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Original Article

Physicochemical Diversity Among Barberry (*Berberis vulgaris* L.) Fruits from Eastern Anatolia

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Abstract

Wild edible fruits have been gaining much interest more recently because of their better biochemical content and widely use in ethno medicine treating common disease such as cold, fever and other medicinal claims are now supported with sound scientific evidences. In this study, diversity of some important physicochemical characteristics (plant growth habit, fruit shape, fruit color, fruit weight, pulp ratio, soluble solid content, total phenolics, total anthocyanin, antioxidant activity, sugars, organic acids) of fruits from fourteen promising barberry (*Berberis vulgaris* L.) selections grown in Erzurum province in Turkey were investigated. Significant differences were observed between the studied genotypes for most of the physicochemical parameters. Fruit weight and pulp ratio were found between 0.102 (25ERZ5) and 0.342 g (25ERZ7) and 60.81% (25ERZ2) and 75.41% (25ERZ11). Total phenolic and anthocyanin content ranged from 2281 (25ERZ5) to 3462 (25ERZ7) as mg GAE per liter fruit juice and 360 to 874 mg as cyanidin-3-glucoside per liter fruit juice. Glucose and fructose were found to be dominant sugars in all barberry accessions analyzed.

Keywords: diversity; less known fruits; human health content

Introduction

Cultivation of fruit crops plays an important role in the prosperity of any nation. It is generally stated that the standard of living of the people can be judged by per capita production and consumption of fruits. They are the important exportable commodities in many countries. Fruits form a significant part of the total agricultural produce in the Turkey as well and the country able to growth over 100 fruit species mostly includes temperate fruits and some subtropical and even tropical ones (Caliskan *et al*., 2017). Along with cultivated fruits, the country has numerous natural populations of wild edible fruits (Yildiz *et al*., 2010; Cuce and Sokmen, 2017).

Wild edible fruits are grown in particular rural areas around the world under poor cultivation conditions and currently in considerable significance view due to their special attributes as a large source of phytochemicals, which is important for human health and welfare (Kamiloglu *et al*., 2009; Tosun *et al*., 2009). Among the compounds with antioxidant properties found in horticultural crops including fruits, grapes and vegetables, phenolic compounds stand out. They account for the largest part of the antioxidant activity of many horticultural plants (Duthie and Crozier, 2000). They occur naturally in plants as secondary metabolites, and are present in different parts of horticultural crops including fruits, leaves, nuts, seeds, and flowers. They are an integral part of the human diet and have also been intentionally added to some medication preparations (Wu *et al*., 2004). Small fruits, known as berries, are very rich in phenol compounds and present high antioxidant activity (Seeram, 2008; Wolfe *et al*., 2008), and are interesting as ingredients for use in juices, jams, ice cream, and cake icing, in addition to being used successfully in the development of functional foods with the objective of enhancing health (Potter *et al*., 2007).

Interest in utilizing natural sources in the development and formulation of products, as an alternative to conventional drugs and synthetic products, contribute to increase interest in research and industrial application of wild edible fruits (Ercisli *et al*., 2012; Cuce and Sokmen, 2017). Moreover, in the past decades, many reports have been published on the nutritional composition and medicinal value of wild edible fruits growing in different

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regions of the world. Most of these studies indicate that indigenous fruits have important socio-economic value. Cultivation and consumption of indigenous fruits could reduce malnutrition and poverty in rural peoples worldwide (Kamiloglu *et al*., 2009; Tosun *et al*., 2009).

Berberis is a wide genus that including about 450-500 species mostly naturally grown in temperate and subtropical regions of the world. *Berberis vulgaris*, European barberry is the most known species and dominated natural barberry production in the world (Gundogdu, 2013). The fruit of *B. vulgaris* is a small berry 5-15 mm long, ripening to red or dark blue, often with a pink or violet waxy surface bloom; in some species, they may be either long or narrow, but are spherical in other species (Ozgen *et al*., 2012; Ahmed *et al*., 2013; Gundogdu, 2013).

