Pak. J. Bot., 51(1): 165-170, 2019. DOI: 10.30848/PJB2019-1(5)

CHEMICAL AND PHYTOCHEMICALS CONTENT OF BARBERRY (*BERBERIS VULGARIS* L.) FRUIT GENOTYPES FROM SIVASLI DISTRICT OF UŞAK PROVINCE OF WESTERN TURKEY

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Abstract

Turkey is one of the most outstanding places with the highest potential to grow barberry crop. The barberry possesses nutritional and health supplements and that can be grown naturally in almost all regions of Turkey. The goal of this paper was to determine chemical and phytochemical contents of barberry genotypes collected fromSivaslı district of Uşak province of Western Turkey. We identified 16 different barberry genotypes from the studied sites which were subjected to phytochemical analysis. We analyzed barberry juices in terms of chemical (fruit skin color, soluble solid content, acidity and pH) and phytochemical (antioxidant activity, phenol, flavonoids and ascorbic acids) values. The results indicated that total flavonoids contents were ranged from 261.66 to 965.97 mg CAT/100 g FW and the highest ascorbic acid values were found as 444.35 with the lowest ones as 120.36 mg/100 g. The antioxidant activity between genotypes was varied from 126.27 to 34.20% and total phenol contents were from 1198.53 to 2616.78 mg GAE/100 g FW. Statistical analysis showed that there was a significant difference (p<0.01) for all measurements between genotypes handled in this study and that a major correlation occurs between total antioxidant activity and total flavonoids, SSC, pH, L (brightness).

Key words: Barberry genotypes, Phytochemical analysis, Antioxidant activity, Phenol contents, Western Turkey.

Introduction

Plants are a unique source of nutrients both for man and animals. Many plant species have medicinal value due to the presence of many chemical compounds (Joseph & Raj, 2010). The data of chemical analysis have proved the importance of wild and semi-wild edible fruits, because they contain high amount of antioxidants such as flavonoids, anthocyanins, organic acids and many others (Perveen *et al.*, 2015; Okatan *et al.*, 2016). Consumption of such fruits inhibits cancer cell proliferation, lowers fat oxidation and lowers cholesterol. In addition, chemical compounds display a broad spectrum of pro-health activities including protection against DNA oxidation, prevention of cancer diseases and reducing the risk of cholesterol and cardiovascular diseases (Boyer & Liu, 2004; Skrajda, 2017; Ersoy *et al.*, 2017). Antioxidants are mainly the phenolic compounds that are found plentiful in several fruits and have scavenging activity by linking to free radicals to remove them from the human body (Dai & Mumper, 2010). Flavonoids help in preventing diseases such as Alzheimer, coronary heart failure, cancer, bacterial ills and inflammation (Shen & Li, 2014; Han *et al.*, 2015; Gundogdu *et al.*, 2017).

Consumers and researchers have showed great interest in medicinal plants (Rahim *et al.*, 2013; Munazir *et al.*, 2015), semi-wild and wild edible fruits due to their striking value on health in recent times. Berries are rich in chemical compounds and are one of the most valuable horticultural crops with regard to their sensory qualities and nutritional value. Barberry is one of the berry fruits that have not so far been under much research compared to the studies on strawberry, currant, raspberry and blackberry.

The barberry genus is commonly found across all parts of Europe and Asia that belongs to Berberidaceae family. *Berberis vulgaris* L. is well known for pharmacological properties and its consumption as food in most of the world (Imanshahidi & Hosseinzadeh, 2008; Gundogdu, 2013). It is a 100-180 cm thorny bush with obovate leaves, yellow flowers and rectangle red fruits. The shiny flowers are androgynous, which are typically found in composite pendant clusters or panicle with 10-20 flowers in each panicle. Barberry fruits have reddish-brown color and can reach up to 13 mm (Kafi *et al.*, 2004; Khadivi-Khub, 2009).

The objective of this study was to study chemical-phytochemical contents of barberry genotypes collected from Sivaslı district of Uşak province in Turkey.

Material and Method

Plant material: The study was carried out in Sivaslı district of Uşak province in Western Turkey in 2017. The climate of the region is temperate (Csa) according to Köppen's classification. Different sixteen barberry genotypes growing in different parts of Sivaslı were collected and their coordinates and altitudes were noted via GPS (Magellan Triton 2000, USA). Coordinates and altitude of genotypes were shown in Table 1. Fruits of collected genotypes were analysed for phytochemicals in the pomology laboratory of Usak University.

