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Research Report

Antioxidant Capacity and Chemical Properties of Selected Barberry (*Berberis vulgaris* L.) Fruits

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Abstract. Six purple-black barberry accessions (*Berberis vulgaris* L.) were obtained from various sites in Sivas province of Turkey. Fruits were analyzed for their total phenolic (TP), total monomeric anthocyanin (TMA) contents and for their antioxidant capacity by the ferric reducing antioxidant power (FRAP) and TEAC assays. Sugar and organic acid composition were also determined by HPLC. TP and TMA contents were measured using the Folin-Ciocalteu reagent and pH differential methods, respectively. Variability between the accessions was not high. Variability among accessions was greatest for FRAP content (C.V. 17.9%); individuals ranged from 41.0 to 65.6 μmol TE·g^{-1} on a fresh weight basis. Variation among the accessions was also evident for TP, TMA, and TEAC values (C.V. 16.21%, 14.85%, and 7.13%, respectively). The major sugars of barberry fruits were glucose (8.84 g·100 mL^{-1}) and fructose (6.12 g·100 mL^{-1}) and dominant organic acids were malic (7.59 g·100 mL^{-1}) and citric (1.34 g·100 mL^{-1}). Within this limited study of six different accessions, chemical profile and antioxidant capacity of barberry fruits have rich source of natural antioxidant substances.

***Additional key words*:** anthocyanin, bioactive compounds, phenolics

Introduction

A significant number of wildly growing fruits available in our environment have been neglected as technological options focus on major crops. This picture has been started to change as recent findings are begin to demonstrate rich source of natural antioxidant substances in most of the highly colored wild fruits (Cekic and Özgen, 2010; Serce et al., 2010). One of these valuable fruit is *Berberis vulgaris* belongs to Berberidaceae family and is commonly known as "barberry". Some varieties are evergreen, some are deciduous with yellow wood and yellow flowers. It is best grown in temperate zones. Deciduous barberries are hardy for colder zones than evergreens. Barberry shrubs grow around 1-2 m tall and wide, do best in full sun to part shade and only need average water, able to tolerate drought for short times once well established. The berries are edible, purple in color with sharp flavor. It is usually consumed as juice due to presence of hard seed in each berry. Native barberries are found on many continents, including America, Europe, Middle East, and Asia. Barberry is well known and naturally adapted in Turkey, and it has been extensively used as a medicinal plant in traditional medicine.

For medicinal purposes, *Berberis vulgaris* has been studied in terms of its fruit and as well as its root. When evaluating research studies of barberries, it is very important to note the distinction between root and fruit extract. The *Berberis vulgaris* root has a yellow color which indicates content of berbamine and berberine (Kathleen, 2000). Berberine is isolated as a main alkaloid from the root and bark of *Berberis vulgaris*. Fatehi et al. (2005), demonstrated pharmacological attributes of barberries suggesting that aqueous extract of barberry has beneficial effects on both cardiovascular and neural system suggesting a potential use for treatment of hypertension, tachycardia and some neuronal disorders, such as epilepsy and convulsion. The aqueous extract from *Berberis vulgaris* fruit was tested to evaluate its antihypertensive effects on DOCA-induced hypertension in the rats. The results suggest that the antihypertensive and vasodilatory effects of *Berberis vulgaris* fruit extract are mainly endothelial-

independent and it may be used to treat hypertension, a status with endothelial dysfunction (Fatehi-Hassanabad et al., 2005). Substances in the *Berberis vulgaris* plant include carbohydrates, organic acids, some vitamins, polyphenolic compounds, pectin, tannin, and minerals, jatrorrhizine, palmatine. Extracts from *Berberis vulgaris* fruit appear to have natural antihistamine and anti-allergy potential and its juice is recommended to cure cholecytitis (Shamsa et al., 1999; Zargari, 1983).

