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Some selected physico-chemical characteristics of wild and cultivated blackberry fruits (*Rubus fruticosus* L.) from Turkey

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Abstract

In this study, some selected physico-chemical properties such as fruit weight, fruit length, fruit width, total soluble solids (TSS), titratable acidity, TSS/acidity ratio, pH, total phenolic content, antioxidant activity and free radical scavenging capacity of 9 cultivated and 16 selected wild blackberry (Rubus fruticosus L.) genotypes grown in Turkey were investigated. The total phenolic content, antioxidant activity and free radical-scavenging capacity of blackberry cultivars and genotypes were determined by using Folin–Ciocalteu, β-carotene bleaching and DPPH radical assays. The results showed that, average fruit weight and fruit dimensions were higher in cultivated blackberries than wild materials. However, TSS, acidity and pH values were higher in wild materials. The total phenolic contents of blackberry cultivars and wild genotypes were in a range of 584 (cv. Bartin) to 788 (cv. Chester) mg/100 g and 610 (Genotypr R2) to 1455 mg/100 g (Genotype R16), expressed as gallic acid equivalents (GAE), on a fresh weight basis. Antioxidant activity of cultivated and wild growing blackberry fruits was found between 72.15 (cv. Arapaho)-89.75% (cv. Bursa 3) and 59.85 (R1)-87.42% (R10), respectively. The antioxidant activity of standard BHA was 85.07%. Different cultivars grown in same location consistently showed differences in antioxidant capacity. The results of this study outlines that the blackberry fruits tested are good sources of natural antioxidants.

Keywords**:** Antioxidants, blackberries, total phenolics, wild material

Introduction

Turkey, situated between Asia and Europe, is a very important area for plant genetic resources and plant diversity. Its rich biological diversity, in particular for wild edible fruits including wild blackberries, is a result of the extreme variations in climate within a very small area and varying altitudes of the valleys where the difference between the lowest and the highest point can reach up to 3,000 meters [1].

Consumption of fresh and frozen blackberries has increased in the past few years in Turkey. In search for alternative crops for farmers, blackberry appears as a potential crop of high market value. Turkey is one of the origins of blackberries and blackberry growing can be done in all parts of Turkey where irrigation is possible. Blackberry cultivation started in the

Marmara region several decades ago and now has been introduced as a new crop in the Mediterranean region [2].

A large number of epidemiological studies provide convincing evidence of the beneficial role of fruits and vegetables in the diet for the maintenance of health and the prevention of degenerative diseases, indicating an association between diets rich in fresh fruits and vegetables and a decreased risk of cardiovascular disease and certain forms of cancer [3,4,5].This protection has been attributed to the fact that these foods may provide an optimal mix of phytochemicals, such as antioxidants, fibre and other bioactive compounds [3]. The phytochemicals in plant tissues responsible for the antioxidant capacity are thought to be mainly vitamins C, flavonoids and the other phenolics [6]. The antioxidant activity of phenols is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, singlet oxygen quenchers and metal chelators [7].

Among fruits, berries constitute a good source of natural antioxidant substances [8]. Extracts of berries from various blackberry, raspberry and gooseberry cultivars act effectively as free radical inhibitors [9]. Berry crops, specifically blackberry fruits, are significant sources of polyphenolic compounds in the human diet [10]. The antioxidant activity of fruits and vegetables varies considerably. Differences gauged between cultivars may be explained by genotype [11, 12], growing temperature [13], growing season, maturity at harvest, environmental stress, and other factors [14].

Accumulating evidence exists, suggesting that in particular genotype may have a profound influence on the content of bioactive compounds in berries [8,15]. However, scientific information on antioxidant properties of various berries, particularly those that are less widely used in culinary and medicine is still scarce. Therefore, the assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants, functional foods and nutraceuticals [16].

There are studies on some fruit characteristics such as vitamin C, pH, acidity, total soluble solids, aroma profiles etc. in cultivated blackberries; however, no available information concerning the antioxidant activity, total phenolic content and free radical-scavenging capacity of cultivated and wild blackberry fruits (*R. fructicosus*) has been done in Turkey. Therefore, in the present study an attempt has been made to know the variability in physico-chemical characteristics of wild and cultivated blackberries.

