

LESS KNOWN VACCINIUM: ANTIOXIDANT AND CHEMICAL PROPERTIES OF SELECTED CAUCASIAN WHORTLEBERRY (*Vaccinium arctostaphylos*) FRUITS NATIVE TO BLACK SEA REGION OF TURKEY

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Abstract. There is an increasing interest in the commercialization of native fruits for food and medicinal extract utilization. This study was undertaken to determine the antioxidant and chemical properties of selected caucasian whortleberry from the native region of Northeastern Turkey. Caucasian whortleberry (*Vaccinium arctostaphylos* L.), a close relative to cultivated blueberries, were harvested from various sites of Black sea region of Turkey and were analyzed for their total phenolic (TP), anthocyanin (TA) contents, and their antioxidant capacity by FRAP and TEAC assays. Specific sugar and organic acid composition were also determined by the help of HPLC. ARC-1, and ARC-6 displayed the highest TP contents (5780 and 5754 $\mu\text{g GAE g}^{-1}$ fw). The TA values were spectrophotometrically estimated and they were varied between 74.7 (ARC-6) and 194.4 (ARC-4) $\mu\text{g del-3-glu g}^{-1}$ fresh weight basis. ARC-1 had the highest amount (19.5 and 23.4 $\mu\text{mol TE g}^{-1}$ fw) of antioxidant capacity for both TEAC and FRAP assays. Fructose and glucose were found to be predominant sugars in all genotypes analyzed. The concentrations of fructose and glucose were averaged at 45.1 and 41.2 g kg^{-1} fw respectively. The organic acid distribution of berries was dominated by citric acid (mean of 9.85 g kg^{-1} fw). These results may provide evidence that Caucasian whortleberry has strong antioxidant capacity. Also, results of this study may be used for utilization of selected genotypes in the breeding studies of close relative vaccinium plants.

Key words: Anthocyanin, bioactive compounds, fruit, FRAP, phenolics, TEAC

INTRODUCTION

Vaccinium is an ancient genus of shrubs in the plant family of *Ericaceae*. Although several species of *Vaccinium* are important commercially, most production comes from the species in the section *Cyanococcus* including the highbush blueberry (*V. Corymbosum* L.), lowbush blueberry (*V. Angustifolium* Ait.), and rabbiteye blueberry (*V. ashei* R. syn. *V. Virgatum* Ait.). The other important species from *Ericaceae* family are cranberry (*V. Macrocarpon* Ait.) from the *Oxycoccus* section, lignonberry (*V. vitis idaea* L.) which belongs to the *Vitis-idaea* section, billberry (*V. myrtilillus* L.) from *Myrtilillus* section, and lastly Caucasian whortleberry (*V. arctostaphylos* L.) which belongs to the *Hemimyrtillus* section. While these crops are well known throughout the world, in many cases, their individual distributions are quite narrow. Almost all *vaccinium* plants require acidic soils and as wild plants they live in habitats such as bog and acidic woodland. The fruit of many *Vaccinium* species are edible and cultivated for commercial use. Among these, Caucasian whortleberry are known locally but often not distinguished from other *Vaccinium* species. It is naturally adapted on the hilly sides and slopes of Black sea region of Northern, particularly Northeastern Turkey (Caucasian area). It has been utilized as fresh, juice, jam, and also a medicinal plant in traditional medicine by local people [Çelik 2009].

Recently, consumers interest in *Vaccinium* species has risen due to increasing evidence for health benefits and antioxidant properties of the berries. Health benefits associated with consumption of some *Vaccinium* species have been recognized for centuries, but it is just within the past decade that anticancer, antioxidant, cardioprotective, and other bioactive properties have been scientifically demonstrated and widely appreciated [Bomser et al. 1996, Zafra-Stone et al. 2007, Monavar et al. 2011]. The anthocyanins and proanthocyanidins are some of the specific polyphenols that have been reported as the bioactive ingredients of the *Vaccinium* species [Stintzing et al. 2002].

Identification and quantification of anthocyanins, phenolics and antioxidant properties of *Vaccinium* berries are well defined [Prior et al. 1998, Moyer et al. 2002, Çelik et al. 2008]. There are numbers of detailed studies showing health benefits from the individual species. However, the studies on characterization and quantification of phytochemical and antioxidant properties of Caucasian whortleberry are very limited.

