Phytochemical Characterization of Blue Honeysuckle in Relation to the Genotypic Diversity of *Lonicera* sp.

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**Abstract:** The phytochemical characteristic analysis of a group of 30 haskap berry genotypes was carried out in mind the concern for the consumption of food with high nutraceutical value that helps maintain good health. Phytochemical fruit composition and antioxidant activity were assessed by the Folin–Ciocalteau, spectrophotometric, DPPH (1,1-diphenyl-2-picrylhydrazyl) as well as ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) method. Evaluation of antioxidant activity was referred to as the Trolox equivalent. The observed differences in the content of phenolics, flavonoids, vitamin C and antioxidant activity allowed us to select genotypes which, due to the high level of the analyzed compounds, are particularly recommended in everyone’s diet. In addition, the analysis of the prospects of increasing the analyzed phytochemical properties, estimated by parameters such as heritability and genetic progress, indicates the effectiveness of breeding in relation to each of the analyzed traits. The results of the presented research can be used in the implementation of future breeding programs for this valuable species.

**Keywords:** ABTS; bioactive compounds; DPPH; flavonoids; genetic distance; genotypic and phenotypic correlation; nutraceutical value; phenolics; UPGMA

1. **Introduction**

The constantly growing awareness of consumers regarding the need for healthy eating and consuming food with health-promoting properties leads to the search and characterization of new species with this direction of utilization. Species belonging to the genus *Lonicera* such as *Lonicera caerulea* var. *edulis*, *L. caerulea* var. *kamtschatica*, *L. caerulea* var. *altaica*, *L. caerulea* var. *byarnikovae* and *L. caerulea* var. *emphylocalyx*, as well as their hybrids, collectively known as *Lonicera caerulea* L., also known as haskap, blue honeysuckle, honeyberry or sweet berry honeysuckle, are representative of such plants. The fruits of this species, like strawberry, blueberry, blackberry or blackcurrant, belong to the so-called superfruits—fruits, which due to the presence of bioactive compounds, are very desirable in the human diet [1,2]. Health-promoting properties of the haskap berries include protective effects against cardiovascular and neurodegenerative diseases, osteoporosis, type 2 diabetes as well as antimicrobial, anticarcinogenic and anti-inflammatory activity [3]. Combining biological traits of individual species allowed us to obtain cultivars with large fruits without a bitter taste, not falling off the bush after ripening, fertile, more resistant to diseases, suitable for mechanical harvesting...
and storage, as well as significantly different in terms of phytochemical content. The assessment of the content of health-promoting phytochemical compounds, such as phenolic acids, flavonoids and anthocyanins has so far been performed usually for a small group of cultivars. Cehula et al. [4] studied 14 cultivars, Wang et al. [5] 7 cultivars, Senica et al. [6] 4 cultivars, Auzanneau et al. [7] 7 cultivars, Khattab et al. [8] 3 cultivars, Sochor et al. [9] 19 cultivars, Wojdylo et al. [10] 8 cultivars, Rupasinghe et al. [11] 3 cultivars and Kusznierewicz et al. [12] 6 cultivars. So far, the largest group of 30 cultivars has been analyzed by Kucharska et al. [13]. The level of the analyzed phytochemical properties, as indicated by numerous authors [12,14–16] depends, on one hand, on the properties of the cultivar (genotype), while on the other hand, it is the result of many environmental factors (soil, fertilization, etc.). The analysis of the influence of each of these factors allows us to determine the extent to which a given phytochemical property results from the impact of genetic factors, and to which it is a result of the effect of the environment, which can be expressed by the heritability coefficient, and which has not been presented so far for this species.

The attractive biological value of *Lonicera* fruits, which consists of the values of individual cultivars, causes a rapid increase in the cultivation area of this species. According to the available data, the global cultivation area in 2017 amounted approximately to 5500 ha. The largest planted area was in China and North Korea, in total, 2000 ha, Poland—1800 ha, Canada—1000 ha, Russia—400 ha and Japan—160 ha [17]. The cultivation area of this species is constantly growing and, for example, in Russia, this area increased to 735 ha in 2019 and its further increase to about 2000 ha is planned in 2022 [18]. Poland, with a cultivation area of approximately 4000 ha in 2019, is a world leader in the production of haskap berries [19]. The Commission Implementing Regulation (EU) 2018/1991 of 13 December 2018 authorizing the introduction of *Lonicera caerulea* L. berries to the market as a traditional food from a third country in accordance with the Regulation of the European Parliament and of the Council (EU) 2015/2283 [20] is likely to support a further increase in the cultivation area of this species. The growing area of cultivation, as well as the valuable properties of the fruits in terms of human health, require, as many authors postulate, the analysis of the biological potential of new genotypes of this species, their suitability for breeding, and thus an indication of the possibility of increasing the content of biologically active compounds in the future [11,13,21]. Therefore, the aim of this study was, firstly, to evaluate the content of polyphenols, flavonoids, vitamin C and antioxidant activity of berry in a large group (30) of haskap genotypes of various origin, including 10 that were analyzed for the first time. Secondly, the estimation of variability, as well as the not-yet presented genotypic correlation, heritability and the genetic advance of characteristic in a group of genotypes studied was evaluated. For this purpose, a large group of genotypes was necessary because it increased the precision of inference. Thirdly, this study can be a guide in the implementation of breeding work aimed at increasing the biological potential of this species and helpful in selecting a cultivar for those who want to consume fruits with a high nutraceutical value.

