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Comparison of Antioxidant Properties of Wild Blueberries (*Vaccinium arctostaphylos* L. and *Vaccinium myrtillus* L.) with Cultivated Blueberry Varieties (*Vaccinium corymbosum* L.) in Artvin Region of Turkey

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A B S T R A C T

Vaccinium arctostaphylos L. and *Vaccinium myrtillus* L. which are found naturally in most part of Blacksea Region, and Artvin are generally called bear grape, Trabzon tea, and likapa. In addition, different varieties of blueberry (*Vaccinium corymbosum* L.) have been cultivated in Artvin region for 5 or 6 years. Blueberries contain appreciable levels of phenolic compounds, including anthocyanins and flavonols that have high biological activity. *V. arctostaphylos* and *V. myrtillus* show that natural distrubition with received *V. corymbosum* of different cultured species in Artvin region will be determined antioxidant activity in this study. In this study showed that wild species had a higher antioxidant effect than cultivated species. *V. myrtillus* had high total polyphenols (11.539-20.742 mg GAE/g dry sample), flavonoids (1.182-2.676 mg QE/g dry sample) and anthocyanins (3.305-11.473 mg Cyn/g dry sample) than *V. corymbosum* species. In addition, wild species had high CUPRAC, FRAP and DPPH values. The antioxidant activities found with CUPRAC, expressed as trolox equivalent antioxidant capacity ranged from 0.143 to 0.297 mmol TEAC/g dry sample. Those determined with DPPH expressed as IC50 ranged from 0.229 to 1.178 mg/ml. Those determined with FRAP expressed as $FeSO_4.7H_2O$ equivalent were in 130.719–346.115 µmol Fe/g dry sample range.

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Artvin Yöresine Ait Doğal Yaban Mersini (*Vaccinium arctostaphllos* ve *Vaccinium myrtillus*) ile Kültüre Alınan Yaban Mersini (*Vaccinium corymbosum*) Türlerinin Antioksidan Özelliklerinin Karşılaştırılması

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Ö Z E T

Karadeniz Bölgesi'nde ve Artvin'de doğal olarak bulunan *Vaccinium arctostaphylos* L. ve *Vaccinium myrtillus* L. genellikle ayı üzümü, Trabzon çayı ve likapa olarak adlandırılmaktadır. Ayrıca Artvin'de 5-6 yıldır yaban mersininin farklı türleri (*Vaccinium corymbosum* L.) yetiştirilmektedir. Yaban mersinleri yüksek biyolojik aktiviteye sahip antosiyaninler ve flavonoller gibi dikkate değer seviyede fenolik bileşenler içermektedir. Bu çalışmada Artvin bölgesinde doğal yayılım gösteren *V. arctostaphylos* ve *V. myrtillus* ile kültüre alınan *V. corymbosum* farklı türlerinin antioksidan aktiviteleri belirlenecektir. Bu çalışmada yabani türlerin kültürlere göre daha yüksek antioksidan etkiye sahip olduğunu görüldü. *V. myrtillus* türleri, *V. corymbosum* türlerinden daha yüksek toplam polifenol (11,539-20,742 mg GAE/g kuru örnek), flavonoid (1,182-2,676 mg QE/g kuru örnek) ve antosiyanin (3,305-11,473 mg Cyn/g kuru örnek) değerine sahiptir. Ayrıca yabani türler yüksek CUPRAC, FRAP ve DPPH değerlerine sahiptir. Antioksidan aktiviteler CUPRAC için Troloks eşdeğeri cinsinden 0,143 -0,297 mmol TEAC/g kuru örnek aralığında bulunmuştur. DPPH için IC50 değerleri 0,229 – 1,178 mg/ml kuru örnek aralığında belirlenmiştir. FRAP için $FeSO_4.7H_2O$ eşdeğeri cinsinden 130,719–346,115 µmol Fe/g kuru örnek aralığında bulunmuştur.

