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Phenolic Content and Antioxidant Activity of *Vaccinicum myrtillus* Collected from Rize Highlands, Turkey

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Abstract: The phenolic content and antiocxidant activity of *Vaccinium myrtillus* collected from highlands of Rize (Handüzı, Kavron and Anzer) were determined. The Folşin-Coilteau method was used for the determimation of phenolic contents. The antioxidant activity values were obtained using the FRAP method. The phenolic content of fruits was calculated as 184,2-556,1 mg GAE.100 g-1 and that of dry leaves as 69,9-224,3 mg GAE.100 g-1. Further antioxidant activity ranged between 487,5-1240,2 mg FSO4/gr in dried fruits and between 170,2-250,3 mg FeSO4/gr in dry leaves. The phenolic content and antioxidant activity of fruits and leaves of *Vaccinium myrtillus* collected from the Rize flora showed values displaying rich contents.

Key words: Vaccinium; phenolics; antioxidant; Rize; Turkey

INTRODUCTION

Bilberry (*Vaccinium myrtillus* L.) has several traditional uses in folk medicine. Decoctions and infusions of its leaves are used for their diuretic, astringent, and antiseptic properties of the urinary tract. Bilberry leaf aqueous extracts are also useful as antibacterials and against inflammation, especially inflammation of the oral cavity (Wang and Lin, 2008).

The perennial dwarf shrub bilberry (*Vaccinium myrtillus* L.), also called European blueberry, is a member of the Ericaceae family and widely spread in the northern hemisphere across Europe and Central Asia.

Bilberry [*Vaccinium myrtillus* Linn. (Ericaceae)] fruit (VMF) is known as Çalı Çileği and Çoban Üzümü in Turkey and can be found in mountains and forests. The height of this shrubby perennial plant varies from 30 to 60 cm, its leaves are bright green and branches are alternating and elliptical. Its bell-shaped flowers are reddish or pink in color, bloom between the months of April and June producing blue-black or purple fruit. Even though it is encountered as a precious wild delicacy today, bilberry has been used as food for centuries in Turkey. More recently, bilberry fruit extracts have been used for the treatment of diarrhea, dysentery, and mouth and throat inflammations. Decocted bilberry leaves have been used to decrease blood sugar in diabetes (Baytop, 2000).

Further, its flowers are single on short stems. The fruits are berries, globular, dark purple, juicy and sour (Kovačević, 2002). In many European countries, the bilberry is one of the most economically important wild berry species (Tomićević et al., 2011).

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 are distributed at 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 (Gümüş et al., 2009). Blueberry with a low amount of calories and sodium content are free cholesterol and an excellent source of fiber.

Blueberry (*Vaccinium corymbosum*) 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 comparison to important Vaccinium crops such as highbush blueberry (*V. corymbosum* L.) and semi-cultivated lowbush blueberry (*V. angustifolium* Aiton), the food and nutritional quality of bilberry fruits is recognized due to their content of health-beneficial phytochemicals (Moyer et al., 2002). Bilberries are characterized by the high abundance of anthocyanidins and anthocyanins (Kalt et al., 1999) and other potent natural antioxidants such as flavonols and phenolic acids (Lättı et al., 2011), considerable amounts of stilbenes (resveratrol) (Rimando et al., 2004) and ascorbic acid (Moyer et al. 2002).

The interest in this berry species is due to its high content of phenolic compounds, which are plant secondary metabolites, well-known for their healthprotecting attributes, as anti-inflammatory (Kim et al., 2014), anti-hypertensive (Rodrigo et al., 2012), anti-microbial (Daglia, 2012) and anti-cancer agents (Paller et al., 2013; Wang and Stoner, 2008).

Further, an increasing demand for healthy ingredients by the food industry and changed consumer consciousness have led to investigations of wild berry resources including *V. myrtillus* focusing on genotypic variation, phytochemical content and physiological aspects towards agricultural and industrial exploitation (Nestby et al., 2011).

The purpose of this study was the determination of phenolic content and antşoxşdant activity of *Vaccinium myrtillus* samples collected from Rize highlands.