August was the driest month with the mean rainfall of 10 mm. With an average of 87 mm rainfall, maximum rainfall was observed in December. The region is dry and hot in summers, warm and rainy in winters (Anonim, 2018).

Chemical analysis

Fruit skin color measurements were performed with a Minolta CR-400 tristimulus colorimeter (Konica Minolta, Inc., Sakai, Osaka 590-8551, Japan) calibrated against a white standard calibration plate. The color was determined by the CIE-*L*a*b** color space method. Three measurements were taken in the equatorial area of fruit skin of each genotype and the mean values were

determined. Soluble solids content (SSC) was determined by Model HI-96801 Hanna, German (digital refractometer) having a sensitivity of 0.2 Brix, at room temperature. The pH value was determined by using a pH meter (Hanna-HI 98103, German) and its calibration was done using pH 4.0 and pH 7.0 tampons. Titratable acidity was measured with voltmeter by titrating sample by means of 0.1 NaOH until the pH reached to 8.01 and the resulting value was expressed as citric acid (Anon., 1995).

Table 1. Coordinates and altitude of barberry genotypes.

Genotypes	Coordinates		Altitude (m)
	North	East	
64USAK01	38°30'28.06"	29°35'32.99"	801
64USAK02	38°30'26.68"	29°35'32.02"	803
64USAK03	38°30'34.39"	29°35'14.78"	824
64USAK04	38°28'27.23"	29°35'49.75"	816
64USAK05	38°28'29.23"	29°35'55.02"	819
64USAK06	38°28'22.99"	29°36'28.31"	810
64USAK07	38°29'27.03"	29°39'41.32"	905
64USAK08	38°29'25.92"	29°39'45.42"	903
64USAK09	38°29'23.13"	29°39'55.85"	903
64USAK10	38°29'04.48"	29°40'50.17"	924
64USAK11	38°28'43.04"	29°37'20.21"	816
64USAK12	38°28'55.01"	29°37'57.19"	864
64USAK13	38°29'11.38"	29°38'21.48"	877
64USAK14	38°29'26.51"	29°39'29.85"	904
64USAK15	38°28'56.41"	29°39'04.46"	907
64USAK16	38°28'46.60"	29°39'11.89"	909

Altitude of collected samples was between 801-909 m. The mean climate value is given in Fig. 1.

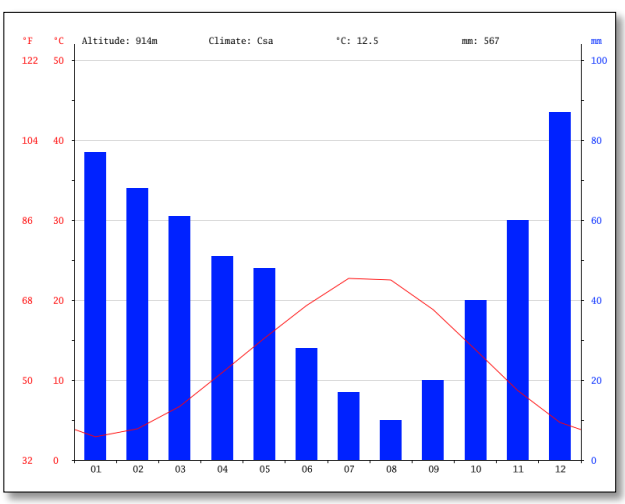

Fig. 1. Mean climate values of Uşak by years (Source: https://tr.climate-data.org/location/193/).

Phytochemical Analysis

Total antioxidant activity (TAA) %: The buffer solution was prepared by mixing 20 mmol/L ferric chloride solution, 10 mmol/L TPTZ (2, 4, 6-tripyridyltriazine) and 0.1 mol/L acetate (pH 3.6) for FRAP (The Ferric Reducing Ability of Plasma) analysis. Finally, the absorbance value was measured by the spectrophotometer (Lamda 35, PerkinElmer, USA) at 593 nm after 10 minutes of absorbance by mixing 2.98 mL of the prepared buffer mixture with 20 µL of the fruit extract. All results from the determination of antioxidant capacity were expressed per gram dry weight as µmol Trolox equivalents (µmol TE) (Benzie & Strain, 1996).

