant in terms of genotype, harvest time, and genotype*harvest time interaction (p<0.05) (Table 1). When the genotypes were analyzed in terms of syringic acid content, it was found that 'Genotype 1' had the highest syringic acid content. Again, in terms of harvest time, it was determined that H1 was more important than the other harvest times. It was determined that the highest amount of syringic acid was in the first harvest (H1) in 'Genotype 1' and differed significantly from other genotypes and harvest times.

Table 1. Effect of different harvest times on phenolic compounds of *Berberis vulgaris* fruits (mg kg^{-1}).

Genotype		Gallic	Catechin	Chlorogenic	Caffeic	Syringic	*p*-coumaric	Rutin	*q*-coumaric	Myricetin	Quercetin
Genotype 1		41.34±5.74a	1547.73±706.86a	8172.35±5308.80a	134.68±78.09a	76.96±64.93a	20.54±7.72a	37.68±27.28a	16.63±7.38a	2441.58±1059.99a	35.48±11.56a
Genotype 2		39.95±4.71b	902.87±340.90b	6072.05±3820.64b	111.04±29.29c	47.91±42.87b	9.92±0.47b	19.74±6.81b	10.57±2.31c	914.86±545.92c	36.01±11.42a
Genotype 3		38.65±3.51c	839.79±217.90c	6170.51±3779.15b	113.89±18.87b	48.75±44.09b	9.26±3.08b	20.12±6.39b	11.12±2.85b	1355.70±1365.81b	29.19±9.00b
Harvest time											
H1		35.44±1.03c	575.33±147.99d	73.09±14.35c	149.84±10.12a	138.00±30.77a	14.65±4.10ab	17.54±3.58c	12.31±0.58b	2993.90±945.77a	40.55±8.80a
H2		39.30±3.35b	1094.43±172.42c	8721.01±842.52b	113.66±14.20c	17.98±2.16d	8.73±0.97c	16.34±2.69c	9.54±2.62c	806.00±406.23d	26.70±12.80b
H3		40.10±3.14b	1187.71±421.40b	8685.16±1155.63b	148.34±64.31b	31.64±6.26c	14.26±9.16b	22.80±7.97b	12.44±4.15b	973.68±576.24c	27.15±4.89b
H4		45.08±4.64a	1529.73±793.42a	9740.60±2994.39a	67.62±30.93d	43.88±20.17b	15.32±9.51a	46.72±26.31a	16.79±8.62a	1509.28±1271.84b	39.81±7.30a
Genotype × Harvest Interaction											
Genotype 1	H1	36.69±0.49de	722.03±74.38h	80.20±1.17ı	157.94±0.98c	177.66±1.06a	19.43±0.11b	21.47±1.60d	12.47±0.19d	3660.11±21.03a	43.50±0.08b
	H2	38.42±0.00cd	1242.87±0.06c	9043.74±0.48d	121.43±0.86f	20.61±0.50h	9.30±0.03de	16.09±3.03e	9.31±0.29f	1255.79±14.46f	20.39±0.77e
	H3	39.99±0.26c	1706.01±3.83b	10004.35±15.35b	231.08±3.33a	39.68±1.99e	25.89±0.30a	32.51±0.06b	17.18±0.39b	1704.14±33.02e	30.43±0.39d
	H4	50.24±1.54a	2520.02±7.55a	13561.10±56.86a	28.25±0.63l	69.90±0.45d	27.54±0.10a	80.66±0.19a	27.55±0.04a	3146.29±2.25c	47.63±1.87a
Genotype 2	H1	35.04±0.96ef	402.74±6.30j	56.88±16.08ı	154.49±0.16c	116.68±0.08c	10.30±0.01d	14.37±0.05ef	12.80±0.20d	1774.91±11.05d	48.56±0.46a
	H2	36.19±0.12ef	877.85±37.10f	9454.23±179.22c	95.41±0.75ı	15.90±0.05ı	9.40±0.03de	19.46±0.13d	12.58±0.05d	814.56±5.43g	43.05±0.10b
	H3	43.43±2.42b	1070.45±29.39e	7422.18±15.59gh	112.68±2.69g	28.29±0.30g	9.73±2.69de	15.07±1.03ef	7.92±0.45g	487.65±28.56j	20.88±1.27e
	H4	45.13±0.13b	1260.44±6.56c	7354.92±3.02h	81.59±0.27k	30.77±0.09f	10.25±0.07d	30.07±0.36bc	8.96±0.07f	582.34±10.28ı	31.57±0.06d
Genotype 3	H1	34.58±0.38f	601.21±23.39ı	82.20±0.43ı	137.10±1.49d	119.66±0.41b	14.21±0.05c	16.79±0.42e	11.66±0.31e	3546.68±15.98b	29.58±0.14d
	H2	43.29±0.40b	1162.57±29.11d	7665.06±78.89g	124.16±0.14e	17.42±1.52ı	7.49±0.30f	13.47±1.25f	6.73±0.44h	347.64±33.32k	16.67±0.07f
	H3	36.86±0.05de	786.66±4.29g	8628.96±29.55e	101.25±0.39h	26.95±0.21g	7.16±0.64f	20.82±0.29d	12.23±0.22de	729.26±10.22h	30.13±0.18d
	H4	39.88±0.39c	808.73±25.00g	8305.79±363.50f	93.04±0.93j	30.97±0.45f	8.18±0.24ef	29.41±0.97c	13.85±0.19c	799.22±14.09g	40.22±2.79c
ANOVA											
$F_{genotype}$		16.71***	2068.72***	750.24***	1255.63***	3053.30***	488.30***	594.54***	1221.30***	21338.88***	100.24***
$F_{harvest}$		109.52***	1575.57***	8163.85***	8441.12***	24827.73***	84.31***	854.60***	730.62***	25667.14***	302.29***
$F_{genotype*harvest}$		34.71***	513.14***	360.61***	3817.14***	605.25***	79.08***	257.07***	709.81***	3717.58***	192.48***

