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# Basic Chemical Composition and Concentration of Selected Bioactive Compounds in Leaves of Black, Red and White Currant

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**Abstract:** The aim of this study was to determine the basic chemical composition, the content of bioactive compounds and antioxidant activity in currant leaves. The leaves of black, red and white currant shrubs were collected in May, and in the beginning of June, July and August, for two years between 2018 and 2019. The proximate analysis, including dry matter, protein, fat, ash and total carbohydrates, was determined. In addition, the content of the polyphenols and the total antioxidant activity using ABTS, DPPH and FRAP assays were conducted. The highest concentration of protein was detected in the whitecurrant leaves harvested in May in both years, while the highest content of crude fat was found in the blackcurrant leaves harvested in both years, with the exception of the August harvest. Extracts from the blackcurrant leaves collected in June/July 2019 had the highest antioxidant activity that was measured by the ABTS method (about 7000 µmol Trolox/g DM) and confirmed by other methods, while extracts from the whitecurrant leaves produced from the August 2018 collection had the lowest antioxidant activity (1884 µmol Trolox/g DM). Currant leaves are a rich source of bioactive compounds and contain higher amounts of polyphenols as compared with currant fruits. These compounds may play a very important role in the risk reduction and even prevention of the most chronic non-communicable diseases. Therefore, further research is needed to identify currant leaves as a source of bioactives for functional foods and natural health products. The highest antioxidant activity was in the redcurrant leaves from all the harvest times in 2018 as measured by the ABTS and FRAP methods. On the contrary, blackcurrant leaves from all the harvest times in 2019 had the highest antioxidant activity.

**Keywords:** blackcurrant; redcurrant; whitecurrant; leaves; antioxidant activity; basic chemical composition



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## 1. Introduction

Currants (*Ribes* L.) are shrubs of the order *Saxifragaceae*, belonging to the gooseberry family (*Grossulariaceae*) [1]. They are cultivated throughout Northern Europe, Asia, North America, in the mountainous areas of South America and North-West Africa [2]. Red and white currant shrubs, and above all, blackcurrant, are cultivated mainly for their tasty and rich bioactive fruits. The fruit is eaten in both fresh and unprocessed forms, as well as in preserves such as frozen berries, juices, jams, jellies, etc. [1]. Other parts of the currant shrubs, such as seeds, buds and leaves, are an underappreciated source of numerous bioactive ingredients. Currant leaves contain more polyphenolic compounds than their fruits and are considered a good source of these compounds [3]. Although currant phenolics were the subject of many studies, these constituents in currant leaves are best characterized in blackcurrant leaves, which are a very good source of these bioactives [4–6]. The main compounds from this group that are detected in the leaves are quercetin (1.359 mg/g DM), myricetin (0.831 mg/g DM), rutin (0.266 mg/g DM) and catechins. The presence of phenolic acids was also detected. This included salicylic (0.236 mg/g DM), p-coumaric (0.266 mg/g DM), gallic and quinic as well as small amounts of chlorogenic, sinapinic, ferulic and coffee acids. It has been reported that the average concentration of the total amount of phenolics in blackcurrant leaves is in the range of 32 to 44 mg/1 DM [1,5–8]. Currant leaves have greater antioxidant activity and stronger anti-inflammatory properties than the fruit and other parts of this plant. As with the leaves of other berries, such as strawberries, blackberries and raspberries, they contain large amounts of polyphenolic compounds [5,7]. Moreover, they contain valuable fatty acids, minerals and essential oils [5].

The fatty acids contained in blackcurrant leaves are mainly unsaturated fatty acids such as $\alpha$-linolenic, $\gamma$-linolenic, *cis*-7,10,13-hexadecatiene and stearidine, as well as phosphatidylcholine and phosphatidylglycerol. They have anti-cancer, antibacterial and anti-inflammatory properties [9]. Blackcurrant leaves contain significant amounts of micronutrients such as iron, manganese and boron. They have also shown a dietary advantage in the potassium to sodium ratio. The high amount of potassium in relation to the amount of sodium causes the diuretic effect of the leaves [7]. The aromatic smell of fresh blackcurrant leaves is related to their content of essential oils that includes monoterpenes and sesquiterpenes [10]. According to our best knowledge there are no references concerning the basic chemical composition and antioxidant capacity of various types of currant leaves.