Total phenolics: Total phenolics of barberry genotypes were determined employing the Folin Ciocalteu phenol reagent procedure (Singleton & Rossi, 1965; Rahim *et al.,* 2013; Munazir *et al.,* 2015). Values of absorbance were detected at 765 nm on a spectrophotometer (Lamda35, PerkinElmer, USA). The values of total phenolics were expressed as mg of Gallic acid equivalents (GAE/l) of extract.

Total flavonoids: The total flavonoid value was measured by the aluminum chloride colorimetric method (Cordenunsi *et al.*, 2003; Youngjae *et al.*, 2007). One ml of fruit ethanolic extract or standard catechin solution (20, 50, 100, 250 mg/L) was added to a 10 ml measuring cylinder, followed by addition of 5 ml of 5 % $NaNO_2$, and after 5 minutes, 4 ml of ddH_2O with 0.3 ml of 10 % $AlCl_3$ was added to the mixture. Then, on the 6th minute, 2 ml of 1 M NaOH was added to the mixture and then the total amount was made to 10 ml of dd H_2O. After thoroughly mixing the solution, the absorbance of the samples was read against the free extract at 510 nm (Lamda 35, PerkinElmer, USA). The total flavonoid scales of the samples were stated as mg catechin equivalent fresh weight using the catechin standard curve (y = 0.0038x-0.0247).

Ascorbic acid: After mashing and filtering, samples of fruit juices of barberry genotypes were obtained. The juice of samples was utilized to determine of vitamin C values. The specimens were homogenized by centrifuge and 4.5 ml 2,6-diclorofenolindofenol solution and 400 µL oxalic acid (0.4 %) was put to the supernatant. The results were read at the spectrophotometer (Lamda 35, PerkinElmer, USA) at the wavelength of 520 nm against the blank (Cilla *et al.,* 2012).

Statistical analysis

The implications of the experiment were analyzed via the SigmaPlot 12 statistical software (Systat Software Inc., San Jose, CA, USA) along with the randomized full block design (RCBD). Values were compared by analysis of variance (ANOVA) and differences among the mean values were determined using Tukey's HSD test. Correlation analyses were used to explain the contacts between chemical and phytochemicals.

Result and Discussion

Fruit skin color: Color of fruit mainly provides essential and multipart quality characteristics for fruits. Due to the presence of a varied carotenoid pigment system exposed to both genetic characteristic and environmental conditions, some poor aspect of fruit colour may be witnessed (López Camelo & Gómez, 2004). In this study, the color lightness (*L**) between genotypes was found to be at significant level

according to statistical analysis at $p<0.01$ (Table 2). The maximum value of color lightness (L^*) (17.52) was found in 64USAK04 genotype, while the lowest (12.59) was obtained in 64USAK12 genotype. The greenness (a^*) and the yellowness (b^*) of fruit skin were measured between 4.84 (64USAK03)-3.09 (64USAK06) and 5.43 (64USAK04)-3.46 (64USAK13) respectively. Ozgen *et al.,* (2012) found values of L^*, $a^*$and b^* of different barberries genotypes ranging from 3.28 to 4.94 for a^* value, from 10.36 to 12.27 for L^* value and from 2.37 to 2.63 for b^* value in Sivas province of Turkey. Color is a significant quality standard for most of the agricultural products. Unwanted changes in fruit color may lead to a decline in its quality value and marketing price (Gorjian *et al.*, 2011). In Iran, Haji (2010) found that average L^* (79.14), a^* (1.86) and b^* (43.31) which were different from our study. Ghaen in northeast of Iran (Ardestani *et al.*, 2013) reported that the values of barberry fruit color indexes came out as (L^* 16.85 - 20.82, a^* 5.69 - 34.84 and b^*-1.01 - 18.91). These differences among the results could be due to the variations in cultivars, soil type, climate conditions such as temperature, rainfall and position of regions and growing seasons.

