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Comparative Evaluation of the Phytochemical Composition of Fruits of Ten Haskap Berry (*Lonicera caerulea* var. *kamtschatica* Sevast.) Cultivars Grown in Poland

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Abstract: The aim of this study was to investigate the qualitative and quantitative fruit profiles of ten cultivars (cvs.) of haskap berry (*Lonicera caerulea* var. *kamtschatica* Sevast.) to determine their antioxidant activity (ABTS test, CUPRAC test, ability to capture superoxide ($O_2^{\cdot-}$) and hydroxyl radicals ($OH^{\cdot}$)), cytotoxic activity (against cancer cell lines breast, MCF-7; colon, HT-29; and melanoma, SK-Mel-28) and physicochemical properties. Most of the selected cultivars had not previously been analyzed for these properties. A total of 19 polyphenolic compounds were identified in the fruits of the tested genotypes, with a quantitative range of 2166.3–3597.0 µg/g. The polyphenol profile was dominated by anthocyanins (90.0–92.4%), and the remaining classes occurred in the following order: phenolic acids > flavonols > flavan-3-ols. The highest concentrations of these polyphenol groups were found in the cultivars 'Honeybee', 'Sinij Uties' and 'Usłada'. The fruits of these cultivars were also characterized by the highest antioxidant activity (546.6–683.5 µg/mL for $O_2^{\cdot-}$ and 541.2–652.1 µg/mL for $OH^{\cdot}$) and cytotoxic activity (103.6–649.2 µg/mL). The data obtained indicate that the fruits of the new haskap cultivars are a good source of bioactive compounds with possible health-promoting properties.

Keywords: *Lonicera caerulea* var. *kamtschatica* Sevast.; haskap berry; antioxidant activity; cytotoxic activity; UPLC-PDA-MS/MS; physicochemical properties

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1. Introduction

One of the berry fruit species that has recently gained popularity is the blue honeysuckle, syn. Haskap berry (*Lonicera caerulea* var. *kamtschatica* Sevast.), which is a shrub belonging to the family Caprifoliaceacea and the genus of honeysuckle (*Lonicera* L.) [1,2]. The haskap fruit composition is dominated by sugars (glucose, fructose), organic acids (citric, malic, quinic), vitamins (C, A, B1, B2, B6, B9, P), pectin and tannins as well as natural macronutrients (magnesium, sodium, potassium, calcium, phosphorus) and micronutrients (manganese, copper, barium, silicon, boron, iodine) [1,3]. Regarding phenolic compounds of haskap berries, anthocyanins are the dominant group, constituting 51 to 95% of all polyphenols. In this group, the dominant compound is cyanidin 3-*O*-glucoside (71 to 93% of the total anthocyanins). Cyanidin 3,5-*O*-diglucoside; cyanidin 3-*O*-rutinoside; and peonidin, pelargonidin, and delphinidin glycosides are also present in smaller amounts [3–7]. Among other polyphenolic compounds present in these fruits, we can distinguish phenolic

acids, flavonols and flavan-3-ols [1,3,4,6–9]. Thanks to the above composition, haskap berries are often called "superfruits" due to their potential health-promoting properties, namely, antibacterial and antidiabetic effects and ability to reduce the risk of osteoporosis, hypertension, anemia, ischemic heart disease and gastrointestinal disorders [4,10]. However, there are few scientific reports on the antioxidant and cytotoxic properties of haskap berry fruit extracts. Previous works concern the anthocyanin fraction isolated from fruits, which has been shown to have a good ability to remove $ABTS^{\cdot+}$ (2.2-azinobis-3-ethylbenzthiazoline-6-sulphonic acid) synthetic radicals and inhibit in vitro and in vivo the growth of hepatocarcinoma cells (SMMC-7721 cell line) as a result of DNA damage and apoptosis [11]. To date, reports have not demonstrated cytotoxic activity against cancer cells or the ability to capture ROS (reactive oxygen species) by unpurified haskap fruit extracts, which constitutes an undiscovered research field.

Haskap berry as a useful and medicinal plant is a native species of Russia, Japan, China and, to a lesser extent, Europe and North America, which is probably due to the popularity of this plant. There are many different cultivars grown on commercial plantations in Poland and other countries in the world. Recently, efforts have also been made to release new edible haskap berry cultivars, especially in North America and Russia, with higher fruit production and more favorable sensory characteristics than older varieties. The newest varieties recently introduced in Canada include 'Honeybee', 'Boreal Blizzard', 'Boreal Beauty' and 'Boreal Best' [1,2,12]. However, work on obtaining new varieties may affect the quality of the fruit and cause various positive or negative changes in their composition. Therefore, in order to properly direct further breeding work on haskap cultivars, it is important to monitor this process. For these reasons, the above-mentioned new haskap berry cultivars bred in Poland were selected for analysis, comparing them with commonly available varieties, such as 'Aurora', 'Vostarg', 'Jugana', 'Usłada', 'Lawina' and 'Sinij Uties', thus obtaining a combination of five Canadian varieties and five Russian varieties. The aim of this study was to evaluate selected varieties of haskap berries based on analyses of their physicochemical properties; health-promoting activities, including antioxidant and cytotoxic activities; and profiles of polyphenolic compounds using the UPLC-PDA-MS/MS (ultra performance liquid chromatography equipped with a photodiode array (PDA) detector, a tandem quadrupole mass spectrometer (TQD) and an electrospray ionization (ESI) source, Waters, Milford, MA, USA) method. The obtained results will provide new information on the biological properties and polyphenol compositions of previously unexplored varieties of haskap berries, providing a scientific basis for their wider use in the food, pharmaceutical and cosmetics industries and also potentially in plant genetics to work on new varieties of haskap berries.

2. Materials and Methods

2.1. Materials and Reagents

2-Deoxy-D-ribose, 2.2-azinobis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), acetonitrile, ascorbic acid, ethanol, ferrozine, gallic acid, neocuproine, perhydrol, quercetin, sodium acetate and sodium carbonate were purchased from Chempur (Piekary Śląskie, Poland). LiChroprep RP-18 (40–63 μm) and other chemicals were purchased from Sigma-Aldrich (Steinheim, Germany). The CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay was purchased from Promega (Madison, WI, USA). The human colon adenocarcinoma cells (HT-29, ATCC® HTB-38TM) and breast adenocarcinoma cells (MCF-7, ATCC® HTB-22TM) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA), while other cells (SK-Mel-28) were a gift from Prof. Jolanta Redowicz (Nencki Institute of Experimental Biology PAS, Warsaw, Poland).

2.2. Plant Material

The research material consisted of haskap berry (*L. caerulea* var. *kamtschatica* Sevast.) fruits collected from June to July 2021 from five-year-old shrubs grown on the commercial plantation in Muniakowice 100, 32-090 Słomniki (Małopolska vojevodship, South Poland),

50°17′31.0″ N 20°06′59.0″ E. Ten cultivars were selected for fruit analysis, including five Canadian ('Boreal Beauty', 'Boreal Beast', 'Boreal Blizzard', 'Aurora' and 'Honeybee') and five Russian ('Vostarg', 'Jugana', 'Usłada', 'Lawina' and 'Sinij Uties') varieties. The fruits of each cultivar were collected by hand from the upper, middle and lower parts of three bushes. At the time of harvest, all fruits were at optimal ripeness, with no signs of mold or rot and no mechanical damage. After the samples were delivered to the laboratory, the fruits from each batch (bush) were combined and each subgroup was frozen and then lyophilized (ALPHA 1-2 LD plus, Martin Christ GmbH, Harz, Germany). Each fruit subgroup was used for analyses, which were performed in triplicate for each variety.