2. Materials and Methods

The Institute of Plant Genetics and Biotechnology has held a collection of cultivars and breeding clones of the genus *Lonicera* since 2007. The experiment used the bushes in the fourth year of fruiting planted in 2015 at the Experimental Station, University of Life Sciences in Lublin (51°13′59″ ϕN, 22°34′0″ λE, elevation: 225.48 m). The research covered a diverse group of genotypes including cultivars of Polish, Slovak, Russian and Canadian origin, as well as breeding clones, the list of which is presented in Table 1. Thirty genotypes were selected for analyses from which the fruits were harvested in June 2019. Three fruit sub-samples were collected from each genotype (at the start, in the middle and the end of the harvest period selecting fully pigmented fruits). Fruit sub-samples were frozen and stored at −20 °C until analysis (10 days). Directly before the analysis, the samples were mixed and the resulting average sample, weighting 150 g, was the material for testing the content of phytochemical compounds such as phenolics, flavonoids, vitamin C and antioxidant activity.
Table 1. List of genotypes tested.

| No. | Genotype     | Country of Origin | No. | Genotype     | Country of Origin |
|-----|--------------|-------------------|-----|--------------|-------------------|
| 1   | 1-17-59      | RUS               | 16  | Jugana       | RUS               |
| 2   | Amphora      | RUS               | 17  | K100         | POL               |
| 3   | Amur         | SVK               | 18  | Karina       | POL               |
| 4   | Aurora       | CAN               | 19  | LeningradskijVelikan | RUS |
| 5   | BakczarskijVelikan | RUS | 20  | Nimfa        | RUS               |
| 6   | BerryBlue    | CZE               | 21  | Polar Jevel  | CAN               |
| 7   | Blue Velvet  | RUS               | 22  | Siniczka     | RUS               |
| 8   | Borealis     | CAN               | 23  | Sinoglaska   | RUS               |
| 9   | Brązowa      | POL               | 24  | T3           | RUS               |
| 10  | Czarna       | POL               | 25  | T5           | RUS               |
| 11  | DoczVelikana | RUS               | 26  | Uspiech      | RUS               |
| 12  | HoneyBee     | CAN               | 27  | Valhova      | RUS               |
| 13  | Indigo Gem   | CAN               | 28  | Vostorg      | RUS               |
| 14  | IndigoTreat  | CAN               | 29  | Warta        | POL               |
| 15  | Jolanta      | POL               | 30  | Zielona      | POL               |

2.1. Fruit Extract Preparation for Polyphenols and Antioxidant Activity Determination

Before the experiment, whole frozen fruit samples (150 g) were homogenized with a blender (PHILIPS). The appropriate fruit material (2.5 g) was successively extracted three times with 8 mL acidified (0.1% (v/v) formic acid) 80% (v/v) methanol for 30 min (Multi-Rotator RS-60 (bioSan)) at room temperature. The supernatant was filtered using a vacuum pump and was poured together. The final volume was 25 mL. The obtained extract was used for the determination of total polyphenols (phenolics, flavonoids) as well as DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay.

2.2. Total Phenolics Content (TPC)

The total phenolics content of fruit extracts was determined according to the method of Khattab et al. [8]. The sample (0.1 mL each) was mixed with 0.4 mL of distilled water, and 2.5 mL 10% Folin–Ciocalteau reagent (v/v) and incubated at room temperature for 5 min before being neutralized by 2.0 mL of 7.5% (w/v) sodium carbonate solution. The reaction mixture was incubated at room temperature for 2 h and the absorbance was measured at 760 nm using a UV–VIS spectrophotometer (Shimadzu A-160, Shimadzu Corp., Kioto, Japan). The absorbance of each extract was reduced by the absorbance of the blank sample that contained the distilled water instead of the Folin–Ciocalteau reagent. Gallic acid in 50% (v/v) methanol solution at a range of concentrations (10–100 mg·L\(^{-1}\)) was used as a standard and a calibration curve was drawn. The content of total phenolics was expressed as mg gallic acid equivalent (GAE)·100 g\(^{-1}\) of fresh weight. All samples were analyzed in five replicates.

