

Comparative Polyphenolic Content and Antioxidant Activities of Some Wild and Cultivated Blueberries from Romania

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Abstract

Two wild and three cultivated blueberry varieties ('Elliot', 'Bluecrop' and 'Duke') from Romania were analyzed comparatively in order to determine the total polyphenols, total anthocyanins, total flavonoids content and measuring the antioxidant activity using three different single electron transfer-based assays, Trolox equivalent antioxidant capacity (ABTS), ferric reducing ability (FRAP), 2,2-diphenylpicrylhydrazil radical scavenging capacity (DPPH) and one hydrogen atom transfer-assay, oxygen radical absorbance capacity (ORAC). Total polyphenols content ranged from 424.84 - 819.12 mg GAE/100 g FW, total flavonoids ranged from 84.33-112.5 mg QE /100 g FW and total anthocyanins ranged from 100.58-300.02 C3GE/100g FW. The anthocyanins were separated and quantified using RP-HPLC-DAD. In *Vaccinium myrtillus*, petunidin-3-glucoside and delphinidin-3-glucoside have the highest contribution to the anthocyanin content while in *Vaccinium corymbosum*, peonidin-3-galactoside represent the major anthocyanin. Except for ORAC assay ($r=0.765$), all antioxidant activity values obtained were highly correlated with total polyphenol content ($0.923 \leq r \leq 0.986$). Wild blueberries had higher total polyphenols content and also antioxidant activity compared with cultivated ones.

Keywords: *Vaccinium myrtillus*, *Vaccinium corymbosum*, total polyphenols, anthocyanins, antioxidant methods

Abbreviations: TPC, total polyphenols content; TA, total anthocyanins; TF, total flavonoids; FRAP, ferric reducing antioxidant potential; ABTS, trolox equivalent antioxidant capacity; ORAC, oxygen radical absorbing capacity; DPPH, 2,2-diphenyl-1-picrylhydrazyl method, TE, Trolox equivalents, Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

Introduction

The interest for natural antioxidants, especially from fruits and vegetables, has increased in recent years. Epidemiological studies indicated that a higher level of natural antioxidants (ascorbic acids, vitamin E, carotenoids and phenolics) in human everyday diet can protect against cardiovascular diseases, cataract, cancer and aging-related disorders (Steffen *et al.*, 2003).

Berries contain high concentration in bioactive compounds such as polyphenols, including anthocyanins, phenolic acids, tannins, carotenoids, vitamin A, C, E, folic acid and minerals such as calcium, selenium and zinc (Kresty *et al.*, 2001; Pineli *et al.*, 2011). Among them, blueberries became well known and often consumed due to their uses for treating biliary disorders, coughs, tuberculosis, diabetes (Martineau *et al.*, 2006; Valentová *et al.*, 2007) and visual disorders (Canter and Ernst, 2004). Blueberries contain high level of anthocyanin and phenolic compounds with high *in vitro* antioxidant capacities compared with other fruits (Wang and Jiao, 2000). The blueberries phenolic content are affected by genetic differences, the cultivar type, growing location and the degree of maturity

at harvest (Zadernowski *et al.*, 2005). The total amount and the proportion between different classes of phenolic compounds in berries cultivar may vary (Beekwilder *et al.*, 2005).

The methods used to determine the total antioxidant capacity can be divided in two major groups: methods based on single electron transfer (SET) and hydrogen atom transfer reaction (HAT). The SET methods include ABTS/TEAC assay (Trolox equivalent antioxidant capacity), FRAP (ferric reducing ability), CUPRAC assay (copper reduction) and DPPH assay (2,2-diphenylpicrylhydrazil radical scavenging capacity). Hydrogen atom transfer reaction (HAT) assay include ORAC (oxygen radical absorbance capacity) and TRAP (total radical-trapping antioxidant parameter) assay. These assays are frequently used to measure the total antioxidant capacity of food extracts. Because their characteristics and because of the differences in the mechanisms of the reaction, a single assay will not reflect all the antioxidants present in the system (Li *et al.*, 2009). Hence, only by combining different assays, information about the response of the compounds present in samples in different experimental conditions can be achieved (Sariburun *et al.*, 2010).