Most of the antioxidant research on barberries focused on the bark (Tomosaka et al., 2008), twigs and leaves of plant (Koncic et al., 2010). Studies on antioxidant and chemical properties of barberry fruits were not extensively studied. Only recently, Akbulut et al. (2009) documented some of the physico-mechanical and TP, TMA, and ascorbic acid properties of one of the local barberry accession. To our knowledge, antioxidant, sugar and organic acid profile of barberry fruits and variability among the different accessions were not studied. Our objective was to determine the ranges in fruit phytonutrient levels, antioxidant capacity, sugar, and organic acid profile within a small, germplasm pool in Sivas, Turkey. We anticipate these data may be potentially useful during the development of specific health-promoting cultivars if and when an optimal or target phenolic profile for barberries emerges from medical and/or nutritional research.

Materials and Methods

Plant Material

More than 30 wildly growing barberry accessions were surveyed in Sivas province of Turkey during the growing seasons of 2010. Six superior accessions were selected. Approximately 0.5 kg fully matured, fresh barberry fruits were hand harvested and transferred to laboratory for analysis. The pomological analysis was conducted from 3 replicates, each having about 100 fruits. Fruit samples were used to determine total soluble solid (TSS) contents by refractometery (Pal-1, Atago). The fruit color was measured using a Minolta portable chromameter (Minolta, Model CR-400) which provided CIE L*, a*, and b* values. Samples were frozen immediately and then, stored in 100 g batches at -30℃ until analyzed. For each fruit sample, three replicates were thawed at room temperature and then homogenized in a standard food blender; for each replicate, excess fruits were used to minimize possible naturally-occurring fruit to fruit variation.

Analytical Procedures

***Determination of total phenolics (TP)*:** TP content was measured according to Singleton and Rossi (1965) procedure. An aliquot of fruit slurry was extracted with a buffer containing acetone, water and acetic acid (70:29.5:0.5 v/v) for 1 hour in the dark. Three parallel extracts were obtained from each accession. Then, extract, Folin-Ciocalteu's reagent and water were incubated for 8 minutes, followed by adding 7% sodium carbonate. After 2 hours, the absorbance was measured by an automated UV-VIS spectrophotometer at 750 nm. Gallic acid was used as a standard. The results were expressed as mg gallic acid equivalent in liter of fruit juice.

***Total monomeric anthocyanins (TMA)*:** TMA were estimated by a pH differential method (Giusti and Wrolstad, 2005), using a UV-VIS spectrophotometer (model T60U, PG Instruments). Absorbance was measured at 533 nm and 700 nm in buffers at pH 1.0 and 4.5 using $A = (A_{533} - A_{700})$ pH 1.0 - $(A_{533} - A_{700})$ pH 4.5 with a molar extinction coefficient of 29,600. Results were expressed as mg of cyanidin-3-glucoside equivalents per liter of juice.

***Determination of total antioxidant activity*:** Total antioxidant activity was estimated by two standard procedures, FRAP and TEAC assays, as suggested by Özgen et al. (2006). Ferric reducing ability of plasma (FRAP) was determined according to the method of Benzie and Strain (1996). Assay was conducted using three aqueous stock solutions containing 0.1 M acetate buffer (pH 3.6) and 10 mM TPTZ [2,4,6-tris(2-pyridyl)-1,3,5-triazine] acidified with concentrated hydrochloric acid (1000:3.3 v/v), and 20 mM ferric chloride. 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. For the standard trolox equivalent antioxidant capacity (TEAC) assay, ABTS was dissolved in acetate buffer and prepared with potassium persulfate, as described by Özgen et al. (2006). The mixture was diluted in acidic medium of 20 mM sodium acetate buffer (pH 4.5) to an absorbance of 0.700 ± 0.01 at 734 nm for longer stability (Özgen et al., 2006). For the spectrophotometric assay, 2.98 mL of the ABTS solution and 20 μL of fruit extract were mixed and incubated for 10 min and the absorbance was determined at 734 nm.

