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Identification and Quantification of Blueberry Antocyanidin and their Radical-Scavenging Activity

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Abstract. Extracts of phenolic compounds were obtained from different blueberry cultivars, *Vaccinium corymbosum* (Elliot, BlueCrop, and Duke) and genotypes, *Vaccinium myrtillus* (Wild 1, Wild 2). The content of anthocyanin aglycons was determined after acid hydrolysis using RP-HPLC-DAD. The antioxidant activity of the obtained extracts was also investigating using a new method, HPS. *Vaccinium myrtillus*, both wild cultivars had high amount of anthocyanins (940 mg/100 g FW, respectively 531.54 mg/100 g FW) comparing to cultivated ones. The lowest values were obtained for Duke variety (100, 9 mg/100 g FW). In agreement with these results, antioxidant activity showed similar behavior between the samples. Until now the antioxidant method (HPS) was tested for the first time for blueberry varieties.

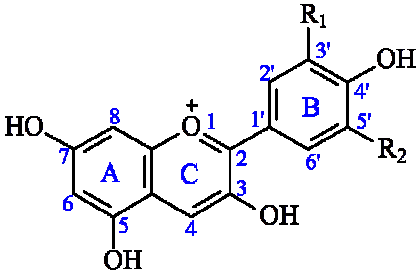
Keywords: blueberry, anthocyanin aglicons, HPLC, antioxidant activity

INTRODUCTION

Blueberry (*Vaccinium myrtillus* L) is a perennial flowering plant, being the most important species of genus *Vaccinium* and is widely known and found in Europe, Asia and North America. Blueberry contains significant concentration of phenolic compounds, mainly anthocyanins. These compounds are believed to be associated with antioxidant and health proprieties (Blumenthal *et al.* 2009, Grace *et al.*, 2009, Wu *et al.*, 2006).

Anthocyanins belong to the class of plant secondary metabolites named flavonoids and they are responsible for red, orange or blue colors in many vegetables and fruits. (Giusti and Wrolstad, 2003). Anthocyanins are sugar residue with aglycones commonly named anthocyanidins (Dandena and Leimane, 2011).

Depending of the number of hydroxyl and methoxyl groups there are 6 common anthocyanidins (sugar free counterparts) found in nature and also in blueberry-cyanidin (Cy), peonidin (Pn), pelargonidin (Pg), malvidin (v), delphinidin (Dp), and petunidin (Pt) (Kong *et al.* 2005) (Fig. 1).



Anthocyanidin	R_1	R_2
Cyanidin	H	OH
Delphinidin	OH	OH
Petunidin	OCH3	OH
Peonidin	OCH3	H
Malvidin	OCH3	OCH3

Figure 1. Structure of blueberry anthocyanidins

Over the recent years many study mention the health benefit of anthocyanidins: suppress grow of tumor cells (Kausar *et al.*, 2012), induce apoptosis (Srivastava *et al*., 2007), have antidiabetic properties (Tsuda, *et al.*, 2003), reduced the proliferation of human colon cancer (Kang *et al.*, 2003).

Regarding anthocyanidins quantification only few data are available in the literature (Oliveira *et al*., 2010, Queiroz *et al.*, 2009, Nyman *et al*., 2001).

The aim of our study was to investigate the anthocyanidins content and antioxidant activity in five blueberry varieties: two wild (*Vaccinium myrtillus*) and three cultivated varieties (*Vaccinium corymbosum*).

MATERIALS AND METHODS

Three varieties of cultivated high bush blueberries (*Vaccinium corymbosum*) Elliot, BlueCrop and Duke were purchased directly from the producers, a farm situated in N-V of Romania. The two types of wild blueberries (*Vaccinium myrtillus,* Wild 1 and Wild 2) were harvested from two different mountainous geographical zones: 45˚24'44"N and 46˚44'37"N of Romania. All berries were picked at the commercially ripe stage. Samples were stored in a freezer at -20 ˚C until analyzed.