MATERIALS AND METHODS

This study was carried out to determine the antioxidant values and total phenolic content of leaf and fruit parts of *Vaccinium myrtillus* samples collected from the Handüzi, Kavron and Anzer highlands of the province Rize in the year 2017. Collected samples were dried at 40°C in the drying oven and their contents were determined using the UV–spectrophotometer device. The pretreatment used in the determination of total phenol and antioxidant content of the samples are the same in both analysis procedure. 0.1 g of each dried sample was completed with methanol (80 %) to reach 10 ml volume. Samples were mixed first in the water bath (50 °C) for a duration of 20 minutes and and the samples were keep waiting after this procedure for 1 h in the dark. The mixture was centrifuged after that for a 20 min, 4000 cycle/min proecess for obtaining the extracts, which are used for the determination of phenolic content and antioxidant activity of the investigated samples.

Determination of Total Phenolic Content

The total phenolic content of collected samples were determined using UV-Vis spectrophotometer as mg GAE/gr DW. The pretreatment of samples was the same as descryibed in the FRAP method. Gallic acid was used as standard, according to the method described by the International Organization for Standardization (ISO) 14502-1. Sample extract as 1/10 of the total volume and 300 µl Na2CO3 was added to tubes containing water involving Folin reagent and all tubes were keep waiting in a ultrasonic shaker (50°C) for 15 min. The measurement were done using a UV spectrophotometer device at a wawe lenght of 765 nm to obtain the absorbance values.

Determination of Antioxidant Activity

A modified version of the FRAP assay described by Izzreen and Fadezelly (2013) was used to determine the antioxidant activity of collected samples as mg FeSO4/gr DW.

For the determination of antioxidant content of the samples as pretreatment, 0.1 g of each dried sample was completed with methanol (80 %) to reach 10 ml volume. Samples were mixed first in the water bath (50 °C) for a duration of 20 minutes and and the samples were keep waiting after this procedure for 1 h in the dark. The mixture was centrifuged after that for a 20 min, 4000 cycle/min process for obtaining the extracts, which are used for the determination of phenolic content and antioxidant activity of the investigated samples.

Collected samples were analyzed regarding their antioxidant activity values. White tea leaves were dried in the drying oven at 40 ^{o}C and its antioxidant activity was determined using the UV-spectrophotometer by the FRAP method. The determination of antioxidant capacity of investigated samples (pretreatments completed) was done using the FRAP method. The FRAP method bases on the colourization after the degradation of the Fe+3 ion, bounded to TPTZ in an acid environment, to Fe+2. 300 mM acetate buffer (pH 3,6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) and 20 mM FeCl3.6H2O solutions were mixed at a proportion of 10:1:1 as FRAP (ferric reducing / antioxidant power) reactives to obtain a buffer solution. A FeSO4.H2O solution was used to prepare different standart probes to obtain a calibration curve. The final samples were obtained with a mix of 1980 µl FRAP dispersive + 20 µl sample and keep waiting after that for 3 min n a ultrasonic shaker (50°C). The measurements were done using a UV Spectrophotometer device at a wave lenght of 595 nm to obtain the final absorbance values.

Statistical analysis

Principal component and Biplot analysis were used to distinguish the collected samples regarding analysed characteristics (Backhaus et al. 1989). Principal component and Biplot analysis was performed using XLSTAT2016 Trial Version. The principal components represent the axes which are the orthogonal projections for the alues representing the highest possible variances in the case of PC1 and PC2. The obtained data were used to create scatter plot diagrams. Therefore, a factor analysis was performed, whereby each variable was used to calculate relationships between sample and investigated trait. Based on the obtained data, the Biplot diagramme was created showing the relationship of investigated samples regarding to their chemical composition.

RESULTS AND DISCUSSION

Obtained results are summaried in Table 1. According to obtained results the antioxidant values using FRAP method were as follows: the phenolic content of fruits was calculated as 184,2-556,1 mg GAE.100 g-1 and that of dry leaves as 69,9-224,3 mg GAE.100 g-1. Further antioxidant activity ranged between 487,5-1240,2 mg FeSO4/g in dried fruits and between 170,2-250,3 mg FeSO4/g in dry leaves.

As shown in Fig. 1 the total phenolic content and antioxidant activity of leaf and fruit samples of *Vaccinium myrtillus* collected from Handüzi Highland were lower than those of Anzer and Kavron highlands. İn all collection sites fruits displayed higher phenolic content and antioxidant acitivity regarding leaf samples.