Introduction

Dark purple fruit of species *Vaccinium* sp. are multi-year plants and cultured plants species are on economic life of the 35-40 years. *Vaccinium* species generally were distributed different regions in North America, Europe and Asia. *Vaccinium arctostaphylos* and *Vaccinium myrtillus* are generally found in Artvin, Rize, Trabzon, Giresun and Ordu, in Turkey and offer a variety of wildlife. In recent years, growing blueberry has become popular, owing to the increasing international demand for its berries (Çelik, 2004; Gümüş et al., 2009)

Blueberry with a low amount of calories and sodium content are free cholesterol and an excellent source of fiber. Blueberry has pectin agent lowering blood sugar. Because of these properties of *Vaccinium* species are grown everywhere in the world where the climate is suitable (Çelik, 2005).

Blueberry is a fruit that has the high antioxidant capacity and includes phenolic compounds. In addition, it has anthocyanins having high biological activity, and includes flavonols. Anthocyanins have protective effect against chronic diseases such as cancer, cardio and cerebrovascular diseases, atherosclerosis and diabetes and possible benefits in terms of health linked to capacity of high antioxidant (Wu et al., 2002). According to the analysis carried out on small-grained many fruits, wild blueberries the capacity to absorb oxygen radicals tend to have the highest antioxidant effect (Atalay et al., 2003).

In the previous studies have been shown to be different antioxidant content of wild and cultivated blueberies (Riihinen et al., 2008; Bunea et al., 2011; Koca and Karadeniz, 2009). But there was not any research which belonged to Artvin region. The aim of the present study is to determine and compare total phenolic content, total flavonoid content, total anthocyanin content and antioxidant activity of native (*V. arctostaphylos* and *V. myrtillus*) and cultivated (*V. corymbosum* cv. Patriot, *V. corymbosum* cv. Bluecrop, *V. corymbosum* cv. Darrow, *V. corymbosum* cv. Brigitta and *V. corymbosum* cv. Blujay) blueberry fruits of Artvin.

Materials and Methods

The fruits of *V. arctostaphylos* were collected from four different regions (Artvin-Sacinka, Borcka-Efeler, Borcka-Efeler-Blueberry Hill, Borcka-Fındık Yaylası) and *V. myrtillus* fruits were collected from Borcka-Fındık Yaylası in Turkey. The fruits of *V. corymbosum* were collected from cultivated fields in Hopa-Kemalpaşa, in Artvin.

Collected ripe fruits were stored at -20°C until the analysis. The fruits dried in the oven at 40°C before treatments. Approximately 10 g of dried sample of the fruits was used preparing methanolic extracts for each specis and cultivars. These preparations were used determining antioxidant activities, and the treatments were done with 3 replications.

Spectrophotometric methods was used on total anthocyanins, total polyphenols, total flavonoids and antioxidant tests. Spectrophotometric methods are frequently used for standardization of natural raw materials.

Total Phenolic Assay

The total phenolic content of fruits were determined by using the Folin-Ciocalteu assay (Slinkard and Singleton, 1977). In this study, gallic acid (1; 0.5; 0.25; 0.125; 0.0625 and 0.03125 mg/ml) was used as a standard. Briefly, 20 µl of various concentrations of gallic acid and 20 µl methanolic samples (1 mg/ml), 400 µl of 0.5 N Folin-Ciocalteu regents and 680 µl of distilled water were mixed and the mixture was vortexed. Following 3-minute incubation, 400 µl of Na_2CO_3 (10%) solution was added and after vortexing, the mixture was incubated for 2 hours. After the incubation period at the room temperature, absorbances of the mixtures were measured at 760 nm. The concentrations of total phenolic compounds were calculated as mg of gallic acid equivalents per g of the dry weight of samples.