***Extraction of individual sugars and organic acids*:** Fruit slurries (5 g) were diluted with purified water or meta-phosphoric acid (2.5%) solution for individual sugar and organic acid analysis, respectively. The homogenate was centrifuged at 6000 rpm for 5 min. Supernatants were filtered through a 0.45 μm membrane filter (Iwaki Glass)

before HPLC analysis, and the mobile phase solvents were degassed before use. All the samples and standards were injected three times each and mean values were used.

***Chromatographic conditions*:** The HPLC analyses were carried out using a Perkin Elmer HPLC system with Totalchrom navigator 6.2.1 software, a pump and UV detector (Perkin Elmer series-200) (Waltham, MA, USA). Separation and determination of organic acids were done by a modified method of Shui and Leong (2002). The separation was carried out on a SGE wakosil C18RS 5 μm column (250 × 4.6 mm ID). Detection was performed at 215 nm. Optimum efficiency of separation was obtained using pH 2.5 sulfuric acid solution (solvent A), and methanol (solvent B). Other parameters adopted were as follows: injection volume 20 μL; column temperature 30℃; detection wavelength 215 nm. Analysis of sugars was performed according to the method described by Bartolome et al. (1995), using a refractive index (RI) detector (Perkin Elmer series-200). The separation was carried out on a SGE SS Exsil amino column (250 × 4.6 mm ID). The elution solvent used was 80% acetonitrile and 20% deionised water. The column was operated at 30℃ with 0.9 mL·min^{-1} flow rate.

***Statistical analysis*:** Data was analyzed using SAS procedures and software (SAS, Cary, NC, USA). Mean and standard deviations were obtained using PROC TABULATE. Coefficients of variation (CV) were calculated, dividing relevant standard deviations by means and multiplying by 100.

Results and Discussion

Our results show that the purple-black fruits of barberries are one of the richest sources of anthocyanins and phenolic compounds among small fruits and have strong antioxidant capacity (Moyer et al., 2002; Sun et al., 2002) (Table 1). Accession B16 displayed the highest TP and TMA levels (GAE at 3629 mg·L^{-1} and Cy-3-glu at 803.6 mg·L^{1}, respectively). Ranges of TP and TMA were GAE at 2512-3629 mg·L^{-1} and Cy-3-glu at 506.7-803.6 mg·L^{1}, respectively. TMA values of our accession were similar to those found in barberry juice samples by Akbulut et al. (2009). However, our TP values were found to be lower; this may be due to the ripening stages of accessions or some of the environmental factors (Özgen et al., 2008, 2012). Variability of phytonutrients was not very high among the barberry accessions. C.V. values of TP and TMA was 16.21% and 14.85%. Antioxidant capacity of C.V. values for TEAC and FRAP was 7.13% and 17.9%. Average total antioxidant capacities were TE at 44.2 and 52.0 mmol·L^{-1} for TEAC and FRAP respectively (Table 1). These values were much higher than most of the dark red fruits documented in the literature (Moyer et al., 2002; Özgen et al., 2006; Sun et al., 2002). These results may provide evidence that barberry fruits have a strong antioxidant capacity. Since barberry fruits are high in antioxidants, consumption of these fruits should help prevent oxidative stress, and may therefore help prevent chronic diseases.

The combination and the ratio of specific sugars and organic acids have been related to flavor quality of fruits. Glucose and fructose were found to be dominant sugars in all barberry accessions analyzed. The concentrations of

Table 1. Total phenolic content (TP), Total anthocyanin (TMA), antioxidant capacity (TEAC and FRAP) of barberry accessions.