2. Materials and Methods

2.1. Collection of blackberry fruits

Blackberry (*Rubus fruticosus* L) fruits of nine cultivars (Chester, Jumbo, Ness, Bartin, Bursa 2, Bursa 3, Navaho, Arapaho and Bursa 1) were grown together at the Fruit Research Station, Malatya. The station is located on 38° 19' 28" N and 038° 17' 11" E with the elevation of 1002 m. Plants were grown in rows in a single block and were trained to a three-wire trellis. Wild blackberry genotypes were grown on a diverse environment covering the most of the southern Turkey. The exact sampling locations of the genotypes were presented in Table 1. The wild genotypes were pre selected according to their higher yield capacity, attractive fruit properties and free of pest and disease characters. Those selections are named as R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11, R12, R13, R14, R15 and R16. The both cultivated and wild fruits were harvested at commercial maturity stage. The cultivars were

harvested at 26 July 2007 and wild materials harvested between 26 August to 5 September 2007. The fruits were selected according to uniformity of shape and color and then transported to laboratory for analysis. Samples divided into two groups and first groups of fruits used for fruit dimensions and fruit weight, pH, total soluble solids and titratable acidity analysis. The other group was dried at 45^0C in an oven in laboratory and ground to a fine powder with a mortar and pestle and kept at room temperature prior to extraction. The dried samples were packed into new plastic bags and stored in a dessicator for a maximum of 3 days until antioxidant activity, total phenolic content and free radical scavenging analysis.

Table 1. Sampling locations of the wild blackberry genotypes used in the study.

Source	Latitude	Longitude	Altitude (m)
R1	37° 23' 14"	031° 52' 07"	1103
R2	36° 43' 57"	031° 36' 41"	71
R3	36° 52' 13"	038° 32' 28"	5
R4	37° 53' 00"	030° 42' 56"	953
R5	38° 11' 18"	031°08' 17"	102
R6	37° 11' 18"	032° 13' 23"	1171
R7	36° 47' 21"	033° 20' 30"	1064
R8	36° 23' 12"	033° 58' 48"	6
R9	36° 12' 01"	035° 44' 08"	221
R10	36° 47' 38"	036° 12' 30"	24
R11	38° 19' 27"	038° 17' 46"	1043
R12	38° 19' 30"	038° 16' 18"	964
R13	38° 24' 42"	038° 12' 38"	789
R14	37° 45' 05"	035° 00' 46"	1365
R15	36° 36' 54"	034° 19' 21"	2
R16	38° 00' 18"	036° 29' 11"	1338

2.2. Determination of fruit weight, dimensions, total soluble solids, pH and acidity of blackberry fruits

Fifty fruits from each cultivar/genotype were used for analysis. Fruit weight was measured by using a digital balance with a sensitivity of 0.001 g (Scaltec SPB31). Linear dimensions of fruits as length and width were measured by using a digital calliper gauge with a sensitivity of 0.01 mm. Total soluble solid contents (TSS) were determined by extracting and mixing one drop of juice from each fruit into a digital refractometer (Model RA-250HE, Kyoto Electronics Manufacturing Co. Ltd., Japan,) at 22^0C. The pH measurements were made using a digital pH meter (WTW Inolab Level 1, Germany) calibrated with pH 4 and 7 buffers. Titratable acidity (TAc) was measured by the titrimetric method. Titratable acidity of blackberry was expressed as % citric acid. TSS/TAc results are also determined.

2.3. Preparation of the methanol extracts

The sample weighing about 100g was extracted in a soxhlet with methanol (MeOH) at 60^0C for 6 h. The extract was then filtered and concentrated in vacuo at 45^0C. Finally, the extracts were then lyophilized and kept in the dark at $+4^0$C until tested.

2.4. Determination of total phenolic, antioxidant activity and radical scavenging capacity in blackberry fruits

In β-Carotene–linoleic acid assay, antioxidant capacity of wild and cultivated blackberry fruits is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [17].

A stock solution of β-carotene/linoleic acid (Sigma–Aldrich) was prepared as follows. First, 0.5 mg of β-carotene was dissolved in 1 ml of chloroform (HPLC grade), then 25 µl of linoleic acid and 200 mg of Tween 40 (Merck) were added. The chloroform was subsequently evaporated using a vacuum evaporator. Then 100 ml of distilled water saturated with oxygen (30 min at 100 ml/min) was added with vigorous shaking. Aliquots (2.5 ml) of this reaction mixture were transferred to test tubes, and 350 µl portions of the extracts (2 g/l in ethanol) were added before incubating for 48 h at room temperature. The same procedure was repeated with butylated hydroxyanisole (BHA) at the same concentration and a blank containing only 350 µl of ethanol. After the incubation period the absorbance of the mixtures were measured at 490 nm. Antioxidant capacities of the samples were compared to those of BHA and the blank.