The literature data about blueberries antioxidant activity and total polyphenol content are diverse, more on cultivated than wild varieties (Castrejón *et al.*, 2008; Dragović-Uzelac *et al.*, 2010; Garzón *et al.*, 2010; Giovanelli and Buratti, 2009; Howard *et al.*, 2003; Koca and Karadeniz, 2009; Prior *et al.*, 1998; You *et al.*, 2011).

The objectives of this study were (1) to investigate total polyphenols, total flavonoids, total anthocyanins content of blueberry extracts, (2) to separate and characterize the anthocyanin content using RP-HPLC-DAD, (3) to measure the antioxidant activity using three different SET-based assays (ABTS, FRAP, DPPH) and one HAT-based method (ORAC), (4) to correlate the antioxidant methods applied with total polyphenol, total flavonoid and total anthocyanin content.

Materials and methods

Chemicals

The standard compounds, including cyanidin-3-*O*-galactoside (purity 90%), cyanidin-3-*O*-glucoside (purity 95%), cyanidin (purity 95%), gallic acid (GAE) (purity 97.5%), quercetin (purity 98%) and 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) 97% purity, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) 98% purity, fluorescein 97% purity, 2,9-dimethyl-1,10-phenanthroline (Neocuproine) 99% purity, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), diammonium salt (ABTS) 98% purity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) 95% purity, 2,4,6-tripyridyl-S-triazine (TPTZ) 98% purity, potassium persulfate were obtained from Sigma-Aldrich (Darmstadt, Germany). Folin-Ciocalteu's phenol reagent, HCl, Na₂CO₃, AlCl₃, NaNO₂, H₂O₂, CuCl₂ were purchased from Merck (Darmstadt, Germany).

Samples extraction

Three varieties of cultivated highbush blueberries (*Vaccinium corymbosum*) 'Elliot', 'Bluecrop' and 'Duke' were purchased directly from the producers, a farm situated in North-West 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"E 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 Micra D-9 KT Digitronic, Germany) with 10 ml of acidified methanol (85:15 v/v, MeOH:HCl) (El-Sayed and Hucl, 2003). 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 redissolved in 10 ml of methanol, the samples were centrifuged at 5000 rpm and filtered through 0.45 µm nylon filter (Millipore).

Total phenolics

The amount of total polyphenol in the blueberry extracts was determined using modified Folin-Ciocalteu colorimetric method (Singleton *et al.*, 1999). Stock solution of sample extracts (25 µl each) were dissolved in methanol and further dilution were performed to obtain readings within the standard curve made with gallic acid (R=0.997). The extracts were oxidized by the Folin-Ciocalteu reagent (120 µl) and the neutralization was made with Na₂CO₃ (340 µl), after 5 minutes. The absorbance was measured at 750 nm after 90 minute in the dark, at room temperature. The results were expressed as milligram of gallic acid per 100 grams.

Total flavonoids

The total flavonoids content was determined using a colorimetric method (Kim *et al.*, 2003). The alcoholic extract was diluted to a final volume of 5 ml with distilled water. After adding 300 µl 5% NaNO₂, the mixture was allowed to stay 5 min. Then 300 µl AlCl₃ 10% was added and, after 6 minutes, 2 ml NaOH 1N. The solution was mixed well and the absorbance was measured against prepared water blank at 510 nm. Total flavonoid content was expressed as mg quercetin equivalents/100 g fresh weight basis.

