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Short Communication

Morphological and Chemical Variability of Wild Populations of Bilberry (*Vaccinium myrtillus* L.)

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Abstract

Eleven bilberry (*Vaccinium myrtillus* L.) populations were evaluated regarding usable traits (content of biologically active compounds in leaves and fruits). Habitat conditions and abundance in the above species also were characterized. The content of phenolic acids and tannins in leaves was distinctly higher in comparison to fruits for each analyzed population. The amount of arbutin in leaves varied from 1,000 mg·100 g^{-1} dw (in Świerże Górne and Zakopane) to 2,400 mg·100 g^{-1} dw (Piduń). The content of flavonoids amounted from 400 to 600 mg·100 g^{-1} dw. The total amount of biologically active compounds in dry fruits was different for each investigated population. The content of anthocyanins varied from 490 to 1,640 mg·100 g^{-1} dw, organic acids from 3,300 to 8,800 mg·100 g^{-1} dw, total sugar from 4,800 to 8,500 mg·100 g^{-1} dw.

Keywords: natural sites, arbutin, anthocyjanins, flavonoids, tannins, polyphenolic acids

Introduction

Bilberry is an undershrub commonly found in Poland mainly in pine and mixed forests. For many years its raw materials (leaves and fruits) have been collected from natural sites and used as components of therapeutic mixtures in Polish folk and traditional medicine.

Leaves, among other items are used in anti-inflammatory mixtures, especially for the urinary tract, as well as mixtures decreasing blood sugar concentration. Fruits have protective properties with reference to blood vessels, antiedema, and immunostimulatory properties. Pharmacological activity of both raw materials is connected with the presence of phenolic components such as tannins, polyphenolic acids, and flavonoids. Moreover, arbutin (hydroquinone β-D-glucopyranoside) is present in leaves, thanks to which the raw material has anti-inflammatory properties. In fruits, anthocyanins are responsible, among other things, for improvement of blood micro-circulation within the eyeball [1-6]. The content of the above components depends on many factors, including the place of raw materials collection. It is known that plants from different habitats differ significantly both in morphological features that influence the final appearance of raw material (size, shape, colour of leaves, or fruits), as well as in the content of pharmacologically active components [7-12]. In Poland, leaves and fruits of bilberry are collected only from natural habitats. The final product gained in this way is hard to be standardized as a result of the impact of habitat conditions on the growth, development, and accumulation of active compounds in herbs.

The aim of this work was evaluation of 11 natural habitats of bilberry (*Vaccinium myrtillus* L.) from the point of view of the possibility of profitable raw material collections with high biological activity. The habitat conditions and abundance in the above species were characterized. The variability within the species was assessed on the grounds of the chosen morphological features and the content of chosen biologically active components in leaves and fruits.

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Experimental Procedures

Investigations were performed in natural habitats of bilberry placed within the Polish regions Mazowieckie, Kujawsko-Pomorskie, Warmińsko-Mazurskie, and Małopolskie (Table 1). The coverage of the habitat by plants of the examined species was determined by means of a 5-point scale (1 – coverage in 10-20%; 2 – coverage in 25-40%; 3 – coverage in 45-60%; 4 – coverage in 65-80%; 5 – coverage in 85-100%).

Morphological Characteristics of Plants

For biometrical analyses, 50 plants were randomly collected from each habitat. For each plant the height, number of leaves on the shoot, number of branches, and the shape and surface of the leaf blade were determined. The surface of the leaf blade was determined by outlining the shape of the leaf on graph paper and calculating its surface. Leaves for this measurement were collected from the upper, middle, and bottom part of the plant. The result was a mean from these three leaves.

Plant Material

As plant material for chemical analyses, leaves and fruits of bilberry were used. On all habitats, both raw materials were collected at the turn of June and July. After harvesting, the leaves were dried in natural conditions in shaded, airy room, and the fruits were initially dried in natural conditions and later dried in drying chamber at 50ºC.

Chemical Assessment of Raw Materials

In leaves the content of phenolic acids, tannins, flavonoids, and arbutin (hydroquinone β-D-glucopyranoside), and in fruits the content of phenolic acids, tannins, anthocyanidins, organic acids, and total sugars were determined.