Total soluble phenolics in the fruit extracts were determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton [18] using gallic acid as a standard. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of fresh weight (FW).

Determination of the radical scavenging activity 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) has been widely used to evaluate the free radical scavenging activity of natural antioxidants [19].

In this study, increasing aliquots of each methanol/HCl 2% extract (3 replicates) were mixed with a methanolic solution of $DPPH^{\bullet}$ (1 m*M*, 300 µl) in 4-ml cuvettes and brought to 3.0 ml with methanol. To eliminate the interference of the extract pigments on the $DPPH^{\bullet}$ reaction, blanks of the fruit extracts were performed using 300 µl methanol instead of the $DPPH^{\bullet}$ solution. After incubation in the dark at room temperature for 15 min, the spectrophotometric determination was assayed at 517 nm using a UV spectrophotometer (Nicolet 100 UK). A $DPPH^{\bullet}$ blank sample (containing 2.7 ml of methanol and 300 µl of $DPPH^{\bullet}$ solution) was prepared and measured daily. The $DPPH^{\bullet}$ solution was freshly prepared daily, stored in a flask covered with aluminum foil, and kept in the dark at 4°C between measurements. The percent decrease in absorbance was recorded for each concentration, and percent quenching of $DPPH^{\bullet}$ radical was calculated on the basis of the observed decrease in absorbance of the radical. Percent inhibition/µl of extract change curves were used to find the concentration at which 50% radical scavenging occurred (EC_{50}).

Percent inhibition was calculated according to the formula:

% inhibition = $[(A_{DPPH} - A_{Extr})/A_{DPPH})] \times 100$ where A_{DPPH} is the absorbance value of the $DPPH^{\bullet}$ blank sample and A_{Extr} is the absorbance value of the test solution. A_{Extr} was evaluated as the difference between the absorbance value of the test solution and the absorbance value of its blank. The EC_{50} values are reported as mg FW in Table 4.

2.5. Statistical analyses

Fruit characteristics data as well as antioxidant activity, total phenolic content and radical scavenging activity were analyzed using SAS procedures. Analysis of variance

(ANOVA) tables were constructed using GLM procedure and the means and standard deviations were calculated using TABULATE. ANOVAs included two factors group (cultivar vs. wild genotypes) and accessions which were nested in group. Both factors were treated as random; thus, a random effect model was used. To evaluate overall similarities of the accessions, they are subjected to principle component analysis using PRINCOMP procedure. In this analysis, the relationships are developed from a covariance matrix derived from standardized morphological fruit characteristics means. The output data sets consisted of eigenvalues, eigenvectors, and standardized principal component scores. The eigenvalues are presented in Table 2 and the genotypes were plotted using their standardized principal component scores in Figure 1.

Table 2. Results of principle components analysis of fruit characteristics for blackberry cultivars and wild genotypes from southern Turkey.

Source	PC 1	PC 2	PC 3
Fruit weight (g)	0.43	0.02	0.04
Fruit width (mm)	0.42	0.25	0.29
Fruit length (mm)	0.44	0.21	0.29
Total soluble solids (TSS)	-0.37	-0.26	0.78
Titratable acidity (TAc)	0.11	-0.74	0.11
TSS/TAc	-0.33	0.52	0.35
pH	-0.43	0.11	-0.29
Eigenvalue	4.50	1.68	0.43
Difference	2.82	1.26	0.20
Proportion	0.64	0.24	0.06
Cumulative	0.64	0.88	0.95