Quantification of the anthocyanin content

The monomeric anthocyanin content of the blueberry extract was measured using the pH-differential method (Giusti and Wrolstad, 2001). The blueberry extracts dissolved in methanol were diluted with 0.025 mol/l potassium chloride (adjusted with HCl to pH 1.0) and 0.4 mol/l sodium acetate (pH 4.5). Each sample and the standard (cyanidin-3-glucoside) were diluted with the buffer solution pH 1 and the absorbance was measured at 520 nm and 700 nm using a UV spectrophotometer (Jasco V-630, International Co. Ltd, Japan). A second aliquot of each sample was diluted to the same value with the buffer solution pH 4.5 and measured at 520 and 700 nm. The absorbance values were calculated as follows:

$$A = (A_{\lambda 520} - A_{\lambda 700})_{\text{pH}1.0} - (A_{\lambda 520} - A_{\lambda 700})_{\text{pH}4.5}$$

The total anthocyanin content was calculated according to the formula:

$$TA = \frac{A \times MW \times DF \times 1000}{\epsilon \times l}$$

The results were expressed as mg cyanidin-3-glucoside chloride per 100 g fruit using the molar absorptivity (ϵ)

in methanol/HCl of 34300 ($M^{-1} \text{ cm}^{-1}$), molecular weight (MW) of 484.8 and dilution factor (DF).

Anthocyanin 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 SPD20 A UV-VIS detector (DAD) and a Luna Phenomenex C-18 column ($5 \mu\text{m}$, $25 \text{ cm} \times 4.6 \text{ mm}$). 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°C . The chromatograms were monitored at 520 nm. The anthocyanins identification and peak assignments are based on their retention times, UV-VIS spectra comparing with standards and published data. The anthocyanin quantification was performed using cyanidin 3-O-galactoside.

Oxygen radical absorbance activity (ORAC)

The oxygen radical absorbance capacity (ORAC) measure the peroxy radical scavenging activity using as standard 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Huang *et al.*, 2002). A fluorescein stock solution ($4 \times 10^{-3} \mu\text{M}$) was made in phosphate buffer (75 mM, pH 7.4) and kept in the dark at 4°C . Before utilization, the fluorescein stock solution was diluted with the phosphate buffer. The fluorescein solution was added to each Trolox standard and blueberry sample (25 μl) made in phosphate buffer and incubated for 30 min, at 37°C . The reaction was initiated by adding 25 μl 2,2'-azobis-2-amidinopropane (AAPH) and the fluorescence was measured kinetically at excitation wavelength 485 nm and emission wavelength 535 nm, every minute using a fluorescence microplate reader BioTek (Synergy HT, BioTek Instruments, Winooski, VT). The ORAC values for each blueberry extract were calculated using the net area under the decay curves and were expressed as micromoles Trolox equivalents per gram sample ($\mu\text{mol TE/g}$).

ABTS radical cation decolorization assay (ABTS)

The ABTS assay is based on the capacity of a sample to scavenge the ABTS radical cation (ABTS^+) compared to a standard antioxidant (Trolox), adapted to 96 wells microplate, described by Arnao *et al.* (2001). The ABTS^+ solution was produced by reacting 7mM ABTS stock solution with 2.45 mM potassium persulfate (final concentration) for 12-16 h, in the dark, at room temperature. Prior to use, the ABTS^+ working solution was prepared by diluting the stock solution with EtOH to an absorbance of 0.70 ± 0.02 at 734 nm. The samples and Trolox standards (20 μl) were combined with the ABTS^+ working solution (170 μl , absorbance 0.70 ± 0.02) in 96-well microplate. After 6 min of incubation at 30°C , the absorbance at 734 nm was read with a microplate reader. If the absorbance

of ABTS value was higher than the standard, the samples were diluted and re-evaluated. The results were expressed as micromoles Trolox equivalents per gram sample (TE $\mu\text{mol/g}$).