Considering literature search, there were a few reports by using very limited number of European barberry genotypes has been reported (Motalleb *et al*., 2005; Akbulut *et al*., 2009; Ozgen *et al*., 2012; Ahmed *et al*., 2013; Gundogdu, 2013). Thus, more data are needed about the biodiversity of morphological and bioactive content of this less known fruit. Thus, the aim of this study was to determine some physicochemical characteristics of fourteen barberry genotypes from Turkey.

Materials and Methods

Plant material

The research was conducted in 2016 on harvested fruits from fourteen promising barberry genotypes grown from Erzurum provinces located in Eastern Anatolia of Turkey.

Determination of physicochemical characteristics

Barberry fruits were harvested from fourteen genotypes at fully maturates stage. Approximately 0.5 kg fully matured, fresh barberry fruits were hand harvested and transferred to laboratory for analysis. The pomological analysis was conducted from three replicates, each having 50 fruits. Fruit weight was measured with an electronic balance of 0.01 g sensitivity. Fruit skin were determined by using a portable chromometer (Minolta CR-400). Colour measurements were recorded using the CIE *L*, *a*, *b* color space. The soluble solid content (SSC) was measured in the filtered juice using a digital refractometer. The intake fruit samples were also used for fruit pulp ratio analysis (AOAC, 1995).

Determination of bioactive contents

For the total phenolic, total anthocyanin and total antioxidant capacity analyses, harvested fruit samples were frozen and stored at -20 °C until analysis. After thawing to room temperature, triplicate of 100 g lots of barberry fruits from each genotype were homogenized in a blender and they were screened for their total phenolic, total anthocyanin and antioxidant activity following a single extraction procedure (Singleton and Rossi, 1965). For this procedure, 3 g aliquots of each homogenate were transferred to polypropylene tubes and extracted with 20 mL of extraction buffer containing acetone, deionized water, and acetic acid (70:29.5:0.5 v/v), for one hour. Total phenolic contents were measured according to Singleton and Rossi (1965). To determine the levels of total phenolics, 1 mL of each extract was combined with Folin-Ciocalteu's phenol reagent and water 1:1:20 (v/v) and incubated for eight minutes, followed by the addition of 10 mL of 7% (w/v) sodium carbonate. After two hours, the absorbance of each was measured at 750 nm. The values of total phenolic were estimated by comparing the absorbance of each with those of a standard response curve generated with gallic acid. The results were expressed as mg gallic acid equivalent in liter of fruit juice.

Total monomeric anthocyanins (TMA) were determined by a pH differential method (Giusti and Wrolstad, 2001), using a UV-VIS spectrophotometer. Absorbance was measured at 533 nm and 700 nm in buffers at pH 1.0 and 4.5 using A = (A533 - A700) pH 1.0 - (A533 - A700) pH 4.5 with a molar extinction coefficient of 29,600. Results were expressed as mg of cyanidin-3-glucoside equivalents per liter of fruit juice.

Antioxidant activity were determined with FRAP (Ferric reducing antioxidant power) method. In FRAP (Ferric reducing ability of plasma) assay, 2.95 mL aliquot of a FRAP reagent, a mixture of 0.1 mol/L acetate buffer, 10 mmol/L TPTZ (2,4,6-tris(2-pyridyl)-1,3,5-triazine), and 20 mmol/L ferric chloride (10:1:1 v/v/v), were combined with 50 µL of acetone fruit extract. These solutions were prepared and stored in the dark under refrigeration. Stock solutions were combined (10:1:1 v/v/v) to form the FRAP reagent just prior to analysis. For each assay laboratory duplicate, 2.98 mL of FRAP reagent and 20 µL of sample extract were mixed. After 10 min, the absorbance of the reaction mixture was determined at 593 nm in a spectrophotometer. The antioxidant capacity values were expressed as mmol trolox equivalent per liter of fruit juice (Benzie and Strain, 1996).