The aim of this study was to investigate the basic chemical composition, total polyphenolic compounds content and antioxidant activity of black (*Ribes nigrum*), red (*Ribes rubrum*) and white currant (*Ribes niverum*) leaves.

## 2. Materials and Methods

### *2.1. Plant Material and Basic Chemical Composition*

The research material was black, red and white currant leaves from private crops located in the Małopolska region of Poland (Figure 1). The plants were not protected by any herbicides, insecticides or pesticides during the growing. The material was collected for two years (2018 and 2019) in May, June and August. These dates were selected based on the plants' development. The samples were collected before the blooming (May), after the blooming when the fruit had already appeared (June/July) and after harvesting the fruits (August). After the collection, the leaves were thoroughly cleaned. Dry mass in the fresh samples was determined based on the AOAC method. Samples were frozen then freeze dryed in a lyophilizer (Christ Alpha 1-4, 37520 Osterode am Harz, Gefriertrocknungsanlangen, Germany). The basic chemical composition of the freeze-dried samples were measured according to the AOAC official methods [11]. The concentration of protein was determined with the Kjeldahl method, crude fat content with the Soxhlet method and ash content using the electric AOAC methods no. 978.04, 935.38, 930.05, respectively. The total carbohydrate content of the dry matter was calculated using the following formula: total carbohydrates = 100 − (protein + raw fat + ash) [12].

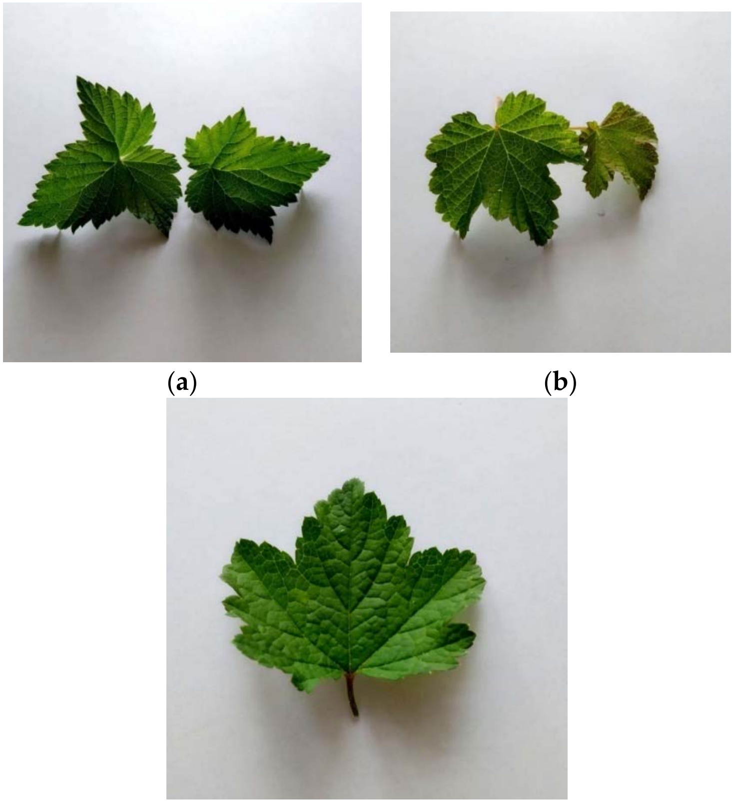

**Figure 1.** Leaves of black (**a**), red (**b**) and white currant (**c**) shrubs [own sources].

### 2.2. Extract Preparation

About 0.5 g of the samples was used for the acidified methanolic extract preparation (70% methanol acidified with formic acid). Samples were extracted by shaking in a water bath shaker (Elpan, type 357, Lubawa, Poland) at room temperature for 2 h without light. After this time, the solutions were centrifuged (Centrifuge type MPW-340, Warsaw, Poland) and then filtered. For the extraction of phenolic compounds from various type plant material 70% acidified (formic acid) methanol is widely used [13,14], hence this method was employed to extract the phenolic compounds from the samples. Prepared samples were stored at −22 °C for further analysis. The total amount of phenolic compounds and antioxidant activity were measured in the extracts.

