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CHEMICAL VARIABILITY AND ANTIOXIDANT ACTIVITY OF THE LEAVES OF CHOSEN HIGHBUSH BLUEBERRY (*VACCINIUM CORYMBOSUM* L.) CULTIVARS

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Abstract. The paper deals with the chemical composition and antioxidant activity of aqueous extracts from leaves of two highbush blueberry varieties: '*Bluecrop*' and '*Northland*'. The study revealed differences in the content of the analyzed components. Leaves of cv. '*Bluecrop*' were characterized by a higher content of chlorophyll, flavonoids and anthocyanins, while the leaves of cv. '*Northland*' contained more reducing sugars and total phenolic acids, tannins, and essential oils. Capacity of neutralizing the free radicals (DPPH) in leaves of both tested cultivars was found at comparable levels.

Key words: *Vaccinium corymbosum*, chemical components, antioxidants, DPPH, essential oils

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Introduction

The use of plant products in dietetics, care, and prevention of many diseases has significantly increased in recent years (Ball 2001). Plant-origin substances having no nutritional value (non-nutritive phytochemicals) are characterized by diverse chemical structures and are classified as vitamins, terpenoids, polyphenols, pigments, alkaloids, glucosilanes, saponins and others (Grajek 2004). Their biological activity is often associated with antioxidant capacity. Many of these compounds are not synthesized by human body, thus providing them with food is some kind of prevention, particularly in protecting against free radicals.

Highbush blueberry (*Vaccinium corymbosum* L.) belongs to Ericaceae family and Vaccinium genus. Its berries have very rich and valuable chemical composition along with diet and taste qualities as well as medicinal properties (Moyer *et al.* 2002; Ścibisz & Mitek 2002). In medicine, both blueberry leaves and fruits are used, especially those of cranberry (*Vaccinium vitis-idaea* L.). Decoctions made of the leaves are used for treating kidney stones and inflammation of the renal pelvis, and are useful for intestine and stomach catarrh (Kalt *et al.* 1999).

Due to the significant increase in blueberry acreage, it is advisable to examine the content of primary and secondary metabolites in the leaves of this species.

The purpose of this study was to determine the chemical characteristics and antioxidant activity of leaves from two popular highbush blueberry cultivars – '*Bluecrop*' and '*Northland*'.

Material and methods

Material for study consisted of leaves of highbush blueberry cultivars '*Bluecrop*' and '*Northland*' obtained in 2010-2011 from the commercial plantation within Lublin area. Laboratory analysis involved healthy and undamaged leaves, in which following parameters were determined directly after the harvest: dry matter (Chmielewska 1955), *chlorophyll a, b,* and their sum by means of

MacKinney method (MacKinney 1941), as well as total and reducing sugars applying Schoorl-Luff procedure (Charlampowicz 1966). Some of the leaves were dried at 35°C (in the shade and on open air) to obtain air-dry raw material, which was analyzed by specifying the content of moisture (Farmakopea Polska 2006); phenolic acids using Arnova reagent (Farmakopea Polska 2002); flavonoids applying Polish Pharmacopoeia method (Farmakopea Polska 2006); anthocyanins according to Miłkowska & Strzelecka (1995), tannins by means of Farmakopea Polska (2006), and essential oils due to indirect distillation using xylene (Farmakopea Polska 2008). Furthermore, the capacity to neutralize free radicals (DPPH) in aqueous extracts made of air-dried leaves using the method described by Chen & Ho (1997) was determined. Analyses of the chemical composition of two highbush blueberry varieties leaves were carried out in the Laboratory of Vegetable and Herbal Resources Quality of the Department of Vegetable Crops and Medicinal Plants at the University of Life Sciences in Lublin.

Results and disscusion

The chemical analyses of plant material revealed differences in the content of primary and secondary metabolites in the leaves of tested highbush blueberry cultivars. Wach (2004) showed that fruits of cv. '*Northland*' accumulate 13.3%, while the cv. '*Bluecrop*' – 14.4% of dry matter. The dry matter content in leaves of tested varieties was almost twice as high reaching the level of 36.5% through 34.4% (Tab. 1).

Leaves of many plants are a substantial source of natural pigments. The content of chlorophyll a and b in the analyzed raw materials depended on the cultivar and averaged to 2.274 mg · g^{-1}. The leaves of cv. '*Bluecrop*' had more *chlorophyll a* (0.909 mg · g^{-1}) and *chlorophyll b* (1.335 mg · g^{-1}).

Mean content of total and reducing sugars in the leaves of highbush blueberry plants amounted to 3.45% and 1.12%. It has been reported that cv. '*Northland*' was more abundant in these substances. According to Wach (2004) total sugars in blueberry fruits of these cultivars are formed at the level of about 14.6% and 13.9%.