Analysis of organic acids

Malic, citric and tartaric acid composition of berries were identified by Bevilacqua and Califano (1989). Juice extracts were obtained by mashing the berries in cheesecloth, after which the samples were stored at -20 °C until analyzed. 5 ml of each sample was mixed with 20 ml of 0.009 N H_2SO_4 (Heidolph Silent Crusher M, Germany), then homogenized for 1 hour with a shaker (Heidolph Unimax 1010, Germany). The mixture was centrifuged for 15 min at 15000 rpm, and supernatants were filtrated twice with 0.45 µm membrane filter following filtration with coarse filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA) and run through a SEP-PAK C18 cartridge. Organic acid readings were performed with HPLC using Aminex column (HPX - 87 H, 300 mm × 7.8 mm, Bio-Rad Laboratories, Richmond, CA, USA) at 214 and 280 nm wavelengths, on Agilent package program (Agilent, USA).

Sugar analysis

The modified method of Melgarejo *et al*. (2000) was used for sugar analyses. 5 mL of fruit extracts was centrifuged at 12000 rpm for 2 minutes at temperature of 4 °C. Supernatants were passed by SEP-PAK C_{18} cartridge. HPLC readings were made with µBondapak-NH2 column using 85% acetonitrile as liquid phase with refractive index detector (IR). Fructose and glucose standards were used for sugar calculations.

Statistical analyses

All data were analyzed using SPSS software and procedures. Analysis of variance tables were constructed using the Least Significant Difference (LSD) method at $p<0.01$.

Results and Discussion

Physicochemical characteristics

Plant growth habit, fruit shape, *L*, *a* and *b* color indices of 14 barberry genotypes are shown in Table 1.

The majority of genotypes had upright plant growth habit (nine genotypes); four genotypes had pyramidal and only one genotype had broad spreading growth habit. In terms of fruit shape, nine genotypes had ovate fruit shape and five genotypes had oblong fruit shapes (Table 1).

There were significant differences among barberry genotypes for *L* (lightness) fruit external color values ($p<0.01$) but *a* (greenness) and *b* (yellowness) color values of 14 genotypes was not statistically differed each other (Table 1). The lightness (*L*), the greenness (*a*) and the yellowness (*b*) for fruit skin were found between 11.30 (25ERZ6)-17.41 (25ERZ11); 2.83 (25ERZ3)-5.62 (25ERZ11) and 2.02 (25ERZ7)-5.28 (25ERZ12) among genotypes, respectively. *L* value of the barberry genotypes for fruit skin were found to be different from each other at $p<0.01$, while *a* and *b* values of genotypes were found not to be different from each other at $p<0.01$ (Table 1). Ozgen *et al*. (2012) revealed genotype dependent fruit skin color in barberry genotypes (*B. vulgaris* L.) grown in middle parts of Turkey, which ranged from 10.36-12.27 for *L* value, 3.28 to 4.94 for *a* value and 2.37 to 2.63 for *b* value and Ahmed *et al*. (2013) reported a wide variation on exterior color of barberry fruits belongs to *B. vulgaris* L. Akbulut *et al*. (2009) also reported average fruit color indices 17.05% for *L* value, 2.06 for *a* value and -0.20 for *b* value. Fruit skin color is effective indicator of appearance, external quality and maturity in barberry fruits.

The average fruit weight, pulp ratio and SSC (Soluble Solid Content) values of barberry genotypes ranged from 0.102 (25ERZ5) to 0.342 (25ERZ7); 60.81% (25ERZ2) to 75.41% (25ERZ11) and 16.95% (25ERZ3) to 20.85% (25ERZ9) respectively (Table 2).

In Iran and Pakistan, average fruit weight of barberry genotypes were reported between 0.051-0.348 (Ardestani *et al*., 2013) and 0.115-0.122 g (Ahmed *et al*., 2013), respectively. Akbulut *et al*. (2008) reported average fruit weight in barberry as 0.070 g from Turkey. All those studies indicating that barberry genotypes grown different parts of the world differs each other in terms of fruit weight. This variability may be due to different growing conditions, genetic background, climate, geography, cultural applications and maturation stage.