Total Flavonoid Assay

The total flavonoid content was measured by using the aluminum chloride assay (Chang et al., 2002). Quercetin was used as a standard. 0.5 ml of Quercetin (1; 0.5; 0.25; 0.125; 0.0625 and 0.03125 mg/ml), 4.3 ml methanol 0.1 ml 10% $Al(NO_3)_3$ and 0.1 ml 1 M NH_4CH_3COO were added in test tubes and mixed. Mixtures were incubated for 40 minutes. After incubation, absorbance was measured at 415 nm. The total flavonoid contents of fruits were expressed as mg quercetin equivalents per g of dry weight sample.

Total Anthocyanin Assay

Determinations of total anthocyanin were made with pH-differential method (Giusti and Wrolstad, 2001; Fuleki and Francis, 1968). 20 mL KCl solution (pH 1) was added first tube, 20 ml CH3COONa (pH 4.5) was added other tube and waited for about 30 minutes. In the meantime, diluted clear sample was spectrum of the 250-600 nm. At pH 1.0 and pH 4.5 the sample solution in the buffer solution was measured absorbance at 520 and 700 nm and calculated by the total monomeric anthocyanin content.

Determination of Antioxidant Activity

The antioxidant activities of the samples were determined using by FRAP, CUPRAC and DPPH methods.

The FRAP method was used for the determination of total antioxidant capacity, based on the reduction of yellow Fe^{3+}-TPTZ complex to the blue Fe^{2+}-TPTZ complex by electron donating substance under acidic condition (Benzie and Szeto, 1999). The 3 ml of FRAP reagent (containing TPTZ, $FeCl_3$, and acetate buffer) and 100 µl of the test sample or the blank (solvents used for extraction) were added test tube and mixed. Maximum absorbance values at 593 nm were recorded for 4 min at 25°C. The final absorbance was compared with the standard curve (100-1000 µmol/l). The data were expressed as µmol $FeSO_4.7H_2O$ equivalents per gram of dry matter.

The CUPRAC method is comprised of mixing the antioxidant solution (directly or after acid hydrolysis) with a copper (II) chloride solution, a neocuproine alcoholic solution, and an ammonium acetate aqueous buffer at pH 7, and subsequently measuring the developed

absorbance at 450 nm after 60 minutes (Apak et al., 2004).

1ml 10 mM $CuCl_2$, 1ml 7.5 mM Neocuproine and 1ml 1M NH_4Ac were added test tubes, than 0.2 ml sample and 0.9 ml H_2O added and mixed. End volume was 4.1 ml. Measure final absorbance at 450 nm. The test results obtained Trolox ® equivalent antioxidant capacity (TEAC) were given.

Radical scavenging activity of methanolic extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was spectrophometrically at 517 nm. The assay is based on the color change of the DPPH solution from purple to yellow as the radical is deactivated by the antioxidants (Pokorny et al., 2001). Briefly, various concentrations 0.75 ml of parts of sample extracts was mixed with 0.75 ml of a 0.1 mM of DPPH in methanol. Radical scavenging activity was measured by using Trolox as standards and the values are expressed as IC50 (mg or mg sample per ml), the concentration of the samples that causes 50% scavenging of DPPH radical.

Statistical analysis:

The data were expressed as mean ± standard deviation (SD) from three parallel measurements. In order to determine the significant differences between values, analysis of variance (ANOVA) and Duncan's multiple range tests were performed. Significance of difference was defined at $\alpha = 0.01$.

Results and Discussion

In general, phenolic acids, flavonoids and anthocyanins are antioxidant molecules. When the value of these molecules is high, antioxidant properties of plants is high (Al-Mamary et al., 2002; Robards et al., 1999). The total anthocyanin, total phenolic content and total flavonol contents of blueberry fruits were shown in Table 1, and FRAP and CUPRAC values were shown in Table 2.

Results showed the highest phenolic content value obtained from V. arc. ebh and V. myrt ($P<0.01$). V. myrt and V. arc. sc showed the highest flavonoid contents and also V. myrt showed anthocyanins contents ($P<0.01$). Between cultivated species, the highest content of polyphenols and anthocyanins were observed Patriot, and the highest flavonoid content was observed Bluejay ($P<0.01$; Table 1). In addition to these, the fruit of V. myrt showed maximum activity according to the FRAP and the fruits of V. myrt and V. arc. ebh showed maximum activity according to the CUPARC ($P<0.01$). Between cultivated species Patriot has the highest activitiy at FRAP. Darrow and Patriot had also the same activity at CUPRAC ($P<0.01$; Table 2).