2.3. Preparation of Extract

The lyophilized fruits were ground and mixed with ethanol (70% with 1% formic acid). Extractions were assisted by ultrasonic waves (ultrasonic bath, Sonic 10, Polsonic, Warsaw, Poland) at 25 °C for 20 min. The extracts were then centrifuged at 10,000× *g* for 15 min (Centrifuge 5430, Eppendorf, Hamburg, Germany), and the resulting supernatant was used for analysis. To assess cell viability, the extracts were additionally extracted into the solid phase using the LiChroprep RP-18 adsorber, according to our previous work [13,14]. The obtained extracts were evaporated (R-215 Rotavapor System, Buchi, Switzerland) and lyophilized, thus obtaining preparations that were used in the cell tests.

2.4. Assessment of Physicochemical Properties

The soluble solids content (SSC) in the fruits was determined using a refractometer (Type Pal-1, Conbest, Kraków, Poland). Dry matter (DM) was determined in accordance with PN-90/A-75101/03 [15], titratable acidity (TA) was determined in accordance with the PN-EN 12147:2000 [16] and total ash value was determined in accordance with the PN-90/A-75101/08 standard [17]. The pH value was measured with a pH meter (Type CP-411, Elmetron, Zabrze, Poland).

2.5. Phenolic Composition by UV–VIS Spectrophotometry and UPLC-PDA-MS/MS

Total polyphenol content (TPC) was assessed using the method described by Gao et al. [18]. The total content of flavonoids (TFC) and total content of anthocyanins (TAC) were determined using the method of Żurek et al. [19] and Lee et al. [20]. The absorbance was measured using a UV–VIS spectrometer (Type UV2900, Hitachi, Japan). The results were expressed in mg of gallic acid (mg GAE/g dry matter (dm)) for the TPC test, mg of quercetin (mg QE/g dm) for the TFC test and mg of cyanidin 3-*O*-glucoside (mg C3G/g dm) for the TAC test.

The analysis of phenolic compounds was performed by ultra performance liquid chromatography (UPLC) (Waters, Milford, MA, USA), as reported by Żurek et al. [14]. The UPLC was equipped with a photodiode array (PDA) detector, a tandem quadrupole mass spectrometer (TQD) and an electrospray ionization (ESI) source. Each sample (5 µL) was separated on a UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 µm, Waters, Warsaw, Poland) operating at 50 °C, with a flow rate of 0.35 mL/min. The elution solvents in the analysis of anthocyanins were 2% formic acid in water (A) and 2% formic acid in 40% acetonitrile (B), while the analysis of other polyphenols used water (A) and 40% acetonitrile (B). The optimized TQD conditions were as follows: capillary voltage 3500 V; cone voltage 30 V; source and dissolution temperatures 120 °C and 350 °C, respectively; nitrogen flow rate 800 L/h. The standards used are listed in Table S1. The collected data were processed using Waters MassLynx v.4.1 software (Waters, Milford, MA, USA). The results are expressed in µg/g dm.

2.6. Determination of Antioxidant Activity

2.6.1. $ABTS^{\bullet+}$ Radical Scavenging Activity

The activity of the extracts for scavenging $ABTS^{\bullet+}$ radicals was assessed according to the method of Re et al. [21]. Fruit extracts were mixed with 0.03 mL of $ABTS^{\bullet+}$ solution. After 6 min, absorbance was measured at 734 nm.

2.6.2. Determination of Copper Ion Reduction

The copper ion reduction test (CUPRAC) was performed according to the method of Apak et al. [22]. Fruit extracts were mixed with 1.0 mL of neocuproine (0.0075 M), 1.0 mL of copper chloride (0.01 M) and 1.0 mL of acetate buffer (1 M). After 30 min, absorbance was measured at 450 nm.

The results for the ABTS and CUPRAC tests are expressed as Trolox Equivalents (mmol TE/g dm).

2.6.3. Superoxide Radical Scavenging Activity Assay

The superoxide ($O_2^{\cdot-}$) radical scavenging activity was assessed according to the method of Robak and Gryglewski [23], with modifications by Pawłowska et al. [24]. Fruit extracts were mixed with 1.0 mL nitrotetrazolium blue chloride (0.15 mM), 1.0 mL β-nicotinamide adenine dinucleotide (0.468 mM) and 1.0 mL phenazine methosulfate (0.06 mM). After 5 min, absorbance was measured at 560 nm.

2.6.4. Hydroxyl Radical Scavenging Activity Assay

Hydroxyl ($OH^{\cdot}$) radical scavenging activity was assessed according to the method of Halliwell et al. [25], with modifications by Żurek et al. [26]. Fruit extracts were mixed with 0.1 mL 2-deoxyribose (0.2 mM), 0.1 mL ferrous ammonium sulfate (1.0 mM), 0.1 mL ethylenediaminetetraacetic acid disodium salt dihydrate (1.04 mM), 0.01 mL ascorbic acid (1.0 mM), 0.01 mL hydrogen peroxide (0.1 M), 1.0 mL trichloroacetic acid (2.8%) and 0.5 mL thiobarbituric acid (1%) and heated to 100 °C for 10 min. After this time, the samples were cooled to room temperature and the absorbance was measured at 532 nm.

The results for the ability of the fruit extracts to inhibit the production of $O_2^{\cdot-}$ and $OH^{\cdot}$ radicals are expressed as the IC_{50} (μg/mL).

2.7. Cell Viability Assay

Three cancer cell lines were selected for this study: breast adenocarcinoma (MCF-7), colon adenocarcinoma (HT-29) and melanoma (SK-Mel-28). Cells were grown in 5% CO_2 at 37 °C in an incubator (CB170 incubator, Binder, Tuttlinen, Germany) in Dulbecco-GlutaMAX-1 modified Eagle medium supplemented with an antibiotic solution and inactivated fetal serum.

The cytotoxicity of the polyphenolic fractions of the fruits of ten haskap berry cultivars was assessed on the basis of earlier reports by Żurek et al. [27]. Three selected tumor cell lines were plated at a concentration of 8.0×10^3 cells/well (200 μL) in 96-well plates. After the adhesion period, the cells were treated with the tested extracts for 48 h at concentrations ranging from 10 to 750 μg/mL. After this time, a mixture of PMS and MTS was added to each well and the absorbance at 490 nm was measured after 2 h on a microplate reader (SmartReader 96, Accuris Instruments. Edison, Rosemead, CA, USA). The results are expressed as IC_{50} (μg/mL).

2.8. Statistical Analysis

The results are expressed as the mean (n = 3–9) and SD. Statistical analysis was performed using Statistica 13.3 software (StatSoft, Kraków, Poland). Significant differences ($p < 0.05$) between means of triplicates were assessed by using Duncan's test. Pearson's correlation and principal component analysis (PCA) were also performed to highlight relationships between variables.

3. Results and Discussion

3.1. Physicochemical Properties

This study assessed the main physicochemical characteristics such as pH value, soluble solids content (SSC), total titratable acidity (TA), dry matter (DM) and ash of 10 haskap berry cultivars (Table 1). The pH value, SSC and dry matter content ranged from 3.04 to 3.35, 10.82 to 14.69 °Brix and 13.63 to 16.94%, respectively. The highest values for these three parameters were found for the 'Honeybee' and 'Usłada' varieties, while the lowest were found for the 'Vostorg' (pH), 'Borael Blizzard' (SSC) and 'Boreal Beast' (DM) varieties. The obtained values are consistent with previous findings [3,28–31].

In general, haskap berries are rich in organic acids, which give them a specific sour taste, reminiscent of blueberries [30]. Our own analyses showed that the TA ranged from 2.13 ('Boreal Beast') to 3.36 ('Boreal Blizzard') g of citric acid/100 g. Higher values have only been shown by Ochmian et al. [28] for fruit of the 'Wojtek' cultivar (4.4%) harvested in the early season. The total acidity of the same variety of late-harvest berries was already within the above range (2.7%). The maturity index (MI) calculated from SSC and TA values is one of the most important factors that determine consumer preference and acceptance of products. The MI value ranged from 3.22 ('Boreal Blizzard') to 6.04% ('Boreal Beast'). On this basis, it can be assumed that the fruit of the 'Boreal Beast' variety has better sensory characteristics among all the varieties analyzed, especially the 'Boreal Blizzard' variety. The last physicochemical parameter analyzed, ash content, ranged from 0.49 ('Aurora') to 0.63% ('Lawina'). These values are consistent with the reports of Wojdyło et al. [3], who emphasized the dependence of this parameter on cultivar and growing conditions, including climate and soil.