### 2.3. Total Polyphenols Content and Antioxidant Activity

The total polyphenol level in each of the acidified methanolic extracts was determined using the Folin–Ciocalteu reagent (Sigma-Aldrich, Saint Louis, MO, USA) [15]. The results were shown as the chlorogenic acid equivalent (CGA) in mg per 100 g of dry sample.

The antioxidant activity of the methanolic extracts of the currant leaf samples was determined with the use of the following methods: $ABTS^{\bullet+}$ (2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid), FRAP (ferric-reducing antioxidant power), as well as DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate). The antioxidant activity using $ABTS^{\bullet+}$ free radicals was measured using a method described by Re et al. [16]. The FRAP analysis was performed according to Benzie and Strain [17]. Determination of antioxidant activity with the DPPH method was performed in accordance with Miliauskas et al. [18].

### 2.4. Statistical Analysis

The results obtained for each sample using the $ABTS^{\bullet+}$, FRAP and DPPH methods were compared to the concentration–response curve of the standard Trolox solution and presented as micromoles of Trolox equivalent per gram of dry weight (TEAC) of the samples.

Basic chemical composition was carried out in duplicate, and the total phenol content as well as antioxidant activity analyses were carried out in triplicate. Results were expressed as the means +/− S.D. Differences between samples were analyzed using the Statistica software v. 13.0 (Tulsa, OK, USA). Duncan's multiple range test was used for testing these differences. $p$ values < 0.05 were regarded as significant.

## 3. Results

### 3.1. Basic Chemical Composition

Dry matter content of currant leaves harvested in both years was significantly higher in August compared to May in all types of leaves. Generally, the dry matter content did not change significantly between the first and the second harvest. In whitecurrant leaves harvested in 2018, the dry matter content did not change significantly between July and August. Redcurrant leaves collected in August 2018 had the highest amount of dry matter among all analyzed samples, while whitecurrant leaves harvested in May 2019 had the lowest content (Table 1).