Mean moisture content in leaves of highbush blueberry was at the level of 8.03%. A larger percentage of water content in the leaves was recorded for cv. '*Northland*' (Tab. 2). The content of secondary metabolites in air-dry leaf tissues varied. Leaves of cv. '*Northland*' were characterized by a higher content of phenolic acids, tannins, and essential oils, while leaves of cv. '*Bluecrop*' – by flavonoids and anthocyanins.

According to study conducted by Turhan *et al.* (2009) content of polyphenols in blueberry leaves was 8567.53 mg · 100 g^{-1}, while in fruits – 2318.40 mg · 100 g^{-1}. The results obtained by Skupień (2006) and Moyer *et al.* (2002) suggest that concentration of polyphenols in fruits varies and depends on a cultivar, growing location, harvest date, and size of fruits.

Numerous studies have shown that anthocyanins content has a significant effect on the antioxidant activity of highbush blueberries (Sapers *et al.* 1984a, b; Prior *et al.* 1998; Zheng & Wang 2003; Skupień 2004). The fruits studied by Ścibisz & Mitek (2002) showed the amount of anthocyanins at the level of 159.4 mg%. Concentration of anthocyanins in blueberries is highly dependent on the size of fruit, because the pigments are located mainly in the skin (Lee & Wrolstand 2004). According to the study by Lenartowicz et al. (1990), content of anthocyanins in the fruits of cv. '*Bluecrop*' was lower and amounted to 105 and 116 mg%. In this paper, the content of anthocyanins in highbush blueberry leaves was recorded at average level of 50 mg · 100 g^{-1}, while in cv. '*Bluecrop*' – more than twice as many of these compounds in comparison to cv. '*Northland*'.

Applying the DPPH method, the antioxidant properties of aqueous extracts made from the leaves of two highbush blueberry cultivars, were compared. The examinations have shown that the ability to neutralize the free radicals was comparable for both analyzed varieties, reaching 82.1%, although extract made of cv. '*Bluecrop*' leaves was more effective. Turhan *et al.* (2009) reported slightly different results. The capacity of eliminating the free radicals within blueberry

Table 1. Content of basic components in leaves of two highbush blueberry cultivars in 2010-2011.

Cultivars	'Bluecrop'	'Northland'	Mean
Dry matter, %	35.4	36.5	35.95
Chlorophyll a, mg · g^{-1}	0.909	0.788	0.849
Chlorophyll b, mg · g^{-1}	1.335	1.319	1.327
Sum of *chlorophylls a+b*, mg · g^{-1}	2.440	2.107	2.274
Reducing sugars, % FM	0.90	1.34	1.12
Total sugars, % FM	2.71	4.19	3.45

Table 1. Content of basic components in leaves of two highbush blueberry cultivars in 2010-2011.

Cultivars	'Bluecrop'	'Northland'	Mean
Moisture, %	7.84	8.23	8.03
Phenolic acids, mg · $100g^{-1}$	0.481	0.581	0.531
Flavonoids, mg · $100g^{-1}$	0.123	0.114	0.119
Anthocyanins, mg · $100g^{-1}$	68	32	50
Tannins, % DM	5.2	7.5	6.4
Essential oils, % DM	0.25	0.35	0.30
DPPH, %	82.9	81.3	82.1

leaf extracts was 69.6%, whereas fruit extracts – 83.9%.

It is difficult to univocally conclude, on a basis of analyzing the content of individual phenolic groups, which have played a decisive role in the antioxidant system. Studies upon the relationship between the content of phenols and antioxidant properties of berry fruits (Kalt *et al.* 1999) have shown a strong correlation between the sum of phenols and anthocyanins vs. the total antioxidant capacity. Similar regularity was found also by Proteggente *et al.* (2002).

It is known that the efficiency, composition, and physicochemical properties of essential oil depend on many factors, among which the most important are: geographic area, from which the raw material originates, raw material harvest time, age, as well as the manner of raw materials preparation for processing. According to the data contained in Tab. 2, the average content of essential oil in studied raw materials amounted to 0.30%. Leaves of cv. '*Northland*' appeared to be the material with higher efficiency of essential oils (0.35%) as compared to cv. '*Bluecrop*' (0.25%). There are no data on this issue in available literature. Therefore, it seems to be interesting to carry out the research aimed at determining the qualitative composition of essential oils from tested materials.

Conclusions

1. It was a large variation in the content of analyzed components and biological activity of leaves of two highbush blueberry cultivars.
2. The average content of total and reducing sugars in leaves of blueberry plants amounted to 3.45% and 1.12%, respectively.
3. Leaves of cv. '*Northland*' were characterized by a higher content of phenolic acids, tannins, and essential oils, while leaves of cv. '*Bluecrop*' – by flavonoids and anthocyanins.
4. Extracts made of highbush blueberry leaves were characterized by high antioxidant activity in the range of 81.3 through 82.9%.

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