Soluble solid content (SSC), acidity and pH: Soluble solid content (SSC), acidity and pH are a significant quality attribute for fruit owing to its contribution to the flavor of fruit products (Young *et al.*, 1993). The highest value for SSC was found in 64USAK13 genotype and the lowest value was in 64USAK01 genotype and the ^{o}Brix rate was 20.83 and 15.47 respectively. Acidity values were in the range of 2.21 % and 2.98 % for 64USAK04 and 64USAK14 genotypes respectively. The highest and lowest pH values were measured in 64USAK04 as 3.21% and 64USAK10 as 2.59%, respectively. In a previous research conducted by Ardestani *et al.*, (2013) in Ghaen in north-east of Iran, pH was detected as 3.16 -3.06 and SSC was measured as 11.17 - 17.33. Awan *et al.*, (2014) reported that titratable acidity was found as 2.25, 2.17 and citric acid as 1.35%, TSS as 20.22, 18.18 and ^{0} Brix as 15.56 and pH as 3.91, 3.52 and 3.33. pH values of B. integerrima and B. vulgaris were found to be 3.16 and 3.06 respectively. In another study, the highest and the lowest SSC values were obtained as 12.7 % and 9.7 %, respectively (Moghaddam *et al.*, 2013). Fallahi *et al.*, (2010) indicated that past-harvests caused an increase in SSC, pH values and fruit sweetness taste but diminish in titratable acidity, which was a sign of negative regression between acidity and pH. In conclusion, some differences between our studies and other studies in terms of SSC, acidity and pH can be seen, the reason of which may be climatic factors, harvest time variations and total temperature fluctuations during the growing season (Table 3).

Correlation of chemical values between barberry genotypes were shown in Table 4. SSC had a optimistic and important correlation with pH (r=0.000, $p<0.01$), L (r=0.000, $p<0.01$) and a* (r=0.000, $p<0.01$). Acidity had a important correlation with b* (r=0,023, $p<0.05$). pH had a positive and important correlation with L (r=0.000, $p<0.01$) and a* (r=0.003, $p<0.01$). L had a positive and important correlation with a* (r=0.000, $p<0.01$).

Table 2. Fruit skin color indices of barberry genotypes.

Genotypes	*L*	*a*	*B*
64USAK01	13.26 l*	2.95 n	4.36 f
64USAK02	15.10 f	4.31 b	3.78 j
64USAK03	16.96 b	4.84 a	4.83 b
64USAK04	17.52 a	3.89 f	5.43 a
64USAK05	15.87 d	3.53 h	3.30 n
64USAK06	13.89 i	3.09 m	3.83 i
64USAK07	14.85 g	3.30 k	4.06 g
64USAK08	16.91 b	4.23 c	4.48 e
64USAK09	15.54 e	3.88 f	4.73 c
64USAK10	13.69 j	3.42 i	4.35 f
64USAK11	13.52 k	3.38 j	3.95 h
64USAK12	12.59 m	3.15 l	4.66 d
64USAK13	16.95 b	4.24 c	3.46 m
64USAK14	15.98 d	4.00 e	3.70 k
64USAK15	14.49 h	3.62 g	4.75 c
64USAK16	16.65 c	4.16 d	3.52 l

Different letters within a column indicate significant differences *$p<0.01$

Table 3. Chemical values of barberry genotypes.

Genotypes	SSC Brix	Acidity %	pH %
64USAK01	15.47 l	2.52 h	2.74 i
64USAK02	17.61 h	2.44 j	2.82 h
64USAK03	19.56 d	2.80 d	3.13 c
64USAK04	20.08 b	2.21 l	3.21 a
64USAK05	18.18 g	2.60 g	2.91 f
64USAK06	15.91 k	2.87 b	2.82 h
64USAK07	17.33 i	2.70 e	3.07 d
64USAK08	19.73 c	2.60 g	2.99 e
64USAK09	18.18 g	2.69 e	2.75 i
64USAK10	17.10 j	2.27 k	2.59 k
64USAK11	17.38 i	2.65 f	2.63 j
64USAK12	19.79 c	2.48 i	3.00 e
64USAK13	20.83 a	2.48 i	3.15 b
64USAK14	18.57 f	2.98 a	2.81 h
64USAK15	18.84 e	2.82 c	2.85 g
64USAK16	18.92 e	2.83 c	2.87 g

Different letters within a column indicate significant differences *$p<0.01$

Table 4. Correlation between chemical value.

