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Determination of anthocianins bilberry (*Vaccinium myrtillus* l.) on North East of Albania

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Abstract

Bilberry (*Vaccinium myrtillus* L., Ericaceae family) is a perennial subshrub and very important plants resource in North of Albania. The fruits of the bilberry are recognized for their bioactive properties and distinctive aroma and flavour. In the present study, the fruits collected from 10 different mountain regions of North and North East of Albania were analysed in order to determine their quantitative and qualitative features. The total amount of biologically active compounds in fresh fruits, were identified by LC-ESI/MS and their individual antioxidant capacities were evaluated by on-line HPLCABTS [1]. To data, 32 anthocyanins and flavonoids compounds have been isolated and identified from the fruits of *Vaccinium myrtillus.* The total amount of anthocyanins (%) varied from 0.15 to 0,51. Higher amounts of total phenolic and total anthocyanins (0.51 % and 1027.1 mg 100 g^{-1} dw) were detected in population collected from Laver Dardh zone of Puka district and the lowest amounts were detected in the population of Vermosh area of Malesia e Madhe district.

Keywords: *Vaccinium myrtillus* L., North of Albania, anthocyanins, phenolics, flavonoids

1. Introduction

Bilberry, also known as blueberry, blue berry, is a Ericaceae (Ericaceae) Vaccinium subfamily (Vaccinioideae) Vaccinium genus (Vacciniodeae) plants, deciduous or evergreen perennial shrub or small shrub, widely distributed in the northern hemisphere. *Vaccinium* is the family of all blueberries and includes more than 450 plants. This plant grows wild around the world and there are many names given to different blueberries. These Blueberries only reach a height of 20 or 40 cm, which is found mainly in the north east of Albania [2]. Bilberry species produce berries of a smaller size than highbush and even though they can be found growing wild in many parts of the U.S. are not commonly found in supermarkets [3].

Berries in general are considered low in terms of their glycemic index (GI). GI is a common way of identifying the potential impact of a food on our blood sugar level once we've consumed and digested that food. In general, foods with a GI of 50 or below are considered "low" in terms of their glycemic index value [4]. When compared to other berries, blueberries are not particularly low in terms of their GI. Studies show the GI for blueberries as falling somewhere in the range of 40-53, with berries like blackberries, raspberries, and strawberries repeatedly scoring closer to 30 than to 40. However, a recent study that included blueberries as a low-GI fruit has found that blueberries, along with other berries, clearly have a favorable impact on blood sugar regulation in persons already diagnosed with type 2 diabetes. Participants in the study who consumed at last 3 servings of low-GI fruits per day (including blueberries) saw significant improvement in their regulation of blood sugar over a three-month period of time. (Their blood levels of glycosylated hemoglobin, or HgA1C were used as the standard of measurement in this study) [5].

Blueberries are phytonutrient superstars. These fruits contain significant amounts of anthocyanadins, antioxidant compounds that give blue, purple and red colors to fruits and vegetables. Included in blueberry anthocyanins are malvidins, delphinidins, pelargonidins, cyanidins, and peonidins. In addition to their anthocyanins, blueberrries also contain hydroxycinnamic acids (including caffeic, ferulic, and coumaric acid), hydroxybenzoic acids (including gallic and procatechuic acid), and flavonols (including kaempferol, quercetin and myricetin). Blueberries also contain the unique, phenol-like antioxidants pterostilbene and resveratrol. Blueberries are among a small number of foods that contain measurable amounts of oxalates, naturally-occurring substances found in plants, animals, and human beings. Laboratory studies have shown that oxalates may also interfere with absorption of calcium from the body [4]. Yet, in every peer-reviewed research study, the ability of oxalates to lower calcium absorption is

relatively small and definitely does not outweigh the ability of oxalate-containing foods to contribute calcium to the meal plan [6].

The area of north east of Albania where the bilberry is located in general, is characterized by high rain precipitation that moves between 800 and 1100 mm per year. The average annual temperature is around 11.9 °C. Average temperatures range from 3.9 to extremes 25.4 °C. Climatic zones are with average annual temperature higher, are between 10.7 and 12.3 ° C, located in the Has district and partially in Tropoja and Vermoshi. Climatic zones with average annual temperature lower, contained between 7.5 and 10.7^{o}C are mountainous areas in north of Albania [7].