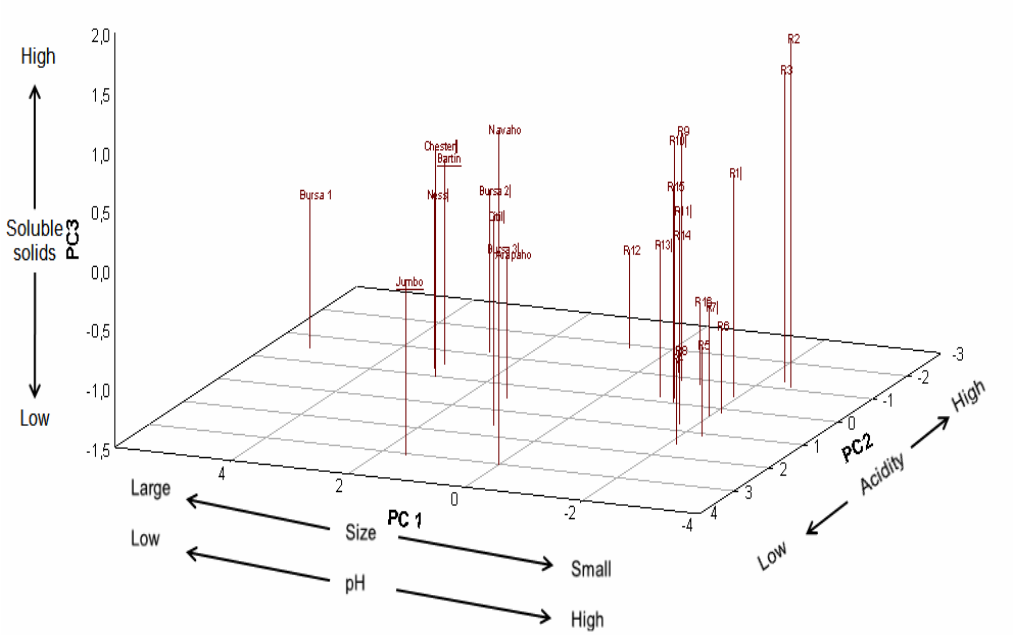


Figure 1. Principle component (PC) analysis plot of the first three PCs calculated from fruit characteristics for blackberry cultivars and wild genotypes from Southern Turkey

3. Results and Discussion

3.1. Fruit weight, dimensions, total soluble solids, pH and acidity of blackberry fruits

The fruit weight, fruit dimensions, TSS (Total soluble solids), pH and titratable acidity (TAc) contents of wild and cultivated blackberry fruits are given in Table 3. Statistically significant differences were recovered between the means of the cultivars and wild genotypes for all the traits tested except acidity and total soluble solid/titratable acidity ratio (Table 3). Fruit weight of blackberry cultivars ranged between 1.2 g (cv. Arapaho) and 5.4 g, with cv. Bursa 1 having the biggest fruits. However, the average fruit weight of wild genotypes were from 0.4 g (R3) to 1.2 g (R12). The accessions were significantly different for all fruit characteristics. The mean fruit weight was higher for the cultivars when compared to those of wild genotypes. The same trend was present for the fruit width and length variables. Indeed, on average, the cultivar had 72% heavier, 29% wider and 49% longer fruits. Among the cultivars, Bursa 1 had heaviest and longest berries while the widest berries were sampled from cv. Chester. For fruit weight and width, the smallest means were from cv. Arapaho while the shortest berries were from cvs. Bursa 2 and Bursa 3. For the wild genotypes, the greatest means were recorded in R12 genotype for all three fruit-size traits.

Total Soluble solids (TSS) were higher in wild genotypes than the cultivars. The overall means were 16.2% vs. 11.6% with the ranges of 12.9-22.3% and 8.6–14.1%. The wild genotypes R2 and R3 had total soluble solid means higher than 20%. Among all accessions titratable acidity (TAc) ranged from 0.5 to 1.5 with the overall average of 1.1. The overall means for TSS/TAc was 14.5 and the lowest and highest means were from the cultivars (6.6 for cv. Bursa 1 and 22.5 for cv. Bursa 3). The pH means of the wild genotypes were slightly but significantly higher than the cultivars. When all the accessions were considered pH average was the lowest in Bursa 1 the greatest fro R4, R5 and R7.

The results of the principle component (PC) analysis clearly separated the cultivars and wild genotypes (Figure 1). The first three PC accounted 64%, 24% and 6% of the total variation making a total of 95% (Table 2). The PC1 mainly consisted of fruit weight, width and length and pH while acidity and soluble solids were the most important traits constructing PC2 and PC3, respectively.

Table 3. Means, standard deviations and significances of fruit characteristics for blackberry cultivars and wild genotypes from Turkey.