**Table 1.** Basic composition of the leaves of three currant species harvested at different dates in 2018 and 2019.

| Harvest Date | Sample | Dry Matter [g/100 g] | Protein [g/100 g DM] | Fat [g/100 g DM] | Total Ash [g/100 g DM] | Carbohydrates [g/100 g DM] |
|---|---|---|---|---|---|---|
| May 2018 | Redcurrant leaves | 33.26 ± 0.21 $^{efg}$ | 15.23 ± 0.30 $^{cd}$ | 4.00 ± 0.08 $^{f}$ | 7.69 ± 0.04 $^{a}$ | 73.08 ± 0.18 $^{k}$ |
| | Whitecurrant leaves | 32.91 ± 0.41 $^{def}$ | 21.42 ± 0.34 $^{i}$ | 4.03 ± 0.04 $^{f}$ | 8.44 ± 0.11 $^{b}$ | 66.11 ± 0.19 $^{fg}$ |
| | Blackcurrant leaves | 32.60 ± 0.06 $^{de}$ | 15.41 ± 0.29 $^{d}$ | 7.75 ± 0.01 $^{m}$ | 11.90 ± 0.03 $^{e}$ | 64.94 ± 0.32 $^{d}$ |
| June/July 2018 | Redcurrant leaves | 38.37 ± 0.23 $^{k}$ | 14.95 ± 0.11 $^{cd}$ | 6.85 ± 0.11 $^{l}$ | 11.75 ± 0.02 $^{de}$ | 66.44 ± 0.02 $^{g}$ |
| | Whitecurrant leaves | 33.87 ± 0.09 $^{gh}$ | 16.58 ± 0.06 $^{e}$ | 4.03 ± 0.05 $^{f}$ | 13.94 ± 0.04 $^{f}$ | 65.45 ± 0.03 $^{e}$ |
| | Blackcurrant leaves | 36.37 ± 0.14 $^{i}$ | 15.51 ± 0.06 $^{d}$ | 6.91 ± 0.05 $^{l}$ | 13.78 ± 0.02 $^{f}$ | 63.80 ± 0.01 $^{c}$ |
| August 2018 | Redcurrant leaves | 41.38 ± 0.07 $^{l}$ | 14.79 ± 0.18 $^{c}$ | 6.10 ± 0.02 $^{k}$ | 11.09 ± 0.10 $^{cd}$ | 68.03 ± 0.27 $^{i}$ |
| | Whitecurrant leaves | 34.54 ± 0.12 $^{hi}$ | 16.29 ± 0.12 $^{e}$ | 3.65 ± 0.11 $^{de}$ | 16.74 ± 0.04 $^{g}$ | 63.32 ± 0.27 $^{ab}$ |
| | Blackcurrant leaves | 30.56 ± 0.16 $^{c}$ | 19.50± 0.32 $^{g}$ | 2.45 ± 0.00 $^{c}$ | 11.89 ± 0.37 $^{e}$ | 66.16 ± 0.05 $^{g}$ |
| May 2019 | Redcurrant leaves | 28.99 ± 0.70 $^{b}$ | 17.90 ± 0.25 $^{f}$ | 1.91 ± 0.10 $^{b}$ | 10.74 ± 0.32 $^{c}$ | 69.45 ± 0.17 $^{j}$ |
| | Whitecurrant leaves | 28.04 ± 1.14 $^{a}$ | 20.72 ± 0.04 $^{h}$ | 1.70 ± 0.01 $^{a}$ | 11.88 ± 0.32 $^{e}$ | 65.69 ± 0.28 $^{ef}$ |
| | Blackcurrant leaves | 32.32 ± 0.52 $^{d}$ | 13.45 ± 0.57 $^{b}$ | 4.34 ± 0.05 $^{g}$ | 16.52 ± 0.75 $^{g}$ | 65.68 ± 0.22 $^{ef}$ |
| June/July 2019 | Redcurrant leaves | 33.75 ± 0.11 $^{fgh}$ | 12.37 ± 0.34 $^{a}$ | 3.76 ± 0.07 $^{e}$ | 16.47 ± 0.67 $^{g}$ | 67.40 ± 0.26 $^{h}$ |
| | Whitecurrant leaves | 31.33 ± 0.03 $^{c}$ | 13.07 ± 0.23 $^{b}$ | 3.55 ± 0.01 $^{d}$ | 18.36 ± 0.29 $^{g}$ | 65.02 ± 0.06 $^{d}$ |
| | Blackcurrant leaves | 33.49 ± 0.24 $^{fg}$ | 12.88 ± 0.18 $^{ab}$ | 5.28 ± 0.15 $^{j}$ | 18.15 ± 0.41 $^{h}$ | 63.68 ± 0.08 $^{bc}$ |
| August 2019 | Redcurrant leaves | 35.01 ± 0.16 $^{i}$ | 12.92 ± 0.25 $^{ab}$ | 4.52 ± 0.06 $^{h}$ | 16.89 ± 0.44 $^{g}$ | 65.67 ± 0.12 $^{ef}$ |
| | Whitecurrant leaves | 32.24 ± 0.35 $^{d}$ | 13.18 ± 0.05 $^{b}$ | 5.01 ± 0.06 $^{i}$ | 18.58 ± 0.15 $^{h}$ | 63.23 ± 0.04 |
| | Blackcurrant leaves | 33.11 ± 0.05 $^{defg}$ | 17.65 ± 0.39 $^{f}$ | 5.19 ± 0.03 $^{j}$ | 9.10 ± 0.02 $^{b}$ | 68.06 ± 0.40 $^{i}$ |

Letters $^{a–m}$ indicate statistically significant differences ($p \leq 0.05$); data are shown as the mean ± SD (standards deviation).