Variable	SSC %	Acidity %	pH	L
Acidity	-0,135	-	-	-
pH	0,698**	-0,125	-	-
L	0,644 **	0,048	0,623**	-
a	0,637**	0,111	0,423**	0,808**
b	0,215	-0,327*	0,244	0,014

*: $p\leq0.05$, **: $p\leq0.01$

Phytochemical Contents: The level of phytochemical compositions in fruits and vegetables is affected by number of conditions such as climatic, cultivation practices, time of harvest, storage terms, genetic variability etc. (Ninfali & Bacchiocca 2003). Barberry fruits have high antioxidant levels and consuming such fruits can help in reducing oxidative stress and may, thus, help in obstructing chronic illness (Ozgen *et al.*, 2012). In

genotypes, the highest value of total antioxidant activity (TAA) (126.27 %) was achieved in 64USAK14 genotype, while the lowest value (34.20%) was obtained in 64USAK10 genotype. Yıldız *et al.,* (2014) reported that total antioxidant activity between genotypes ranges from 62.43 to 66.45. According to Hassanpour & Alizadeh (2016), the level of total antioxidant activity of barberry genotypes was found higher. The average total phenol value in genotypes was measured as the highest (2616.78 mg GAE/100 G FW) in 64USAK09 genotype, while the lowest value (1198.53 in mg GAE/100 G FW) was found in 64USAK04 genotype. Yıldız *et al.,* (2014) stated total phenolic values of barberry fruits ranged from 2500 mg to 3720 mg GAE/L of fruit juice. Awan *et al.,* (2014) reported total phenolics as 689.82, 675.68 and 702.94 in barberry genotypes. In Central Anatolia region of Turkey, according to Ozgen *et al.,* (2012), total phenolic content of fruits of barberry genotypes was found between 2560-3630 mg GAE per L. Akbulut *et al.,* (2009), found the value of total phenolic as 789.32 ± 88.50 mg/100 g for the fresh barberry fruits in Turkey. These results were close to the values obtained by Motalleb *et al.,* (2005), Kiselova *et al.,* (2005) and Ardestani *et al.,* (2013).

Flavonoids such as quercetin and quercetin glycoside have shown a good capability in inhibiting free radicals (Lu & Foo, 2000). The highest content was found as 965.97 mg CAT/100 g FW in 64USAK10 genotype, and the lowest content as 120.36 mg CAT/100 g FW in 64USAK15 genotype in terms of total flavonoids. Awan *et al.,* (2014) reported total flavonoid as 385.52, 376.93 and 395.09. Sasikumar *et al.,* (2012) determined total flavonoid content of 320 mg equal to Eq quercetin/100 g for fresh fruit barberry genotypes. In genotypes, the levels of ascorbic acid were determined between 120.36 (64USAK15)-444.35 (64USAK02) mg/100 g. Awan *et al.,* (2014) reported ascorbic acid as 10.70, 14.92 and 13.59 %. Ardestani *et al.,* (2013) determined ascorbic acid content of barberry genotypes as 10.83 % mg/100gm in Iran (Table 5).

Phytochemical contents of barberry genotypes were subjected to principle component analysis (PCA) using the PRINCOMP procedure (Figs. 2 and 3). The relationships were determined from a covariance matrix derived from standardized phytochemical contents of barberry genotypes characteristics means and the output data sets consisted of eigen-values, eigenvectors, and standardized principal component scores.

Correlation of phytochemical contents of barberry genotypes was shown in Table 6. Total antioxidant activity had a significant correlation with total flovanoids (r=0.014, p=<0.05), SSC (r=0.003, p<0.05), pH (r=0.007, p<0.01) and L(r=0.002, p<0.01). Total phenol had a positive and significant correlation with acidity (r=0.000, p<0.01). Total flovanoids had a significant correlation with SSC (r=0.02, p<0.01), acidity (r=0.033, p<0.05), pH (r=0.032, p<0.05), L (r=0.001, p<0.01) and a (r=0.000, p<0.01). And ascorbic acid had a positive and important correlation with a* (r=0.006, p<0.01).

Table 5. Phytochemical contents of barberry genotypes.