Source	Fruit weight (g)	Fruit width (mm)	Fruit length (mm)	Soluble solids (SS)	Acidity (A)	SS/A	pH
Cultivar							
Arapaho	1.2 ± 0.50	16.4 ± 0.83	18.9 ± 0.94	11.7 ± 5.72	1.0 ± 0.01	11.9 ± 5.72	3.4 ± 0.02
Bartin	3.2 ± 0.36	16.9 ± 0.53	19.8 ± 1.18	12.0 ± 0.35	1.2 ± 0.04	9.9 ± 0.63	3.1 ± 0.07
Bursa 1	5.4 ± 0.59	18.0 ± 0.32	26.4 ± 1.28	8.6 ± 0.46	1.3 ± 0.09	6.6 ± 0.26	3.0 ± 0.06
Bursa 2	2.6 ± 0.44	17.0 ± 0.19	17.1 ± 0.76	11.7 ± 0.70	1.4 ± 0.13	8.6 ± 0.88	3.2 ± 0.05
Bursa 3	1.5 ± 0.35	16.6 ± 1.02	17.0 ± 1.24	12.3 ± 0.61	0.5 ± 0.03	22.5 ± 1.73	3.6 ± 0.14
Chester	2.6 ± 0.36	19.8 ± 0.82	22.3 ± 1.31	12.3 ± 0.50	1.2 ± 0.05	10.3 ± 0.83	3.3 ± 0.17
Jumbo	2.0 ± 0.68	18.2 ± 0.73	19.7 ± 1.04	9.7 ± 0.46	0.5 ± 0.01	19.2 ± 0.57	3.3 ± 0.02
Navaho	1.5 ± 0.69	17.5 ± 0.43	19.4 ± 1.28	14.1 ± 0.79	1.0 ± 0.06	14.2 ± 0.06	3.3 ± 0.04
Ness	3.1 ± 0.88	17.4 ± 1.57	19.7 ± 2.63	11.1 ± 0.23	1.2 ± 0.06	9.4 ± 0.39	3.1 ± 0.03
Mean	2.4 ± 1.32	17.4 ± 1.21	19.8 ± 2.89	11.6 ± 2.15	1.0 ± 0.30	12.9 ± 4.37	3.3 ± 0.19
Wild							
R1	0.6 ± 0.07	10.0 ± 0.24	8.6 ± 0.14	18.2 ± 0.67	1.1 ± 0.11	16.8 ± 2.10	3.7 ± 0.06
R2	0.6 ± 0.10	9.9 ± 0.89	9.6 ± 1.03	22.3 ± 1.53	1.3 ± 0.05	17.9 ± 0.92	3.8 ± 0.07
R3	0.4 ± 0.02	9.8 ± 0.07	8.5 ± 0.26	21.3 ± 1.10	1.3 ± 0.06	16.8 ± 0.91	3.7 ± 0.10
R4	0.9 ± 0.04	10.9 ± 0.84	8.9 ± 0.36	14.1 ± 1.01	0.7 ± 0.04	19.9 ± 1.79	4.0 ± 0.06
R5	0.7 ± 0.02	9.9 ± 0.22	7.9 ± 0.21	14.7 ± 0.58	0.8 ± 0.02	19.2 ± 0.83	4.0 ± 0.11
R6	0.6 ± 0.03	9.4 ± 0.30	7.8 ± 0.21	15.2 ± 0.71	0.9 ± 0.03	16.4 ± 1.28	3.9 ± 0.03
R7	0.7 ± 0.04	10.3 ± 0.54	9.0 ± 0.09	15.5 ± 0.50	0.9 ± 0.07	16.8 ± 1.81	4.0 ± 0.06
R8	0.9 ± 0.03	11.0 ± 0.48	9.1 ± 0.55	12.9 ± 0.91	1.2 ± 0.20	10.5 ± 1.99	3.7 ± 0.09
R9	1.1 ± 0.15	12.4 ± 0.53	11.0 ± 0.69	17.7 ± 0.64	1.2 ± 0.05	14.5 ± 0.48	3.6 ± 0.05
R10	1.1 ± 0.15	13.0 ± 0.53	10.4 ± 0.08	17.8 ± 0.69	1.1 ± 0.09	16.5 ± 1.67	3.6 ± 0.12
R11	0.7 ± 0.06	11.2 ± 0.28	9.4 ± 0.30	14.6 ± 0.59	1.5 ± 0.09	9.9 ± 0.91	3.3 ± 0.04
R12	1.2 ± 0.31	13.0 ± 0.88	11.4 ± 0.81	13.0 ± 0.00	1.5 ± 0.07	8.9 ± 0.43	3.4 ± 0.03
R13	0.8 ± 0.06	11.3 ± 0.33	9.3 ± 0.28	15.0 ± 0.05	1.0 ± 0.02	15.0 ± 0.24	3.5 ± 0.06
R14	0.7 ± 0.12	11.6 ± 0.75	9.4 ± 0.50	16.1 ± 0.22	0.9 ± 0.02	18.8 ± 0.49	3.7 ± 0.03
R15	0.9 ± 0.10	11.5 ± 0.52	10.2 ± 0.47	16.7 ± 0.61	1.0 ± 0.03	16.5 ± 1.13	3.5 ± 0.04
R16	0.7 ± 0.04	10.2 ± 1.30	7.8 ± 0.85	14.5 ± 0.42	1.1 ± 0.06	12.9 ± 0.83	3.7 ± 0.05
Mean	0.8 ± 1.16	11.0 ± 1.22	9.3 ± 1.13	16.2 ± 2.70	1.1 ± 0.23	15.5 ± 3.41	3.7 ± 0.21
Overall mean	1.4 ± 1.16	13.5 ± 3.38	13.3 ± 5.55	14.5 ± 3.36	1.1 ± 0.26	14.5 ± 4.37	3.5 ± 0.29

