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

Özlem Saral¹, Zafer Ölmез², Hüseyin Şahin³

¹Department of Nutrition and Dietetics, College of Health, Artvin Çoruh University, 08000 Artvin, Turkey,
²Department of Forest Engineering, Faculty of Forestry, Artvin Çoruh University, 08000 Artvin, Turkey,
³Espiye Vocational School, Giresun University, 28600, Espiye/Giresun, Turkey.

ARTICLE INFO

Article history: Received 05 May 2014 Accepted 21 July 2014 Available online, ISSN: 2148-127X

Keywords: Vaccinium sp., Antioxidant activity Wild blueberry Cultivated blueberry Polifenol

C A R T I N E I N F O

ABSTRACT

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 distribution with received V. corymbosum of different cultivated 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 Cy/n/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 FeSO₄.7H₂O equivalent were in 130.719–346.115 μmol Fe/g dry sample range.

M A K A L E B İ L G İ S İ

Geliş 05 Mayıs 2014
Kabul 21 Temmuz 2014
Çevrimiçi baskı, ISSN: 2148-127X

Anahtar Kelimeler: Vaccinium sp., Antioxidan aktivite Yaban yabanmersini Kültür yabanmersini Polifenol

Ö Z E T

Karadeniz Bölgesi’nde ve Artvin’de doğal olarak bulunulan Vaccinium arctostaphylos L. ve Vaccinium myrtillus L. genellikle aynı görüntü arı çıkartıcı, Trabzon çayı ve likapa olarak adlandırılmaktadır. Ayrıca Artvin’de 5-6 yıl yarı yaban mersinini farklı türleri (Vaccinium corymbosum L.) yetiştirilmektedir. Yaban mersinileri yüksek biyolojik aktiviteye sahip antioxiyaniner ve flavonoller gibi dikkate değer seviyede fenolik bileşenler içerimektedir. Bu çalışmada Artvin bölgesinde doğal yayılımı gösteren V. arctostaphylos ve V. myrtillus ile kültüre alınan V. corymbosum farklı türlerinin antioksidan aktiviteleri belirlenecektir. Bu çalışmada yaban türlerin kültürlere göre daha yüksek antioksidan etkisi sahip olduğu görüldü. V. myrtillus, 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 antosiyaniner (3.305-11.473 mg Cy/n/g kuru örnek) değerine sahiptir. Ayrıca yaban türlerin yüksek CUPRAC, FRAP ve DPPH değerlerine sahip. Antioksidan aktiviteler CUPRAC için Troloks eşdeğerleri cisindende 0.143–0.297 mmol TEAC/g kuru örnek aralığında bulunmuştur. DPPH için IC50 değerleri 0.229 – 1.178 mg/ml kuru örnek aralığına belirlenmiştir. FRAP için FeSO₄.7H₂O eşdeğerleri cisindende 130.719–346.115 μmol Fe/g kuru örnek aralığına bulunmuştur.
Introduction

Dark purple fruit of species Vaccinium sp. are multi-year plants and cultivated 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 blueberries (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 species 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 Na2CO3 (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(NO3)3, and 0.1 ml 1 M NH4Cl, COO 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 Fe3+-TPTZ complex to the blue Fe2+-TPTZ complex by electron donating substance under acidic condition (Benzie and Szeto, 1999). The 3 ml of FRAP reagent (containing TPTZ, FeCl3, 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 FeSO4/7H2O 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 neocuprine alcoholic solution, and an ammonium acetate aqueous buffer at pH 7, and subsequently measuring the developed
in red color 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 α= 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 anthoacyanins 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 CUPRAC (P<0.01).

Between cultivated species Patriot has the highest activity 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, blueberry fruits have a high antioxidant content. Its fresh and dried fruits are sold in many markets. In addition, leaves of blueberries can be found in markets as tea. According to the results of some previous researches, fruits of blueberries prevent memory losing and aging since they include antiaging features (Celik, 2005). Anthoacyanins, phenolic compounds and flavonoids have 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 blueberry 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, Giovaneli and Buratti (2009), collected V. myrtillus (lot 1 and lot 2) and V. corymbosum (Bluecrop, Goldraube, 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*

<table>
<thead>
<tr>
<th>Samples**</th>
<th>Total phenolics (mg GAE/g DS)</th>
<th>Total flavonoid (mg QE/g DS)</th>
<th>Total anthocyanin (mg Cyn/g DS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. arc. ebh</td>
<td>20.74 ± 0.24d</td>
<td>1.93 ± 0.10c</td>
<td>6.13 ± 0.01e</td>
</tr>
<tr>
<td>V. arc. fy</td>
<td>19.19 ± 0.20f</td>
<td>1.18 ± 0.16b ±e</td>
<td>3.38 ± 0.19e</td>
</tr>
<tr>
<td>V. arc. ep</td>
<td>16.22 ± 0.49c</td>
<td>2.20 ± 0.28d</td>
<td>5.54 ± 0.00b</td>
</tr>
<tr>
<td>V. arc. sc</td>
<td>11.54 ± 0.63c</td>
<td>1.16 ± 0.06b</td>
<td>1.67 ± 0.00</td>
</tr>
<tr>
<td>V. myrt</td>
<td>20.06 ± 0.33c</td>
<td>2.67 ± 0.01d</td>
<td>11.47 ± 0.00c</td>
</tr>
<tr>
<td>Dr</td>
<td>13.49 ± 0.56e</td>
<td>0.74 ± 0.46a</td>
<td>4.46 ± 0.01c</td>
</tr>
<tr>
<td>Bc</td>
<td>5.56 ± 0.26h</td>
<td>0.88 ± 0.05b</td>
<td>1.71 ± 0.00b</td>
</tr>
<tr>
<td>Br</td>
<td>11.54 ± 0.30d</td>
<td>2.53 ± 0.58d</td>
<td>3.87 ± 0.00b</td>
</tr>
<tr>
<td>Pt</td>
<td>15.88 ± 0.57b</td>
<td>1.67 ± 0.166c</td>
<td>5.36 ± 0.01b</td>
</tr>
<tr>
<td>Bj</td>
<td>8.329 ± 0.362e</td>
<td>5.650 ± 0.372c</td>
<td>1.03 ± 0.003a</td>
</tr>
</tbody>
</table>

*aValues within column followed by different letters are significantly different α =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-Blueberry Hill among all species their cultivars. Compared with other contents of total flavonoids and total anthocyanins *V. myrtillus* 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. arct. 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. Brigatora 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.

### Acknowledgements

The authors would like to thank the Artvin Çoruh University for its financial support of this research (2011.F10.02.08).

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