The content of phenolic acids, tannins, flavonoids, anthocyanidins, and arbutin was determined according to Polish Pharmacopoeia VI [13]. The content of organic acids according to Polish Standard PN-90/A-75101/04 [14], the content of total sugars using the method of Luff-Schoorl (Polish Standard PN-90/A-75101/07) [15]. In all analyses, air-dried raw materials were used in three trials.

Total content of phenolic acids (calculated as caffeic acid) was made from aqueous solution with Arnov reagent in the presence of 1n HCL and 1n NaOH. Absorbance of the obtained solution was measured at 490 nm wave length. Flavonoids contents were quantified using the chelating properties of the molecules toward $AlCl_3$, with quercetin used as standard. The raw materials were extracted with acetone. The hydrolysis of flavonoids was performed using hydrochloric acid (28 g·1000 ml^{-1}). Flavonoids were isolated from acetone extract witch ethyl acetate. Absorbance of obtained flavonoid solution was measured at 425 nm wavelength.

Tannin content (calculated as pyrogalol) was made from aqueous solution with Folin-Ciocalteus reagent. Absorbance measurements were made at 760 nm wavelength.

For determining anthocyanidins, raw materials were extracted using a mixture of methanol and water (7:3). Obtained extracts were condensed. The extracts were shaken three times with butane-1-ol. Absorbance of gained solution was measured at 545 nm, applying butane-1-ol as reference. The content of anthocyanidins was passed in count on cyaniding chloride. To determine arbutin (hydroquinone β-D-glucopyranoside) content we prepared an aqueous extract from leaves. Absorbance of solution was measured at 455 nm, applying water as reference. All spectrophotometric measurements were performed using a PV-8740 UV- VIS Philips spectrophotometer.

The organic acids were determined according to Polish Norms PN-90 and 75101/04. The plant material was extracted with water and titrated by hydroxide of sodium. The content of total sugars was determined using the Luff-Schoorl method that relies on the reduction of Cu (II) contained in Luff reagent by carbohydrate present in the solution. The plant material was heated with water. The Carres I and Carres II liquids were added to solution to clarify. Hydrolysis of the obtained solution was carried out with concentrated HCl, then the liquid Luff was added. The sample was titrated 0.1 n $Na_2S_2O_3$. Total sugars were calculated from the difference of the two titrations (blank and appropriate).

The results were statistically analyzed using STATGRAPHIC PLUS 4.1, with the level of significance equal to 0.05. The significance was assessed by the HSD-Tukey test.

Results and Discussion

The habitats described in this work were placed in four regions located in the northern, central, and southern parts of Poland (Fig. 1) so that they differed in climate and soil conditions, insolation level, soil humidity, and the presence of strong winds. The above factors could influence the density of berry plants and the character of growth, including morphological features such as height, number of branches, total number, and size of leaves. The highest were the plants growing in habitats placed in the mountains. Those plants grew in strong density protecting themselves in this way from strong insolation, strong wind and excessive transpiration (habitats: Obidza – 29.4; Zakopane – 36.6 cm). The populations growing in lowland forests (Pyry, Celestynów, Kozienice) were clearly lower (on average 19 cm), and plants most often grew in irregularly dense patches. Different results were obtained by Woodward [7], who assessed morphological features of bilberry growing at altitudes of 200, 610, and 1,100 m. That author demonstrated that heights of bilberry plants decreased with altitude and the lowest plants grew at 1,100 m.

The density of plants could have influenced the number of branches formed by this species. The performed obser-

Table 1. Index of examined habitats of bilberry: characteristics and locations.