Reducing power (FRAP)

The reducing capacity of methanolic blueberry extracts was measured as ferric reducing antioxidant power (FRAP). Antioxidants are evaluated as reducers of Fe^{3+} to Fe^{2+} , which is chelated by TPTZ to form Fe^{2+} - TPTZ complex, with a maximum absorbance at 593 nm (Benzie and Strain, 1996). The absorbance of the colored product was monitored by Biotek Synergy HT spectrophotometer. All solutions were used in the day of preparation. Briefly, 2.5 ml TPTZ (10 mM in 40 nM HCl), 2.5 ml acetate buffer (300 mM, pH=3.6) and 2.5 ml FeCl_3 (20 mM) were mixed and after adding 180 μl FRAP reagent the mixture was incubated for 3 min. Then, 20 μl of each sample added to each well and the absorbance was read immediately at 593 nm with a microplate reader. Samples dilution were performed when the values were over the linear range of the curve of 0 to 1 $\mu\text{M Fe}^{2+}/\text{ml}$, using $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$.

DPPH· scavenging activity assay

The DPPH scavenging activity assay was done according to a method reported by Brand-Williams *et al.* (1995). A DPPH· solution (80 μM) was freshly prepared in 95% methanol. A volume of 250 μl of this solution was allowed to react with 35 μl sample and the absorbance was measured at 515 nm, for 30 minutes. The chemical kinetics of blueberries extract was recorded. The antioxidant activity was calculated as follows:

$$\% \text{ DPPH} \cdot \text{ scavenging activity} = (1 - [A_{\text{sample}} / A_{\text{control} = 0}]) 100$$

Statistical analysis

The data's are expressed as mean \pm 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 the 5% level ($p < 0.05$). All statistical analysis was carried out using Graph Pad Version 4.0 (Graph Pad Software Inc; San Diego, CA, USA). Pearson's correlation coefficient was calculated using Microsoft Excel 2003.

Results and discussion

Total polyphenols content

The comparative data about total polyphenols, flavonoids and anthocyanins content in wild and cultivated blueberries are presented in Tab. 1.

The total polyphenols content (TPC) was determined using the Folin-Ciocalteu method. Gallic acid was used as calibration standard and the results (expressed as gallic acid equivalents) were expressed as means \pm standard deviation of triplicate analysis. The TPC values in the blueberry extracts analyzed was in the range of 424.84 - 819.12 mg

Tab. 1. Total polyphenols, total flavonoids and total anthocyanins content in wild and cultivated blueberries

	Total polyphenols GAE mg/100 g	Total flavonoids mg QE/100 g	Total anthocyanins C3GE mg/100 g
<i>Vaccinium corymbosum</i>			
'Elliot'	526.3 ± 26 ^d	92.82 ± 8.4 ^a	163.40 ± 16.4 ^c
'Bluecrop'	652.27 ± 30 ^c	103.18 ± 10.2 ^a	160.76 ± 13.9 ^c
'Duke'	424.84 ± 20 ^c	84.33 ± 8 ^b	100.58 ± 13.5 ^d
<i>Vaccinium myrtillus</i>			
Wild 1	819.12 ± 36 ^a	110.36 ± 12.3 ^a	300.02 ± 27.9 ^a
Wild 2	672.59 ± 30 ^b	112.50 ± 15 ^a	252.23 ± 18 ^b

GAE mg/100g, QE mg/100g, and C3GE mg/100g represent mg of gallic acid equivalents, mg of quercetin equivalents and mg of cyanidin-3-glucoside equivalents per 100g of fresh blueberry, respectively. For each measurements, the data marked by different letters indicate significant difference ($p < 0.05$)

GAE/100 g fresh weight. Among all the varieties analyzed, the Wild 1 blueberry revealed the highest TPC at 819.12 gallic acid equivalents/100 g FW followed by Wild 2 blueberries (672.59 mg GAE/100g). Between the cultivated blueberries, 'Bluecrop' has the highest TPC at 652.27 mg GAE/100 g while the lowest value was found for 'Duke' variety (424.84 mg GAE/100g). Significant differences were found in total phenolic content when compared all the varieties analyzed ($p < 0.05$).

