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Phytochemical Properties of Some Blackberry Genotypes Grown in Van Ecology

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Abstract In this study, biochemical compositions of three different blackberry genotypes found in the ecology of Bahçesaray district of Van province were identified. Five different organic acids and eight different phenolic compounds were identified in these genotypes. In the fruits, most common phenolic compound was rutin (4.490 mg/g) and most common organic acid was citric acid (1.132 g/100 g). Citric acid was followed by malic, succinic, fumaric and oxalic acid. The 65BS02 blackberry genotype was found to be rich in vanillin, rutin, gallic, chlorogenic, kaffeic and p-coumaric acid. In conclusion, it was seen that the components varied depending on the genotype. In addition, the 65BS02 genotype emerged as a valuable genotype due to its superior characteristics.

Keywords: Blackberry, phenolic compounds, organic acids

Van Ekolojisinde Yetiştirilen Bazı Böğürtlen Genotiplerinin Fitokimyasal Özellikleri

Öz: Bu çalışmada, Van’ın Bahçesaray ekolojisindeki üç farklı böğürtlen genotipinin biyokimyasal bileşikleri tespit edilmiştir. Genotiplerde 5 farklı organik asit ve 8 farklı fenolik bileşik belirlenmiştir. Meyvelerde, fenolik bileşiklerden en fazla rutin (4.490 mg/g), organik asitlerden ise en fazla sitrik (1.132 g/100g) asit ön plana çıkmıştır. Sitrik asidi malik, süksinik, fumarik ve okzalik asit takip etmiştir. 65BS02 böğürtlen genotipi vanilik, rutin, gallik, klorojenik, kaffeik ve p-kumarik asit yönünden zengin bulunmuştur. Sonuç olarak genotipe bağlı olarak bileşenlerin değişkenlik gösterdiği görülmüştür. Ayrıca 65BS02 genotipi üstün özellikleri ile değer kazanan genotip olarak ortaya çıkmıştır.

Anahtar kelimeler: Böğürtlen, fenolik bileşikler, organik asitler

1. Introduction

Blackberry (*Rubus spp*.) is part of the *Rubus* species within the *Rosaceae* family of the *Rosales* order. Blackberries are rich in vitamins A, B and C, and potassium, and more than 350 species of indigenous blackberries have been identified (Crandall, 1995).

Different opinions have been proposed by many researchers on the spread of blackberries on the earth. According to some sources, the origin of this fruit is South, West and Central

Europe (Gerçekçioğlu, 1999). It is also known that almost all of the cultivars are of North American origin (Ağaoğlu, 1986). According to some sources, however, the origin of blackberries is reported as Western India and Pakistan, and it is said to spread from here to Western Europe and North Africa, aneventually to North America (Anonymous, 2005). Turkey is an important country in terms of plant diversity and it is one of the leading regions in the world. Many of the fruit species that naturally grow on Earth continue to exist on these lands (Artık and Eksi, 1988). Blackberry, which easily adapts to different climatic conditions and is not selective in terms of soil characteristics, is a plant species that grows easily in Turkey. Almost all of the wild forms of blackberry grow in Turkey. However, it is known to be concentrated in Central Anatolia and Black Sea regions (Gerçekçioğlu, 1996).

The importance of blackberry, one of the berry fruits, is increasing in Turkey. Blackberry breeding around the world began 150 years ago, with the first blackberry breeding program in Texas (Moore, 1984). In 1964, the blackberry breeding program conducted at the University of Arkansas patented 10 of 13 species and all these species spread to the world. In this breeding program, the researchers worked on varieties that were thornless, had high fruit quality characteristics and yielded "primocane" fruits (Clark, 1999). Some commercially grown blackberries (*Rubus sp*. L.) were obtained from *Rubus* species in North America. Within this species are thorned, thornless, creeping, erect and semi-erect blackberry varieties (Poling, 1997).