No.	Site	Habitat characteristics	Region/location/altitude of habitat
1	Pyry	Kabacki Forest Medium-insolated, placed between a clearing and a tourist tract. Bilberry plants grew in irregularly dense patches, poorly fruiting, forming small, but firm and juicy berries. Habitat coverage – 4	Mazowieckie N 52º07.950 E 21º01.739 13 m a.s.l.
2	Tabor	Forest in the vicinity of Celestynów Medium-shaded, ground strongly grown through with moss, dump. Plants grew in small dense clumps. Poor fruiting, small berries. Habitat coverage – 4	Mazowieckie N 52º02.553 E 21º21.665 95 m a.s.l.
3	Tumanek	Forest in the vicinity of Wyszków Placed on the verge of the forest, medium-insolated. Plants grew in dense patches. Abundantly fruiting, fruits large and firm. Bilberry plants grew in irregularly dense patches.	Mazowieckie N 51º3353.49 E 21º3003.46 73 m a.s.l.
4	Świerże Górne	Kozienicka Forest Poorly insolated habitat, plants grew in fairly dense to dense patches. Abundant fruiting, fruits medium and fairly firm. Habitat coverage – 5	Mazowieckie N 51º3922.33 E 21º2705.86 105 m a.s.l.
5	Czarnów	Chojnowski Forest Placed next the blue tourist tract, medium-insolated. Plants covering large surfaces, growing in irregular density. Poor fruiting, fruits badly shaped and wrinkled (probably because of the drought in the period of fruiting). Habitat coverage – 5	Mazowieckie N 51º02.917 E 21º05.344 209 m a.s.l.
6	Koronowo	Forests in the vicinity of Koronowskie Lake Habitat placed on a slope on a dump ground, moderately insolated. Bilberry plants covering densely large surfaces. Abundant fruiting, fruits large, elongated, firm, and juicy. Habitat coverage – 5	Kujawsko-Pomorskie N 53º3027.12 E 20º4858.23 150 m a.s.l.
7	Stanowo	Forrest between Iława and Ostróda Habitat placed in beech forest on a dumping ground. Bilberry plants grew in irregularly dense patches. Good fruiting with large, elongated, juicy berries Habitat coverage – 4	Warmińsko-Mazurskie N 53º1814.74 E 18º0245.96 160 m a.s.l.
8	Piduń	Mazurian Plane in the vicinity of Galwica National Park Habitat placed in mixed wood, shaded. Plants covering large surfaces, growing in poor density. Abundantly fruiting: fruits large and fleshy. Habitat coverage – 5	Warmińsko-Mazurskie N 53º4000.28 E 19º4721.18 132 m a.s.l.
9	Nowe Borowe	Mazurian Plane, forests in the vicinity of Jedwabno Habitat placed in a hilly area, light, and dump. Bilberry plants grew in irregularly dense patches. Abundant fruiting: fruits large and sweet. Habitat coverage – 4	Warmińsko-Mazurskie N 53º3110.36 E 20º4138.18 84 m a.s.l.
10	Obidza	Forest in Beskid Sądecki Mountainous area, well insolated. Plants grew in small dense clumps. Satisfactory fruiting: fruits large and juicy. Habitat coverage – 4	Małopolskie N 49º25.068 E 020º37.248 888 m a.s.l.
11	Zakopane	Kondratowa Alp Habitat along a tourist tract surrounded by a mountain range. Area strongly insolated. Plants grew in small dense clumps. Abundantly fruiting, fruits large, delicate, and soft. Habitat coverage – 5	Małopolskie N 49º15.046 E 019º57.330 1313 m a.s.l.

Table 2. Morphological characteristics of bilberry plants.

Site	Plant height* (cm)	Number of branches* (item)	Number of leaves* (item)	Surface of leaf blade* (mm^2)
Pyry	18.6 j**	6.1 a	6.6 de	210.0 b
Tabor	19.1 i	2.7 e	7.0 cd	341.0 a
Tumanek	19.8 h	2.7 e	7.1 cd	272.0 ab
Świerże Górne	19.7 hi	3.1 e	7.3 c	310.0 a
Czarnów	25.2 e	6.4 a	7.3 c	295.0 a
Koronowo	21.8 g	4.8 bc	6.6 de	329.0 a
Stanowo	27,3 c	4.7 bc	8.2 a	212.0 b
Piduń	27.4 c	4.5 c	7.7 b	318.0 a
Nowe Borowe	26.3 d	6.4 a	6.0 f	312.0 a
Obidza	29.4 b	3.8 d	6.9 d	347.0 a
Zakopane	36.6 a	4.1 c	6.4 e	329.0 a
Mean	24.6	4.5	6.3	254.8

* Mean from 50 plants

** Means marked with the same letters do not differ according to Tukey's HSD test at p=0.05

vations showed that the greater the plant density the fewer branches they form. For example, in Czarnów, Nowe Borowe, and Pyry, where plants grew in irregular moderate density, the number of branches per plant was on average 6. In Tabor, Tumanek, and Świerże Górne (high density) branches were around 3. A big difference between populations also was present in the number of leaves (from 6.4 to 8.2 per shoot) and the leave blade surface (210.0-347.0 mm^2) (Table 2).