2. Material and methods

To determine the anthocianins bilberry (*vaccinium myrtillus* l.) are taken 10 samples from different location on north east of Albania end concretely: three samples on Puka region, two on Tropoja region, two on Kuksi region and three on Malesia e Madhe region. Together with fruit and leaves samples are taken also soil samples on two different layer depth: on 0-30 cm and 30 - 60 cm.

Total anthocyanins content

Total anthocyanin content was investigated according to the procedure described in Ph. Eur. 6.0 (European Pharmacopoea 6.0., 2008) [6]. Briefly, 50g of fresh berries were crushed extemporaneously. To 5g of crushed, accurately weighed drug, 95 ml of methanol were added and mechanically stirred for 30 min then filtered into a 100 ml volumetric flask. Filter was rinsed and diluted to 100 ml with methanol. A 50-fold dilution of this solution in a 0.1 per cent v/v solution of hydrochloric acid in methanol was prepared. The absorbance of the solution was measured at 528 nm, using a 0.1 per cent v/v solution of hydrochloric acid in methanol as the compensation liquid. The percentage content of anthocyanins, expressed as cyanidin-3-glucoside chloride, was calculated from the expression: A x 5000/718 x m (A = absorbance at 528 nm; 718 = specific absorbance of cyanidin-3-glucoside chloride at 528 nm; m = mass of the substance to be examined in grams) [8].

LC/UV/MS analysis

LC/MS analysis was performed on an Agilent MSD TOF coupled to an Agilent 1200 series HPLC. The same column and gradient program used as for HPLC-DAD analysis. Formic acid (1%) was used instead of 1 % solution of orthophosphoric acid in water, for analysis of flavonoids and phenolic acids. Mass spectra were acquired using an Agilent ESI-MSD TOF. Drying gas (N2) flow was 12 L/min; nebulizer pressure was 45 psig; drying gas temperature was 350 ^{o}C. For ESI analysis, the parameters were: capillary voltage, 4000 V; fragmentor, 140 V; skimmer, 60 V; Oct RF V 250 V, for negative (flavonoids) and positive modes (anthocyanins) (11). The mass range was from 100 to 2000 m/z. Processing of data was carried out with the software Molecular Feature Extractor.

3. Results and discussion

Agro-chemical land evaluation

Land agrochemical testing was conducted by Agriculture Technology Transfer Cecnter of Fushe Kruje in main plots with bilberry in north eastern Albania (1) [9][10]. Samples were taken in two layr levels 0-30 cm and 30 -60 cm. For each sample in the analytic testing were determine the reaction of soils, mechanical composition, level of humus, nitrogen general, phosphorus assimilable, potassium, capacity rate cationic, state of cations exchangeable (Na^{+}, K^{+}, Ca^{++} and Mg^{++}) and electrical conductivity.

For determining the soils physical attributes were done the partical metricr analysis. The clay content in them varies in values ranging from 26.8 to 43.0 % , with an overall average of 35.2 %. The data show that 91.3 % of these lands have clay content of over 30 % and only 8.7 % of them below this value. In the second layer of clay content retains the same value as that of the first layer. According to higher content of clay have Malesia Madhe district lands and less Tropojë district. The data show that 82.6 % of the land belonging to the balanced type of clay soils (clay loam), 8.7 % of the land is so balanced and clay soils.

The soil reaction result that 95.7% of lands are slightly basic and only 4.3% of them are easily acids. This indicator ranges in values ranging from 6.9 to 7.7, with an overall average of 7.3. In the second layer retains the same pH value.

The electric conductivity values show that the EC ranging from 0107 to 0260 mS/cm, with an average total of 0.185 mS/cm. In the second layer, this index suffers a decrease of 12.1% versus the first layer. All lands have value K.E. under 0:45 mS/cm, which considers them without salinisation problems that can restrict normal cultivation of vegetation.

Cationic exchange capacity (CEC) show that 69.6% of the soils have a high level of CEC and only

30.4% have a medium level. CEC values fluctuate from 15:17 - 32.15 g mek/100 ground. In first class average of this indicator amounts to 23.7 g mek/100 ground, while the second layer does not have its changes, regardless of the depth change.

The humus content ranges from 0.96-2.72%, with an overall average of 1.73%. In the second layer of humus content suffers reduced at 39.1%, compared with the upper layer. According to the poorest lands occupy 39% of the soils studied and the level of their average 61%.

Base saturation stage show that 73.9% of the land have studied this indicator very high, above 80%, but in the remainder it appear higher in value ranging from 70 to 80%. According to the calculations of all lands have value of this indicator over 70%, which indicates that these lands fall under the category of too little land shplashme.