Table 1. Physicochemical characteristics; total content of polyphenols, flavonoids and anthocyanins; and antioxidant and cytotoxic activity of fruits of ten haskap berry cultivars.

	Cultivar									
	Boreal Beauty	**Boreal Beast**	**Boreal Blizzard**	**Aurora**	**Honeybee**	**Vostorg**	**Jugana**	**Usłada**	**Lawina**	**Sinij Uties**
Physicochemical properties										
pH	3.14 ± 0.09 ^{ab}	3.16 ± 0.10 ^{ab}	3.09 ± 0.02 ^{ab}	3.14 ± 0.06 ^{ab}	3.35 ± 0.08 ^{c}	3.04 ± 0.05 ^{a}	3.15 ± 0.08 ^{ab}	3.13 ± 0.07 ^{ab}	3.37 ± 0.06 ^{c}	3.21 ± 0.07 ^{b}
SSC	12.54 ± 0.39 ^{b}	12.88 ± 0.30 ^{bc}	10.82 ± 0.85 ^{a}	13.46 ± 0.74 ^{cd}	14.38 ± 0.31 ^{e}	11.54 ± 0.35 ^{a}	13.24 ± 0.20 ^{bc}	14.69 ± 0.27 ^{e}	14.07 ± 0.08 ^{de}	13.33 ± 0.06 ^{bcd}
TA	2.94 ± 0.04 ^{e}	2.13 ± 0.05 ^{a}	3.36 ± 0.04 ^{h}	2.84 ± 0.09 ^{d}	3.03 ± 0.03 ^{f}	3.12 ± 0.03 ^{g}	2.95 ± 0.01 ^{e}	2.54 ± 0.04 ^{b}	2.70 ± 0.01 ^{c}	2.85 ± 0.05 ^{d}
MI	4.27 ± 0.08 ^{c}	6.04 ± 0.24 ^{f}	3.22 ± 0.28 ^{a}	4.74 ± 0.19 ^{d}	4.75 ± 0.07 ^{d}	3.69 ± 0.14 ^{b}	4.48 ± 0.05 ^{cd}	5.78 ± 0.16 ^{f}	5.22 ± 0.04 ^{e}	4.68 ± 0.07 ^{d}
DM	13.79 ± 1.69 ^{a}	13.63 ± 0.68 ^{a}	15.45 ± 1.47 ^{ab}	15.25 ± 1.10 ^{ab}	16.94 ± 1.03 ^{b}	15.92 ± 0.70 ^{ab}	15.21 ± 1.02 ^{ab}	14.23 ± 1.81 ^{a}	14.15 ± 0.87 ^{a}	15.08 ± 1.03 ^{ab}
Ash	0.50 ± 0.02 ^{ab}	0.51 ± 0.08 ^{abc}	0.53 ± 0.06 ^{abc}	0.49 ± 0.01 ^{a}	0.54 ± 0.04 ^{abc}	0.59 ± 0.06 ^{bcd}	0.60 ± 0.02 ^{cd}	0.59 ± 0.08 ^{bcd}	0.63 ± 0.05 ^{d}	0.54 ± 0.04 ^{abc}
Content of polyphenolic compounds										
TPC	20.90 ± 0.33 ^{a}	45.09 ± 0.21 ^{f}	43.69 ± 0.29 ^{e}	32.13 ± 0.33 ^{b}	42.98 ± 0.33 ^{e}	32.15 ± 0.57 ^{b}	39.37 ± 0.57 ^{d}	35.75 ± 0.33 ^{c}	32.70 ± 0.87 ^{b}	46.76 ± 0.57 ^{g}
TFC	7.47 ± 0.04 ^{c}	6.56 ± 0.04 ^{a}	8.06 ± 0.03 ^{d}	8.50 ± 0.07 ^{e}	15.86 ± 0.05 ^{j}	8.81 ± 0.04 ^{f}	7.22 ± 0.03 ^{b}	12.61 ± 0.02 ^{i}	12.19 ± 0.04 ^{h}	10.81 ± 0.04 ^{g}
TAC	14.05 ± 0.16 ^{b}	22.03 ± 0.23 ^{f}	14.26 ± 0.11 ^{b}	18.50 ± 0.02 ^{d}	21.77 ± 0.13 ^{e}	14.11 ± 0.06 ^{b}	13.01 ± 0.39 ^{a}	18.76 ± 0.33 ^{d}	17.27 ± 0.29 ^{c}	18.74 ± 0.15 ^{d}
Antioxidant activity										
ABTS	42.62 ± 0.53 ^{a}	53.29 ± 0.21 ^{e}	45.12 ± 0.29 ^{b}	49.60 ± 0.08 ^{d}	58.69 ± 0.14 ^{g}	48.35 ± 0.08 ^{c}	43.02 ± 0.24 ^{a}	55.62 ± 0.14 ^{f}	49.94 ± 0.21 ^{d}	53.29 ± 0.08 ^{e}
CUPRAC	36.52 ± 0.05 ^{a}	48.71 ± 0.05 ^{e}	40.56 ± 0.05 ^{c}	49.95 ± 0.08 ^{f}	53.92 ± 0.10 ^{i}	47.22 ± 0.05 ^{d}	37.53 ± 0.08 ^{b}	51.30 ± 0.08 ^{g}	49.36 ± 0.05 ^{f}	52.37 ± 0.13 ^{h}
$O_2^{\cdot -}$	1003.05 ± 0.03 ^{e}	622.27 ± 0.02 ^{b}	760.00 ± 0.01 ^{d}	1149.66 ± 0.05 ^{f}	546.62 ± 0.02 ^{a}	770.25 ± 0.02 ^{d}	651.34 ± 0.02 ^{bc}	683.47 ± 0.01 ^{c}	1458.16 ± 0.06 ^{g}	668.47 ± 0.01 ^{bc}
$OH^{\cdot}$	1163.00 ± 0.03 ^{e}	703.55 ± 0.01 ^{c}	734.58 ± 0.02 ^{c}	717.85 ± 0.01 ^{c}	639.60 ± 0.02 ^{b}	1297.15 ± 0.04 ^{f}	894.38 ± 0.03 ^{d}	541.22 ± 0.00 ^{a}	1174.06 ± 0.01 ^{e}	652.08 ± 0.01 ^{b}
Cytotoxic activity										
MCF-7	610.00 ± 9.12 ^{e}	594.04 ± 6.30 ^{de}	610.34 ± 9.74 ^{e}	575.74 ± 7.99 ^{d}	305.16 ± 3.47 ^{b}	588.63 ± 3.63 ^{de}	675.81 ± 8.67 ^{f}	310.25 ± 3.62 ^{b}	416.42 ± 3.54 ^{c}	244.55 ± 1.25 ^{a}
HT-29	299.04 ± 5.31 ^{d}	279.80 ± 1.83 ^{d}	478.16 ± 6.61 ^{f}	395.86 ± 1.00 ^{e}	103.62 ± 4.51 ^{a}	228.86 ± 1.04 ^{c}	290.38 ± 4.32 ^{d}	169.53 ± 3.45 ^{b}	386.72 ± 10.69 ^{e}	158.28 ± 6.32 ^{b}
SK-Mel-28	489.20 ± 2.99 ^{b}	634.76 ± 1.14 ^{c}	622.75 ± 1.89 ^{c}	>750	427.35 ± 1.84 ^{a}	>750	>750	464.31 ± 0.52 ^{b}	>750	649.22 ± 3.20 ^{c}

Results are expressed as the mean and SD. Measurements were made in 3–9 repetitions. Values marked with the same letter (in the same row) did not differ significantly ($p < 0.05$) according to Duncan's test. The results for soluble solids content (SSC) are expressed in °Brix; titratable acidity (TA) in g of citric acid/100 g; dry matter (DM), maturity index (MI) and Ash in %; total polyphenol content (TPC) in mg GAE/g; total flavonoids content (TFC) in mg QE/g; total anthocyanins content (TAC) in mg C3G/g; $ABTS^{\bullet+}$ radical scavenging activity (ABTS) and copper ion reduction (CUPRAC) in mmol TE/100 g; and superoxide ($O_2^{\cdot -}$) and hydroxyl ($OH^{\cdot}$) radical scavenging activity and cytotoxic activity as IC_{50} (μg/mL).