For sample extraction, 5 g of blueberries, in three replicated each, was extracted by grinding the sample 1 min at 20,000 rpm in a blender (Ultra-Turrax Miccra D-9 KT Digitronic, Germany) with 10 ml of acidified methanol (85:15 v/v, MeOH:HCl). The homogenate was centrifuged at 3500 rpm for 10 min. The extract was separated and the residual tissue was re-extracted until the extraction solvents became colorless (the total solvent volume was between 100-250 ml). After adding 10 ml of the same solvent mixture, the extraction was carried out under stirring. The filtrates were combined in a total extract, which was dried by vacuum rotary evaporator at 40 ˚C. Prior to each analysis, the dry residues were redisolved in 10 ml of methanol; the samples were centrifuged at 5000 rpm and filtered through 0.45 μm nylon filter (Millipore).

Acid hydrolysis of anthocyanin. 2 mL extract was mixed with 2 ml of 2 M HCl in a screw-cap test tube. The mix was capped and hydrolyzed for 1 h at 90˚C, then cooled in an ice bath.

Anthocyanidine determination by RP-HPLC-DAD. Analyses were performed on a Shimadzu HPLC system equipped with a binary pump delivery system LC-20 AT (Prominence), a degasser DGU-20 A3 (Prominence), diode-array SPDM20 A UV–VIS detector (DAD) and a Luna Phenomenex C-18 column (5μm, 25 cm x 4.6 mm). The mobile phase consisted in: solvent A -formic acid (4.5%) in bidistilled water and solvent B-acetonitrile. The gradient elution system was: 10% B, 0-9 min; 12% B, 9-17 min; 25% B 17-30 min; 90% B, 30-50 min; 10% B, 50-55 min. The flow rate was 0.8 ml/min and the analyses were performed at 35 ºC. The chromatograms were monitored at 520 nm.

HPS scavenging activity. The hydrogen peroxide scavenging activity was done following the method described by Ruch *et al.* (1989). A solution of 40 mM H_2O_2 was prepared in phosphate buffer (1 M; pH=7.4). 3.4 ml phosphate buffer solution, 5 μl of blueberry extracts or Trolox solution, 0.6 ml of H_2O_2 solution (40 mM) was mixed together and the absorbance was read at 230 nm, against a blank solution (phosphate buffer without H_2O_2).

The results were expressed as micromoles Trolox equivalents per gram sample (TE μmol /g).

RESULTS AND DISCUSSIONS

Anthocyanidine determination by RP-HPLC-DAD. The chromatogram of anthocyanidine separation in analysed samples is presented in Figure 2. Based on their retention time, UV-VIS spectra compared with standards and published data, five anthocyanidins we identified: delphinidin, cyanidin, petunidin, peonidin and malvidin.