An increase in production and economic importance of Caucasian whortleberry crop has necessitated definitive selection of genotypes and analysis of their fruit pomological and phytonutrient quality. However, there were very limited studies available for earlier references regarding the selection or phytonutrient studies on these berries. Ayaz et al. [2005] determined phenolic acid composition of some Caucasian whortleberry selections. Caffeic acid was found to be the predominant phenolic acid one of the seven hydroxybenzoic acid and four hydroxy cinnamic acid derivatives determined. Results from Latti et al. [2009] indicated that the most predominant anthocyanins were delphinidin (41%), petunidin (19%) and malvidin (19%).

There is an increasing interest in the commercialization of native fruits for food or medicinal extract and the utilization in breeding studies. Less well-known species of *Vaccinium*, particularly wild plantings of Caucasian whortleberry, are limitedly distributed. This study was undertaken to determine the antioxidant and chemical properties of selected caucasian whortleberry from native region of Northeastern Turkey.

MATERIALS AND METHODS

Plant material. Wildly grown six genotypes of Caucasian whortleberry (*V. arctostaphylos*) were sampled across from Rize (northeastern part of Anatolia), Turkey (fig. 1). Mature, dark purple-black color berries were harvested and transferred to laboratory for chemical and phytochemical analysis. Fruit samples were frozen after weight and size determination. Then, about 100 g fruit samples were frozen at -40°C until analyzed. For each fruit sample, three replicates were thawed at room temperature and homogenized in a standard food blender; for each replicate, excess fruits (more than 100 individual fruits) were used to minimize possible naturally-occurring fruit to fruit variation.



Fig. 1. Caucasian whortleberry (*Vaccinium arctostaphylos* L.) plants and fruits

Determination of total phenolics (TP). TP content was determined according to Singleton and Rossi [1965] procedure. An aliquot of slurry was extracted with buffer containing acetone, water, and acetic acid (70:29.5:0.5 v/v) for 2 h in the dark. Three parallel extracts were obtained from each genotype. Then, extracted, Folin-Ciocalteu's reagent and water were incubated for 8 min, followed by adding 7% sodium carbonate. After 2 h, 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 μg gallic acid equivalent in g of fresh weight (GAE g^{-1} fw).

Total anthocyanins (TA). Total anthocyanin (TA) were estimated by a pH differential method [Giusti and Wrolstad 2005], using a UV-VIS spectrophotometer (model T60U, PG Instruments). Absorbance was measured at 543 nm and 700 nm in buffers at pH 1.0 and 4.5 using $A = (A_{533} - A_{700}) \text{ pH } 1.0 - (A_{533} - A_{700}) \text{ pH } 4.5$ with a molar extinction coefficient of 29,000. Results were expressed as μg of delphinidin-3-glucoside equivalents per g ($\mu\text{g del-3-glu g}^{-1}$ fw) fresh weight.

Determination of total antioxidant activity. The total antioxidant activity was estimated by two standard procedures, FRAP (Ferric reducing ability of plasma) and TEAC (trolox equivalent antioxidant capacity) assays, as suggested by Özgen et al. [2006]. FRAP was determined according to the method of Benzie and Strain [1996]. An 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] 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 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, 3 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. Slurries (5 g) were diluted with purified water or metaphosphoric acid (2.5%) solution for individual sugar and organic acid analysis, respectively. The homogenate was centrifuged at 10000 rpm for 10 min. Supernatants were then 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 the 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] and Özgen et al. [2009]. The separation was carried out on a SGE wakosil C18RS 5 μm column (250 \times 4.6 mm ID). Optimum efficiency of separation was obtained by using a 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°C; detection wave-

length, 215 nm. An analysis of sugars was performed according to the method described by Bartolome et al. [1995] and Gündüz and Saraçoğlu [2012] using a refractive index (RI) detector (Perkin Elmer). The separation was then 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°C with 0.9 mL/min flow rate and the sample injection volume was at 20 µL.

Statistical analysis. Means and standard deviations were obtained using PROC TABULATE. Coefficients of variation (CV) were calculated, dividing the relevant standard deviations by means and multiplied by 100. The data was analyzed using SAS procedures and software (SAS, Cary, NC, USA).