Akbulut *et al*. (2009) reported average pulp ratio in barberry genotype grown in Turkey as 75.59%. Ardestani *et al*. (2013) reported average SSC values of barberry genotypes as 17.33%, which is indicating a good agreement with our present results. Ahmed *et al*. (2013) reported average SSC in barberry genotypes between 23.60 and 27.63%. Ozgen *et al*. (2012) reported SSC between 19.27-21.80% among six barberry genotypes.

Genotypic effects might explain the significant differences in our study because all genotypes were grown in a single location and similar cultural and technical practices were applied.

Table 1. Plant growth habit, fruit shape and fruit skin colour indices of the tested barberry (*Berberis vulgaris* L.) genotypes

Genotypes	Plant growth habit	Fruit shape	*L*	*a*	*b*
25ERZ1	Upright	Oblong	13.22ab	3.07^{NS}	4.11^{NS}
25ERZ2	Broad -spreading	Oblong	11.94ab	4.40	4.42
25ERZ3	Upright	Oblong	15.45ab	2.83	3.25
25ERZ4	Upright	Ovate	14.22ab	2.99	2.45
25ERZ5	Pyramidal	Ovate	11.35b	3.11	3.64
25ERZ6	Upright	Ovate	11.30b	4.46	4.21
25ERZ7	Pyramidal	Oblong	12.25ab	3.83	2.02
25ERZ8	Upright	Ovate	13.42Ab	5.02	3.11
25ERZ9	Upright	Ovate	11.98Ab	4.91	4.67
25ERZ10	Upright	Ovate	16.44ab	3.77	3.80
25ERZ11	Pyramidal	Oblong	17.41a	5.62	4.29
25ERZ12	Upright	Ovate	15.02ab	3.15	5.28
25ERZ13	Upright	Ovate	13.43ab	3.71	3.03
25ERZ14	Pyramidal	Ovate	17.05ab	5.22	3.73

Means within a column followed by the same letter are not significantly different at p<0.01.

Table 2. Physicochemical parameters of the tested barberry (*Berberis vulgaris* L.) fruits

Genotypes	Fruit weight (g)	Pulp ratio (%)	SSC (%)
25ERZ1	0.241ab	72.41ab	17.15bc
25ERZ2	0.310ab	60.81c	20.07ab
25ERZ3	0.204ab	70.22ab	16.93c
25ERZ4	0.191ab	69.61b	19.63ab
25ERZ5	0.102b	69.42b	18.83b
25ERZ6	0.221ab	73.66ab	17.41bc
25ERZ7	0.342a	63.30bc	19.42ab
25ERZ8	0.302ab	74.22ab	17.68bc
25ERZ9	0.156ab	68.38bc	20.85a
25ERZ10	0.324ab	67.11bc	17.09bc
25ERZ11	0.223ab	75.41a	20.18ab
25ERZ12	0.265ab	61.67bc	17.88bc
25ERZ13	0.188ab	64.87ab	19.20ab
25ERZ14	0.141ab	71.41ab	20.40ab

Means within a column followed by the same letter are not significantly different at $p<0.01$.

Bioactive content

Table 3 indicates bioactive contents of 14 barberry genotypes. There were statistically significant differences on total phenolic, total monomeric anthocyanin and antioxidant activity among the barberry genotypes ($p<0.01$).

The genotypes exhibited total phenolic contents between 2281 (25ERZ5) and 3462 (25ERZ7) mg GAE per liter of fruit juice (Table 3). The results showed that total phenolic content of barberry genotypes are strongly genotype dependent because all genotypes are found same climatic and geographical and similar cultivation conditions. This phenomena previously was also determined by some researchers on barberry genotypes. For example, Ozgen *et al*. (2012) found that total phenolic content of barberry fruits were between 2565-3629 mg GAE per liter among 6 barberry genotypes grown in inner part of Turkey. In Iran, Motalleb *et al*. (2005) reported 3450 mg GAE per 100 g of total phenolic content in barberry fruits. Our results were in agreement with those studies.