The IC50 values determined from analysis of DPPH were showed in Fig. 1. When the fruits of V. arc. ep. had the highest DPPH radical cleaning, the lowest activity was obtained from Bj fruits.

In general, bluberry fruits have a high antioxidant content. Its fresh and dried fruits are sold in many markets. In addition, leaves of bluberries can be found in markets as tea. According to the results of some previous researches, fruits of blueberries prevent memory lossing and aging since they includes antiageing features (Çelik, 2005). Anthocyanins, phenolic compounds and flavonoids hfave the ability to neutralize free radicals. Blueberries contain high amounts of phenolic compounds and have a high antioxidant activity (Prior et al., 1998).

According to the total polyphenols, anthocyanins, flavonoids and antioxidant activity analyses of the dry bluberry fruits, the results showed that the dry natural blueberries (*V. arctostaphylos* and *V. myrtillus*) had a higher activity than dry cultured blueberries in the study.

Bunea et al. (2011), compared total anthocyanins, total polyphenols, total flavonoids, DPPH and FRAP for *V. myrtillus* (Wild 1, Wild 2) and *V. corymbosum* (Bluecrop, Elliot, Duke) which were collected in Romania. In this study, it was reported that wild blueberries had higher antioxidant activity than cultivated blueberries. In Italy, Giovanelli and Buratti (2009), collected *V. myrtillus* (lot 1 and lot 2) and *V. corymbosum* (Bluecrop, Goldtraube, Darrow and Patriot) fruits and made total anthocyanins, total polyphenols, DPPH and FRAP analyses. They reported that the wild blueberries had a high antioxidant capacity than the cultivated species. In addition to this, they found that the fruits of cv. Patriot showed the highest activity among the all cultivars. It can be said that the similar results were determined from our study for *V. myrtillus* and *V. corymbosum.*

Table 1 Results of statistical analyses showing the relationship of phenolic contents, flavonoid contents and anthocyanin with different blueberry species*

Samples**	Total phenolics (mg GAE/g DS†)	Total flavonoid (mg QE/g DS)	Total anthocyanin (mg Cyn/g DS)
V. arc. ebh	20.74 ± 0.24^{g}	1.93 ± 0.10^{c}	6.13 ± 0.01^{i}
V. arc. fy	19.19 ± 0.20^{f}	$1.18 \pm 0.16^{a,b}$	3.30 ± 0.00^{d}
V. arc. ep	16.22 ± 0.49^{e}	2.20 ± 0.28^{d}	5.54 ± 0.00^{h}
V. arc. sc	11.54 ± 0.63^{c}	$2.16 \pm 0.46^{c,d}$	1.16 ± 0.00^{b}
V. myrt	20.06 ± 0.33^{g}	$2.67 \pm 0.01^{c,d}$	11.47 ± 0.00^{j}
Dr	13.49 ± 0.56^{c}	0.74 ± 0.46^{a}	4.43 ± 0.01^{f}
Bc	5.56 ± 0.26^{b}	0.88 ± 0.05^{a}	1.71 ± 0.00^{c}
Br	11.54 ± 0.30^{d}	2.53 ± 0.58^{d}	3.89 ± 0.00^{e}
Pt	15.88 ± 0.57^{e}	$1.67 \pm 0.16^{b,c}$	5.36 ± 0.01^{g}
Bj	8.329 ± 0.362^{a}	5.650 ± 0.372^{e}	1.03 ± 0.003^{a}

*Values within column followed by different letters are significantly different α at =0.01

**: V. arc. ebh: *V. arctostaphylos* (Borçka-Efeler-Blueberry Hill), V. arc. fy: *V. arctostaphllos* (Borçka-Fındık Yaylası), V. arc. ep: *V. arctostaphllos* (Borçka-Efeler), V. arc. sc: *V. arctostaphylos* (Artvin-Saçinka), V. myrt:*V. myrtillus*,Dr: *V. corymbosum* cv. Darrow, Bc: *V. corymbosum* cv. Bluecrop, Br: *V. corymbosum* cv. Brigitta, Pt: *V. corymbosum* cv. Patriot, Bj: *V. corymbosum* cv. Bluejay. †DS: dry sample.