Besides their pleasant taste and flavor, fruits are very beneficial to human health by preventing diseases with the vitamins and minerals they contain. Blackberry fruit also contains these functional ingredients which are effective in protecting health against many diseases. This is why blackberry is highly used in food and medicine industry (Berk and Tuna, 2017). Just like its fruit, blackberry leaves are also very important for health and have strong antioxidant properties due to the flavanoids and phenolic acids they contain. They are one of the major ingredients of herbal mixtures used in the treatment of vascular stiffness, hypertension, and neural diseases (Melkadze et al., 2008). It is also known that blackberry extract has a positive effect when it is given to obese patients. It reduces oxidative stress in direct proportion to the amount of antioxidants it contains, and accelerates fat burning in obese people with exercise (Niloofari et al., 2014). In addition, this extract has significant neurodegenerative activity due to its high antioxidant content and also prevents diseases such as Alzheimer and Parkinson (Tavares et al., 2012). Moreover, it is known that fruit and leaves of blackberries show antiviral effects. Depending on the concentration, it destroys virus populations by up to 99%. Because of this feature, it has been used since the 16th century in the treatment of intraoral wounds (Danaher et al., 2011).

Phenolic compounds have anticarcinogenic, antiviral, antimicrobial, antimutagenic and antioxidant properties. It is therefore very important that phenolic compounds are obtained from natural sources by extraction. Extraction of phenolic compounds from natural products is associated with many parameters (Juntachote et al., 2006).

Eastern Anatolia is a region with terrestrial clime and there are many fruit species growing naturally in the region. Therefore, the presence of many blackberry genotypes is known in the region. This study aimed to determine the phytochemical properties of blackberry genotypes found in Bahçesaray district of Van province.

2. Materials and Methods

In this study, organic acid and phenolic compound contents of the fruits of blackberry genotypes grown in Bahçesaray (Van) district were determined. Bahçesaray district has area of 576 km² and 1670 meters above sea level. It is 110 km away the center of Van province. Bahçesaray is surrounded by steep high mountains and hills. The region has microclimate property and fruit species such as walnut, apple, pear, grape and apricot are grown. The fruits of the blackberry genotypes examined in this study were harvested during ripening period. The fruits were then transported to the laboratory for necessary analysis and stored at -80 °C.

Phenolic compounds extraction and determination

The HPLC separation method for phenolic compounds determination was employed following the procedure described by Rodriguez-Delgado et al. (2001). Samples of about 100 g were minced and 5 g from each sample was transferred to centrifuge tubes. The specimen was mixed uniformly and diluted with distilled water (1:1) and sedimented at 15000 × g for 15 min. The supernatant was filtered by 0.45 µm membrane filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA), then filtrate was injected into HPLC system (gradient). The separation of chromatographic data in Agilent 1100 series HPLC took place in DAD detector (Agilent, USA) with 250 mm × 4.6 mm, 4 µm ODS column (HiChrom, USA). The solvents as mobile phase solvent A, methanol–acetic acid–water (10:2:88) and Solvent B (Table 1), methanol–acetic acid–water (90:2:8) in water with a flow rate of 1 mL min^{-1} and 20 µL injection volume were used for spectral determination at 254, and 280 nm.

Table 1. Vanillic, rutin, gallic and catechin contents of three blackberry genotype fruits (mg/g).
Çizelge 1. *Üç böğürtlen genotipinin vanilik, rutin, gallik ve kateşin içerikleri (mg/g).*

Genotypes	Vanillic	Rutin	Gallic	Catechin
65BS01	0.035 ± 0.001 b*	2.505 ± 0.015 b	0.535 ± 0.009 b	0.064 ±0.002 b
65BS02	0.068 ± 0.003 a	4.490 ± 0.025 a	0.915 ± 0.011 a	0.011 ± 0.000 c
65BS03	0.036 ± 0.001 b	1.211 ± 0.013 c	0.215 ± 0.003 c	0.087 ± 0.005 a

*: The difference between the means indicated by the same letter in the same column is insignificant ($P < 0.05$).