In terms of genotype, harvest time, and genotype*harvest time interaction, *p*-coumaric acid was found to be statistically significant ($p<0.05$) (Table 1). 'Genotype 1' was found to have higher *p*-coumaric content compared to other genotypes. In addition, it was noted that 'Genotype 1' showed a significant increase in *p*-coumaric content at H3 and H4 times compared to other genotypes.

In terms of rutin content, 'Genotype 1' was found to have higher rutin content than the other genotypes (Table 1). In terms of harvest time, H4 was found to be more prominent in terms of rutin content compared to other harvest times. Rutin content was found to be statistically significant in terms of genotype, harvest time, and Genotype*Harvest time interaction. Genotype*harvest time interaction showed variation. The highest rutin content was found in 'Genotype 1' at H4 time.

Significant differences were found between genotypes in terms of *q*-coumaric acid content ($p<0.05$) (Table 1). The highest *q*-coumaric acid content was found in 'Genotype 1'. In terms of harvest time, it was determined that H4 was more effective on *q*-coumaric acid content compared to other harvest times. In addition, *q*-coumaric acid content was significant in terms of genotype, harvest time, and Genotype*Harvest time interaction. The highest *q*-coumaric acid content was found in 'Genotype 1' at H4 time.

Significant differences were found between genotypes in terms of myricetin acid content ($p<0.05$) (Table 1). The highest myricetin content was found in 'Genotype 1' and the lowest in 'Geontype 2'. It was found that H1 was more prominent on myricetin content compared to other harvest times. It was also found that myricetin content decreased significantly in H2 among the genotypes. In all genotypes, myricetin content was higher in H1. The highest myricetin content was detected in 'Genotype 1' at H1.

Quercetin content was found to be significant in terms of genotype, harvest time, and genotype*harvest time interaction ($p<0.05$) (Table 1). Among the genotypes, 'Genotype 1' and 'Genotype 2' in the same group were found to be significant in terms of quercetin content. In terms of harvest time, it was determined that H1 and H2 had the same effect on quercetin content. The highest quercetin content was found in 'Genotype 1' at H4 and in 'Genotype 2' at H1.