The TPC data's obtained are comparable to previous findings which reported values between 251-310 mg GAE/100 g for some cultivated blueberries and between 577 and 614 mg GAE/100 g for wild Italian blueberries (Giovannelli and Buratti, 2009). Lee *et al.* (2004) obtained between 367-1286 mg GAE/100 g total polyphenols for *V. membranaceum* and for *V. ovalifolium* species 677-1054 mg GAE/100 g. Prior *et al.* (1998) reported values between 181 and 390 mg/100g for *V. corymbosum* L species. Dragovič-Uzelac *et al.* (2010) reported for 'Bluecrop' variety higher amount compared with 'Duke', while Prior *et al.* (1998) obtained higher TPC values for 'Duke' vari-

ety. It is known that phenolics show quantitative variation at different genetic levels within species.

Total flavonoids content

The total flavonoids content of the extracts was determined using aluminium chloride colorimetric method and the results are shown in Tab. 1. The total flavonoids content ranged from 84.33 mg QE/100 g in 'Duke' variety to 112.5 mg QE/100 g in Wild 2 blueberries. For the other blueberry varieties the TFC were as follows: Wild 1 (110.36 mg QE/100 g), 'Bluecrop' (103.18 mg QE/100 g), 'Elliot' (92.82 mg QE/100 g) and 'Duke' (84.33 mg QE/100 g). There were no significant differences in total flavonoid content among 'Elliot', 'Bluecrop', Wild 1 and Wild 2 ($p < 0.05$). 'Duke' variety has the lowest total flavonoid content, significantly different than other varieties analyzed. There are no data regarding the total content of flavonoids in blueberries. In blackberry varieties, the TFC content range between 29.07-82.21 mg QE/100 g (Sariburun *et al.*, 2010).

Total anthocyanins content

The total anthocyanins content (TAC) of the blueberries extract were also determined (Tab. 1). The highest anthocyanins content was found in wild blueberries Wild 1 (300.02 mg/100g), followed by wild blueberries Wild 2 (252.23 mg/100g), 'Elliot' (163.4 mg/100g), 'Bluecrop' (160.76 mg/100g) and the lowest TAC was found in 'Duke' variety (100.58 mg/100g). There were significant differences ($p < 0.05$) in anthocyanins content between Wild 1, Wild 2 and 'Duke'. However, significant differences in the total anthocyanin content were not observed between 'Elliot' and 'Bluecrop' ($p < 0.05$). The levels of TAC in TPC are between 23% and 37% and they are in agreement with the data's reported by other authors (Giovannelli and Buratti, 2009). The present TAC values obtained are in agreement with other studies (Dragovič-Uzelac *et al.*, 2010; Prior *et al.*, 1998).

Tab. 2. Concentration of individual anthocyanins in blueberries (expressed in mg/100 g fruit)

Compound	Retention time	Maximum absorbance	<i>Vaccinium myrtillus</i>		<i>Vaccinium corymbosum</i>		
			Wild 1	Wild 2	'Bluecrop'	'Elliot'	'Duke'
Delphinidin-3-galactoside	9.2	217; 277; 523	113.67±11	73.43±8.1	53.29±7.2	ND	ND
Delphinidin-3-glucoside	10.6	217; 277; 523	119.86±14	87.14±8.2	24.53±2	53.62±7	23.69±2.4
Cyanidin-3-galactoside	12.4	217; 279; 518	91.85±10	37.95±4.3	9.96±1.8	ND	ND
Delphinidin-3-arabinoside	13.01	217; 277; 529	66.64±9.2	76.35±8.8	31.78±2.6	41.07±5.1	13.64±1.1
Cyanidin-3-glucoside	14.4	217; 279; 518	96.48±11	43.34±6.1	2.08±0.4	3.09±0.6	0.11±
Petunidin-3-galactoside	15.09	217; 277; 524	38.36±5.2	17.53±0.9	28.54±2.5	ND	ND
Petunidin-3-glucoside	16.9	217; 278; 521	146.27±18	85.19±7.8	25.14±2.5	21.35±1.9	8.18±0.9
Peonidin-3-galactoside	19.4	217; 278; 520	8.71±1.3	ND	ND	125.79±12	37.114.3
Petunidin-3-arabinoside	20.3	217; 277; 525	12.80±1.9	9.66±1.2	12.70±1.9	ND	ND
Peonidin-3-glucoside	22.4	217; 278; 521	108.81±12	28.91±2.2	54.37±7.5	12±0.9	12.14±1.1
Malvidin-3-galactoside	25.3	217; 278; 527	119.53±12	39.00±4.3	37.97±2.6	67.45±9	27.55±2.4
Malvidin-3-glucoside	27.4	217; 277; 528	17.51±3.2	6.04±1.1	34.75±2.5	ND	ND
Malvidin-3-arabinoside	29.5	217; 277; 528	tr	tr	ND	ND	ND