3.2. Content of Polyphenolic Compounds

Polyphenolic compounds are secondary metabolites that are commonly found in a plant's world and involved in their protection against UV radiation and antifungal and antibacterial activity. Recently, these compounds have attracted attention due to their potentially broad health-promoting effects, including anti-inflammatory, antioxidant and antibacterial activities [32,33]. As shown in Table 1, for the tested haskap berry cultivars, the total content of polyphenols ranged from 20.90 to 46.76 mg GAE/g, flavonoids from 6.56 to 15.86 mg QE/g and anthocyanins from 13.01 to 22.03 mg C3G/g. The highest contents of these three groups of compounds were found in the fruits of cvs. 'Sinij Uties', 'Honeybee' and 'Boreal Beast'. In turn, the lowest concentrations of these compounds were found in the fruits of cv. 'Boreal Beauty' (in the TPC test), cv. 'Boreal Beast' (in the TFC test) and cv. 'Jugana' (in the TAC test).

According to the results presented in the available literature, the TPC content of haskap fruit ranges from 0.64 to 138.37 mg GAE/g [8,30,31,34–40], which is consistent with our own results. However, in the papers cited, information on the polyphenolic compounds in fruits could only be found for four haskap cultivars. Pažereckaite et al. [31], Gawroński et al. [35], Dziedzic et al. [40] and Česonienė et al. [41] for the cv. 'Vostarg' reported contents 1.7 times lower, 5.9 times lower, 4.3 times higher and 50.2 times lower, respectively, compared to our own results. Gawroński et al. [35] and Dziedzic et al. [40] for the cv. 'Aurora' showed contents 7.6 times lower and 3.9 times higher and, for the cv. 'Honeybee', 8.6 times lower and 3.0 times higher. However, for the fourth cv., 'Jugana', Gawroński et al. [35] reported a concentration 5.9 times lower. The above comparison indicates a high variability of TPC in haskap berry extracts.

As described in the literature, the TFC content in haskap fruit ranges from 4.37–15.83 mg QE/g [34,35,38]. These values are similar to those obtained in this study. However, to date, results have only been published for four of the ten varieties tested. Gawroński et al. [35] reported TFC contents in the fruit of the cv. 'Aurora' that were 1.4 times lower; for the cv. 'Honeybee', 2.1 times lower; for the cv. 'Jugana', 1.2 times higher; and for the cv. 'Vostorg', 1.1 times lower than those reported in this study. A number of experimental reports on the TAC content of haskap fruit have been reported, according to which, the values ranged from 0.39 to 48.21 mg C3G/g [30,31,34,36,37,40,42]. However, so far, only three of the ten cultivars studied in these works have been analyzed, such as 'Aurora', 'Vostorg' and 'Honeybee'. In our research, for the cv. 'Aurora', the TAC content was 1.8 times higher than in the work of Dziedzic et al. [40] and 6.3 times higher than that reported by De Silva and Rupasinghe [42]. For the cv. 'Vostorg', the content was 3.5 times higher than that presented in the work of Česonienė et al. [41] and, for the cv. 'Honeybee', the content was 1.3 times lower than in the work of Dziedzic et al. [40]. However, the results of an analytical study by De Silva and Rupasinghe [42] showed that the TAC content depended on the harvest date of haskap berries. The fruit of the cv. 'Aurora' harvested on five different dates (June to July) showed an increase in TAC content with harvest date, reaching the highest content on the fifth date (July 2nd). Hence, there might be differences in the results obtained and the referenced work, not only for the TAC content but also in terms of TPC and TFC content. Also, the different methodologies of the analyses used, especially the method of sample preparation, as well as the variances of cultivars tested, could significantly affect the differences between the results obtained. So far, no changes in the content of polyphenolic compounds in haskap berry extracts have been assessed depending on the extraction method used. However, it is commonly known that for the dominant anthocyanins, a change in the pH value of the extractants significantly affects the extraction efficiency. This was confirmed in a study by Kang et al. [43]; using subcritical extraction with water with 1% citric acid as a solvent, a 3 times higher concentration of TAC was obtained in blueberry and chokeberry extracts compared to the control sample (water as a solvent). The study also showed that even the type of acid used can significantly affect the TPC extraction efficiency. Therefore, all these aspects mentioned can explain the differences between this work and the cited reports.

3.3. Polyphenol Profiles

The qualitative profiles of polyphenolic compounds are presented in Table 2 and the quantitative profiles are presented in Table 3 and Figure 1. The detected polyphenolic compounds were characterized on the basis of their UV maxima, *m/z*, the fragmentation ions formed, retention time, peak areas, the standards used and the available literature data. Using the UPLC-PDA-MS/MS method, 19 polyphenolic compounds were identified in tested haskap berry cultivars, of which 9 belonged to the flavonols class, 6 to anthocyanins, 3 to phenolic acids and 1 compound to the flavan-3-ols group. It is noteworthy that the qualitative profiles of the identified polyphenols were not dependent on the analyzed fruits of the haskap cultivar; the same number of individual classes was found for each genotype. The polyphenol content ranged from 2166.25 ('Boreal Beauty') to 3597.02 ('Honeybee') µg/g. In the quantitative profiles, 90.0–92.4% were anthocyanins, 3.5–6.1% phenolic acids, 2.8–4.1% flavonols and 0.9–1.1% flavan-3-ols (Table 3, Figure 1).

Table 2. Individual phenolic compounds identified in ten haskap berry cultivars using the UPLC-PDA-MS/MS method.

	Compound	**Rt**	**λ_{max}**	**[M − H] *m/z***	
		min	**nm**	**MS**	**MS/MS**
	Anthocyanins				
1	Cyanidin 3,5-*O*-diglucoside	2.23	279, 515	611	449, 287
2	Cyanidin 3-*O*-glucoside	2.66	279, 514	449	287
3	Cyanidin 3-*O*-rutinoside	2.85	279, 515	595	287
4	Pelargonidin 3-*O*-glucoside	3.09	278, 504	433	271
5	Peonidin 3-*O*-glucoside	3.35	279, 517	463	301
6	Peonidin 3-*O*-rutinoside	3.43	279, 517	609	301
	Other phenolics				
7	Neochlorogenic acid	2.21	288sh, 324	353	191
8	Chlorogenic acid	2.85	288sh, 324	353	191
9	Procyanidin dimer B-type	3.01	279	577	289
10	Quercetin 3-*O*-rutinoside-7-*O*-rhamnoside	3.85	255, 354	755	301
11	Quercetin 3-*O*-pentoside-glucoside I	4.03	255, 355	595	301
12	Quercetin 3-*O*-pentoside-glucoside II	4.18	255, 355	595	301
13	Quercetin 3-*O*-rutinoside	4.30	255, 355	609	301
14	Quercetin 3-*O*-glucoside	4.51	255, 355	463	301
15	Quercetin 3-*O*-rhmanoside	4.66	255, 355	447	301
16	Quercetin 3-*O*-pentoside	4.73	255, 355	433	301
17	Kaempferol 3-*O*-rutinoside	4.86	264, 338	593	285
18	3,4-di-*O*-caffeoyl-quinic acid	5.00	288sh, 324	515	353
19	Quercetin 3-*O*-(6″-acetyl)-glucoside	5.23	255, 335	505	301

Table 3. The content of polyphenolic compounds (μg/g dm) identified in fruits of ten haskap berry cultivars.