Organic Acids extraction and determination

For organic acids extraction, the procedure described by Bevilacqua and Califano (1989) was followed with modification. The sample of 200 g was fragmented and 10 g from each sample was transferred to centrifuge tubes. The samples were mixed with 10 mL of 0.009 N H_2SO_4 and then samples were homogenized with Heidolph Silent Crusher M, Germany. Thereafter, the samples were mixed by gentle shaking for an hour (Heidolph Unimax 1010, Germany) and centrifuged at 14.000 × rpm for 15 min. The supernatant was filtered by coarse filter paper, then twice through 0.45 µm membrane filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA), and finally in the SEP-PAK C18 cartridge. The content of organic acids was determined by HPLC using an Aminex column (HPX-87H, 300 mm × 7.8 mm, Bio-Rad) fitted on an Agilent 1100 series HPLC G 1322 A, Germany) (Bevilacqua and Califano, 1989). Organic acids were determined at 214 and 280 nm wavelengths. As the mobile phase, 0.009 N H_2SO_4 was used.

Statistical analysis

The data of three replications were analysed by SPSS (ver. 20) according to one-way ANOVA based on completely randomized design. Mean comparisons were carried out by Duncan's multiple range test at $P \leq 0.05$ probability level.

3. Results and Discussion

Phenolic compounds and organic acid levels were determined in blackberry fruits of 3 different genotypes and these values were presented in Tables 1 and 2. Significant differences were found between the groups ($P < 0.05$).

When the phenolic compound contents of the fruits were examined, a genotype-dependent variability was found. The 65BS02 blackberry genotype stood out with 6 different phenolic substances (vanillic, rutin, gallic, chlorogenic, kaffeic, p-kumaric). Rutin and chlorogenic acid were the most frequent phenolic compounds, whereas p-coumaric acid and catechin were the less frequent. It was determined that 65BS02 genotype had the highest rutin and chlorogenic content among the genotypes, and these values were measured as 4.490 mg/g and 3.505mg/g, respectively. The lowest rutin content was found in 65BS03 with 1.211 mg/g. It was also found that this genotype had the lowest p-coumaric acid. The lowest catechin content was found in 65BS02 genotype (Tables 1 and 2). Sellappan et al. (2002) reported that the level of p-coumaric acid varied between 0.4 and 2.08 mg/100 g. In addition, researchers obtained the highest catechin value as 312.86 mg/100g in blackberry fruits. In present study, catechin values were higher and p-coumaric acid were lower than the findings of Sellappan et al. (2002). Another study in Croatia found that the rutin level was 16 mg/kg (Jakobek et al., 2009) whereas a study in US found it as 17.8 mg/100g (Siriwoharn and Wrolstad, 2004). In a study on blackberry fruits, Gündoğdu et al. (2016) found that the amount of chlorogenic acid was 1.175 mg/100 g in the Bursa 3 variety and 1.165 mg/100 g in Bursa 2 variety. The findings in these studies are lower compared to ours. The different results were attributed to the fact that studies were carried out in different regions and on different varieties. However, in accordance with our study, Türkben et al. (2010) reported the highest p-coumaric acid level as 877.45 mg/kg in the Chester variety. In addition, p-coumaric acid level in Bursa 1 variety was reported as 287.15 mg/kg. Differences in vanillic and gallic acid values were obtained in our study between the genotypes. Vanillic acid was found to vary between 0.035 mg/g (65BS01) and 0.068 mg/g (65BSO2). In addition, highest gallic acid content was measured as 0.915 mg/g (65BS02) (Table 1). Some researchers investigated phenolic compounds in blackberry fruit and obtained a gallic acid level of 89.0 mg/kg. However, the researchers reported vanillic acid level as 45.1 mg/kg (Zadernowski et al., 2005).