Genotypes	Total antioxidant activity (TAA) %	Total phenol (tp) (mg GAE/100 G FW)	Total flavonoids (tf) (mg CAT/100 g FW)	Ascorbic acid (aa) (mg/100 g)
64USAK01	46.41 m	1236.60 n	584.41 f	185.96 k
64USAK02	52.84 k	1683.82 i	318.50 n	444.35 a
64USAK03	76.30 f	2043.72 g	432.35 j	300.72 e
64USAK04	96.36 c	1198.53 o	529.73 g	146.50 n
64USAK05	87.27 d	1633.31 j	404.25 k	208.27 i
64USAK06	76.39 f	1982.41 h	667.62 d	268.83 f
64USAK07	51.98 l	1582.06 k	653.74 e	182.93 l
64USAK08	59.19 i	2155.96 d	764.87 b	339.09 c
64USAK09	85.46 e	2616.78 a	355.06 m	198.88 j
64USAK10	34.20 o	1550.42 l	965.97 a	307.10 d
64USAK11	60.82 h	2112.84 e	737.15 c	214.03 h
64USAK12	69.25 g	2564.44 b	583.70 f	243.68 g
64USAK13	99.98 b	1507.43 m	397.67 l	163.33 m
64USAK14	126.27 a	2054.26 f	452.76 i	351.84 b
64USAK15	56.52 j	2493.34 c	465.27 h	120.36 o
64USAK16	41.63 n	2156.55 d	261.66 o	268.50 f

Different letters within a column indicate significant differences *p<0.01

Table 6. Correlation between chemical and phytochemical contents.

Variable	Total antioxidant	Total phenol	Total flavonoids	Ascorbic acid
Total phenol	0,021	-	-	-
Total flavonoids	-0,354*	-0,164	-	-
Ascorbic acid	-0,086	0,082	0,018	-
SSC	0,423*	0,213	-0,334**	-0,106
Acidity	0,208	0,545**	-0,308*	0,087
pH	0,387**	-0,199	-0,309*	-0,248
L	0.444**	-0,169	-0,463**	0,049
a	0.261	0.099	4.495**	0,393**
b	-0,058	0,123	0,223	-0,265

*: $p\leq0.05$, **: $p\leq0.01$

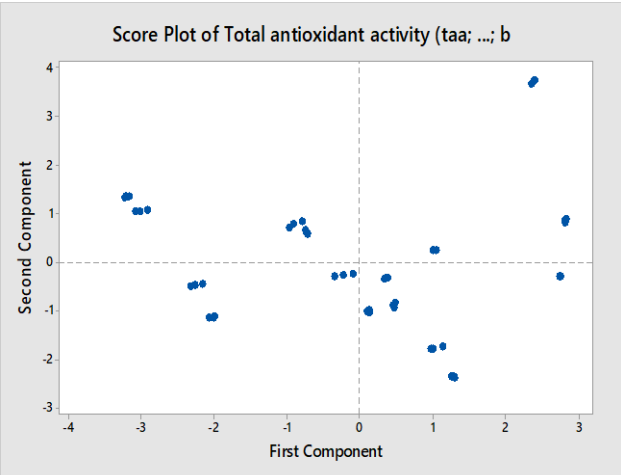

Fig. 2. Multivariate principal component analysis (PCA) plot of sixteen barberry genotypes; based on evaluated parameters in the present study see Tables 3 and 5.

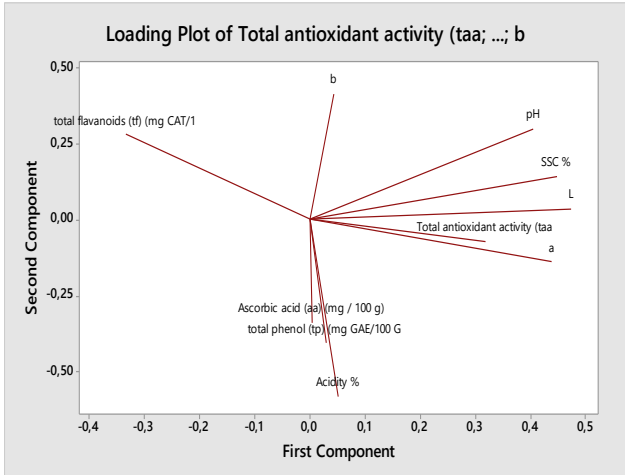

Fig. 3. Multivariate principal component analysis (PCA) plot of sixteen barberry genotypes; based on evaluated parameters in the present study see Tables 3 and 5.

Conclusion

The genotypes of barberry from Sivasli district were phytochemically analysed. The studied genotypes were superior to those of many previous studies. Among the genotypes, 64USAK02, 64USAK09 and 64USAK14 were found of having the highest phytochemical content values that might be promising for future studies.

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(Received for publication 22 January 2018)