Leaves, the basic raw material gained from bilberry, contain tannins, polyphenolic acids, and flavonoids [9, 16-18].

Fig. 1. Locations of investigated sites (sites numbered as in Table 1).

They also contain arbutin, thanks to which the raw material has anti-inflammatory properties within the urinary tract. Suchorska-Tropiło and Olszeska-Kaczyńska [19] name arbutin as a component present in leaves of bilberry in trace quantities. Leaves collected from the described habitats contained from 1,000 to 2,400 mg·100 g^{-1} dw of arbutin, more than herb of heather (500-1,000 mg·100 g^{-1} dw) [20], which is a used source of arbutin. Tannins are the second group of leaf components with ant-iflammatory properties [21, 22].

Rosłon and Suchorska-Tropiło [9] show that the content of tannins in bilberry leaves can reach 7.5% (e.g. 7,500 mg·100 g^{-1}). Considerably higher results were obtained by Gallet and Lebreton [23]. In their study the content of tannins in bilberry leaves amounted to 220-230 mg·g^{-1}. In the presented work the content of tannins amounted on average to 7,480 mg·100 g^{-1} dw. Examined populations differed significantly in respect to this feature (from 5,100 to 12,400 mg·100 g^{-1} dw). Significant differences were also observed in the content of polyphenolic acids in leaves (from 1,700 mg·100 g^{-1} dw – Obidza to 3,400 mg·100 g^{-1} dw Piduń and Świerże Górne), but obtained results are distinctly lower compared with data demonstrated by Gallet and Lebreton [23] (8,750-9,840 mg·100 g^{-1} dw). Content of the next group of phenolics – flavonoids averaged 540 mg·100 g^{-1} dw, which is in accordance with above authors [23]. There was no observed difference in the content of flavonoids.

Fruits, the second raw material gained from bilberry, contain (similar to leave) tannins and polyphenolic acids, and also anthocyanin dyes, organic acids, and sugars [24, 25]. This raw material has also been well known for years (thanks to the presence of tannins), and has valuable anti-diarrhoea, and anti-inflammatory properties. According to Strzelecka and Kowalski [25], for example, the content of

Table 3. Content of determined phenols in bilberry leaves and fruits (mg·100 g^{-1} dw).

Site	Polyphenolic acids		Tannins		Arbutin leaves	Flavonoids leaves	Anthocyanins fruits
	leaves	fruits	leaves	fruits			
Pyry	2,500 b*	700 cd	6,300 e	2,600 a	1,500 b	600 a	960 e
Tabor	3,200 a	800 c	8,500 d	900 c	1,300 bc	400 a	690 f
Tumanek	3,300 a	600 cd	8,800 c	1,600 bc	1,700 b	500 a	610 f
Świerże Górne	3,400 a	500 d	12,400 a	1,000 c	1,000 c	600 a	1,470 b
Czarnów	2,600 b	500 d	9,700 b	900 c	2,100 a	400 a	1,280 c
Koronowo	2,800 b	1,200 b	5,800 fg	900 c	1,200 c	600 a	490 h
Stanowo	2,800 b	600 cd	6,000 f	2,000 b	1,500 b	500 a	1,490 b
Piduń	3,400 a	1,000 bc	5,100 h	800 c	2,400 a	500 a	490 h
Nowe Borowe	3,200 a	600 cd	5,800 fg	1,300 bc	1,600 b	600 a	1,640 a
Obidza	1,700 c	1,500 a	5,600 g	1,800 b	2,200 a	600 a	640 g
Zakopane	2,500 b	1,300 ab	8,300 d	1,200 bc	1,000 c	600 a	1,120 d
Mean	2,850	850	7,480	1,360	1,590	540	990

* Means marked with the same letters do not differ according to Tukey`s HSD test at p=0.05.

tannins in bilberry fruits can amount to even 12%. Fruits of examined populations contained from 800 mg·100 g^{-1} dw (Piduń) to 2,600 mg·100 g^{-1} dw (Pyry) of tannins, so clearly less than results seen by other authors.