The relationships between harvest time and phenolic compounds examined in principal components (PCA) biplot analysis are shown in Figure 2. Principal component 1 (PC1) was 45.4%, principal component 2 (PC2) was 32.9% and 78.3% in total. Accordingly, caffeic acid and syringic acid were positively correlated with each other and negatively correlated with other properties (gallic, catechin, chlorogenic, *p*-coumaric, rutin, *q*-coumaric, myricetin, quercetin). 'Harvest 1' was found to have the highest levels of caffeic and syringic acid, while 'Harvest 4' stood out for its catechin, gallic acid, and chlorogenic acid content. It was also noted that 'Genotype 1' stood out in terms of myricetin, quercetin, *p*-coumaric, *q*-coumaric, and rutin content.

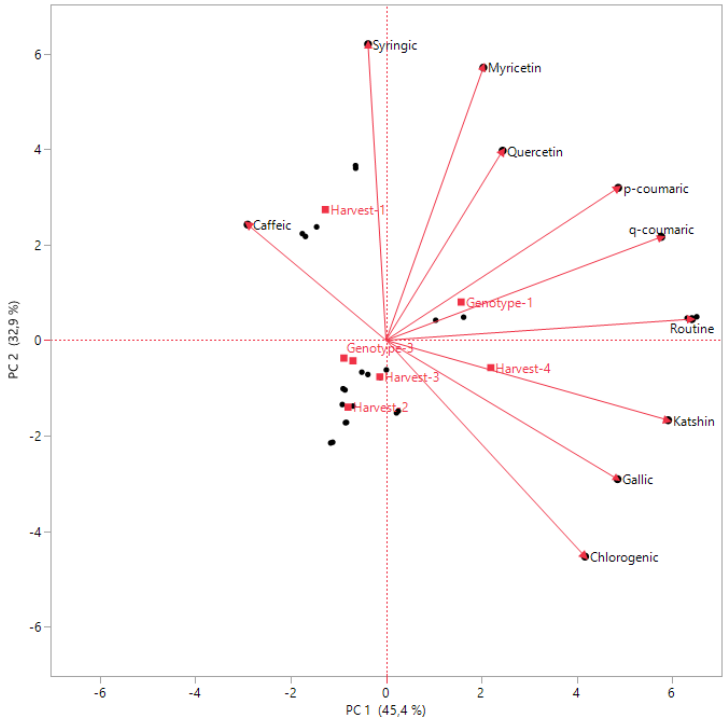

Figure 2. Principal component analysis (PCA) for phenolic compounds analyzed in genotypes fruit samples.

The correlation analysis of phenolic compounds among the genotypes is illustrated in Figure 3. The analysis revealed a strong positive correlation between gallic acid and both catechin and rutin, a moderate positive correlation with chlorogenic acid, and a moderate negative correlation with caffeic acid. As the concentration of gallic acid increases, the levels of catechin, chlorogenic acid, and rutin also rise, while the level of caffeic acid decreases. Upon examining the figure, a strong positive correlation was observed between catechin and both chlorogenic acid and rutin, along with a moderate positive correlation with *p*-coumaric and *q*-coumaric acids. Similarly, chlorogenic acid exhibited a weak negative correlation with caffeic and myricetin acid content, and a strong negative correlation with syringic acid. A robust positive correlation was identified between syringic acid and myricetin. Additionally, there was a notable positive correlation between *p*-coumaric acid and both rutin and *q*-coumaric acid. Furthermore, an exceptionally strong positive correlation was observed between rutin and *q*-coumaric acid.

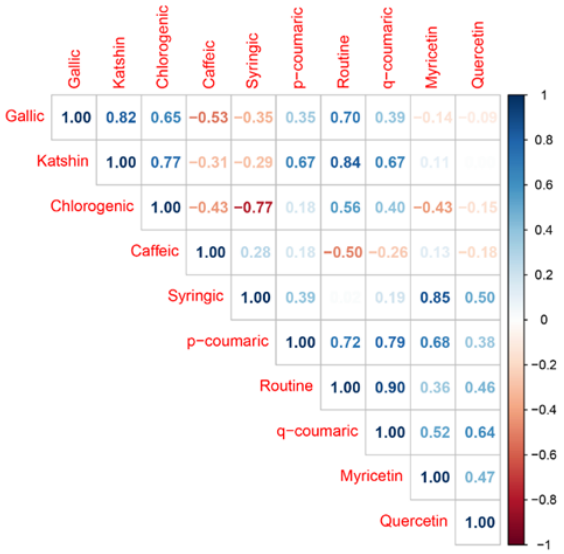

Figure 3. Correlation between phenolic compound contents analyzed in fruit samples of genotypes ($p \leq 0.05$).