RESULTS AND DISCUSSION

Pomological characteristics. Fruit pomological characteristics of six selected genotypes are reported in Table 1. The native Caucasian whortleberry population from present study showed variability regarding berry size and shape. ARC-5 (1.19 g) had the largest size of berries follow by ARC-6 (1.05 g) and ARC-4 (0.87 g). The shape of ARC-4 berries were longer than other genotypes as the shape index value was 1.36. The other berries were mostly round in shape. ARC-5 had the highest number of berries per

Table 1. Several fruit characteristics of 6 Caucasian whortleberry genotypes selected from Rize (northeastern part of Anatolia), Turkey

Traits	ARC-1	ARC-2	ARC-3	ARC-4	ARC-5	ARC-6
Berry width (mm)	9.48 ±0.27	10.38 ±0.19	9.89 ±0.15	9.85 ±0.19	12.24 ±0.18	11.71 ±0.32
Berry length (mm)	10.52 ±0.33	11.95 ±0.28	10.95 ±0.40	13.43 ±0.45	13.17 ±0.30	11.72 ±0.25
Shape index	1.11 ±0.07	1.15 ±0.07	1.11 ±0.06	1.36 ±0.07	1.08 ±0.09	1.00 ±0.08
Berry weight (g)	0.52 ±0.04	0.77 ±0.07	0.64 ±0.05	0.87 ±0.07	1.19 ±0.06	1.05 ±0.10
Berry/cluster	5.30 ±0.42	6.00 ±0.51	5.80 ±0.26	5.80 ±0.26	7.90 ±0.54	5.50 ±0.33
Yield (g plant ⁻¹)	468.8 ±73	371.4 ±45	304.0 ±50	292.2 ±41	285.6 ±51	174.2 ±29
Surface wax	absent	absent	absent	mild	dense	absent

Values represent triplicate means ± standard deviations from the mean.

cluster (7.90). However, larger berry size and more berries in each cluster of ARC-5 did not reflect the highest yield among the 6 genotypes selected. ARC-1 was displayed the highest yield (468.78 g) but the smallest berry size (10.52 g). ARC-5 was dense and ARC-4 had a mild surface wax formation on the berries while no significant wax formation was observed on rest of the berries. All berries were deep dark purple-black color when the time of harvest. No visual difference was observed.

Antioxidant capacity. Results from this study showed that the dark color fruits of Caucasian whortleberry are one of the richest sources of anthocyanins and phenolic compounds and have a strong antioxidant capacity among small fruits (tab. 2). ARC-1 and ARC-6 were displayed the highest TP contents (5780 and 5754 $\mu\text{g GA g}^{-1}$ fw) respectively. Range of TP content was 3888–5780 $\mu\text{g GAE g}^{-1}$ fw. Although their findings are in dry weight basis, similar results for TP content was reported by Hasanloo et al. [2011] when they compared 4 different wildy grown Caucasian whortleberry genotypes in Iran (11.48–42.69 mg GAE g^{-1} dw). In another study, Ayaz et al. [2005] was quantified and characterized, phenolic acid profile of Caucasian whortleberry was determined a caffeic acid and is the predominant phenolic acid in addition to 7 other hydroxybenzoic and 4 hydroxycinnamic acid derivatives.

Table 2. Total phenolic content (TP), total anthocyanin (TA), antioxidant capacity (TEAC and FRAP) of Caucasian whortleberry genotypes

Accession	^a TP ($\mu\text{g GAE g}^{-1}$ fw)	^b TA ($\mu\text{g del-3-glu g}^{-1}$ fw)	^c TEAC ($\mu\text{mol TE g}^{-1}$ fw)	^d FRAP ($\mu\text{mol TE g}^{-1}$ fw)
ARC 1	5780 \pm 430	185.6 \pm 7.3	19.5 \pm 0.8	23.4 \pm 2.3
ARC 2	4279 \pm 332	128.2 \pm 1.9	17.7 \pm 0.6	20.8 \pm 1.8
ARC 3	3888 \pm 474	90.5 \pm 1.0	13.8 \pm 0.4	18.3 \pm 1.4
ARC 4	4637 \pm 433	194.4 \pm 4.3	15.8 \pm 0.2	20.4 \pm 1.2
ARC 5	4660 \pm 978	95.8 \pm 2.1	16.7 \pm 0.5	19.4 \pm 0.9
ARC 6	5754 \pm 832	74.7 \pm 1.6	17.1 \pm 0.7	14.9 \pm 0.9
Grand mean	4833	128.2	16.8	20.5
CV (%)	18.86	37.2	11.02	10.8

Values represent triplicate means \pm standard deviations from the mean; population variability is indicated by the grand mean and its associated coefficient of variability (i.e., the population standard deviation expressed as a percentage of the mean).