	Cultivar									
	Boreal Beauty	**BorealBeast**	**Boreal Blizzard**	**Aurora**	**Honeybee**	**Vostorg**	**Jugana**	**Usłada**	**Lawina**	**Sinij Uties**
1 *	74.60 ± 1.71 ^{e}	163.42 ± 14.7 ^{c}	51.35 ± 0.03 ^{f}	159.24 ± 1.52 ^{c}	131.94 ± 4.37 ^{d}	258.77 ± 10.62 ^{a}	60.87 ± 0.05 ^{c}	68.85 ± 2.89 ^{c}	76.85 ± 0.01 ^{e}	188.43 ± 3.28 ^{b}
2	1753.07 ± 23.10 ^{d}	2033.01 ± 92.57 ^{c}	1746.0 ± 16.61 ^{d}	2009.09 ± 66.60 ^{c}	2696.21 ± 20.4 ^{a}	1894.91 ± 1.41 ^{d}	1856 ± 78.0 ^{d}	2263.33 ± 25.04 ^{b}	2040.56 ± 34.21 ^{c}	2418.93 ± 83.80 ^{a}
3	55.90 ± 0.04 ^{c}	161.1 ± 6.28 ^{b}	256.9 ± 8.52 ^{a}	197.53 ± 1.50 ^{a}	272.40 ± 10.1 ^{a}	140.10 ± 5.89 ^{b}	132.45 ± 0.01 ^{b}	297.76 ± 5.00 ^{a}	271.12 ± 9.40 ^{a}	238.28 ± 3.62 ^{a}
4	15.30 ± 0.15 ^{b}	13.33 ± 1.94 ^{b}	8.67 ± 0.07 ^{c}	9.93 ± 0.37 ^{c}	21.21 ± 0.86 ^{b}	6.21 ± 0.00 ^{c}	12.13 ± 0.20 ^{b}	26.44 ± 0.92 ^{a}	11.98 ± 0.18 ^{b}	11.55 ± 0.33 ^{b}
5	88.80 ± 2.95 ^{c}	107.4 ± 2.24 ^{b}	63.6 ± 2.37 ^{d}	80.91 ± 3.29 ^{c}	102.9 ± 1.26 ^{b}	72.87 ± 1.22 ^{c}	106.4 ± 3.69 ^{b}	125.8 ± 1.88 ^{a}	98.90 ± 2.95 ^{b}	98.90 ± 3.47 ^{b}
6	5.86 ± 0.66 ^{c}	7.45 ± 2.05 ^{c}	13.8 ± 0.56 ^{b}	11.98 ± 0.15 ^{b}	12.25 ± 0.67 ^{b}	9.24 ± 0.32 ^{b}	10.56 ± 0.16 ^{b}	22.80 ± 0.68 ^{a}	19.16 ± 0.65 ^{a}	14.03 ± 0.25 ^{b}
7	5.37 ± 0.20 ^{c}	5.53 ± 0.84 ^{c}	13.1 ± 0.16 ^{b}	10.83 ± 0.59 ^{b}	13.16 ± 0.17 ^{a}	20.29 ± 0.30 ^{a}	10.01 ± 0.30 ^{b}	10.94 ± 0.37 ^{b}	11.19 ± 0.16 ^{b}	14.18 ± 0.45 ^{b}
8	61.20 ± 2.49 ^{d}	113.6 ± 7.37 ^{c}	118.5 ± 6.50 ^{c}	71.64 ± 0.94 ^{d}	187.0 ± 14.9 ^{a}	106.5 ± 3.18 ^{c}	97.34 ± 3.31 ^{c}	131.5 ± 1.86 ^{b}	107.7 ± 2.73 ^{c}	95.53 ± 0.99 ^{d}
9	20.50 ± 1.85 ^{c}	26.23 ± 3.63 ^{c}	21.84 ± 0.29 ^{c}	24.72 ± 1.98 ^{c}	40.36 ± 1.66 ^{d}	26.00 ± 0.89 ^{c}	25.53 ± 0.36 ^{c}	33.63 ± 0.85 ^{b}	28.72 ± 0.27 ^{b}	34.92 ± 0.86 ^{b}
10	3.18 ± 0.17 ^{c}	3.36 ± 0.18 ^{c}	2.42 ± 0.19 ^{c}	5.19 ± 0.21 ^{b}	1.65 ± 0.00 ^{c}	2.82 ± 0.04 ^{d}	3.81 ± 0.10 ^{c}	7.87 ± 0.07 ^{a}	7.92 ± 0.18 ^{a}	8.41 ± 0.01 ^{a}
11	5.63 ± 0.63 ^{d}	8.11 ± 0.97 ^{c}	7.18 ± 0.29 ^{c}	4.73 ± 0.00 ^{d}	8.13 ± 0.34 ^{c}	16.36 ± 0.41 ^{b}	10.91 ± 0.10 ^{b}	14.15 ± 0.33 ^{b}	34.03 ± 0.17 ^{a}	7.30 ± 0.01 ^{c}
12	1.83 ± 0.15 ^{c}	1.52 ± 0.14 ^{c}	1.65 ± 0.00 ^{c}	2.06 ± 0.09 ^{c}	1.36 ± 0.00 ^{a}	2.03 ± 0.02 ^{c}	1.74 ± 0.04 ^{c}	2.49 ± 0.01 ^{b}	5.44 ± 0.00 ^{a}	2.73 ± 0.01 ^{b}
13	46.60 ± 1.91 ^{c}	57.2 ± 1.48 ^{b}	50.01 ± 2.10 ^{b}	54.81 ± 0.01 ^{b}	59.03 ± 0.99 ^{a}	45.75 ± 1.05 ^{b}	49.20 ± 0.25 ^{b}	63.63 ± 0.04 ^{a}	36.72 ± 0.35 ^{c}	76.11 ± 2.52 ^{a}
14	9.21 ± 0.01 ^{b}	8.31 ± 0.53 ^{b}	6.39 ± 0.00 ^{c}	6.80 ± 0.11 ^{c}	18.79 ± 0.65 ^{b}	12.71 ± 0.06 ^{a}	8.43 ± 0.01 ^{b}	15.94 ± 0.15 ^{a}	15.35 ± 0.51 ^{a}	10.20 ± 0.16 ^{b}
15	1.51 ± 0.06 ^{c}	1.61 ± 0.21 ^{c}	0.80 ± 0.01 ^{d}	2.71 ± 0.09 ^{b}	3.73 ± 0.06 ^{c}	2.03 ± 0.00 ^{b}	1.35 ± 0.01 ^{c}	4.62 ± 0.15 ^{a}	2.35 ± 0.02 ^{b}	4.05 ± 0.11 ^{a}
16	1.38 ± 0.00 ^{c}	1.31 ± 0.14 ^{c}	0.73 ± 0.03 ^{d}	1.92 ± 0.03 ^{c}	1.30 ± 0.04 ^{c}	2.79 ± 0.03 ^{b}	1.22 ± 0.04 ^{c}	2.10 ± 0.02 ^{b}	4.49 ± 0.17 ^{d}	2.92 ± 0.14 ^{b}
17	1.90 ± 0.03 ^{a}	1.61 ± 0.58 ^{b}	1.75 ± 0.03 ^{b}	1.07 ± 0.03 ^{d}	1.44 ± 0.05 ^{a}	0.45 ± 0.02 ^{e}	0.85 ± 0.01 ^{e}	2.46 ± 0.09 ^{a}	0.94 ± 0.04 ^{e}	1.47 ± 0.09 ^{a}
18	9.31 ± 0.32 ^{d}	10.9 ± 0.87 ^{c}	10.79 ± 0.32 ^{c}	10.94 ± 0.37 ^{c}	20.29 ± 0.29 ^{a}	16.05 ± 0.12 ^{b}	13.86 ± 0.52 ^{c}	14.02 ± 0.57 ^{c}	12.00 ± 0.15 ^{c}	15.90 ± 0.90 ^{b}
19	5.17 ± 0.08 ^{b}	4.29 ± 0.87 ^{c}	2.15 ± 0.07 ^{d}	4.37 ± 0.06 ^{c}	4.12 ± 0.10 ^{a}	8.32 ± 0.31 ^{a}	4.95 ± 0.20 ^{c}	4.38 ± 0.05 ^{c}	5.91 ± 0.32 ^{b}	6.32 ± 0.14 ^{a}
Total (μg/g)	2166.25 ± 17.30 ^{c}	2729.26 ± 96.71 ^{b}	2377.58 ± 38.18 ^{c}	2670.34 ± 73.30 ^{b}	3597.02 ± 52.50 ^{a}	2643.22 ± 25.76 ^{b}	2407.56 ± 78.75 ^{c}	3112.32 ± 40.88 ^{a}	2790.70 ± 52.46 ^{b}	3248.90 ± 77.20 ^{a}

* List of compounds as given in Table 2. Results are expressed as mean and SD. Measurements were made in 3 repetitions. Values marked with the same letter (in the same row) did not differ statistically significantly ($p < 0.05$) according to Duncan's test.