The results of 'hierarchical clustering and heat map analysis' between genotypes and harvest times and phenolic compounds are given in Figure 4. When the dendrogram obtained is examined, it is seen that genotype and harvest time are divided into 2 clusters (A and B). It was found that only G1 and H4 were included in main cluster B. It is seen that the main cluster A is divided into two sub-clusters A1 and A2, which are divided into many sub-clusters within themselves. It was determined that G1 and H1, G3 and H1, and G2 and H1 were collected in sub-cluster A1, while the other genotypes and harvest times were found in sub-cluster A2. When the figure was analyzed in terms of phenolic compounds, it was determined that the dendrogram was divided into two main clusters, X and Y. Of these, X was subdivided into X1 and X2, and Y was subdivided into Y1 and Y2. It was determined that gallic acid, catechin, and chlorogenic acid were included in subcluster X1, and *p*-coumaric, rutin, and *q*-coumaric acid were included in subcluster X2. Similarly, only caffeic acid was found to be in subcluster Y1, while the other phenolic acids (syringic, myricetin, and quercetin acid) were found to be in subcluster Y2. When the heat map was analyzed, G1 and H4 were found to be prominent in terms of gallic, catechin, chlorogenic, *p*-coumaric, rutin, *q*-coumaric, myricetin and quercetin acid content; G1 and H3 in terms of *p*-coumaric and caffeic acid; G2 and H1 in terms of quercetin; G3 and H1 in terms of myricetin; and G1 and H1 in terms of syringic, myricetin and quercetin acid content.

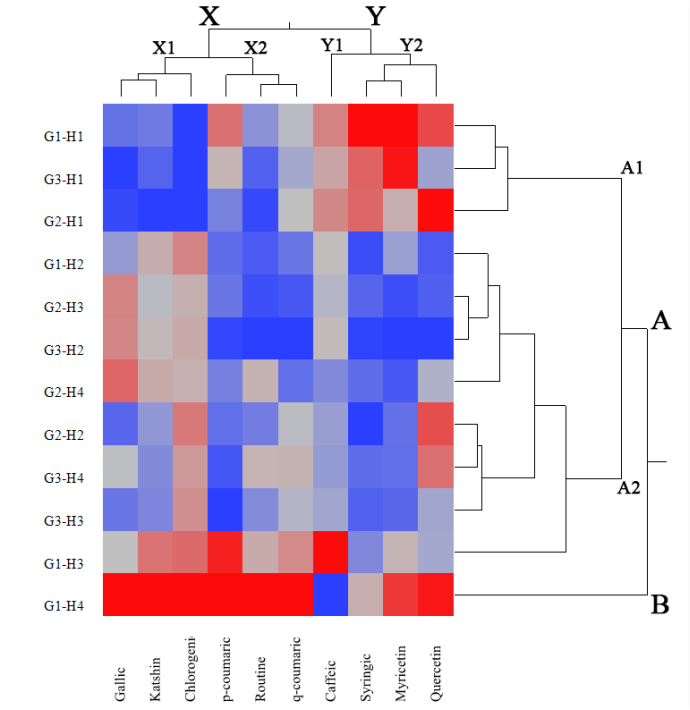

Figure 4. Heat map obtained as a result of hierarchical cluster analysis between harvest time and phenolic compounds analyzed. In the heat scale, colors shifting to red indicate an increase, and colors shifting to blue indicate a decrease.

4. DISCUSSION and CONCLUSION

The high antioxidant capacity of *B. vulgaris* plant extracts can be attributed to the abundance of active phenolic compounds within them. Likewise, the antibacterial effects observed in berberis fruit extracts are also ascribed to these biologically active phenolic constituents, which play a crucial role in their efficacy (Yang *et al*., 2022).