^aTP contents were estimated by the Folin-Ciocalteu assay of Singleton and Rossi [1965]. Values are expressed as $\mu\text{g GAE g}^{-1}$ fw

^bTA were determined by the pH-differential method of Giusti and Worlsted [2005]. Values are expressed as $\mu\text{g del-3-glu g}^{-1}$ fw

^cFRAP values were determined by the method of Benzie and Strain [1996]. Values are expressed as $\mu\text{mol of TE g}^{-1}$ fw

^dTEAC values were determined by the method of Özgen et al. [2006]. Values are expressed as $\mu\text{mol TE g}^{-1}$ fw

TA values spectrophotometrically estimated herein for our 6 samples were varied between 74.7 (ARC-6) and 194.4 (ARC-4) $\mu\text{g del-3-glu g}^{-1}$ fresh weight basis (tab. 2). Variability of TA content was very high among the selected Caucasian whortleberry (C.V. 37.2%). ARC-4 contained 2.6 fold higher TA values than ARC-6 genotype. TA and TP in the mature berries was primarily found in skins, but was also found in flesh. Thus, smaller berries with higher specific surface area usually have higher TA

and TP. This may be the one reason that ARC-6 has the lowest amount of TA with the largest berry size among the other genotypes. In addition to fruit size, skin thickness, and light condition, other ecological factors might also influence TA, TP and antioxidant activity when comparing different genotypes. Results from Latti et al. [2009] indicated that the most predominant anthocyanins in this species were delphinidin (41%), petunidin (19%), and malvidin (19%). Also, similar conclusion was raised from Caucasian whortleberry samples originated from Iran [Nickavar and Amin 2004].

The antioxidant potential among Caucasian whortleberry samples in our study averaged 16.8 and 20.5 $\mu\text{mol TE g}^{-1}$ on a fresh weight basis as determined by the TEAC and FRAP methods respectively (tab. 2). The variability of antioxidant capacity was not very high among the genotypes. C.V. values of TEAC and FRAP was 11.02% and 10.8%. ARC-1 had the highest amount (19.5 and 23.4 $\mu\text{mol TE g}^{-1}$ fw) of antioxidant capacity for both TEAC and FRAP assays. This is closely related to ARC-1 having the highest amount TP and rich TA content. These values were higher than most of the berries documented in the literature [Moyer et al. 2002, Sun et al. 2002]. These results may provide evidence that Caucasian whortleberry has strong antioxidant capacity. In general, berry crops exhibit elevated antioxidant capacities and are effective scavengers of several reactive oxygen species primarily due to the high levels of phenolics, flavonoids and other polyphenolic compounds.

Table 3. Mean individual specific sugar contents (g kg^{-1}) \pm standard deviation of different Caucasian whortleberry genotypes

Accession	Sugar (g kg^{-1})		
	fructose	glucose	total
ARC 1	38.3 \pm 0.4	30.9 \pm 0.6	69.2 \pm 0.6
ARC 2	47.0 \pm 0.6	37.7 \pm 0.7	84.7 \pm 1.4
ARC 3	33.0 \pm 1.1	27.0 \pm 1.1	60.0 \pm 2.4
ARC 4	48.6 \pm 0.6	46.2 \pm 2.1	98.4 \pm 2.8
ARC 5	58.2 \pm 1.1	60.6 \pm 1.5	118.8 \pm 2.1
ARC 6	45.2 \pm 0.9	44.7 \pm 1.1	89.9 \pm 1.4
Grand mean	45.1 \pm 6	41.2 \pm 8	86.8 \pm 2.1
CV (%)	19.46	29.44	23.99

Values represent triplicate means \pm standard deviations from the mean; population variability is indicated by the grand mean and its associated coefficient of variability (i.e., the population standard deviation expressed as a percentage of the mean)

Specific sugars and organic acids. The combination and the ratio of specific sugars and organic acids have been related to flavor quality of fruits. Fructose and glucose were found to be predominant sugars in all genotypes analyzed (tab. 3). The concentrations of fructose and glucose were averaged at 45.1 and 41.2 g kg^{-1} fw respectively. Organic acid distribution of berries was dominated by the citric acid (mean of 9.85 g kg fw^{-1})

Table 4. Mean individual organic acid contents (g kg^{-1}) \pm standard deviation of different Caucasian whortleberry genotypes

Accession	Organic Acid (g kg^{-1})				
	tartaric acid	malic acid	ascorbic acid	citric acid	total
ARC 1	3.19 \pm 0.15	0.42 \pm 0.05	0.09 \pm 0.0	10.93 \pm 0.45	14.63 \pm 0.65
ARC 2	2.77 \pm 0.04	0.12 \pm 0.03	0.07 \pm 0.0	8.36 \pm 0.02	11.31 \pm 0.03
ARC 3	3.40 \pm 0.08	0.34 \pm 0.02	0.10 \pm 0.0	8.56 \pm 0.58	12.40 \pm 0.68
ARC 4	3.77 \pm 0.04	0.34 \pm 0.05	0.05 \pm 0.0	14.20 \pm 0.49	18.35 \pm 0.43
ARC 5	3.02 \pm 0.05	0.53 \pm 0.15	0.04 \pm 0.0	8.88 \pm 0.36	12.47 \pm 0.18
ARC 6	3.34 \pm 0.06	0.03 \pm 0.00	0.16 \pm 0.0	8.16 \pm 0.12	11.68 \pm 0.13
Grand mean	3.25 \pm 0.34	0.29 \pm 0.19	0.08 \pm 0.04	9.85 \pm 2.36	13.81 \pm 2.63
CV (%)	10.50	65.51	51.82	23.92	19.02