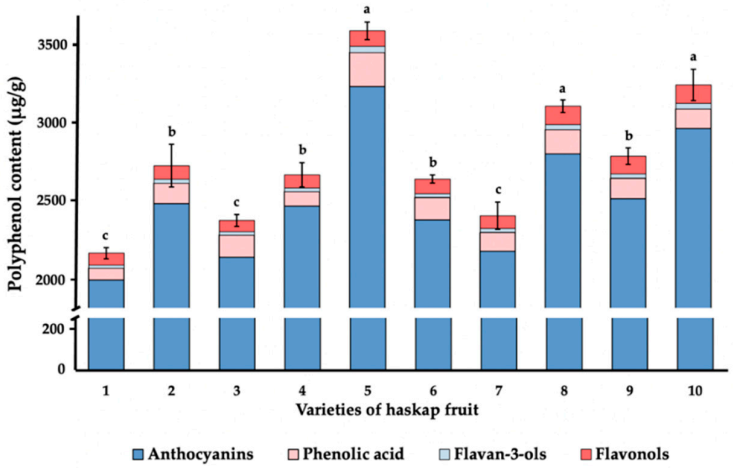

Figure 1. The content of individual classes of polyphenols (anthocyanins, phenolic acids, flavan-3-ols, flavonols) estimated by the UPLC-PDA-MS/MS method in fruits of ten haskap berry cultivars: **1**. 'Boreal Beauty'; **2**. 'Boreal Beast'; **3**. 'Boreal Blizzard'; **4**. 'Aurora'; **5**. 'Honeybee'; **6**. 'Vostorg'; **7**. 'Jugana'; **8**. 'Usłada'; **9**. 'Lawina'; **10**. 'Sinij Uties'. Values are presented as mean and SD. Measurements were made in 3 repetitions. Statistical analysis (a–c) was performed using Duncan's test ($p < 0.05$).

Our results on the types and total content of polyphenolic compounds were similar to those previously reported by Senica et al. [9], Senica et al. [8], Ochmian et al. [44], Rupasinghe et al. [34], Orsavova et al. [36] and Khattab et al. [37] for different genotypes of haskap berry. In turn, other researchers obtained higher concentrations of polyphenols ranging from 3750.0 to 13,590.0 µg/g [3,6,42]. The differences with respect to the works cited, as well as the differences shown between the varieties tested, may be due to the different environmental conditions for plant growth and the genotypes tested. Senica et al. [8] found that the varying content of polyphenolic compounds in haskap berries depends on the exposure of the fruit to abiotic stresses. Fruits of bushes growing higher, which were more exposed to increased UV radiation, had a higher total polyphenol content compared to fruits located lower. Also, in a study by Ochmian et al. [44], the type of growing conditions was the main factor determining the overall polyphenolic compound profile of haskap berries.

The main class of polyphenolic compounds detected in haskap fruits were anthocyanins. These compounds, which belong to the flavonoids class, are natural pigments of plant origin, occurring in shades of red, violet and blue. High concentrations of anthocyanins are mainly found in berries, including haskap fruits, where they are the predominant group of polyphenolic compounds [32,34,37,45]. The content of anthocyanins in the fruits of the ten haskap berry cvs. ranged from 1993.46 ('Boreal Beauty') to 3236.66 ('Honeybee') µg/g (Table 3), which corresponded to previously provided results [6,7,9,34] for other haskap berry genotypes. According to previous reports, the content of anthocyanins in haskap berries depends mainly on the date of fruit harvest [42] and ambient temperature [8]. Our observations suggest that the factors determining the content of this class of polyphenols in haskap berry fruit may also include the date of fruit ripening. For the cvs. 'Honeybee' and 'Sinij Uties', which fruit at the end of June and the beginning of July, a significantly higher content of anthocyanins was shown in comparison to the cvs. 'Boreal Beauty' and 'Boreal Blizzard', which fruit at the end of July and the beginning of August. The key marker of the anthocyanin profile of the haskap fruit was the cyanidin 3-*O*-glucoside content. In reports by Wojdyło et al. [3], Kucharska et al. [7], Rupasinghe et al. [34], Khattab et al. [37] and Raudone et al. [45], this compound accounted for between 71.2 and 93.8% of the total anthocyanins. In our study, cyanidin 3-*O*-glucoside was also the dominant anthocyanin (79.5–87.9%), while other compounds present in high concentrations included cyanidin 3-*O*-rutinoside (2.8–12.0%) and cyanidin 3,5-*O*-diglucoside (2.4–10.7%) (Table 3, Figure 1).

The second identified group of polyphenolic compounds in the fruit of the haskap berry cultivars studied were phenolic acids. These compounds were detected in all raw materials of plant origin, mainly in bound form in the form of esters and glycosides, including hydrolyzing lignins and tannins [32]. These are also important components of haskap berry fruits due to their characteristic taste [1]. Their content in the analyzed fruit samples of the tested cultivars ranged from 75.88 ('Boreal Beauty') to 220.45 ('Honeybee') µg/g. Chlorogenic acid (74.6–87.4%), 3,4-di-*O*-caffeoyl-quinic acid (7.6–12.7%) and neochlorogenic acid (4.2–14.2%) were identified at the highest concentrations. Chlorogenic acid was also reported by other authors to be predominant in haskap fruit (68.7–89.1%) [3,6–9,34,36,37,45,46]. Moreover, De Silva and Rupasinghe [42] showed a significant difference in the concentration of this acid depending on the genotype, indicating a higher fruit content in Polish than in Canadian haskap berry cultivars. In our study, no differences were found between the groups of cultivars from Russia and Canada (Table 3, Figure 1).

The next group consisted of flavan-3-ols, whose presence in plants in single and oligomeric forms is closely related to their health properties. Only one compound from this group, namely, procyanidin dimer B-type, was identified in the haskap berry cultivars studied. Its content ranged from 20.50 ('Boreal Beauty') to 40.36 ('Honeybee') µg/g. Significantly higher concentrations of flavan-3-ols, ranging from 71.40 to 915.71 µg/g, were found by Oszmiański et al. [6], Kucharska et al. [7], De Silva and Rupasinghe [42] and Raudone et al. [45]. Depending on the cultivar studied, (+)-catechin [6,7], (−)-epicatechin [42] and procyanidin dimer B-type proved to be the dominant compounds [7,45]. The profiles and contents of individual flavan-3-ols in the haskap fruit were closely related to the geographical origin of the cultivated plant [47], genotype [7], and stage of fruit maturity [1]. Hence, there may be differences in the amounts of these compounds in our study and the works cited, as well as the absence of a single dominant compound, which is characteristic of the polyphenol classes described above.