It was shown that the amount of protein was significantly higher in the whitecurrant leaves in both years in May as opposed to samples harvested in other months. The lowest content of protein was determined in redcurrant leaves harvested in July and August in both years (Table 1). The percentage of fat in the dry matter of the currant leaves increased significantly between the first and second harvest in all samples except that obtained from the whitecurrant leaves in 2018, where the fat content did not increase significantly between these dates. In the period from July to August, the fat content in the dry matter increased significantly in the 2018 blackcurrant leaves and in all leaves harvested in 2019. The level of fat was significantly higher in the blackcurrant leaves in all harvests in both years with the exception of the August harvest. In this term, there was no difference in crude fat content in the leaves of the black and red currants. It was shown that out of all plants, blackcurrant leaves had the significantly highest fat content in their dry matter. The largest content was recorded in the samples at the turn of June and July 2018. It was found that the ash content was significantly highest in all tested currant leaves in August compared to the ones harvested in May in both years. Between the harvest times (June, July and August), this value increased significantly in the leaves of black and white currants. In the leaves of redcurrant, there were no statistically significant differences between the amount of ash in the dry matter of the material from July and August. The highest amount of ash was found in the leaves of the whitecurrant from July and August of 2019 and the leaves of the blackcurrant from August 2019. The total carbohydrate concentration in the dry leaves significantly decreased in almost each harvested sample in both years. The exception was the redcurrant leaves collected in 2018. It was shown that the leaves of the redcurrant contain the significantly highest total carbohydrate content among all cultivars tested in each harvest. The largest amount of this component in the dry matter of leaves was contained in the redcurrant leaves from May 2018, and the smallest in the whitecurrant leaves collected in August 2018 and 2019.

### 3.2. Total Phenolic Content and Antioxidant Activity

The concentration of polyphenolics was significantly lower in the leaves of the whitecurrant harvested in all three months in both years with the exception of July 2019. The level of total polyphenols increased significantly in the black and white currant leaves harvested between May and July 2019. The blackcurrant leaves, collected at the turn of June and July in 2019, had the highest level of total polyphenols, while whitecurrant leaves from August 2018 contained the lowest concentration of these compounds.

The highest antioxidant activity was found in the leaves of redcurrant from all harvest times in 2018 as measured by the ABTS and FRAP methods. On the contrary, the blackcurrant leaves from all harvest times in 2019 had the highest antioxidant activity measured by the ABTS method (Table 2).

**Table 2.** Polyphenol content and antioxidant activity of the leaves of three currant species harvested at different dates in 2018 and 2019.