Gallic acid (GA), belonging to the hydroxybenzoic acid group, is part of a vast family of secondary metabolites that are extensively found across the plant kingdom. It plays a significant role in preventing oxidative damage (Embuscado, 2015) and functions as a natural antioxidant (Lu *et al*., 2006). Previous studies have reported that gallic acid is the most abundant phytochemical compound in *B. vulgaris* fruit extracts (Gholizadeh-Moghadam *et al*., 2019; Yang *et al*., 2022; Outaki *et al*., 2023). In studies, the highest amount of gallic acid in *B. vulgaris*

fruit samples was found as 132 mg kg^{-1} (Gundogdu, 2013); 334.82 mg kg^{-1} (Gholizadeh-Moghadam *et al*., 2019); 182 mg kg^{-1} (Yang *et al*., 2022); 330.407 mg kg^{-1} (Eroğlu *et al*., 2020); 1037.994 mg kg^{-1} (Çakır & Karabulut, 2020); 132 mg kg^{-1} (Sayın & Balcı, 2022). In our study, the highest amount of gallic acid was 41.34 mg kg^{-1} in G1 and the best harvest time was 45.08 mg kg^{-1} in H4. In genotype*harvest interaction, G1*H4 (50.24 mg kg^{-1}) had the best effect.

Catechin, a secondary metabolite, is a potent source of antioxidants (Akbulut *et al*., 2009; Arakawa, 2004). In a comprehensive study carried out across three distinct states, catechin emerged as the most prevalent phenolic compound in *B. vulgaris* fruits, with a concentration of 640 mg kg^{-1}, consistently observed in all three locations (Yang *et al*., 2022). Gundogdu (2013) determined catechin as the second-highest phenological compound in *B. vulgaris* fruits (218 mg kg^{-1}). In our study, catechin was the third-highest phenolic compound. The highest amount of catechin 1529.73 mg kg^{-1} was obtained from the interaction of G1 and H4. Our results were consistent with the predominance of catechin in other studies.

Chlorogenic acid, one of the important phenolic compounds, is found in many fruits and vegetables. Chlorogenic acid, along with its related hydrolysates, functions as an antioxidant, contributing to the neutralization of free radicals and the reduction of oxidative stress (Zuo *et al*., 2015; Chiang *et al*., 2015). The plant content of chlorogenic acid has some pharmacological properties and is used as a medicinal drug (Zeiger, 1998). In many studies, it has been reported that chlorogenic acid is the main phenolic compound in *B. vulgaris* fruits (Gundogdu, 2013; Eroğlu *et al*., 2020; Sayın & Balcı, 2022; Yang *et al*., 2022). The highest chlorogenic acid concentrations obtained were 752 mg kg^{-1} (Gundogdu, 2013); 1990.482 mg kg^{-1} (Eroğlu *et al*., 2020); 624 mg kg^{-1} (Yang *et al*., 2022); 1017.320 mg kg^{-1} (Çakır & Karabulut, 2020); 45.98 mg kg^{-1} (Sayın & Balcı, 2022). Similarly, Tan *et al*., (2018) determined that polyphenols, flavonoids, and alkaloids were present in berberis edible fruits and the amount of chlorogenic acid was 47.1 mg kg^{-1}. As presented in Table 1, chlorogenic acid emerged as the predominant phenolic compound in the fruit samples of the genotypes analyzed in our study. Overall, the greatest concentrations of chlorogenic acid were observed in the 'G1' and 'H4' genotypes, with the most significant interaction recorded at 13,561.10 mg kg^{-1} in the G1*H4 combination. The findings of our study were in parallel with the findings of other studies. Chlorogenic acid was the dominant phenolic compound, and the highest amount was obtained from our study.

Caffeic acid, a crucial intermediate in the biosynthesis of lignin, is found in all plants. This hydroxycinnamic acid derivative and polyphenol is orally bioavailable and possesses potential antioxidant, anti-inflammatory, and antineoplastic properties. Additionally, it plays a role in preventing DNA damage induced by free radicals by mitigating oxidative stress (Espíndola *et al*., 2019). Caffeic acid in berberis fruits, which has an important potential in terms of phytochemical plants, was determined as 51.78 mg kg^{-1} by Gholizadeh-Moghadam et al. (2019); 152.225 mg kg^{-1} by Eroğlu et al. (2020) and 904.432 mg kg^{-1} by Çakır and Karabulut (2020). In our study, the highest caffeic content was observed in G1 and H1. The best genotype*harvest interaction was 157.94 mg kg^{-1} in G1xH1.