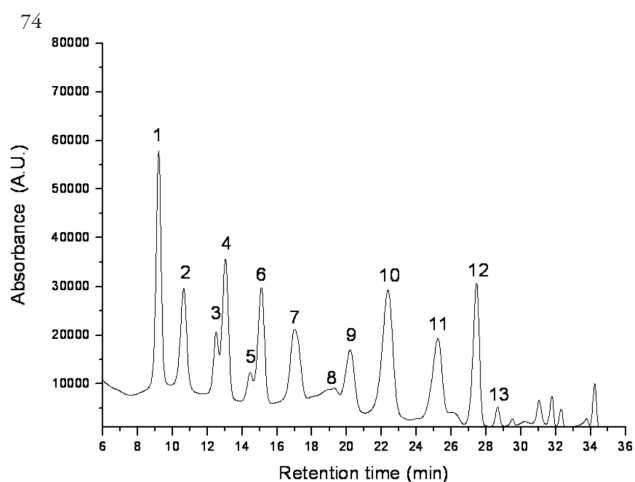


Fig. 1. Anthocyanin HPLC profiles for 'Bluecrop' variety. Peak identification: 1- Delphinidin-3-galactoside; 2- Delphinidin-3-glucoside; 3- Cyanidin-3-galactoside; 4- Delphinidin-3-arabinoside; 5- Cyanidin-3-glucoside; 6- Petunidin-3-galactoside; 7- Petunidin-3-glucoside; 8- Peonidin-3-galactoside; 9- Petunidin-3-arabinoside; 10- Peonidin-3-glucoside; 11- Malvidin-3-galactoside; 12- Malvidin-3-glucoside; 13- Malvidin-3-arabinoside

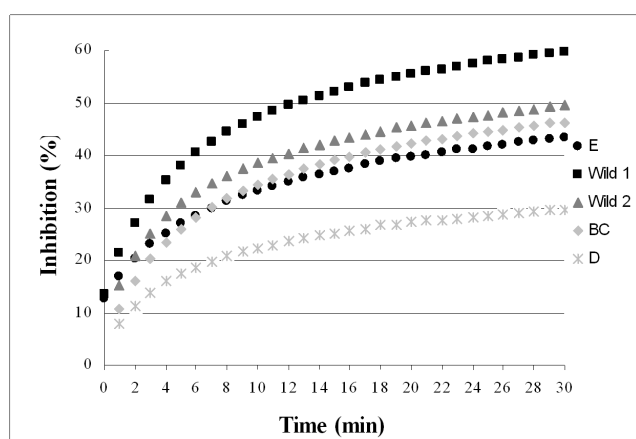


Fig. 2. Kinetics of antioxidant activity of blueberry extracts using DPPH method. The inhibition percentage represents the antioxidant activity

Identification and quantification of anthocyanins

Using HPLC-DAD, 13 anthocyanins were identified based on their retention time, UV-VIS spectra compared with standards and published data. HPLC chromatogram for anthocyanins separation in 'Bluecrop' variety is presented in Fig. 1.