Table 2 shows that the level of ferulic and caffeic acid changes depending on the genotype. 65BS02 genotype was identified as the genotype with the highest amount of caffeic acid (0.890 mg/g). The lowest amount of caffeic acid was obtained in 65BS03 genotype. Moreover, this genotype also contained the highest amount of ferulic acid (0.030 mg/g) (Table 2). Zadernowski et al. (2005) found that the amount of caffeic acid in blackberry fruits was 105.1 mg/kg and that of ferulic acid was 55.3 mg/kg. The amount of caffeic acid detected in our study was higher compared to that reported by Zadernowski et al. (2005). In another study conducted in Bursa, the amount of ferulic acid was found to be between 413.82 and 757.69 mg/kg. In addition, the researchers stated that the amount of caffeic acid was between 377.89 and 736.85 mg/kg (Türkben et al., 2010). While our findings were consistent with Türkben et al. (2010) in terms of cafeic acid content, the ferulic acid values in both studies were higher than our findings.

Table 2. Chlorogenic, caffeic, *p*-coumaric and ferulic contents of three blackberry genotype fruits (mg/g)

Çizelge 2. *Üç böğürtlen genotipinin klorogenik, kafeik, p-kumarik ve ferulik içerikleri (mg/g)*

Genotypes	Chlorogenic	Caffeic	*p*-coumaric	Ferulic
65BS01	2.580 ± 0.012 b*	0.665 ± 0.023 b	0.265 ± 0.006 b	0.012 ± 0.001 c
65BS02	3.505 ± 0.021 a	0.890 ± 0.019 a	0.905 ± 0.026 a	0.017 ± 0.002 b
65BS03	2.670 ± 0.016 b	0.545 ± 0.012 c	0.105 ± 0.006 c	0.030 ± 0.001 a

*: The difference between the means indicated by the same letter in the same column is insignificant ($P < 0.05$).

When we compared our findings with previous studies, we observed differences in phenolic acid levels. Our findings were higher than some studies, and lower than some other studies. The difference was thought to be due to the fact that the studies were conducted in different countries or different regions of the same country. Therefore, it can be said that ecological factors, effects of genotype differences and climatic factors affect phenolic acid levels and cause this variability. In addition, the differences in the methods used in these studies should not be overlooked.

Table 3. Organic acid content of three blackberry genotype fruits (g/100g).
Çizelge 3. *Üç böğürtlen genotipinin organik asit içeriği (g/100g).*

Genotypes	Citric	Oxalic	Malic	Succinic	Fumaric
65BS01	1.132 ± 0.013 a*	0.033 ± 0.001 c	0.647 ± 0.018 c	0.455 ± 0.010 a	0.065 ± 0.002 b
65BS02	1.090 ± 0.021 a	0.085 ± 0.006 a	0.953 ± 0.026 a	0.301 ± 0.019 b	0.030 ± 0.001 c
65BS03	0.945 ± 0.009 b	0.055 ± 0.005 b	0.815 ± 0.014 b	0.509 ± 0.013 a	0.093 ± 0.006 a

*: The difference between the means indicated by the same letter in the same column is insignificant ($P < 0.05$).

When organic acid contents of blackberry fruits were examined, variability was observed between the genotypes. It was determined that citric acid levels were the highest in blackberry fruits. It was followed by malic, succinic, fumaric acid and oxalic. While 65BS01 genotype had the lowest organic acid content, 65BS02 genotype had the highest values. The amount of citric acid varied between 0.945 and 1.132 g/100g (65BS01). 65BS02 genotype had the highest malic and oxalic acid content. When the amounts of succinic and fumaric acid were examined, it was found that 65BS03 genotype had the highest values and 65BS02 genotype had the lowest values (Table 3). In a study conducted with different fruits, the researchers determined fumaric acid level as 34.1 mg/kg in blackberry variety whereas this value was 30.7 mg/kg in wild blackberry (Mikulic-Petkovsek et al., 2012). Kafkas et al. (2006) measured different malic acid values depending on blackberry variety in the study they performed. According to the researchers, lowest malic acid level was determined as 0.6 mg/g in C. Thornless cultivar and the highest value was found as 11.0 mg/g in Loch Ness cultivar. In another study, Gazioglu Sensoy et al. (2015) found that the dominant acid was citric acid and reported that this was followed by malic acid. In another study conducted on different varieties, succinic acid value varied between 2.021 g/kg (Bursa 2) and 0.953 g/kg (Jumbo) (Gündoğdu et al., 2016). While our study was consistent with Gündoğdu et al. (2016), the values we obtained were higher than the findings of other studies. It was considered that these differences were due to genotype and variety differences, as well as ecological factors, i.e. changes in organic acid contents due to rainfall and air temperature.