Values represent triplicate means \pm standard deviations from the mean; population variability is indicated by the grand mean and its associated coefficient of variability (i.e., the population standard deviation expressed as a percentage of the mean)

and followed by tartaric acid (mean of 3.25 g kg^{-1} fw) (tab. 4). Both high sugar and relatively high organic acid content of fruit have good indication of taste and flavor. ARC-4 genotype with 18.35 g kg^{-1} fw displayed the highest total acid concentration.

CONCLUSIONS

This study yielded information on variability of some of the antioxidant and chemical properties of Caucasian whortleberry genotypes grown in the Blacksea region of Turkey. There are wide genetical differences within the same species, since they propagate by seeds over hundreds of years. These results may provide evidence that Caucasian whortleberry has strong antioxidant capacity. Since these berries are high in antioxidants, consumption of these berries should help prevent oxidative stress, and may therefore help prevent chronic diseases. Especially, the less well-known species of *Vaccinium*, including the wild plants of caucasian whortleberry. With high antioxidant capacity, this may increase its popularity among the other *vaccinium* species. Results of this study may be also used for utilization of selected genotypes in breeding studies of close relative *vaccinium* plants. Infact, Ehlenfeldt and Ballington [2012] pointed out that Caucasian whortleberry is important and closely allied species to *Vaccinium* gene pool as a breeding material.

Within this limited study of six different genotypes, the variability for chemical profile and antioxidant capacity suggests its high potential of health benefits. However, more detail biological and pharmacological studies are needed for demonstration and clarification of health benefits of Caucasian whortleberry.

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MNIEJ ZNANA BORÓWKA: WŁAŚCIWOŚCI ANTYOKSYDACYJNE I CHEMICZNE WYBRANYCH OWOCÓW BORÓWKI KAUKASKIEJ (*Vaccinium arctostaphylos*) Z REJONU MORZA CZARNEGO W TURCJI

Streszczenie. Obserwuje się rosnące zainteresowanie handlem rodzimymi owocami przeznaczonym do spożywania i użytku w medycynie. Niniejsze badanie podjęto w celu określenia antyoksydacyjnych i chemicznych właściwości wybranych borówek kaukaskich z północno-wschodniego regionu Turcji. Borówki kaukaskie (*Vaccinium arctostaphylos*), bliski kuzyn borówek hodowlanych, zostały zebrane w różnych miejscach rejonu czarnomorskiego Turcji i zostały zanalizowane pod kątem całkowitej zawartości fenoli (TP), antocyjanin (TA) oraz ich zdolności antyutleniających za pomocą testów FRAP i TEAC. Oznaczono też skład cukrów i kwasów organicznych za pomocą HPLC. ARC-1 oraz ARC-6 wykazywały największą zawartość TP (5780 i 5754 $\mu\text{g GAE g}^{-1}$ świeżej masy). Wartości TP oszacowano metodą spektrofotometryczną. Mieściły się w zakresie 74,7 (ARC-6) i 194,4 (ARC-4) $\mu\text{g del-3-glu g}^{-1}$ świeżej masy. ARC-1 miał największą (19,5 i 23,4 $\mu\text{mol TE g}^{-1}$ świeżej masy) zdolność antyoksydacyjną w testach TEAC I FRAP. Stwierdzono, że fruktoza i glukoza były dominującymi cukrami we wszystkich analizowanych genotypach. Stężenia fruktozy i glukozy wynosiły odpowiednio średnio 45,1 i 41,2 g kg^{-1} świeżej masy. Zawartość organicznych kwasów w borówkach była zdominowana przez kwas cytrynowy (średnio 9,85 g kg^{-1} świeżej masy). Wyniki te mogą dowodzić, że borówka kaukaska ma silne właściwości antyoksydacyjne oraz mogą sugerować użycie wybranych genotypów w badaniach nad hodowlą roślin pokrewnych.

Słowa kluczowe: antocyjaniny, związki bioaktywne, owoce, FRAP, fenole, TEAC

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