2.3. Flavonoids Content

Total flavonoids content of fruit extracts was determined using a spectrophotometric method [11]. The extract or standard (quercetin) (0.5 mL) was diluted to 3.7 mL with distilled water and mixed with 0.15 mL of 5% (w/v) sodium nitrite (NaNO\(_2\)) solution at time zero. After incubation at room temperature for 5 min, 0.15 mL of 10% (w/v) aluminum chloride (AlCl\(_3\)) was added and the mixtures were vigorously shaken. After 1 min, 1 mL of 1 M sodium hydroxide (NaOH) was added, mixed, and the absorbance at 510 nm was measured immediately versus a blank. The blank consisted of 50% (v/v) methanol instead of sample. Quercetin in 50% (v/v) methanol solution at a range of concentrations (10–100 mg·L\(^{-1}\)) was used as a standard and a calibration curve was drawn. Total flavonoid content in the extract was expressed as mg quercetin equivalent (QE)·100 g\(^{-1}\) of fresh weight. All samples were analyzed in five replicates.
2.4. Antioxidant Activity

As suggested by Schlesier et al. [22] that the assessment of antioxidant activity should be carried out using at least two methods, the DPPH and ABTS methods were used in this work.

2.4.1. DPPH Assay

The antiradical activity of extracts was determined with the DPPH• radical (1,1-diphenyl-2-picrylhydrazyl), according to the method of Brand-Williams et al. [23] slightly modified by Khattab et al. [8]. Fruit extracts or standard (Trolox) (0.1 mL) were mixed with 2.9 mL of 0.1 mM DPPH solution and incubated in the shade at room temperature for 10 min. The absorbance was measured at 515 nm. Solution with 100% methanol, instead of samples, was used as a control. The absorbance of each extract was reduced by the absorbance of the blank sample that contained the extract and 100% methanol instead of the DPPH solution. Antioxidant activity was calculated based on Trolox standards at a range of concentrations 0.4–1.0 mM and expressed as a mmol Trolox equivalent (TE)·100 g⁻¹ fresh sample. All samples were analyzed in five replications.

2.4.2. ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) Assay

The antioxidant activity was also determined with the cation radical ABTS•⁺ (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), in line with the methodology described by Re et al. [24]. ABTS•⁺ was generated by reacting ABTS aqueous solution (7 mM) with potassium persulfate (2.45 mM) in the dark for 12–16 h. The initial solution was diluted until absorbance reached 0.7 (±0.02) at 734 nm. The reaction mixture contained a 3 mL ABTS•⁺ solution and a 0.03 mL extract or standard (Trolox). Measurements were made after 10 min at room temperature. Antioxidant activity was calculated based on Trolox standards at a range of concentrations 0.1–1.0 mM and expressed as a mmol Trolox equivalent (TE)-100 g⁻¹ fresh sample. All samples were performed in five replicates.

2.5. Fruit Extract Preparation for Vitamin C Content Determination

From the homogenized fruits (150 g) was prepared a 5 g sample which was mixed with 40 mL of 3% metaphosphoric acid. After stirred for 30 min (Multi-Rotator RS-60 (bioSan)) at room temperature the solution was filtered using a vacuum pump. The final volume was made up to 50 mL with the 3% metaphosphoric acid and the obtained extract was used for the determination of vitamin C content.

Vitamin C was determined with the spectrofluorimetric method [25] with necessary modifications. The oxidizing solution (100 mL) was prepared by mixing of I₂ (1.3 g), KI (40%, 10 mL) solution, HCl (7 M, 0.1 mL) and distilled water. The derivatization reagent was prepared by dissolving OPDA (o-phenylenediamine, 10 mg) in 10 mL of 0.005 M H₂SO₄. Sample (2 mL) was mixed with 0.3 mL of a 0.005 M solution of iodine in potassium iodide. After being vortexed for 1 min, 0.3 mL of 0.01 M Na₂S₂O₃ was added. After adjusting the pH of the samples to approximately 6.0, the derivatization was carried out by adding 0.3 mL of OPDA solution and stirring for 30 min at room temperature. The final volume was made up to 100 mL with the distilled water. The analysis was performed on Cary Eclipse (Varian, Palo Alto, CA, USA) spectrophotometer at an excitation wavelength λ = 365 nm and an emission wavelength λ = 425 nm. The vitamin C content was calculated based on a standard curve obtained using an aqueous solution of L-ascorbic acid standard in concentrations of 10, 20, 50 and 100 mg·L⁻¹. The vitamin C content in the extract was expressed as mg L-ascorbic acid-100 g⁻¹ fresh sample. All samples were analyzed in five replications.