Fruit weight, TSS, pH and acidity of fruit of blackberry cultivars grown in a different region of Turkey were previously reported between 2.0-6.6 g, 8.98-20.2 %, 3.3-3.6, and 1.0-3.1%, respectively [20, 21, 22]. Fruit weight, TSS, pH and acidity of fruit of wild growing blackberries in Turkey were between 1.5-2.1 g, 11.3-13.1%, 3.33-3.35 and 0.7-1.0%, respectively [23]. Our fruit weight, TSS, pH and acidity results in general were within the limits of these studies. The variation of fruit weight, TSS, pH and acidity in blackberry fruits could be due to different cultivars used, environmental conditions and the nutritional status of the plantations as well.

3.2. Total phenolic, antioxidant activity and radical scavenging activity in blackberry fruits

Folin-Ciocalteau's method allows the estimation of all flavonoids, anthocyanins, and nonflavonoid phenolic compounds, that is, of all the phenolics present in the samples. Table 4 reports the amounts of total phenolic content quantified in each cultivar and wild genotypes of blackberries.

The total phenolic contents of the fresh blackberry cultivars per 100 g ranged from 584.0 mg GAE in cv. Bartin fruit to 788.0 mg GAE in cv. Chester fruit (Table 4). The average total phenolic content of nine blackberry cultivars was 758.0 mg GAE/ 100 g fresh weight. On the other hand, total phenolic content of wild materials were between 610 mg (R2) to 1455 mg (R16) GAE per 100 g fresh weight. The average total phenolic content of wild genotypes was 951.3 mg GAE/100 g fresh weight indicating higher value than cultivars. The results for total phenolics clearly showed that there were wide variations both cultivar and in particular wild genotypes (Table 4). Earlier, total phenolic content in blackberry cultivars and wild genotypes were reported from 230-978 mg GAE/100 g fresh weight basis. Costantino et al. [24] indicated that plant genotype strongly affects total phenolic content in blackberries. Our results are within the range of the values reported above literatures.

Table 4. Antioxidant activity, total phenolic content and free radical scavenging capacity of blackberry cultivars and genotypes

Source	Total phenolic content mg GAE/100 g FW	Antioxidant activity (%)	Radical scavenging capacity EC_{50} (mg)[a]
Cultivar			
Arapaho	772.7 ± 34.5	72.1 ± 0.9	5.8 ± 0.1
Bartin	584.0 ± 24.0	79.2 ± 0.8	6.6 ± 0.0
Bursa 1	709.7 ± 21.0	75.7 ± 0.7	6.2 ± 0.2
Bursa 2	713.3 ± 10.5	81.7 ± 1.7	7.0 ± 0.1
Bursa 3	748.3 ± 30.5	89.4 ± 0.4	7.7 ± 0.2
Chester	788.0 ± 38.5	85.6 ± 2.0	8.7 ± 0.1
Jumbo	762.0 ± 12.0	82.3 ± 0.2	7.9 ± 0.4
Navaho	730.7 ± 10.5	79.7 ± 0.1	7.2 ± 0.2
Ness	782.0 ± 52.0	84.8 ± 1.0	8.5 ± 0.2
Mean	758.0 ± 99.6	81.9 ± 5.4	7.4 ± 1.0
Genotype			
R1	673.0 ± 34.6	59.8 ± 0.5	6.7 ± 0.1
R2	610.0 ± 21.5	70.0 ± 4.0	6.4 ± 0.5
R3	1364 ± 105.0	79.9 ± 2.7	9.7 ± 0.2
R4	1283 ± 96.0	80.3 ± 2.6	9.5 ± 0.1
R5	1206.3 ± 15.5	77.5 ± 1.4	9.3 ± 0.4