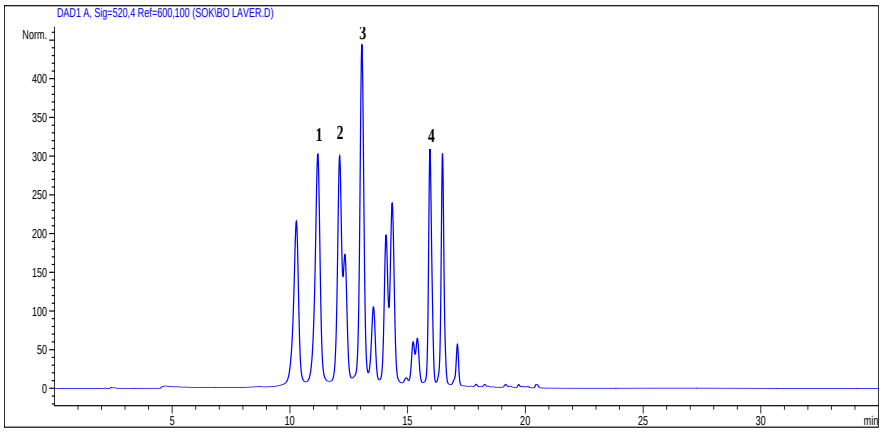
Total anthocyanins

According to the Ph. Eur. fresh bilberry fructus are standardised on min 0.3% of total anthocyanins, expressed as cyanidin 3-glucoside, and calculated on dried fruit. Since water content in bilberry fruits is min 70%, in all of the tested samples total anthocyanin content is above prescribed minimum [11].

Table 1. Total anthocyanins (%)in *Vaccinium myrtillus* samples.

Sample	*Total anthocyanins*
Kabasa	0.47 ± 0.02
Laver daroh	**0.51± 0.02**
Teblim puku	0.46 ± 0.01
Kolosjan	0.51 ± 0.01
Gryke	0.41 ± 0.01
Komuna fierce	0.45 ± 0.02
Cerrem tropoje	0.16 ± 0.02
Komune kelmend	0.45 ± 0.02
Sample 9	0.15 ± 0.00
Sample 10	0.49 ± 0.01

Fingerprint identification of anthocyanins



Legend: **1**, delphinidin–glucoside; **2**, cyanidin-galactoside; **3**,cyanidin-glucoside; **4**, malvidin-glucoside.

Figure 1. HPLC chromatogram (λ = 520 nm) of MeOH extract of bilberry sample.

LC/UV/MS analysis

Table 3. LC/UV/MS analysis of anthocyanins in *Vaccinium myrtillus* samples

Peak	*tR (min)*	*Compound*	*DAD λmax (nm)*	*Mass*	*Molecular formula*
1	10,0	Delphinidin hexoside	526	465,1034	$C_{21}H_{21}O_{12}$
2	10,9	Delphinidin 3-O-glucoside	526	465,1041	$C_{21}H_{21}O_{12}$
3	11,9	Cyanidin 3-O-galactoside	518	449,1096	$C_{21}H_{21}O_{11}$
4	12,1	Delphinidin pentoside	526	435,0911	$C_{20}H_{19}O_{11}$
5	12,9	Cyanidin 3-O-glucoside	518	449,1106	$C_{21}H_{21}O_{11}$
6	13,4	Petunidin hexoside	528	479,1170	$C_{22}H_{23}O_{12}$
7	13,9	Cyanidin 3-O-arabinoside	518	419,0987	$C_{20}H_{19}O_{10}$
8	14,2	Petunidin hexoside	528	479,1176	$C_{22}H_{23}O_{12}$
9	15,1	Peonidin hexoside	518	463,1249	$C_{22}H_{23}O_{11}$

Peak	*tR (min)*	*Compound*	*DAD λmax (nm)*	*Mass*	*Molecular formula*
10	15,3	Petunidin pentoside	528	449,1090	$C_{21}H_{21}O_{11}$
11	15,9	Peonidin hexoside	520	463,1239	$C_{22}H_{23}O_{11}$
12	16,0	Malvidin hexoside	528	493,1348	$C_{23}H_{25}O_{12}$
13	16,5	Malvidin hexoside	530	493,1352	$C_{23}H_{25}O_{12}$
16	17,1	Malvidin pentoside	530	463,1243	$C_{22}H_{23}O_{11}$

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