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 Ölmez², 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.

ABSTRACT

Vaccinium arctostaphylos L. and Vaccinium myrtillus L. which are found naturally in most part of Blacksea Region, 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 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 FeSO₄.7H₂O equivalent were in 130.719–346.115 µmol Fe/g dry sample range.

ARTICLE INFO

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

Corresponding Author:
E-mail: oscarhan@artvin.edu.tr

Abstract

Artvin Yöresine Ait Doğal Yaban Mersini (Vaccinium arctostaphylos ve Vaccinium myrtillus) ile Kültüre Alınan Yaban Mersini (Vaccinium corymbosum) Türlerinin Antiksidan Özelliklerinin Karşılaştırılması

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., Antioksidan aktivite Yaban yabanmersini Kültür yabanmersini Polifenol

Ö Z E T

Karadeniz Bölgesi’nde ve Artvin’de doğal olarak bulunan Vaccinium arctostaphylos L. ve Vaccinium myrtillus L. genellikle ayı主要用于, Trabzon çayı ve likapa olarak adlandırılmaktadır. Ayrıca Artvin’de 5-6 yıldır yaban mersinin farklı türleri (Vaccinium corymbosum L.) yetiştirilmektedir. Yaban mersinleri yüksek biyolojik aktiviteye sahip antiosiyaniner 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 antikсидan aktiviteleri belirlenmiştir. Bu çalışmada yaban türlerin kültürle göre daha yüksek antiksidan etkiye 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 antiosiyana (3.305-11.473 mg Cyn/g kuru örnek) değerlerine sahiptir. Ayrıca yaban türlerin CUPRAC, FRAP ve DPPH değerlerine sahiptir. Antiksidan aktiviteler CUPRAC için troloks eşdeğerleri 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 FeSO₄.7H₂O eşdeğerleri cinsinden 130.719–346.115 µmol Fe/g kuru örnek aralığında bulunmuştur.
Introduction

Dark purple fruit of species Vaccinium sp. are multiyear 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 no 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-Findik Yaylasi) and V. myrtillus fruits were collected from Borcka-Findik Yaylasi 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-Ciocalteau 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 methanicol samples (1 mg/ml), 400 µl of 0.5 N Folin-Ciocalteau 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 NH₄CH₃COO 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

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

1ml 10 mM CuCl₂, 1ml 7.5 mM Neocuproine and 1ml 1M NH₄Ac were added test tubes, than 0.2 ml sample and 0.9 ml H₂O 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 spectrophotometrically 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 (Pokorný 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. eah 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 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 lossing and aging since they includes antiaging features (Çelik, 2005). Anthocyanins, 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 cultivated 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, Elliott, Duke) which were collected in Romania. In this study, it was reported that wild blueberries had higher antioxidant activity than cultivated blueberries. In Italy, Giovannelli 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. eah | 20.74 ± 0.24 | 1.93 ± 0.10 | 6.13 ± 0.01 |
| V. arc. fy | 19.19 ± 0.20 | 1.18 ± 0.16 | 3.30 ± 0.00 |
| V. arc. ep | 16.22 ± 0.49 | 2.20 ± 0.28 | 5.54 ± 0.00 |
| V. arc. sc | 11.54 ± 0.63 | 2.16 ± 0.46 | 1.16 ± 0.00 |
| V. myrt | 20.06 ± 0.33 | 2.67 ± 0.01 | 11.47 ± 0.00 |
| Dr | 13.49 ± 0.56 | 0.74 ± 0.46 | 4.43 ± 0.01 |
| Bc | 5.56 ± 0.26 | 0.88 ± 0.05 | 1.71 ± 0.00 |
| Bb | 11.54 ± 0.30 | 2.53 ± 0.58 | 3.89 ± 0.00 |
| Pt | 15.88 ± 0.57 | 1.67 ± 0.16 | 5.36 ± 0.01 |
| Bj | 8.329 ± 0.362 | 5.650 ± 0.372 | 1.03 ± 0.003 |

*Values within column followed by different letters are significantly different at α=0.01
**V. arc. eah: V. arctostaphylos (Borçka-Efelier-Blueberry Hill), V. arc. fy: V. arctostaphylos (Borçka-Fındık Yaylası), V. arc. ep: V. arctostaphylos (Borçka-Efeiler), 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.
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. Hasanlölo 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. arctostaphylos* 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. Brigata 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).