Table 4. Correlation between phenolic compounds and organic acids.
Çizelge 4. *Fenolik bileşikler ve organik asitler arasındaki korelasyon.*

	(V_2)	(V_3)	(V_4)	(V_5)	(V_6)	(V_7)	(V_8)	(V_9)	(V_{10})	(V_{11})	(V_{12})	(V_{13})
Citric (V_1)	-0.180	-0.273	-0.471	-0.669	0.285	0.655	0.690	-0.557	0.241	0.617	0.471	-0.980^{**}
Oxalic (V_2)		0.972^{**}	-0.777	-0.608	0.880^{*}	0.622	0.581	-0.710	0.890^{*}	0.660	0.780	0.227
Malic (V_3)			-0.672	-0.516	0.833^{*}	0.535	0.483	-0.620	0.866^{*}	0.570	0.704	0.333
Succinic (V_4)				0.964^{**}	-0.951^{**}	-0.963^{**}	-0.956^{**}	0.983^{**}	-0.925^{**}	-0.974^{**}	-0.989^{**}	0.429
Fumaric (V_5)					-0.887^{*}	-0.993^{**}	-0.995^{**}	0.976^{**}	-0.868^{*}	-0.992^{**}	-0.965^{**}	0.619
Vanillic (V_6)						0.908^{*}	0.868^{*}	-0.949^{**}	0.988^{**}	0.922^{**}	0.973^{**}	-0.214
Rutin (V_7)							0.990^{**}	-0.991^{**}	0.878^{*}	0.995^{**}	0.974^{**}	-0.600
Gallic (V_8)								-0.973^{**}	0.840^{*}	0.980^{**}	0.958^{**}	-0.657
Catechin (V_9)									-0.913^{*}	-0.989^{**}	-0.991^{**}	0.503
Cholorgenic (V_{10})										0.900^{*}	0.952^{**}	-0.160
Caffeic (V_{11})											0.978^{**}	-0.550
p-coumaric (V_{12})												-0.420
Ferulic (V_{13})												1.000

*: $P<0.05$, **: $P<0.01$

Table 4 shows that there is a correlation between phenolic compounds and organic acids. The highest positive correlation was found between rutin and caffeic acid values (0.995). This was followed by the correlation between rutin and gallic acid (0.990). It was found that malic acid increased significantly as oxalic acid increased. Positive correlation was found between gallic and chlorogenic acid, and caffeic and p-cumaric acid levels. Succinic acid levels showed parallelism with catechin and fumaric acid values. However, the negative relationship of this acid with vanillic, rutin, gallic, clorogenic, caffeic and p-coumaric acid was found to be statistically significant. As a result, it was found that as succinic acid increased these acids decreased. A similar negative relationship was also found between catechin and caffeic acid.

4. Conclusion

In recent years, the search for food rich in phenolic substances and organic acids in markets has been increasingly favored by consumers. These biochemical characteristics of fruits rich in phenolic and organic substances vary depending on genetic and ecological conditions. Therefore, when the genotypes investigated in this study are evaluated in terms of phenolic compounds and organic acids, the 65BS02 genotype stands out. As a result, the 65BS02 genotype should be considered as a variety candidate and should be made available to consumers, breeders, farmers and even the industry.

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