Accession[z]	TP[y] (GAE at mg·L^{-1})	TMA[x] (cy-3-glu at mg·L^{-1})	TEAC[w] (TE at mmol·L^{-1})	FRAP[v] (TE at mmol·L^{-1})
B3	2565 ± 117	506.7 ± 7.00	49.3 ± 2.2	50.3 ± 2.3
B4	2629 ± 201	624.6 ± 20.2	45.8 ± 2.2	57.4 ± 1.7
B7	2512 ± 246	617.3 ± 12.4	43.9 ± 1.0	56.3 ± 3.4
B12	3005 ± 166	702.5 ± 7.80	42.5 ± 1.9	65.6 ± 2.5
B14	3434 ± 215	737.5 ± 28.5	42.5 ± 1.5	41.1 ± 1.0
B16	3629 ± 240	803.6 ± 17.7	41.1 ± 1.0	41.0 ± 0.8
Grand Mean	2962	665.4	44.2	52
CV (%)	16.21	14.85	7.13	17.9

[z]Accession values represent triplicate means ± standard deviations from the mean; population variability is indicated by the grand mean and its associated CV (coefficient of variability) (i.e., the population standard deviation expressed as a percentage of the mean).
[y]TP contents were estimated by the Folin-Ciocalteu assay of Singleton and Rossi, 1965. Values are expressed as mg gallic acid equivalent in liter of fruit juice.
[x]TMA were determined by the pH-differential method of Giusti and Worlstad, 2005. Values are expressed as mg cy-3-glu in liter of fruit juice.
[w]TEAC values were determined by the method of Özgen et al., 2006. Values are expressed as mmol Trolox equivalent in liter of fruit juice.
[v]FRAP values were determined by the method of Benzie and Strain, 1996. Values are expressed as mmol of Trolox equivalent in liter of fruit juice.

Table 2. Mean individual sugar contents (g·100 mL^{-1}) ± standard deviation of different barberry accessions.

Accession[z]	Sugar (g·100 mL^{-1})		
	Fructose	Glucose	Total
B3	5.80 ± 0.1	8.16 ± 0.2	13.95 ± 0.2
B4	5.73 ± 0.1	8.10 ± 0.0	13.84 ± 0.1
B7	5.25 ± 0.4	7.61 ± 0.7	12.86 ± 1.0
B12	6.26 ± 0.2	9.00 ± 0.2	15.26 ± 0.5
B14	6.92 ± 0.5	10.13 ± 0.8	17.05 ± 1.3
B16	6.78 ± 0.2	10.05 ± 0.5	16.83 ± 0.7
Grand Mean	6.12	8.84	14.96
CV (%)	10.71	12.24	11.58

[z]Accession values represent triplicate means ± standard deviations from the mean; population variability is indicated by the grand mean and its associated CV (coefficient of variability) (i.e., the population standard deviation expressed as a percentage of the mean).

Table 3. Mean individual organic acid contents (g·L^{-1}) ± standard deviation of different barberry accessions.

Accession[z]	Organic Acid (g·L^{-1})				
	Tartaric Acid	Malic Acid	Ascorbic Acid	Citric Acid	Total
B3	0.52 ± 0.2	8.96 ± 1.5	0.03 ± 0.0	0.87 ± 0.3	10.37 ± 2.0
B4	0.63 ± 0.0	1.82 ± 0.1	0.03 ± 0.0	1.38 ± 0.4	3.86 ± 0.4
B7	0.69 ± 0.1	1.86 ± 0.0	0.02 ± 0.0	2.72 ± 0.5	5.30 ± 0.5
B12	0.55 ± 0.0	10.91 ± 1.2	0.03 ± 0.0	0.97 ± 0.2	12.45 ± 1.5
B14	0.62 ± 0.0	12.01 ± 0.1	0.03 ± 0.0	1.00 ± 0.1	13.67 ± 0.2
B16	0.27 ± 0.1	9.97 ± 0.9	0.03 ± 0.0	1.07 ± 0.1	11.34 ± 1.1
Grand Mean	0.55 ± 0.1	7.59 ± 0.6	0.03 ± 0.0	1.34 ± 0.3	9.50 ± 0.9
CV (%)	29.38	57.06	15.06	52.61	40.62