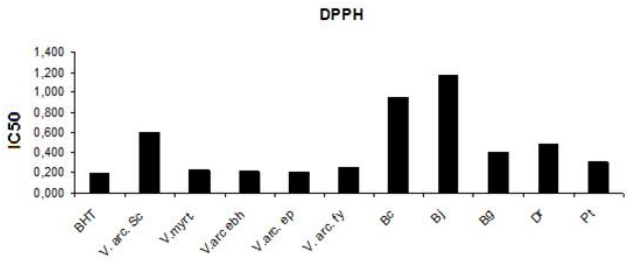

Figure 1 The results of DPPH for dry blueberry fruits

Table 2 Results of statistical analyses showing the relationship of FRAP and CUPRAC analyses for different blueberry species*

Samples	FRAP (µmol Fe/g DS)	CUPRAC (mmol TEAC/g DS)
V. arc. ebh	299.481 ± 0.277^{h}	0.297 ± 0.010^{g}
V. arc. fy	210.307 ± 3.476^{f}	$0.285 \pm 0.014^{e,f}$
V. arc. ep	250.401 ± 3.540^{g}	0.274 ± 0.007^{e}
V. arc. sc	130.719 ± 9.899^{c}	0.143 ± 0.013^{c}
V. myrt	346.115 ± 6.039^{i}	0.304 ± 0.008^{g}
Dr	150.277 ± 5.990^{d}	0.120 ± 0.002^{b}
Bc	90.021 ± 2.960^{b}	0.090 ± 0.005^{a}
Br	188.283 ± 5.132^{e}	0.141 ± 0.007^{c}
Pt	208.251 ± 4.954^{f}	0.200 ± 0.006^{d}
Bj	71.909 ± 3.663^{a}	0.077 ± 0.003^{a}

*Values within column followed by different letters are significantly different α at =0.01

Our study showed that *V. arctostaphylos* berries had higher antioxidant contents than *V. corymbosum*, supporting the findings reported by Koca and Karadeniz (2009). According to results of Burdulis et al.'s (2009) study, *V. myrtillus* had high radical scavenging and antioxidant activity. Hasanloo et al. (2011) also examined the contents of antioxidant *V. arctostaphylos* fruits which were collected from different regions of Iran. They found that the fruits contained high anthocyanin (1 mg/g) and polyphenols (42.73 mg GAE/g), and also stated *V. arctostaphylos* berries were the source of antioxidants.

In the present study, the highest content of total polyphenols content obtained from the fruits of *V. arctostaphylos* collected from Borçka-Efeler-Bluberry Hill among all species their cultivars. Compared with other contents of total flavonoids and total anthocyanins *V. myrtllus* fruits were better than all others. According to FRAP and CUPRAC tests, *V. myrtillus* had higher activity than *V. arctostaphylos*. When radical scavenging capacity of these species considered, radical cleansing of V. arc. ep was better than the other species.

It was found that the contents of the total phenolic, total flavonoids and total anthocyanins of cv. Patriot, cv. Darrow and cv. Brigita were higher among *V. corymbosum* cultivars, respectively. In addition, cv. Patriot had a high level of activity of FRAP and CUPRAC analyses and it was better in view of radical scavenging capacity.

Overall, it can be said that when the fruits of *V. myrtillus* have higher antioxidant activity among all species, *V. corymbosum* cv. Patriot fruits have the highest antioxidant activity among the cultivars in this study.

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