The last group of identified polyphenolic compounds was flavonols, which represent a broad class of flavonoids found in the highest concentrations in flowers, leaves and fruit skin. The range of flavonol concentrations in the haskap berry cultivars studied varied from 73.08 ('Boreal Blizzard') to 119.50 ('Sinij Uties') µg/g, which is consistent with previous reports [8,9,34]. A higher content of this compound, ranging from 106.5 to 478.4 µg/g, was reported by Kucharska et al. [7] and De Silva and Rupasinghe [42]. Senica et al. [8] also showed that the content of this group of compounds correlated with cultivation at lower temperatures and high UV irradiation. In our research, quercetin 3-*O*-rutinoside (32.5–68.4%), quercetin 3-*O*-glucoside (8.1–18.9%) and quercetin 3-*O*-pentoside-glucoside I (6.1–30.1%) were predominant (Table 3, Figure 1). Quercetin 3-*O*-rutinoside was also the major flavonol identified by Senica et al. [8], Rupasinghe et al. [34] and De Silva and Rupasinghe [42], where it accounted for 27.3–88.1% of the total flavonols.

3.4. Antioxidant Activity

Antioxidants of plant origin are considered to be one of the most effective agents for scavenging reactive oxygen species (ROS). Therefore, their content is associated with many biological effects attributed to plant materials. Currently, there are several methods for assessing antioxidant activity [33]. In this study, the ability to scavenge synthetic ABTS radicals, reduce copper ions (CUPRAC) and scavenge superoxide ($O_2^{\cdot -}$) and hydroxyl radicals ($OH^{\cdot}$) was investigated (Table 1). In order to fully characterize the antioxidant capacity of plant raw materials, it is important to use several methods based on different mechanisms of action. The ABTS test involves measuring the ability of antioxidants to neutralize the synthetic $ABTS^{\bullet +}$ cation radical. The equivalents of radicals in biological systems are $O_2^{\cdot -}$ and $OH^{\cdot}$ radicals. The $OH^{\cdot}$ radical is a highly reactive form of oxygen that can damage nucleic acids and contribute to inflammation-related diseases. Superoxide anions are also highly harmful to cellular systems. Moreover, metal ions (Fe^{2+}, Cu^{2+}) are involved in the production of free radicals, indirectly contributing to DNA damage and lipid peroxidation [48].

For the analyzed samples of haskap berries, a significant difference in terms of antioxidant activity was shown ($p < 0.05$). The highest antioxidant capacity for the ABTS, CUPRAC and $O_2^{\cdot -}$ methods was found for the cv. 'Honeybee' (58.69 mmol TE/100 g, 53.92 mmol TE/100 g and 546.62 µg/mL, respectively) and that for the $OH^{\cdot}$ method was found for the cv. 'Usłada' (541.22 µg/mL). In turn, the lowest antioxidant activity measured by four selected methods was shown for the cv. 'Boreal Beauty' (42.62 mmol TE/100 g and 36.52 mmol TE/100 g, respectively, for ABTS and CUPRAC), the cv. 'Lawina' (1458.16 µg/mL for $O_2^{\cdot -}$) and the cv. 'Vostorg' (1297.15 µg/mL for $OH^{\cdot}$).

For comparison, in the previously published reports, the antioxidant activity estimated using the ABTS test ranged from 2.20 to 137.89 mmol TE/100 g [3,30,35,40,45,49]. At the same time, similar to the assessment of the content of three classes of polyphenols, only four cultivars out of ten tested in our studies had been evaluated previously. Gawroński et al. [35] and Dziedzic et al. [40] found the antioxidant activity for the cv. 'Aurora' c to be 19.1 times lower and 1.8 times higher, the cv. 'Honeybee' to be 18.3 times lower and 2.1 times higher, and the cv. 'Vostorg' to be 19.3 times lower and 2.8 times higher, respectively. In turn, Gawroński et al. [35] reported for the cv. 'Jugana', that the activity was 12.7 times lower.

The antioxidant activity of haskap fruit using the CUPRAC method has only been published in one paper so far. Raudone et al. [45], in a fruit analysis of eight haskap berry cultivars obtained, found values in the range of 19.11 to 69.76 mmol TE/100 g. These results are comparable to those obtained in this study; however, the referenced study did not analyze the fruits of cultivars as the subject of their own research.

Only one paper has been published to date on the ability to scavenge $O_2^{\cdot -}$ and $OH^{\cdot}$ radicals. Rop et al. [39] presented the results of fruit analyses for 12 haskap berry cultivars, including none from our study. Nevertheless, the obtained results, expressed as % inhibition, showed a scavenging capacity of $O_2^{\cdot -}$ radicals from 31.0 to 41.1% and that of $OH^{\cdot}$ radicals from 25.9 to 37.2%. The high potential of haskap fruits to capture these two groups of radicals might, in turn, be indicated by research published by Zhao et al. [50], who obtained IC_{50} values of 1.43 µg/mL (for $O_2^{\cdot -}$) and 0.03 µg/mL (for $OH^{\cdot}$) for the isolated anthocyanins fraction.

The superoxide anion radical plays an important role in the formation of ROS, including hydrogen peroxide, hydroxyl radicals and singlet oxygen. The resulting ROS may induce the degradation of lipids, proteins and DNA, contributing to the progression of the aging process or the development of lifestyle diseases. Therefore, their removal is one of the body's most effective defense methods. The four selected methods of assessing antioxidant activity showed that the analyzed fruits of the haskap berry showed multidirectional antioxidant abilities based on different mechanisms of action. Many studies have shown that the mechanisms of action of antioxidant compounds are strongly correlated with anti-inflammatory, antibacterial, cardioprotective and chemopreventive effects and dependent on the total content of polyphenols or their individual classes [13,51–53]. Our own research showed that the content of anthocyanins significantly influenced the antioxidant activity expressed by the ABTS (r = 0.934) and CUPRAC (r = 0.889) methods, where the compound mainly responsible for this activity was cyanidin 3-*O*-glucoside (r = 0.968 and r = 0.899 for the ABTS and CUPRAC methods, respectively) (Figure 2).

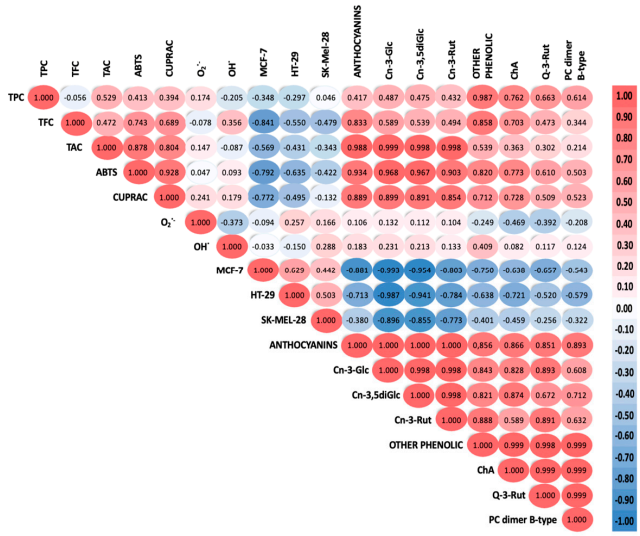

Figure 2. Pearson's correlation index for fruits of haskap berry cultivars, showing the strength of the relationship between variables such as antioxidant activity (ABTS, CUPRAC, $O_2^{\cdot-}$, $OH^{\cdot}$), cytotoxic activity (MCF-7, HT-29, SK-Mel-28), classes of individual polyphenols assessed spectrophotometrically (TPC, TFC, TAC) and chromatographically (anthocyanins, other polyphenols), and compounds identified at the highest concentrations (cyanidin 3-*O*-glucoside, cyanidin 3,5-*O*-diglucoside, cyanidin 3-*O*-rutinoside, chlorogenic acid, quercetin 3-*O*-rutinoside, procyanidin dimer B-type). A red color indicates a positive correlation and blue indicates a negative correlation. Antioxidant and cytotoxic activity was mainly dependent on the content of anthocyanins, especially cyanidin 3-*O*-glucoside.