Total monomeric anthocyanin contents were also strongly influenced by barberry genotypes and varied from 360 mg (25ERZ5) to 874 mg (25ERZ7) per liter of fruit juice as cyanidin 3-glycoside (Table 3). Akbulut *et al*. (2009), Ardesti *et al*. (2013) and Motalleb *et al*. (2005) revealed that barberry fruits are not only rich source for phenolics but also rich source of anthocyanin. One of the richest sources of anthocyanins is the purple-black of barberries, which has strong antioxidant capacity (Ozgen *et al*., 2012). Ozgen *et al*. (2012) also reported total monomeric anthocyanin content between 506-803 mg per liter as cyanidin 3-glycoside among 6 barberry genotypes sampled middle part of Turkey. Our data are in agreement with the reported anthocyanin content. Anthocyanins are not only known as non-toxic and non-mutagenic, but they have positive therapeutic properties such as antioxidant, anti-inflammatory, anticarcinogenic, antiviral, and antibacterial effects (Tall *et al*., 2004).

Antioxidant activity of the fourteen barberry fruits determined by FRAP method is shown in Table 3. The antioxidant activity statistically was significantly differed among barberry genotypes ($p<0.01$) (Table 3). Antioxidant activity were found between 51.1 (25ERZ5) and 69.3 (25ERZ9) expressed as mmol per liter fruit juice among barberry genotypes (Table 3). Previously, barberry fruits have been reported to have a strong antioxidant activity (Ozgen *et al*., 2012; Hoshyar *et al*., 2016). In literature, several studies conducted on antioxidant activities of extracts from B. vulgaris. The fruits, roots, twigs, and leaves provided antioxidant activities. The antioxidant activity was well correlated with the content of main plant antioxidants, phenols, and flavonols in barberry fruits (Koncic *et al*., 2010). The leaves and fruit have good antioxidant activity, which was revealed when using a DPPH free radical scavenging assay, and probably involve the high flavonoid content (Hadaruga *et al*., 2010).

A lots of number of fruit species are used in the cellular and metabolic disease treatment such as diabetes, obesity and cancer etc. due to their high phenolic, vitamins and anthocyanin content. There are some speculations that the generation of free radicals inside the body in some physiological conditions is resulted in the cellular changes and development of cancer etc. and this could be neutralized by the antioxidants from different plants including fruits. Several studies have shown that plant derived antioxidant nutraceuticals scavenge free radicals and

Table 3. Bioactive content ant antioxidant activity of the tested barberry (*Berberis vulgaris* L.) fruits

Genotypes	Total phenolics (mg GAE per L)	Antioxidant activity FRAP (ammol trolox equivalent per L)	Total anthocyanin (Cy-3-glu, mg per L)
25ERZ1	2965ab	57.3cd	490c
25ERZ2	2544bc	54.2de	710ab
25ERZ3	3312ab	63.3bc	411cd
25ERZ4	3378ab	65.1b	453cd
25ERZ5	2281b	51.1e	776ab
25ERZ6	2772bc	50.4e	360d
25ERZ7	3462a	52.8de	530bc
25ERZ8	3007ab	54.9de	707ab
25ERZ9	3155ab	69.3a	587bc
25ERZ10	2910ab	60.6c	846a
25ERZ11	3241ab	62.2bc	874a
25ERZ12	3090ab	54.6de	638b
25ERZ13	2647bc	58.7cd	502bc
25ERZ14	2845c	56.1d	620b

Means within a column followed by the same letter are not significantly different at $p<0.01$.

Table 4. Organic acids of the tested barberry (*Berberis vulgaris* L.) fruits (g/L)

Genotypes	Tartaric	Malic	Citric
25ERZ1	0.41^{NS}	5.98bc	1.21^{NS}
25ERZ2	0.62	4.67bc	1.46
25ERZ3	0.57	5.92bc	1.02
25ERZ4	0.47	5.17bc	1.45
25ERZ5	0.34	6.04b	1.12
25ERZ6	0.50	6.26ab	1.24
25ERZ7	0.58	8.81a	1.41
25ERZ8	0.64	4.95bc	1.16
25ERZ9	0.60	4.51bc	1.91
25ERZ10	0.78	3.86bc	1.45
25ERZ11	0.64	3.41c	1.88
25ERZ12	0.37	4.11bc	1.67
25ERZ13	0.51	4.01bc	1.34
25ERZ14	0.47	5.22bc	1.20