Comparing the content of these components in leaves and fruits, it was observed that in the case of all populations significantly more of those components were present in leaves. Both raw materials (leaves and fruits) differed also in the content of polyphenolic acids. The content of this group of components amounted from 500 mg·100 g^{-1} dw (Czarnów) to 1,500 mg·100 g^{-1} dw (Obidza), and in all populations was lower than in leaves. The smaller differences were observed in plants from Obidza habitat (1.1 times), the highest in plants from Świerże Górne (6.8 times). Bilberry fruits are valued mostly for the presence of anthocyanin components. They have the second largest quantity of those components after chokeberries. Many authors have underlined the great pharmacological activity of these components [4, 5]. According to Noculak-Palczewska [26], the content of anthocyanins in bilberry fruits amounts to around 0.5%, but the quantity increases during the process of ripening. Burdulis et al. [27], analyzing 9 populations of bilberry collected in natural environment in Lithuania, showed that anthocyanin content in fruits was from 0.264% to 0.399%. Rieger et al. [12] considered that the content of these compounds decreases together with rising altitude.

The results gained in the presented work showed a big difference in the content of these component in fruits collected in different habitats in Poland, but did not find relationships between the content of anthocyanins and the position of habitats at altitude.

The smallest amount of anthocyanins was determined in fruits collected in Koronowo. It amounted to 490 mg·100 g^{-1} dw and was three times lower in comparison to the highest content of those components in fruits collected in Nowe Borowe (1,640 mg·100 g^{-1} dw) (Table 3).

The presence of other groups of chemical compounds in this raw material, organic acids and total sugars, influences not only its pharmacological properties (mainly dietary), but also its taste value. The total content of organic acids in fruits is assessed at the level of 1,500 mg·100 g^{-1} dw. In the case of examined populations, the content of those acids was higher and amounted to from 3,300 mg·100 g^{-1} dw (Świerże Górne) to 8,800 mg·100 g^{-1} dw (Stanowo). The content of total sugars amounted to from 44 to 85 mg·g^{-1} dw and was similar to the results available in literature [28]. In the case of 5 populations, the total content of sugars was higher than the content of organic acids. Especially significant differences were observed in plants from Wyszków, where the content of total sugars was twice as high as the content of organic acids (content of sugars 8,500 mg·100 g^{-1} dw; content of organic acids 4300 mg·100 g^{-1} dw). In the case of populations from Piduń, Koronowo, Stanowo, and Obidza the higher content of organic acids in comparison to total sugars was observed, but the differences were statistically irrelevant. In case of fruits collected in Pyry and Zakopane, the content of both analyzed groups of compounds was similar (Fig. 2).

Conclusions

The assessment of habitat abundance in plants considering the coverage of its surface by bilberry plants shows that they are proper for gaining raw materials from this species (the level of coverage was assessed on 4 and 5 points from 60 to 100%).

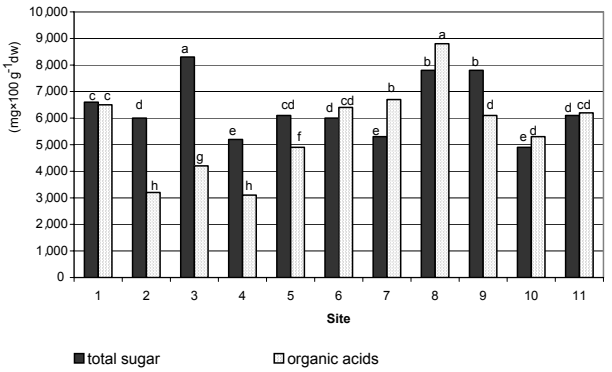

Fig. 2. Organic acids and total sugar content in bilberry fruits (mg·100 g^{-1} dw).
* Means marked with the same letters do not differ according to Tukey's HSD test at p=0.05.

Chemical analyses confirmed that both raw materials of bilberry (leaves and fruits) are valuable sources of biologically active compounds such as arbutin, polyphenolic acids, tannins, anthocyanidins, and others. However, as they originate from natural habitats, sometimes they differ significantly in the quantity of those compounds. This influences their quality and causes problems in standardization.

In the presented work the analyzed populations were characterized by lower content of tannins than in literature. In leaves, this low content of tannins was compensated for by high content of arbutin and polyphenolic acids, and in fruits by anthocyanidins and organic acids.

Especially interesting for the pharmaceutical industry were the populations from Czarnów, Obidza, and Piduń, where the leaves had high content of arbutin, resulting in strong antiseptic and anti-inflammatory properties and populations in Nowe Borowe, where the fruits where characterized by high anthocyanin dyes content (16,400 mg·100 g^{-1} dw).

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