[z]Accession values represent triplicate means ± standard deviations from the mean; population variability is indicated by the grand mean and its associated CV (coefficient of variability) (i.e., the population standard deviation expressed as a percentage of the mean).

glucose and fructose were averaged 8.84 and 6.12 g·100 mL^{-1} respectively (Table 2). Organic acid distribution of barberries was dominated by malic acid (mean of 7.59 g·L^{-1}) followed by citric acid (mean of 1.34 g·L^{-1}) (Table 3). Both high sugar and relatively high organic acid content of the fruit have good indication of taste and flavor. Interestingly B4 and B7 displayed lower total acid 3.86 and 5.30 g·L^{-1} respectively. These values were originated from lower malic acid concentration as B4 and B7 exhibited almost 3 times less total acid concentration than other accessions. One of these accessions could be the choice of specific consumers in the future.

The fruit color characteristics and TSS content were presented in Table 4. TSS content of barberry juice was determined relatively high as averaged 20.20%. Although it is not easy to get high yield of juice from barberry fruit, TSS content of juice displayed the promising values. Variability between the accessions was not high as C.V (%) values exhibited 8.23. The L* values of barberry fruits ranged from 10.36 to 12.27 with the average of 11.37. L* values have greater variability than a* and b* values, when compared by their C.V. values (11.37% to 3.75 and 2.52%, respectively). The highest a* values obtained from accession B16 fruits with 4.94, while B14 had the highest b* values among the barberry accessions. High a* and b* values were the indication of red color. Similar pattern was observed with Chroma values while hue angle of B7 had highest values.

This study yielded information on variability of some of the antioxidant and chemical properties of barberry accessions grown in Sivas province of Turkey. The high antioxidant capacity of barberry fruit is primarily due to the high levels of TP, flavonoids such as anthocyanin and other polyphenolic compounds found in this fruit. Especially, barberry fruits with high amount of anthocyanin and phenolic content in addition to high antioxidant capacity, may increase its popularity among the other wild fruits. Within this limited study of six different accessions, variability for chemical profile and antioxidant capacity suggested its high potential of health benefits. However, more detail biological and pharmacological studies are needed for demonstration and clarification of health benefits of anthocyanin rich barberries.

Table 4. Mean and significance for brix and color parameters of different barberry accessions.

Accession[z]	TSS (%)	L*	a*	b*	Chroma	Hue
B3	20.00 ± 1.68	10.95 ± 2.79	3.71 ± 0.89	2.59 ± 0.45	4.54 ± 0.87	35.62 ± 5.79
B4	20.20 ± 0.90	11.95 ± 2.78	3.30 ± 1.00	2.52 ± 0.34	4.18 ± 0.91	38.35 ± 6.54
B7	19.27 ± 2.28	10.91 ± 2.27	3.28 ± 1.12	2.62 ± 0.29	4.25 ± 0.90	40.15 ± 9.33
B12	21.20 ± 1.43	11.75 ± 2.95	3.28 ± 0.62	2.38 ± 0.57	4.11 ± 0.46	36.30 ± 9.57
B14	21.80 ± 1.18	10.36 ± 3.11	4.00 ± 0.55	2.63 ± 0.53	4.80 ± 0.61	33.28 ± 5.28
B16	19.33 ± 1.03	12.27 ± 1.75	4.94 ± 1.25	2.37 ± 0.38	5.51 ± 1.17	26.50 ± 6.98
Grand Mean	20.20	11.37	3.75	2.52	4.57	35.03
CV (%)	8.23	22.84	28.60	17.02	20.62	23.70

[z]Accession values represent triplicate means ± standard deviations from the mean; population variability is indicated by the grand mean and its associated CV (coefficient of variability) (i.e., the population standard deviation expressed as a percentage of the mean).

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