R6	833.7 ± 23.5	67.5 ± 1.0	6.7 ± 0.1
R7	1209.0 ± 110.5	77.4 ± 0.4	9.2 ± 0.3
R8	851.3 ± 11.5	65.6 ± 2.3	6.8 ± 0.8
R9	1132.0 ± 26.6	71.9 ± 1.8	8.1 ± 0.3
R10	1312.3 ± 14.0	87.4 ± 0.1	9.6 ± 0.3
R11	987.3 ± 86.0	74.9 ± 3.0	7.4 ± 0.2
R12	1066.7 ± 26.5	76.9 ± 2.8	7.2 ± 0.4
R13	1328.3 ± 115.1	85.6 ± 1.2	9.7 ± 0.2
R14	1109.7 ± 9.5	83.3 ± 0.3	7.7 ± 0.1
R15	731.0 ± 21.0	72.8 ± 0.3	6.8 ± 0.1
R16	1455.3 ± 29.0	88.7 ± 2.3	9.8 ± 0.1
Mean	1072.1 ± 262.9	76.2 ± 8.1	8.2 ± 1.3
Overall mean	951.3 ± 263.8	78.4 ± 7.6	7.9 ± 1.3

[a] Radical scavenging activity expressed as EC_{50} (value defined as mg of fruit required to decrease the initial $DPPH^{\bullet}$ concentration by 50%)

It was previously reported that wild blackberry germplasm had a higher total phenolic content than cultivated blackberries [25]. This phenomenon could be due to an induction in the synthesis of antioxidant enzymes and an increase in polyphenolic concentration brought about due to the greater exposure of the unsheltered wild plants to extremes of temperature, and insult by pests and disease organisms, because phenolic compound synthesis is typically a defensive mechanism.

It is well-known that phenolic compounds contribute to fruit quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing health-beneficial effects [26].

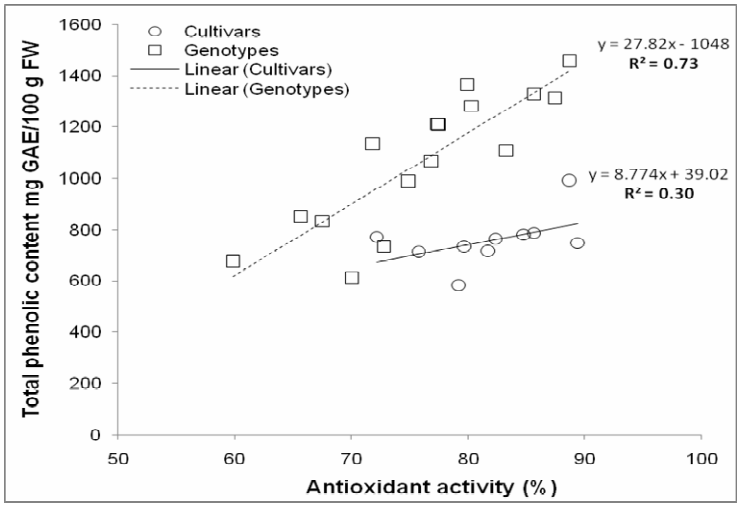
The antioxidant activity in nine cultivar and sixteen wild blackberry genotypes is shown in Table 4. The antioxidant activity of BHA was 85.07%. In general, all of the studied cultivated and wild blackberry fruits had relatively high antioxidant capacity. The average values of antioxidant activity of cultivars and wild materials were 81.9% and 78.4% which indicating very close value to standard BHA (85.07%). Even some cultivars such as Bursa 3 (89.4%), Chester (85.6%) and wild materials R16 (88.7%), R10 (87.4%) and R13 (85.6%) had higher antioxidant activity than standard BHA (85.07%) (Table 4). These results agree with those previously reported for blackberries in which a good antioxidant capacity [9]. However our results were not comparable with other authors because of the method used. It was previously reported that the genotypes effects antioxidant capacity in different fruit species such as strawberries [27] and blueberries [28]. According to our results wild and cultivated blackberries may be good sources of natural antioxidants. The great difference among blackberry cultivars in terms of antioxidant activity is supposed to be largely due to the genetic effect because all plants were grown in the same ecological condition. However, the wide variation occurred among wild materials could be explain not only genetic background but also environmental effect because wild material sampled different agro ecological locations.

Many berry fruit have high concentrations of phenolic acids, anthocyanins, some flavonols, and other phenolic classes, which have antioxidant activity in vitro [7]. Vitamin C, foliate, and carotenoids, which are nonphenolic compounds, possess antioxidant activity as well, and while they are found in high concentration in some fruit, their concentrations in

blackberry are not high compared to other fruit such as rose hip, cornelian cherry, kiwifruit etc. and they probably do not contribute appreciably to the antioxidant activity; however, we did not determine these compounds in our genotypes. Previously it has been reported that blackberries contain high amounts of cyanidin glycosides, a strong antioxidant [29].