2.6. Statistical Analysis of the Data

All data obtained in the experiment were statistically analyzed by ANOVA (analysis of variance) and the significance of differences between means was established by the Fishers LSD test at p ≤ 0.05 with Statistica 13.1 [26] statistical software. The cluster analysis based on the content of phenolics, flavonoids, vitamin C and antioxidant activity was conducted using the Unweighted Pair-Group Method with
Arithmetic Mean (UPGMA) method available in the same software [26]. Fruit phytochemical content and antioxidant activity are quantitative features, so the genetic estimation of these traits may be assessed with the use of genetic parameters like: heritability (H), genetic advance (GA), phenotypic and genotypic coefficient of variability (PCV and GCV respectively). These parameters are commonly used in creative breeding which is focused on obtaining new genetically improved cultivars (genotypes). Estimation of these coefficients will help to know the gene action affecting the concerned traits. Heritability is a genetic parameter used for the evaluation of genetic determination of quantitatively inherited traits. Heritability values are useful to indicate the reliability of the phenotypic value of characteristic to predict its genetic value, high values indicate a greater possibility of genetic advance (GA) obtained with the selection. Generally, heritability indicates the effectiveness with which the selection of genotypes could be based on phenotypic performance. Genetic advance (GA) expected from the selection is a precise indicator of the improvement of features in genotypic value for the new breeding population compared with the base population under one cycle of selection at a given selection intensity. Genetic advance (GA) can be estimated in population by multiplication of the values of heritability (H), phenotypical standard deviation of traits (σp) in population and selection intensity coefficient (k), which value depends on the percent of selection intensity (in our research 20%, k = 1.4), so that GA = k·σ_p·H. In our study, all analyzed traits were assessed by their phenotypic and genotypic value. The phenotypic value of an individual is the values of the traits observed and measured in the test sample. For each quantitative trait, a part resulting from the action of genes can be separated from its phenotypic value and is called the genotypic value. The genotypic and phenotypic coefficient of variation (GCV, PCV) was calculated according to Burton and DeVane [27], heritability (H) and genetic advance (GA) were calculated according to Allard [28] in an Excel spreadsheet. Relationships between fruit characteristics were estimated using Pearson’s correlation coefficient (r). Correlation coefficients measure the mutual relationship between a pair of variables, independently of other variables being considered. Correlation coefficients between characteristics were estimated both on the basis of phenotypic values (phenotypic correlation) and genotypic values (genotypic correlation). Genotypic and phenotypic correlation coefficients were computed using META-R software [29].

3. Results and Discussion

The results obtained in this study (Table 2) clearly indicate that the content of bioactive compounds and antioxidant activity was significantly different in the studied group of genotypes.

Among the analyzed properties, the highest value of the coefficient of variation (CV, 32.8%) was observed for vitamin C, while flavonoid and phenolic compound values were much lower and very similar (19.2 and 19.8, respectively). Antioxidant activity determined by the ABTS method was characterized by greater variability when compared to the DPPH method. Vitamin C content ranged from 8.5 to 29.7 mg L⁻¹ 100 g⁻¹ fw which meant over a three times higher content of this compound in cultivars such as Zielona, Karina or clone T5 compared to the cultivar Brązowa. In the study of Molina et al. [30], the average ascorbic acid level was 24.8 mg 100 g⁻¹ fw; Caprioli et al. [31] recorded an average level of ascorbic acid of 22.5 mg 100 g⁻¹ fw, while Wojdyło et al. [10] obtained 17 mg 100 g⁻¹ fw. Thus, these values differed only slightly from the mean for all genotypes tested in this experiment. Apart from cultivar, works of other authors indicated a possible significant impact on the level of this property of factors such as locality and years of research, resulting in different content of this compound in the range: 17–25 mg 100 g⁻¹ [32]; 31.9–44.5 mg 100 g⁻¹ [33]; 3.19–32.12 mg 100 g⁻¹ [10]. However, the cited values are significantly lower compared to the previously recorded high content in the range from 28.56 to 86.9 mg 100 g⁻¹ by Pokorná-Juríková and Matuškovič [34] for Lonicera kamtschatica, cultivar Gerda 25 and exceptionally high content in the range of 67.66–186.61 mg 100 g⁻¹ fw obtained in the study on cultivars and clones by Juríková et al. [35]. Nevertheless, it can be indicated (in principle) that this genetic background in most cultivars has a decisive influence on the level of synthesis of this compound, while other genotypes are more variable in this respect. For example, vitamin C content tested in Switzerland was the highest in the cultivar Indigo Gem, lower in IndigoTreat and
the lowest in Berry Blue [7] and the same order of cultivars in terms of this compound content was observed in our research. This confirmed the essential role of the genotype of the cultivar in shaping this property, as pointed out by Jurikova et al. [36]. Increasing the level of vitamin C in fruits seems to be the desired direction of changes in new cultivars, and this is related to reports that the consumption of high vitamin C doses results in enhanced antisenescence and anti-atherosclerotic effects [37].