Syringic acid, one of the phenolic compounds, is an important source found in plants. Eroğlu *et al*., (2020) emphasized that syringic acid was the second most abundant phenolic compound (867.850 mg kg^{-1}) in *B. vulgaris* fruit. Gundogdu (2013) stated that less amount of syringic acid (32 mg kg^{-1}) was observed. Çakır and Karabulut (2020) reported it as 957.690 mg kg^{-1}. In our study, the highest amount of syringic acid was obtained from G1 and H1. The highest result was obtained from G1*H1 interaction (177.66 mg kg^{-1}).

In a study on the characterization and investigation of antioxidant activity, a significant amount of *p*-coumaric acid content was observed in *B. vulgaris* (Outaki *et al*., 2023). Çakır and Karabulut (2020) reported it as 938.299 mg kg^{-1}. Although a similar result was emphasized by Gholizadeh-Moghadam *et al*., (2019), Eroğlu *et al*., (2020) did not find the presence of *p*-coumaric acid in the fruit extracts examined. In our study, the amount of *p*-coumaric acid was found in G1 and H4, and the best interaction was found as 27.54 mg kg^{-1} from G1*H4.

In Berberis fruits, rutin was determined as 0.02 mg kg^{-1} by Tan *et al*., (2018); 7.61 mg kg^{-1} by Gholizadeh-Moghadam *et al*., (2019); and 81 mg kg^{-1} by Gundogdu (2013). In our study, the highest amount of rutin was found in G1 and H4, and the best interaction was found as 80.66 mg kg^{-1} in G1*H4. In our research, we identified a positive correlation between the compounds rutin and *q*-coumaric acid. The amounts of *q*-coumaric were also found in G1 and H4, and the best interaction was 27.55 mg kg^{-1} in G1*H4.

Yang *et al*., (2022) reported the amount of myricetin as 640 mg kg^{-1} in their study. In our study, the highest amount of myricetin was detected after chlorogenic acid among the genotypes. Myricetin amounts were also found in G1 and H1, and the best interaction was 3660.11 mg kg^{-1} in G1*H1.

Gundogdu (2013) reported quercetin content as 11 mg kg^{-1}; Gholizadeh-Moghadam *et al*., (2019) found it to be 37.20 mg kg^{-1}. In our study, the amount of quercetin was found in G2 and H1, and the best interaction was 48.56 mg kg^{-1} in G2*H1. The findings of our study align with those reported in prior research, demonstrating consistency with previously established results.

In our study, after chlorogenic acid, myricetin and catechin were the most prevalent compounds detected among the genotypes. These were followed by caffeic acid, rutin, syringic acid, gallic acid, quercetin, *p*-coumaric acid, and *q*-coumaric acid. Previous studies have shown variability in the levels of phenolic acids (such as caffeic acid, chlorogenic acid, *p*-coumaric acid, cinnamic acid, and gallic acid) and flavonoids (including rutin, apigenin, and quercetin) (Gholizadeh-Moghadam *et al*., 2019). The differences observed in phenolic compound content and flavonoid concentrations among plant species can be ascribed to various factors, including genetic composition, environmental conditions (such as soil quality, temperature, fertilization, light, and moisture), and the conditions under which the plants are harvested and stored (Tomosaka *et al*., 2008; Awan *et al*., 2014; Embuscado *et al*., 2015; Gholizadeh-Moghadam *et al*., 2019).