3.5. Cell Viability

Cancer is one of the most devastating and life-threatening chronic diseases in the world. Hence, the search for new phytopharmaceuticals that can be used effectively and safely in their prevention and treatment has recently increased [54]. In our own research, the highest cytotoxic activity against the MCF-7 cell line was found in haskap fruits of the cv. 'Sinij Uties' (244.55 µg/mL) (Table 1). However, in relation to the lines HT-29 and SK-Mel-28, fruits of the cv. 'Honeybee' had 103.62 and 427.35 µg/mL, respectively. In turn, the lowest activity was shown for the cultivars 'Jugana' (675.81 µg/mL, relative to the MCF-7 line) and 'Boreal Blizzard' (478.16 µg/mL, relative to the HT-29 line) as well as for the cvs. 'Aurora', 'Vostorg', 'Jugana' and 'Lawina' (>750 µg/mL, relative to the SK-Mel-28 line). Therefore, a high selectivity of cytotoxic activity of the studied haskap berry varieties can be observed; however, the new 'Honeybee' variety is characterized by the highest activity. It is also worth emphasizing that these are the first published results on the cytotoxic effects of haskap fruit extracts on the three selected cancer cell lines.

From a review of the literature, there is little information on the cytotoxic effect of haskap berry fruit on cancer cells. Wang et al. [46], Caprioli et al. [55], Lee et al. [56] and Rupasinghe et al. [4], in analyses of the anticancer activity of fruit varieties not included in this study, did not show cytotoxicity against hepatocellular carcinoma (HepG2), melanoma (A375), breast cancer (MDA-MB 231) or acute monocytic leukemia (THP-1) cell lines.

Interestingly, our study showed a previously unreported cytotoxic effect of haskap fruits. However, polyphenolic fractions isolated from these fruits were used in our study, which could have contributed to an increase in their cytotoxic effect on cells. Therefore, further studies should be conducted to clarify the selectivity and mechanism of action on cancer cells. For anthocyanins isolated from haskap fruits, in vitro and in vivo studies showed a significant inhibition of the growth of hepatocarcinoma cells (SMMC-7721) by blocking the cell cycle in the G2/M phase, inducing DNA damage and apoptosis [11]. In another study, also for the purified anthocyanin fraction, the ability to reduce DNA damage and fragmentation and increase the ATM-dependent DNA damage repair cascade in lung epithelial cells (BEAS-2B) treated with carcinogenic tobacco nitrosamine (NNKOAc) was

shown, thus demonstrating the therapeutic potential of preventing lung carcinogenesis due to tobacco use [57]. On the other hand, our own studies showed a significant interaction between the content of anthocyanins and MCF-7 and HT-29 cell viability (r = −0.881 and r = −0.713, respectively), especially the content of cyanidin 3-*O*-glucoside (r = −0.993 and r = −0.987, respectively) (Figure 2). Hence, haskap fruits, especially their anthocyanin fractions, may be a potential chemopreventive agent in cancer diseases; however, further studies using a wider range of cancer cell lines as well as in vivo systems are necessary to confirm this thesis. In this context, according to our results, the cv. 'Honeybee' deserves special attention.

3.6. Principal Component Analysis (PCA)

Data on the physicochemical properties, content of phenolic compounds and health-promoting activity of haskap berries were subjected to PCA analysis (Figure 3). The total share of variance of the two main components was 67.84%, with PC1 and PC2 explaining 52.47 and 15.37% of the total variance, respectively. Based on the results obtained, three groups can be distinguished. The first group includes the varieties 'Honeybee', 'Sinij Uties' and 'Usłada', which were characterized by the highest pH, content of soluble substances and antioxidant and cytotoxic activity, which correlated with the highest concentrations of phenolic compounds, including those occurring in the highest concentrations (cyanidin 3-*O*-glucoside, cyanidin 3,5-*O*-diglucoside, cyanidin 3-*O*-rutinoside, chlorogoneic acid, quercetin 3-*O*-rutinoside, procyanidin dimer B-type). The second group includes the varieties 'Boreal Blizzard', 'Vostorg', 'Jugana', 'Boreal Beauty' and 'Aurora', with the highest titratable acidity and ash and dry matter contents. In turn, the last group was created from two varieties of haskap berries, i.e., 'Boreal Beast' and 'Avalanche', with the highest maturity indices. The PCA analysis revealed that the 'Honeybee', 'Sinij Uties' and 'Usłada' varieties are the most valuable source of polyphenols, which is related to their high antioxidant and cytotoxic activity and other potential health-promoting properties.

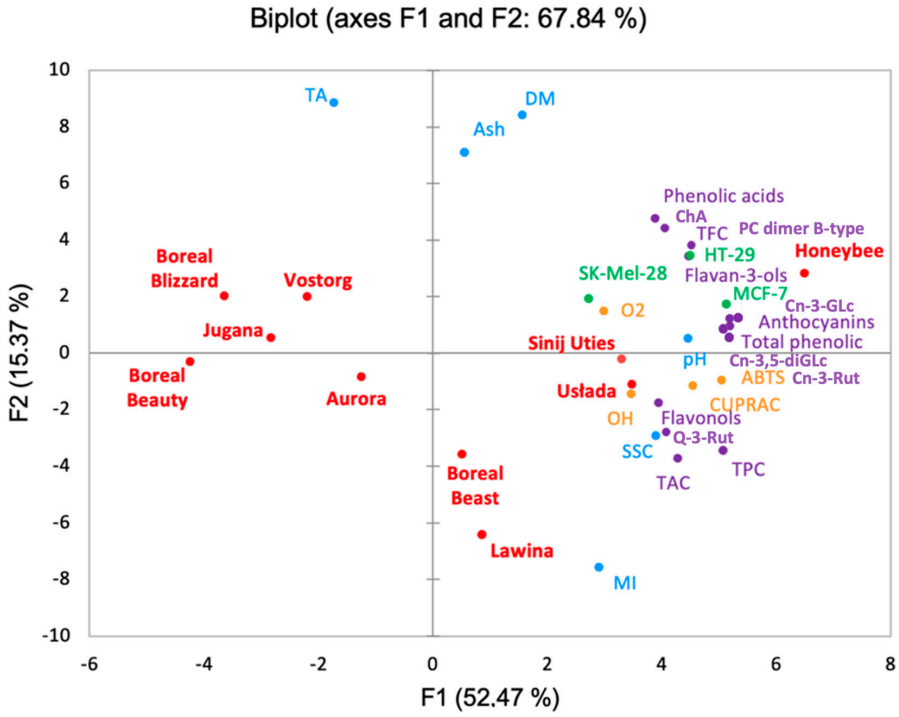

Figure 3. Principal component analysis of data regarding the physicochemical properties, content of phenolic compounds and health-promoting activity of haskap berries. The cultivars 'Honeybee', 'Sinij Uties' and 'Usłada' were characterized by the highest values for antioxidant and cytotoxic activity and phenolic compound content.

4. Conclusions

The evaluated ten haskap berry cultivars differ significantly in physicochemical properties and the content of individual classes of polyphenols, including anthocyanins, phenolic acids, flavan-3-ols and flavonols. Of the above groups, anthocyanins are dominant in haskap berries (90.0–92.4% of all polyphenols), where the compound present in the highest concentration is cyanidin 3-*O*-glucoside. The 'Honeybee' cultivar had the highest content of this class of compounds, as well as all polyphenols. Moreover, in this study, all the cultivars tested showed high antioxidant activity through different mechanisms of action and anticancer properties in vitro against breast cancer, colon cancer and melanoma cell lines. Among the tested cultivars, 'Honeybee' and 'Usłada' had the highest antioxidant activity and 'Sinij Uties' and 'Honeybee' had the highest cytotoxic activity. It has been shown that the above-mentioned properties of haskap fruit depend primarily on their anthocyanin content.

The conducted research on the content of bioactive ingredients and antioxidant and cytotoxic properties of haskap fruits showed a significant advantage of these features in the case of the new 'Honeybee' cultivar, which indicates that its inclusion in diets may play an important role in maintaining the health of society. Nevertheless, further studies, both in vitro and in vivo, are necessary to determine the best approach to using this raw material, especially its cytotoxic potential, taking into account both the efficacy and safety of use.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agriculture14101734/s1, Table S1: Calibration curve parameters of the method developed for each standard.

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