Table 2. Total phenolics, flavonoids, vitamin C content and antioxidant activity determined by the DPPH (1,1-diphenyl-2-pirclyhydrazyl) and ABTS (2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assays in fruits of 30 genotypes of blue honeysuckle. The results are given in mg gallic acid equivalent (GAE)-100 g⁻¹ fw (phenolics), mg quercetin equivalent (QE)-100 g⁻¹ fw (flavonoids), mg L-ascorbic acid-100 g⁻¹ fw (Vitamin C), mmol Trolox equivalent (TE)-100 g⁻¹ fw (DPPH and ABTS).

| Genotypes       | Phenolics | Flavonoids | Vitamin C | Antioxidant Activity |
|-----------------|-----------|------------|-----------|----------------------|
|                 |           |            |           | DPPH | ABTS |
| 1-17-59         | 934.1 ± 21.8 | 1137.5 ± 19.9 | 9.8 ± 3.6 | 2.2 ± 0.1 | 5.5 ± 0.17 |
| Amphora         | 727.9 ± 43.4 | 1030.5 ± 9.2  | 9.6 ± 0.7  | 2.0 ± 0.3 | 4.5 ± 0.28 |
| Amur            | 509.8 ± 24.5 | 722.1 ± 18.0  | 24.1 ± 0.9 | 1.6 ± 0.2 | 2.6 ± 0.10 |
| Aurora          | 422.3 ± 27.8 | 609.8 ± 13.5  | 15.6 ± 1.7 | 1.2 ± 0.05 | 2.6 ± 0.70 |
| Bakczarskij Velikan | 642.1 ± 24.6 | 1054.0 ± 17.2 | 16.5 ± 0.3 | 1.9 ± 0.04 | 4.1 ± 0.16 |
| Berry Blue      | 5010.0 ± 18.7 | 684.1 ± 4.3   | 11.1 ± 0.7 | 1.4 ± 0.03 | 2.5 ± 0.02 |
| Blue Velvet     | 518.5 ± 28.4 | 679.0 ± 20.3  | 22.9 ± 0.6 | 1.4 ± 0.06 | 3.1 ± 0.05 |
| Borealis        | 480.6 ± 30.6 | 679.2 ± 32.1  | 17.3 ± 0.6 | 1.5 ± 0.08 | 3.4 ± 0.05 |
| Brazowa         | 470.2 ± 19.2 | 436.9 ± 6.6   | 8.8 ± 0.9  | 1.4 ± 0.01 | 2.9 ± 0.06 |
| Czarna          | 746.2 ± 10.5 | 1002.5 ± 17.1 | 21.1 ± 2.5 | 1.9 ± 0.06 | 3.3 ± 0.18 |
| Docz Velikana   | 642.2 ± 9.4  | 938.7 ± 8.0   | 17.6 ± 1.1 | 1.8 ± 0.04 | 3.3 ± 0.20 |
| Honey Bee       | 497.1 ± 18.9 | 740.9 ± 9.1   | 21.2 ± 1.2 | 1.6 ± 0.05 | 3.2 ± 0.11 |
| Indigo Gem      | 474.7 ± 18.7 | 680.7 ± 6.6   | 24.3 ± 0.2 | 1.4 ± 0.08 | 3.2 ± 0.06 |
| Indigo Treat    | 756.6 ± 32.0 | 969.8 ± 15.3  | 15.4 ± 3.7 | 1.9 ± 0.05 | 4.4 ± 0.43 |
| Jolanta         | 512.5 ± 6.9  | 626.1 ± 14.6  | 24.7 ± 2.6 | 1.6 ± 0.06 | 2.2 ± 0.84 |
| Jugana          | 659.4 ± 17.3 | 863.8 ± 12.2  | 12.6 ± 4.3 | 1.9 ± 0.03 | 3.4 ± 0.24 |
| K100            | 491.0 ± 14.5 | 727.8 ± 6.0   | 19.0 ± 0.2 | 1.4 ± 0.04 | 3.0 ± 0.05 |
| Karina          | 606.7 ± 33.9 | 899.5 ± 12.8  | 27.7 ± 4.0 | 1.6 ± 0.05 | 3.6 ± 0.08 |
| Leningradskij   | 616.8 ± 9.9  | 966.5 ± 12.3  | 23.8 ± 1.3 | 1.6 ± 0.05 | 3.7 ± 0.02 |
| Velikan         | 591.6 ± 13.6 | 902.0 ± 13.4  | 24.6 ± 1.7 | 1.7 ± 0.04 | 3.5 ± 0.10 |
| Nimfa           | 461.3 ± 34.8 | 707.9 ± 7.9   | 8.5 ± 1.0  | 1.4 ± 0.06 | 2.5 ± 0.08 |
| Polar Jivel     | 610.1 ± 24.1 | 906.8 ± 12.7  | 17.5 ± 0.9 | 1.8 ± 0.06 | 3.3 ± 0.14 |
| Siniczka        | 732.9 ± 20.6 | 960.1 ± 5.1   | 13.9 ± 1.4 | 1.9 ± 0.03 | 3.3 ± 0.27 |
| Sinoglaska T3   | 658.8 ± 36.1 | 924.7 ± 7.5   | 21.9 ± 0.4 | 1.8 ± 0.06 | 3.5 ± 0.35 |
| T5              | 506.6 ± 14.2 | 798.5 ± 12.4  | 26.8 ± 2.3 | 1.4 ± 0.05 | 2.9 ± 0.12 |
| Uspiech         | 616.1 ± 20.4 | 949.8 ± 25.4  | 18.2 ± 0.8 | 1.8 ± 0.07 | 2.9 ± 0.52 |
| Valhova         | 700.3 ± 21.7 | 1023.7 ± 18.6 | 14.1 ± 0.4 | 1.9 ± 0.05 | 3.6 ± 0.18 |
| Vostorg         | 537.5 ± 0.9  | 766.4 ± 4.3   | 22.2 ± 0.3 | 1.6 ± 0.02 | 2.5 ± 0.06 |
| Warta           | 462.7 ± 4.5  | 708.6 ± 15.5  | 17.6 ± 0.7 | 1.3 ± 0.04 | 2.8 ± 0.08 |
| Zielona         | 688.6 ± 24.5 | 929.9 ± 10.3  | 29.7 ± 0.1 | 1.8 ± 0.03 | 3.5 ± 0.21 |
| LSD             | 29.0        | 18.1         | 2.6        | 0.06       | 0.31       |
| Mean            | 592.6       | 834.2        | 18.6       | 1.7        | 3.3        |
| Standard deviation | 118.0    | 160.9       | 6.1        | 0.3        | 0.7        |
| Range           | 422.3–934.1 | 436.9–1137.5 | 8.5–29.7   | 1.2–2.2    | 2.2–5.5    |
| CV (%)          | 19.8        | 19.2         | 32.8       | 15.1       | 21.7       |