**References**

Al-Mamary M, Al-Meei A, Al-Habori M. 2002. Antioxidantactivities and total phenolics of different types of honey. NutritionResearch 22: 1041-047.

Apak R, Güçlü K, Özyürek M, Karademir SE. 2004. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. Journal of Agricultural and Food Chemistry 52: 7970-7981.

Atalay M, Gordillo G, Roy S, Rovin B, Bagchi D, Bagchi M, Sen CK. 2003. Anti-antigonic property of edible berry in a model of hemangioma. FEBS Letters 544: 252-257.

Benzie IF, Szeto YT. 1999. Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. Journal of Agricultural and Food Chemistry 47: 633-636.

Bunea A, Rugina DO, Pintea AM, Sonta Z, Bunea CI, Socaciu C. 2011. Comparative polyphenolic content and antioxidant activities of some wild and cultivated blueberries from Romania, Notulae Botanicae Horti Agrobotanici Cluj-Napoca 39(2):70-76.

Burdulis D, Aarkinas A, Jastien I, Stackevicien E, Nikolajevas A, Rupina DO, Pintea AM, Sconta Z, Bunea CI, Socaciu C. 2011. Comparative polyphenolic content and antioxidant activities of some wild and cultivated blueberries from Romania, Notulae Botanicae Horti Agrobotanici Cluj-Napoca 39(2):70-76.

Çelik H. 2004. Karadeniz için yeni bir meyve türü likapa (yaban mersini) (in Turkish). Ekoloji Magazin 1: 50-53.

Çelik H. 2005. Yaban Mersini (Likapa) Yetiştiriciliği (in Turkish). Hasan Yayınelik.

Fuleki T, Francis FJ. 1968. Quantitative methods for anthocyanins. 2. Determination of total anthocyanin and degradation index for cranberry juice, J. Food Science 3378-83.

Giovaneli G, Buratti S. 2009. Comparison of polyphenolic composition and antioxidant activity of wild Italian blueberries and some cultivated varieties. Food Chemistry 112: 903-908.

Giusti MM, Wrolstad RE (Ed.). 2001. Anthocyanins, Characterization and Measurement with UVisible Spectroscopy, In: Wrolstad RE (Ed.), Current Protocols in Food Analytical Chemistry, John Wiley&Sons, New York, USA.

Gümüş C, Ölmze Z, Hangişi Ölmze G, Kalender Ç. 2009. Arvind’de yaban mersini (Vaccinium sp., Likapa) yetiştiriciliği eğitimi konulu AB prosesinin tanımı ve prosjenin yürütüldüğünde kararlaştırılan güçlükler ve sorunlar (in Turkish). II. Ormancilık Sosyo-Ekonomik Sorunlar Kongresi, 19-21 Şubat, İsparta, 81-88.

Hasanloo T, Sephehrifar R, Hajimehdipoor H. 2011. Levels of phenolic compounds and their effects on antioxidant capacity of wild Vaccinium arctostaphylos L. (Qare-Qat) collected from different regions of Iran. Turkish Journal Biology 35: 371-37

Koca I, Karadeniz B. 2009. Antioxidant properties of blackberry and blueberry fruits grown in the Black Sea Region of Turkey. Scientia Horticulturae 121: 447-450.

Pokorny J, Yanishileva N, Gordon M. 2001. Antioxidants in Food, CRC Press, USA.
Prior RL, Cao GH, Martin A, Sofic E, Mc Ewen J, O’Brien C, Lischner N, Ehlenfeldt M, Kalt W, Krewer G, Mainland CM. 1998. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of Vaccinium species. Journal of the Agricultural and Food Chemistry 46: 2686-2693.

Riihinen K, Jaakola L, Karenlampi S, Hohtola A. 2008. Organ-specific distribution of phenolic compounds in bilberry (Vaccinium myrtillus) and “northblue” blueberry (Vaccinium corymbosum x V. angustifolium). Food Chemistry 110: 156-160.

Robards K, Prenzler PD, Tucker G, Swatsitang P, Glover W. 1999. Phenolic compounds and their role in oxidative processes in fruits. Food Chemistry 66: 401-436.

Slinkard K, Singleton VL. 1977. Total phenol analysis: Automation and comparison with manual methods. American Journal of Enology and Viticulture 28: 49-55.

Wu X, Cao G, Prior RL. 2002. Absorption and metabolism of anthocyanins in elderly women after consumption of elderberry or blueberry. The Journal of Nutrition, ProQuest Agriculture Journals 132(7): 1865-1871.