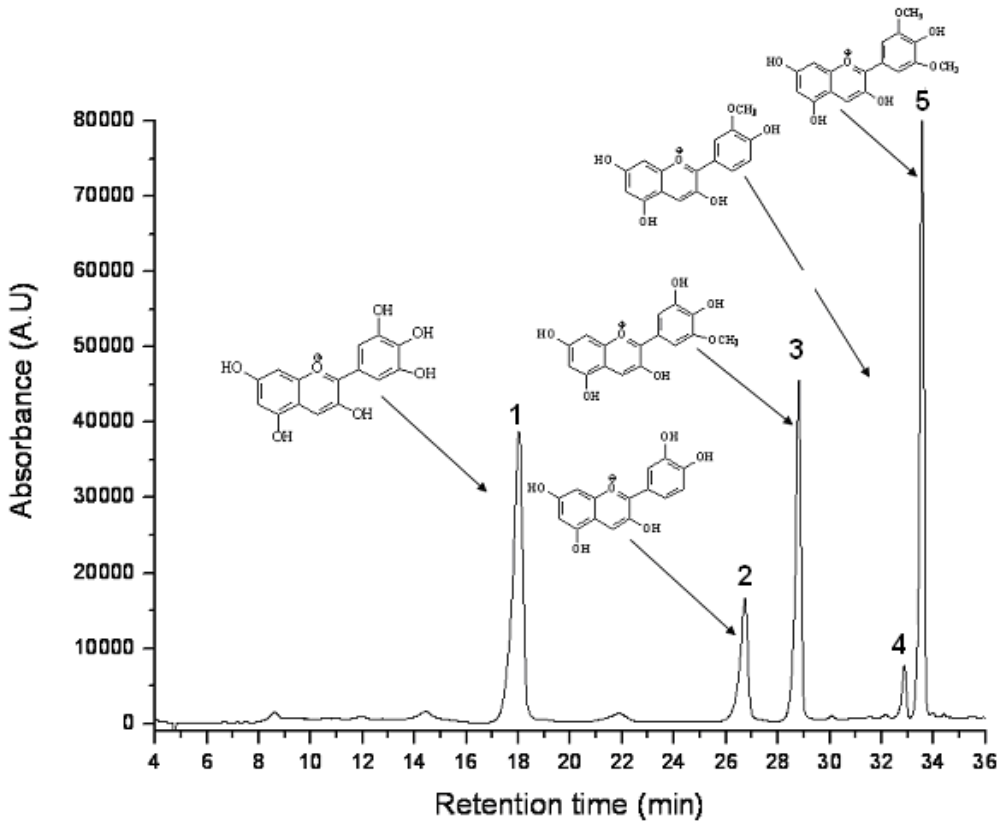


Fig. 2. Anthocyanin aglicons HPLC profiles in BlueCrop variety.
Peak identification: 1- Delphinidin; 2-Cyanidin; 3-Petunidin; 4-Peonidin; 5-Malvidin

Anthocyanin aglycon contents in wild and cultivated blueberries are shown in Table 3. In *Vaccinium myrtillus*, the concentration of anthocyanins aglycones are higher than in cultivated ones which is in agreement with data's obtained for total anthocyanin content; the most representative are delphinidin and petunidin.

In *Vaccinium corymbosum*, delphinidin and malvidin has the highest concentration from all the anthocyanidins identified, which is in accordance with Wu *et al.* (2006).

The highest anthocyanidin content in wild blueberries is represented by delphinidin 300 mg/100g FW respectively 236.92 mg/100g FW. There were obtained significant differences between all the anthocyanidin quantified in all the blueberries analyzed.

The hydrogen peroxide scavenging activity (HPS) depends on the change of the absorbance value at 230 nm, as a consequence of the decrease in H_2O_2 concentration due to the presence of scavenger compounds. The main disadvantage of the method is the possible interference of some phenolics, which absorb in UV region and a inefficient degradation of H_2O_2 (Özyürek *et al.*, 2010). We tested for the first time this method for blueberries varieties.

The values obtained were between 25.30-44.37 μmol TE/g, with statistical differences between all samples ($p<0.05$). This method was applied for the first time on blueberries and higher antioxidant capacity was obtained for wild blueberries compared with cultivated ones.

Tab. 1

Anthocyanin aglycones content (mg/100 g FW) in blueberries

Compound	*Vaccinium myrtillus*		*Vaccinium corymbosum*		
	Wild 1	Wild 2	BlueCrop	Elliot	Duke
Delphinidin	300.17±27[a]	236.92±18[a]	109.60±13.2[a]	30.3±3[b]	30.5±3.4[b]
Cyanidin	188.33±18[b]	81.30±8[c]	12.04±0.5[d]	1.0±0.2[e]	1±0.2[e]
Petunidin	197.44±21[b]	112.38±14[b]	66.39±6[b]	6.8±0.4[d]	6.7±0.4[d]
Peonidin	117.51±14[d]	28.91±5[e]	54.37±5[c]	40.3±5[a]	40.2±8[a]
Malvidin	137.04±10[c]	45.03±9[d]	72.71±5[b]	21.6±2[c]	22.5±4.3[c]

Values are mean ± standard deviation, n=3;
In each column, mean values with different letters are significantly different at $p<0.05$

Tab. 2

Antioxidant activity determinate trough HPS assay

Sample	Antioxidant activity, μM TE/g
Vaccinium corymbosum	
Elliot	25.30 ± 2.3[e]
Bluecrop	36.48 ± 2.1[c]
Duke	27.55 ± 1.9[d]
Vaccinium myrtillus	
Wild 1	44.37 ± 2.6[a]
Wild 2	38.87 ± 2.7[b]

CONCLUSION

There were analyzed two wild and three cultivated blueberries from the anthocyanidine and antioxidant activity point of view. The major anthocyanidine separated using HPLC were delphinidin, cyanidin, petunidin, peonidin and malvidin. Wild blueberries contain higher amount of anthocyanidins and also higher antioxidant activity compared with cultivated ones.

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