The largest and most diverse group of polyphenols are flavonoids, which occur in the form of free molecules or bound to sugars. Flavonoids are characterized by a wide spectrum of health-promoting activities and are used in the pharmaceutical, medical and cosmetic industries. This is due to their antioxidant, anti-inflammatory, antimutagenic and anticarcinogenic properties combined with their ability to modulate key functions of cellular enzymes. They are used to combat diseases such as cancer, Alzheimer’s disease and atherosclerosis [38]. The following genotypes were characterized by a high
content of flavonoids, exceeding 1000 mg·100 g\(^{-1}\) fw: clone 1-17-59, cvs Bakczarskij Velikan, Amphora, Valhova and Czarna, and this value was more than twice as high as that observed in the weakest cultivar in this respect, Brazowa. Different contents of this compound in the analyzed group of 12 genotypes (of Russian origin) were observed by Rop et al. [14], who emphasized the pronounced cultivar variability in this regard. The cultivars Borealis and Indigo Gem tested by Rupasinghe et al. [11] in Canada were characterized by total flavonoid content at the level of 699.29 and 638.55 mg QE·100 g\(^{-1}\) fw, respectively. The same cultivars in Poland showed a similar content of this group of compounds, while flavonoid content in the cultivars Leningradskij Velikan and Nimfa (3.27 and 3.11 g·kg\(^{-1}\) fw, respectively), tested in the Czech Republic [14], was much lower compared to the results obtained in the present work. The opposite was found in Berry Blue, Borealis and Indigo Gem, for which Rupasinghe et al. [16] obtained significantly higher values (1156.6, 1582.8 and 1128.5–1327.0 mg QE·100 g\(^{-1}\) fw respectively) compared to the results in this study. Therefore, it should be noted that a large variation in the content of flavonoids may result from cultivar differences and growing locations and conditions, as also indicated by the authors cited above.

The role of plant phenolics in the prevention against chronic diseases such as cardiovascular, diabetes and neurodegenerative diseases has been suggested in many studies. Honeysuckle berry fruit contains triterpenoic acids, \( \beta \)-carotene, catechol, flavonols, chlorogenic acid and many other acids [39]. In the current study, the group of genotypes with a high total phenolic content included: clone 1-17-59, cvs. IndigoTreat, Czarna, Sinoglaska, Amphora, and Valhova, while low values of this trait were recorded in cultivars Aurora, Polar Jevel and Warta. The list of 19 cultivars in terms of total polyphenol content presented by Gołba et al. [39], indicating that cultivars of Canadian origin such as Aurora, Borealis and Honey Bee were characterized by a lower content of this group of compounds compared to cultivars of Russian origin. This general tendency was also confirmed in our research, with the exception of the cultivar IndigoTreat, where polyphenol content was the highest among the cultivars studied. In the study by Senica et al. [40], the content of this group of compounds in Canadian cultivars ranged from 362.2 mg GAE·100 g\(^{-1}\) fw to 471 mg GAE·100 g\(^{-1}\) fw, while the values observed by us were close to or slightly above the upper range. Kusznierewicz et al. [12] showed that some genotypes analyzed in different locations were characterized by a similar level of total anthocyanin content, total phenolic content and antioxidant activity, while others significantly varied in this respect. For example, the cultivar Berry Blue, tested in Canada, showed a lower level of total phenolic content compared to the cultivar Indigo Gem [8], whereas in our research, it was Berry Blue that was more effective in this regard. In contrast, earlier studies by Orincak et al. [41] suggested that different cultivation conditions did not seem to significantly influence the content of this compound. Therefore, while the variability of chemical compositions in the cultivars tested under the same conditions results from their genetic diversity [42], additionally, changes in environmental conditions may also result in an increased or decreased level of their synthesis. Additional factors affecting the observed level of properties include different years (differences in climatic conditions) [34] and maturity stage of the fruit, as late-harvested fruits had significantly higher polyphenolic content then early harvested berries [43,44]. Moreover, Kithma et al. [15] found that the impact of the harvesting date on polyphenol composition was very distinct. Considering that the total phenolic content for the studied group of genotypes was on average 592 mg·100 g\(^{-1}\) fw, the daily intake of phenolic extract calculated by Jurgoński et al. [45] at the level of 0.8 g can be successfully met by eating fruits of this species.