Studies suggest that *B. vulgaris* is abundant in a diverse array of phytochemicals, such as ascorbic acid, vitamin K, numerous triterpenoids, more than ten phenolic compounds, and over thirty alkaloids (Rahimi-Madiseh *et al*., 2017). Consequently, *B. vulgaris* is noted for its beneficial properties, such as analgesic, anticancer, antimicrobial, anti-inflammatory, antifungal, antidiabetic, antioxidant, antibacterial, anti-nociceptive, and hepatoprotective effects (Mokhber-Dezfuli *et al*., 2014; BenSaad *et al*., 2017; Rahimi-Madiseh *et al*., 2017). Given the traditional medicinal use of various parts of *B. vulgaris* and their validated effects in recent studies, it is evident that different parts of the plant, particularly the fruits, have become valuable sources for developing new therapeutics due to the structural diversity of their active compounds (Tomosaka *et al*., 2008; Rahimi-Madiseh *et al*., 2017; Yang *et al*., 2022). As the global popularity of *B. vulgaris* grows, evidence suggests that this plant serves as an abundant reservoir of compounds beneficial for both medicine and human nutrition, and its significance is expected to increase over time (Yang *et al*., 2022).

When the findings were examined in terms of harvest time, the highest amount of chlorogenic acid, catechin, routine, gallic, quercetin, *q*-coumaric, and *p*-coumaric was in the G1*H4 interaction; Myricetin, syringic and caffeic acid were found in G1*H1 interaction. Moghaddam *et al*. (2013) found that factors such as cluster weight, fruit weight, and size peaked in October, while fresh and dried fruit yield, anthocyanin content, Brix, and maturity index reached their highest levels in November. Fallahi *et al*. (2010) investigated the impact of various harvest dates (9 Sep., 1 Oct., 22 Oct., and 12 Nov.) on *B. vulgaris* and concluded that mid-November was the optimal harvest time, enhancing both fruit quality and yield. Bak *et al*. (2021) suggested that October is the suitable harvest period for *B. vulgaris*, cautioning that relying solely on color characteristics to determine harvest time could be misleading due to the plant's extended harvest period and ability to retain color. Saeidirad and Mazloumzadeh (2012) observed that the coloration of berberis fruits was lowest in mid-October and highest in mid-November, with sensory tests indicating the best quality from the second harvest. Another study by Javadzadeh (2013) comparing different harvest dates (7 Oct., 22 Oct., and 7 Nov.) and methods of collection

and drying found that fruit length, fresh and dry weights of 100 fruits, and fresh and dried fruit yield were lowest in the first harvest and highest in the last. Moghaddam *et al*. (2013) also highlighted that picking and sun-drying berberis fruits during cooler hours of the day can enhance quality indices. Our findings align with these previous studies, which reported varying levels of phenolic content in *B. vulgaris* fruits.

Recent studies have proposed that the phytochemical components of fruits may be influenced by the geographical conditions or the specific growing environments in which the fruits are cultivated (Mariod *et al*., 2010). As seen in studies, delaying the harvest date increased the amount of anthocyanins in the fruit. The reason for this observation is that carbon is allocated to biomass during the initial stage of fruit development; which is the production of secondary metabolites in the final stage of fruit growth. That is, late in growth, large amounts of carbon are not needed for primary metabolism; therefore, secondary compounds are synthesized more actively (Arena & Curvetto, 2008). Therefore, it is important to harvest the fruits at later dates rather than harvesting them immediately when they begin to ripen, in terms of phenolic substances. This may explain why 'Harvest 4' (December) stands out in most of the phenolic compounds analyzed.

The antioxidant activity is primarily attributed to the presence of potent antioxidant compounds. This situation is related to the active phenolic substance they contain. Overall, our findings highlighted the abundant phenolic content present in *B. vulgaris* fruits. It was determined that harvest time significantly affected the amount of phenolic content in fruit samples of *B. vulgaris* genotypes harvested at different times. As a result, as the harvest time was delayed, there were significant increases in gallic, catechin, chlorogenic, routine, and *q*-coumaric acid contents, while notable decreases occurred in the amount of caffeic and syringic acid. Similarly, it was determined that there were fluctuations in the amounts of *p*-coumaric acid, myricetin, and quercetin depending on the harvest time. The findings of this study showed the importance of harvest time in terms of phenolic acid amount and that the most suitable harvest time is H4 (December) in terms of gallic, catechin, chlorogenic, *p*-coumaric, routine, and *q*-coumaric.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

TB, LK, BDÇ, TK: Research, Methodology, Writing, Review and Editing**. BDÇ, LK, TB:** Preparing the samples and performing the experiments. LK: Statistical analysis, Data curation, Data visualization and interpretation.

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