Means within a column followed by the same letter are not significantly different at $p<0.01$.

modulate oxidative stress-related degenerative effects (Ames *et al*., 1993; Joseph *et al*., 1999). Free radicals have been implicated in many diseases such as cancer, atherosclerosis, diabetes, neurodegenerative disorders and aging (Yen and Chen, 1995; Halliwell and Gutteridge, 1999). Previous reports suggest that higher intake of antioxidant rich food is

Table 5. Sugar content of the tested barberry (*Berberis vulgaris* L.) fruits (g/100 ml)

Genotypes	Glucose	Fructose	Total
25ERZ1	8.80ab	6.81bc	15.61b
25ERZ2	8.91ab	7.02cd	17.93ab
25ERZ3	9.07ab	6.01cd	15.08ab
25ERZ4	10.29a	7.11bc	17.38ab
25ERZ5	9.45ab	6.70c	16.15ab
25ERZ6	9.00ab	6.91cd	15.91ab
25ERZ7	10.24a	6.86cd	17.10ab
25ERZ8	10.42a	5.63d	16.09ab
25ERZ9	9.82ab	8.29ab	18.11a
25ERZ10	8.03b	7.37bc	15.40ab
25ERZ11	9.94ab	7.77b	17.71ab
25ERZ12	8.86ab	7.37bc	16.24ab
25ERZ13	8.94ab	7.91b	16.85ab
25ERZ14	9.16ab	8.69a	17.84ab

Means within a column followed by the same letter are not significantly different at $p<0.01$.

associated with decreased risk of degenerative diseases particularly cardiovascular diseases and cancer (Thatte *et al*., 2000).

Organic acids

Statistically important differences ($p<0.01$) occurred between barberry genotypes in terms of malic acid but the differences not significant for citric and tartaric acid contents (Table 4). Malic acid was the dominant organic acids in fruits of all barberry genotypes. This was followed by citric and tartaric acid, respectively. The malic acid and citric acid content were between 3.41 (25ERZ11) and 8.81 (25ERZ7) g per liter and 1.12 (25ERZ5) and 1.91 (25ERZ9) g per liter (Table 4). In parallel to this study Ozgen *et al*. (2012) from Turkey determined that malic acid and citric acid from organic acids found in barberry fruit were intensive. High organic acid content of the barberry fruit has good indication of taste and flavor. In addition giving taste to fruits, organic acids are among the chemicals that also have vital importance for protecting human health. It has been understood in some studies that organic acids especially malic acid, citric acid, and tartaric acid make contributions to health significantly in several issues such as enhancing immunity system, preventing formation of kidney stones, eliminating oral diseases, reducing risks of poisoning caused by toxic metals, glamorization and strengthening of skin, and reducing fibromyalgia symptoms (Abraham and Flechas 1992; Penniston *et al*. 2007).

Sugars

There were statistically differences between barberry genotypes ($p<0.01$) in terms of glucose and fructose content (Table 5). In this study, contents of glucose and fructose, which are essential sugars in barberry fruits, were determined and differences between genotypes were revealed (Table 5). Content of glucose was measured as higher than the fructose. The highest glucose values were observed in 25ERZ8 as 10.42 g per 100 g fruit juice while the lowest value were obtained from 25ERZ10 genotype as 8.03 g per 100 g fresh fruit (Table 5). Previously great differences were observed between barberry genotypes in terms of sugar contents (Ozgen *et al*., 2012). Sugars occur naturally in a wide variety of fruits, vegetables, milk and dairy foods. Glucose is the primary source 17 of energy for the body and is the only fuel used by brain cells.

Conclusions

The results showed that there were enough diversity among barberry genotypes grown naturally in Turkey and also results confirmed the barberry grown in Turkey could be important source of phenolic compounds with high antioxidant activity. Overall, the results of this study show the great potential of barberries for the development of foods rich in compounds with antioxidant properties.

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