The consumption of products containing high levels of antioxidants shows a positive effect against cancers and inflammatory diseases. The honeysuckle berry serves as a rich source of free radical scavengers [39]. The genotypes 1-17-59 and Amphora were characterized by the highest antioxidant activity, determined both by the DPPH and ABTS methods. In turn, the lowest antioxidant activity was found for the cultivar Aurora using the DPPH method and the cultivar Jolanta using the ABTS method. In the group of genotypes studied by Rop et al. [14], the cultivars Leningradskij Velikan and Nimfa had the lowest antioxidant activity. In our research, on the other hand, Nimfa belonged to the group of intermediate activity, and Leningradskij Velikan to a slightly lower antioxidant activity.
In turn, Auzanneau et al. [7] considered IndigoTreat and Uspiech as the best cultivars in terms of antioxidant activity, as determined by DPPH and ABTS, and Berry Blue as the weakest in this respect, which was also demonstrated in our research. Moreover, the latter authors pointed out that different antioxidant activities in individual years of research could be the result of weather conditions. Research by Rupasinghe et al. [11] and Bakowska-Barczak et al. [21] indicated that the antioxidant potential of haskap berries was higher compared to fruits of other berry plants. According to Khattab et al. [8], it resulted from the high phenolic and anthocyanin content, as the highest radical scavenging activity was found in cultivars with a high content of these compounds. In relation to phenolic compounds, this was confirmed by the results of this study listed in Table 3, where high and statistically significant correlations, both at the phenotype and genotype levels, were found between polyphenol content and antioxidant activity analyzed by the DPPH and ABTS methods (phenotypic 0.94, genotypic 0.95; phenotypic 0.72, genotypic 0.82, respectively).

Table 3. Phenotypic (right side) and genotypic (left side) correlation matrix between bioactive compounds studied in 30 blue honeysuckle genotypes.

|          | Flavonoids | Phenolics | Vitamin C | DPPH  | ABTS  |
|----------|------------|-----------|-----------|-------|-------|
| Flavonoids | 1.0000     | 0.8797 *  | 0.0838    | 0.8302 * | 0.7085 * |
| Phenolics  | 0.8850 *   | 1.0000    | -0.0343   | 0.9417 * | 0.7183 * |
| Vitamin C | -0.0246 ns | -0.1843 ns | 1.0000    | -0.0751 ns | -0.0615 ns |
| DPPH      | 0.8551 *   | 0.9520 *  | -0.1911 ns | 1.0000 | 0.6584 * |
| ABTS      | 0.7495 *   | 0.8222 *  | -0.2235 ns | 0.7435 * | 1.0000 |

* significant at $p \leq 0.05$; ns not significant.

Such correlation also concerned flavonoid content and both methods measuring antioxidant activity. In addition, similarly to Rupasinghe et al. [11], a highly positive correlation between total phenolic content and total flavonoid content was observed. Therefore, according to many authors, the high antioxidant activity of the Kamchatka berry, previously determined on the basis of a smaller number of genotypes compared to this study, was caused by both the high level of total flavonoid and total polyphenol contents [9,14,46,47]. On the other hand, correlations between vitamin C and polyphenol content were negative, however, this direction of interactions was confirmed by the study of Senica et al. [40]. Although correlations at the phenotypic level are helpful in determining the relationship between the studied traits, it should be noted that apart from the genetic component, they also contain an environmental determinant, thus the desired direction of trait-level change should not be fully expected during the selection of breeding materials. Therefore, it is important to analyze the relationship directly at the genotypic level. In our research, in most cases, genotypic correlation coefficients were higher than phenotypic ones, which indicated, as reported in the example of other species of berry plants by Mishra et al. [48], that the effects of environment suppressed the phenotypic relationship between these characters. Moreover, knowledge of the degree of genetic correlations between important traits had a great impact on the selection of improved genotypes in breeding programs [49,50] and enabled indirect selection. According to Connor et al. [51], the total phenolic content was suitable for indirect selection for antioxidant activity in blueberry, indicating the possibility of also obtaining new blue honeysuckle genotypes with higher levels of phytochemical compounds.

The content of total phenolics, flavonoids, vitamin C as well as antioxidant activity analyzed by means of cluster analysis (Figure 1) revealed three main cluster groups.