Table 1: Total phenolic content and antoxidant activity of fruit and leaf parts of *Vaccinium myrtillus* samples collected from different highlands

Location	Total Phenolic Content (mg GAE .100 g^{-1} DW)	Antioxidant activity (mg $FeSO_4$ / g DW)
HANDÜZİ Highland 1833 m Fruit	184,2	487,5
HANDÜZİ Highland 1833 m Leaf	69,9	170,2
KAVRON Highland 2300 m Fruit	445,8	1240,2
KAVRON Highland 2300 m Leaf	224,3	250,3
ANZER Highland 2106 m Fruit	556,1	1225,5
ANZER Highland 2106 m Leaf	212,9	243,6

The differentiation of collected *Vaccinium myrtillus* samples using Biplot analysis are shown in Fig.2. The collected samples could be clearly differentiated based on their total phenolic content and antioxidant activity values..

Remarkable differences were detected regarding investigated traits in different plant parts and further investigations should be initiated involving more genotypes from other highlands to screen present diversity in this manner.

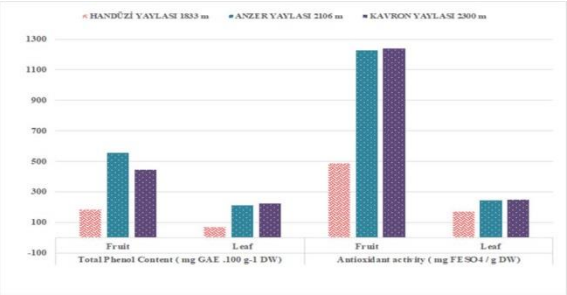

Fig 1. Determination of antioxidant activity and total phenol content of different parts of *Vaccinium myrtillus* genotypes

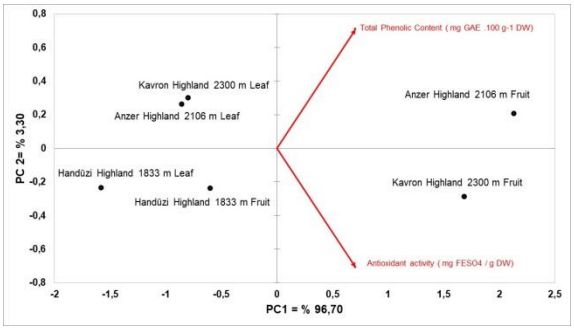

Fig. 2: Differentiation of collected *Vaccinium myrtillus* samples using Biplot analysis. Using the first two principal component 100 % of present variation could be explained.

Genetic and environmental factors interact to determine the amount of total phenolics and total anthocyanins, and thus the antioxidant activity found in berries (Dahlo, 2011). Antioxidant activity often closely traces the concentrations of total phenolics and total anthocyanins (Prior et al., 1998) and factors affecting the concentration of phenolics also influence antioxidant activity. These include, for instance mature of berries, preharvest environmental conditions, postharvest storage conditions and preocessing. There could of course also be genetic differences between populations that could lead to differences in antioxidant activity (Connor et al., 2002; Ehlenfeldt and Prior, 2001; Prior et al., 1998).

İn the present study samples were collected from different highlands of Rize; that means possible genotypic differences and of course the effecte of different climatic conditions should be effective.

Conclusion

Growing interest in the role of antioxidants in human health has triggered intense research in the field of agronomic and food sciences. Recently, studies have been devoted to determining how the content and activity of these compounds can be maintained or improved through cultivar development, production practices, postharvest storage, and plant processing. Moure et al. (2000) also maintain that it is a priority to replace these synthetic substances with natural ones, which is why the search for this type of new plant products has been intensified for their use in the food, pharmaceutical, and cosmetic industry.

There are remarkable differences in phenolic content and antioxidant capacity of fruit and leaf parts of *Vaccinium myrtillus* genotypes collected from different highlands of Rize. Based on study results, future studies have to

be directed to the evaluation of the effect of genotype, altitude and possible collection times on seconder metabolites in *Vaccinium myrtillus*, which can be supported by molecular characterisation of local bilberry genotypes.

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