Total phenolic content and antioxidant activity of both wild and cultivated blackberries showed positive relationship (r=0.86 for wild genotypes and r=0.46 for cultivars) (Figure 2). However, the relationships between two parameters were stronger in wild genotypes rather than cultivars. Phenolic compounds are considered to be the group of compounds that contribute most to antioxidant activity of fruits and vegetables. The results of this study demonstrated quite clearly that the antioxidant activity of crude extracts of blackberry fruits can be expressed in terms of their phenolic content. This positive relationship previously has been found in blackberries [25].

Various phytochemical components, such as flavonoids, phenylpropanoids, and phenolic acids are known to be responsible for the antioxidant capacity of fruits and vegetables. Free radical scavenging is generally accepted to be the means by which antioxidant compounds inhibit lipid peroxidation [19]. In this study, the DPPH$^{\bullet}$ radical scavenging activities of blackberry cultivars and wild genotype fruits were measured and data then correlated to total phenolics and antioxidant activity. A stronger radical quenching agent generally resulted in a lower EC_{50} value. Both blackberry cultivars and wild genotypes significantly differed in their DPPH$^{\bullet}$ quenching activities. As shown in Table 4, the DPPH$^{\bullet}$ scavenging activity was the lowest in cv. Arapaho (8.7 mg) while the highest in cv. Chester (5.8 mg). Among wild genotypes, R16 was the highest radical scavenging activity with the lowest EC_{50} value (4.4 mg), while R1 had the lowest activity with the highest EC_{50} value (7.8 mg) (Table 4). The average EC_{50} values for blackberry cultivars and wild genotypes were between 3.8 and 8.2 mg FW, respectively. Benvenuti et al. [24] reported the average EC_{50} values for blackberry cultivars were between 4.6-9.5 mg FW bases which support our findings. The average values of the DPPH$^{\bullet}$ radical scavenging activity of the cultivars and genotypes were correlated with the average values of total phenolic content and antioxidant activity: the correlation coefficients, r^2, were 0.992 (P = 0.0003) for total phenolics, 0.725 (P = 0.07) for antioxidant activity, respectively. (Figure 2). A significant correlation was observed between the average values of the DPPH$^{\bullet}$ radical scavenging activity and those of total phenolics and antioxidant activity. In the literature, the correlation between radical scavenger activity and total phenolics is reported [6]. Our results confirmed that the radical scavenging activity is related to total phenolics.



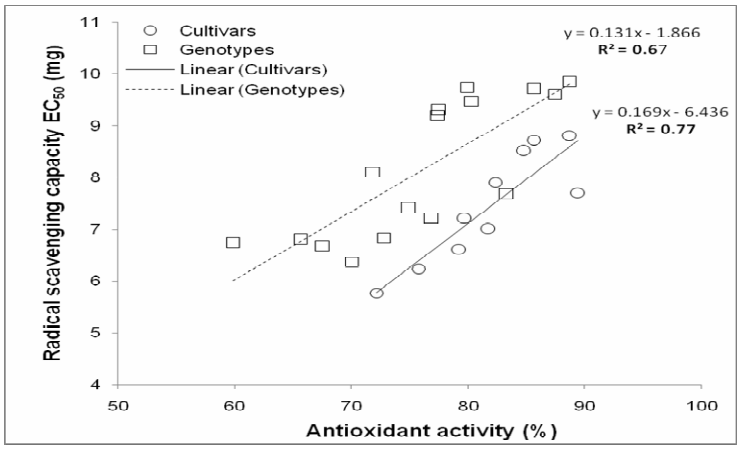


Figure 2. The relationships between antioxidant activity and total phenolic content, radical scavenging capacity and antioxidant activity in blackberry.

Conclusion

The results of our study show large variations on physico-chemical properties of both cultivated and wild growing blackberries. Fruits differ among themselves in the weight, sweetness, acidity, antioxidant activity etc. A wide diversity among genotypes in Turkey, presumably the one of the centre of origin and diversity of *Rubus fruticosus*, offers scope for selecting the better ones. The results also imply that dietary polyphenolic phytochemicals from blackberries may supply substantial antioxidants, which, in turn, may provide health-promoting effects to consumers. This may be the first study to provide comparable data that fruits of cultivated and wild growing *Rubus fruticosus* has high level biological activity. The strong antioxidant activity of fruits also provides scientific justification for the use of the fruits in ethnomedical practice in Turkey.

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