The first one comprised only the breeding clone 1-17-59, which showed the remarkably high content of phenolics, flavonoids as well as antioxidant activity in both testing methods. The second one was composed of thee genotypes—Amfora, IndigoTreat and Bakczarskij Velikan—for which the values of the analyzed parameters were slightly lower. Two sub-clusters could be distinguished within the third cluster, the first one composed of 11 genotypes and the second one composed of 15 genotypes. It should be noted that within the analyzed clusters, there was no genotype clustering with a common country of origin, similar to what was observed by Sochor et al. [9].
The estimation of genetic components of variance allows for the understanding of the function of genes affecting the quantitative traits. Among them, the most important parameters are genotypic and phenotypic variances, heritability [52], and heritability coupled with high genetic advance as a percentage of the mean provides better information than single parameters [53]; the values of these parameters are presented in Table 4.

### Table 4. Estimates of variance, heritability (H) and genetic advance of fruit phytochemical content and antioxidant activity in 30 blue honeysuckle genotypes.

| Traits | Phenotypic Variance ($\sigma^2_P$) | Phenotypic Coefficient of Variation (PCV %) | Genotypic Variance ($\sigma^2_G$) | Genotypic Coefficient of Variation (GCV %) | Heritability (H) | Genetic Advance (GA) | Genetic Advance as Percentage of Mean (GAM %) |
|--------|----------------------------------|------------------------------------------|----------------------------------|------------------------------------------|-----------------|---------------------|---------------------------------------------|
| Flavonoids | 26,615                          | 19.56                                     | 26,406                           | 19.48                                     | 0.99            | 226.61              | 27.16                                       |
| Phenolics | 14,302                           | 20.18                                     | 13,756                           | 19.79                                     | 0.96            | 161.14              | 27.19                                       |
| Vitamin C | 110.21                           | 56.34                                     | 105.72                           | 55.19                                     | 0.95            | 14.09               | 75.67                                       |
| DPPH    | 64,266                           | 15.32                                     | 62,133                           | 15.06                                     | 0.96            | 343.13              | 20.74                                       |
| ABTS    | 528,371                          | 22.08                                     | 468,188                          | 20.78                                     | 0.88            | 901.74              | 27.39                                       |

Phenotypic variance values expressed by the phenotypic coefficient of variation (PCV%) for all traits were only slightly higher than genotypic variance values expressed by the genotypic coefficient of variation (GCV%), which indicated a low environmental impact on the level of these properties. The values of the heritability coefficient ranged from 0.88 for the assessment of antioxidant activity using the ABTS method to 0.99 for flavonoid content. These values should be considered high because Falconer [54] considered heritability above 0.5 already as high. Such high heritability values of the analyzed parameters are, according to Vieira et al. [50], useful to indicate the reliability of the phenotypic value in predicting the genetic value, since high values suggest a greater possibility of
gain with the selection. Moreover, due to the insignificant influence of environmental conditions, they may constitute the basis for direct selection [55]. High heritability coupled with high genetic advance as a percentage of the mean was observed for vitamin C content, and thus selection would be very effective for this trait. For phenolic and flavonoid content and antioxidant activity, satisfactory selection effects can also be obtained, because heritability and genetic gain are still high. Since this is the first report regarding heritability and genetic advance for the content of phenols, flavonoids, vitamin C and antioxidant activity in blue honeysuckle, the results can be applied only to other species of berry plants. For example, in physico-chemical studies of various characteristics of strawberry fruits, Vieira et al. [50] observed heritability in the range 0.79–0.31, while Mishra et al. [48] estimated ascorbic acid heritability in this species at the level of 0.76. In the red raspberry, heritability (narrow-sense) estimates of Connor et al. [56] were $H = 0.54$ for antioxidant activity and 0.48 for total phenolic content; these estimates implied that a rapid response to selection was possible. Connor et al. [56] estimated the heritability of blueberries to be 0.43 for antioxidant activity and 0.46 for total phenolics. Antioxidant capacity and total phenolic content (TPC) were at the same level of heritability (0.55) in blackcurrants [57].

4. Conclusions

Honeysuckle fruits are a valuable source of health-promoting compounds that can be used as nutraceuticals. The use of the genus *Lonicera* fruits in the diet as a potential source of bioactive compounds with health-promoting properties can be extremely beneficial for consumers. The research presented in this article extends and updates the existing reports on honeysuckle berries. Our research revealed that some of the genotypes analyzed for the first time (1-17-59, DoczVelikana, Jugana, Polar Jevel, Valhova) were characterized by a higher level of phenolic compounds, flavonoids and antioxidant activity compared to the already analyzed genotypes. This shows that breeding studies allow for a significant increase in the content of biologically active compounds with health-promoting properties in the fruits of this species. It can be concluded, based on the analysis of the group of genotypes studied, that there is a potential for obtaining new genotypes with increased content of all analyzed properties, including particularly high vitamin C content.

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