In *Vaccinium myrtillus*, petunidin-3-glucoside and delphinidin-3-glucoside have the highest contribution to the anthocyanin content (15.6% and 16.9%; 17.3 and 12.7% respectively). In *Vaccinium corymbosum*, peonidin-3-galactoside represent the major anthocyanin content in 'Elliot' and 'Duke' variety (40.3% and 30.3%) but in 'Bluecrop' peonidin-3-glucoside is absent, peonidin-3-glucoside being the major contributors (17.3%).

Tab. 3. Antioxidant activity for blueberry fruits, using four different complementary assays (FRAP, ABTS, ORAC, DPPH)

	FRAP $\mu\text{M Fe}^{2+}/\text{g}$	ABTS TE $\mu\text{mol}/\text{g}$	ORAC TE $\mu\text{mol}/\text{g}$	DPPH- scavenging activity (%)
<i>Vaccinium corymbosum</i>				
'Elliot'	50.74 ± 1.9^c	36.46 ± 4.26^c	38.05 ± 1.56^c	43.48 ± 1.9^d
'Bluecrop'	60.39 ± 1.6^b	37.96 ± 2.98^{bc}	37.04 ± 1.7^a	46.64 ± 1.65^c
'Duke'	33.03 ± 2.54^d	24.33 ± 3.76^d	34.85 ± 1.3^b	29.96 ± 1.38^c
<i>Vaccinium myrtillus</i>				
Wild 1	73.71 ± 3.2^a	56.65 ± 3.79^a	38.49 ± 1.01^a	59.79 ± 1.24^a
Wild 2	64.87 ± 2.9^b	43.08 ± 2.3^b	37.78 ± 0.89^a	49.93 ± 1.6^b

Total antioxidant activity

For measuring the total antioxidant activity of the blueberry extracts, four different assays were used. Tab. 3 includes the mean values for antioxidant activity measured.

ORAC assay is probably the most widely used HAT-based assay and indicate the free-radical scavenging ability of antioxidant against peroxy radical. The values obtained were not significantly different among samples, ranging from 34.85 - $38.49 \mu\text{mol TE/g}$ fresh weight. The present ORAC results are similar to those obtained by Prior *et al.* (1998) (17 - $37.1 \mu\text{mol TE/g}$) and lower than the values reported for rabbiteye blueberries by You *et al.* (2011) (44 - $55 \mu\text{mol TE/g}$). For 'Duke' variety, it has been obtained $34.85 \mu\text{mol TE/g}$ comparable with the values reported by Wang *et al.* (2009) ($40.4 \mu\text{mol TE/g}$) and Moyer *et al.* (2002) ($32.6 \mu\text{mol TE/g}$). Regarding the ORAC values for 'Bluecrop' variety it is a great discrepancy in the literature data. Prior *et al.* (1998), reported 17 mM TE/kg , Moyer *et al.* (2002), 50 mM TE/kg and Howard *et al.* (2003), between 21 - 38 mM TE/kg , the last values being comparable with the present value. The last mention study indicates that genotypes influence more the TPC and ORAC values than growing season.

ABTS assay is based on the antioxidant ability of the extracts to react with ABTS^+ radical cation generated in the system. In contrast, the ferric reducing antioxidant power assay (FRAP) measures the ability of the extracts to reduce ferric complex to the ferrous form. The averages values obtained for ABTS and FRAP assay are given in Tab. 2. Blueberries Wild 1 extract showed the highest antioxidant activity based on FRAP and ABTS assay ($73.71 \mu\text{M Fe}^{2+}/\text{g}$ and $56.65 \mu\text{mol TE/g}$). The lowest level in both assays was obtained for 'Duke' variety. The antioxidant activity order in both assays, FRAP and ABTS, was: Wild 1 > Wild 2 > 'Bluecrop' > 'Elliot' > 'Duke'. In FRAP assay, there were significant differences found between Wild 1, Wild 2, 'Bluecrop' and 'Duke' ($p < 0.05$). In ABTS assay, significant differences were found between Wild 1, Wild 2, 'Elliot' and 'Duke' ($p < 0.05$). There were no statistical differences between 'Elliot' and 'Bluecrop' varieties.

Tab. 4. Pearson's correlation coefficients of antioxidant activity (FRAP, ABTS, ORAC, DPPH), total polyphenol content (TPC), total flavonoid (TF) and total anthocyanin (TA) in blueberries

	TA	TF	FRAP	ABTS	ORAC	DPPH
TPC	0.923*	0.914*	0.975**	0.966**	0.765 ^{ns}	0.973**
TA	-	0.894*	0.919*	0.961**	0.805 ^{ns}	0.939*
TF		-	0.95*	0.853 ^{ns}	0.739 ^{ns}	0.896*
FRAP			-	0.951*	0.858 ^{ns}	0.985**
ABTS				-	0.853 ^{ns}	0.986**
ORAC					-	0.891*
DPPH						-

*,** significant at $p < 0.05$ and 0.01 , respectively

The range of FRAP values in the present study (33.03-73.71 $\mu\text{M Fe}^{2+}/\text{g}$) was generally higher than those reported by Koca and Karadeniz (2009) (7.41-13.69 $\mu\text{mol/g}$ for cultivated and 34.45-57.92 $\mu\text{mol/g}$ for wild blueberries). The present ABTS values are higher than the values obtained by Sellappan *et al.* (2002) (8.11-38.29 $\mu\text{mol/g}$).

High level of antioxidant activity obtained for Wild 1 blueberries, by all the methods used, could be due to its high level of total polyphenol and total anthocyanin content.

The DPPH· scavenging activity of blueberries extract is presented in Tab. 4. It has been obtained significant differences between all the blueberry extracts analyzed, with the highest value for Wild 1 (59.79%) and the lowest for 'Duke' variety (29.96%). The present data are in agreement with previously reported data: 34.13% of inhibition for wild blueberries (Li *et al.*, 2009) and 28% of inhibition (Ogawa *et al.*, 2008).

The kinetic curves presented in Fig. 2 clearly indicated that Wild 1 and 2 blueberries had the highest scavenging activity during 30 minutes.

In Tab. 4 are presented the Pearson's coefficients, which indicate the possible correlation between polyphenol composition with different antioxidant activity and the correlation of different assays used with each other.

A correlation analysis was done among total polyphenols content, total flavonoid, total anthocyanin and the antioxidant activity values obtained (Tab. 4). The total polyphenols content (TPC), total flavonoids (TF) and total anthocyanins (TA) exhibit a significant correlation ($p < 0.05$ and $p < 0.01$) with different antioxidant methods with a decreasing order of $\text{TPC} > \text{TA} > \text{TF}$. This suggests that antioxidant activity is more correlated to TPC than TA. These results are in agreement with other studies (Giovanelli and Buratti, 2009; Koca and Karadeniz, 2009; Prior *et al.*, 1998). The high correlation between the FRAP and TPC content can be attributed to the fact that both assays rely on the same reaction mechanism.

There were no statistically significant correlation between ORAC values and TPC, TF and TA. Tabart *et al.* (2009) found no correlation between ABTS or DPPH values and ORAC data's applied on different phenolic

standards. The highest Pearson's coefficient between was obtained when it has been compared ABTS and DPPH antioxidant methods (0.986). As mentioned before, these two assays are also based on similar mechanisms.

Conclusions

There were analyzed, for the first time, the antioxidant activity in relation to the polyphenol content in wild and some cultivated blueberry fruits from Romania. Total polyphenol, total anthocyanin, total flavonoid content and antioxidant activity measured with six different assays were higher in wild blueberry varieties, as compared with cultivated ones. The FRAP, ABTS and DPPH assays showed higher correlation, statistically significant, with total polyphenol content compared with total anthocyanin content. To measure adequately the antioxidant potential ABTS and DPPH methods are considered to be most appropriate, in good agreement with the concentrations of phenolic derivatives (polyphenols, anthocyanins, flavonoids).

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