| Harvest Date | Sample | Total Polyphenols [mg CGA/100 g DM] | ABTS [µmol Trolox/g DM] | DPPH [µmol Trolox/g DM] | FRAP [µmol Trolox/g DM] |
|---|---|---|---|---|---|
| May 2018 | Redcurrant leaves | 31 952.06 ± 257.48 $^{n}$ | 4 327.28 ± 68.24 $^{ef}$ | 5 175.42 ± 160.30 $^{e}$ | 9 356.12 ± 73.35 $^{k}$ |
| | Whitecurrant leaves | 20 365.50 ± 133.75 $^{c}$ | 3 593.43 ± 297.49 $^{d}$ | 5 060.14 ± 35.37 $^{de}$ | 7 389.15 ± 97.19 $^{h}$ |
| | Blackcurrant leaves | 22 101.61 ± 170.57 $^{d}$ | 1 800.10 ± 232.49 $^{a}$ | 4 316.47 ± 96.67 $^{c}$ | 4 669.14 ± 54.54 $^{c}$ |
| June/July 2018 | Redcurrant leaves | 23 054.90 ± 137.38 $^{e}$ | 3 031.80 ± 43.69 $^{c}$ | 4 323.85 ± 72.67 $^{c}$ | 8 017.53 ± 71.16 $^{i}$ |
| | Whitecurrant leaves | 20 105.07 ± 175.78 $^{c}$ | 2 197.94 ± 153.74 $^{ab}$ | 3 002.98 ± 17.43 $^{a}$ | 3 969.21 ± 128.06 $^{b}$ |
| | Blackcurrant leaves | 25 616.54 ± 320.22 $^{h}$ | 2 378.48 ± 155.19 $^{b}$ | 7 213.48 ± 120.98 $^{i}$ | 7 248.21 ± 154.92 $^{h}$ |
| August 2018 | Redcurrant leaves | 24 180.49 ± 89.29 $^{f}$ | 4 401.35 ± 42.59 $^{f}$ | 5 492.61 ± 35.42 $^{f}$ | 6 068.05 ± 153.11 $^{f}$ |
| | Whitecurrant leaves | 17 249.70 ± 66.35 $^{a}$ | 1 884.06 ± 28.14 $^{a}$ | 3 233.58 ± 99.27 $^{a}$ | 3 517.63 ± 85.54 $^{a}$ |
| | Blackcurrant leaves | 30 151.01 ± 66.18 $^{m}$ | 4 597.92 ± 54.20 $^{fg}$ | 6 428.35 ± 25.00 $^{h}$ | 7 411.46 ± 92.97 $^{h}$ |
| May 2019 | redcurrant leaves | 29 512.52 ± 177.98 $^{l}$ | 4 246.76 ± 42.45 $^{ef}$ | 5 162.47 ± 52.96 $^{e}$ | 8 862.36 ± 15.21 $^{j}$ |
| | whitecurrant leaves | 24 304.23 ± 199.70 $^{f}$ | 3 946.17 ± 87.01 $^{de}$ | 4 065.84 ± 35.21 $^{c}$ | 8 763.98 ± 42.91 $^{j}$ |
| | blackcurrant leaves | 32 374.29 ± 112.05 $^{o}$ | 6 989.44 ± 81.46 $^{i}$ | 7 877.82 ± 101.60 $^{k}$ | 6 749.45 ± 30.64 $^{g}$ |
| June/July 2019 | Redcurrant leaves | 18 699.40 ± 66.61 $^{b}$ | 4 827.60 ± 202.71 $^{gh}$ | 3 744.63 ± 140.94 $^{b}$ | 5 291.90 ± 56.80 $^{d}$ |
| | Whitecurrant leaves | 26 005.09 ± 135.54 $^{i}$ | 2 859.15 ± 402.33 $^{c}$ | 5 799.54 ± 17.92 $^{g}$ | 6 628.90 ± 57.79 $^{g}$ |
| | Blackcurrant leaves | 27 733.81 ± 197.76 $^{k}$ | 4 918.81 ± 19.97 $^{h}$ | 7 794.50 ± 99.62 $^{j}$ | 5 987.46 ± 53.46 $^{f}$ |
| August 2019 | Redcurrant leaves | 26 734.85 ± 179.40 $^{j}$ | 3 635.08 ± 42.79 $^{d}$ | 6 486.89 ± 480.40 $^{h}$ | 5 590.34 ± 90.2 $^{e}$ |
| | Whitecurrant leaves | 25 976.21 ± 110.58 $^{i}$ | 5 047.38 ± 52.64 $^{h}$ | 6 269.47 ± 111.90 $^{h}$ | 5 948.26 ± 125.30 $^{f}$ |
| | Blackcurrant leaves | 24 917.50 ± 90.24 $^{g}$ | 2 006.18 ± 61.50 $^{ab}$ | 4 820.47 ± 102.28 $^{d}$ | 6 772.73 ± 61.69 $^{g}$ |

ABTS-2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid), DPPH-2,2-diphenyl-1-picryl-hydrazyl-hydrate, FRAP-ferric-reducing antioxidant power; Letters $^{a,b}$ indicate statistically significant differences ($p \leq 0.05$); data are shown as the mean ± SD (standards deviation). CGA-Chlorogenic acid equivalent. Letters $^{a–o}$ indicate statistically significant differences ($p \leq 0.05$); data are shown as the mean ± SD (standards deviation)

The antioxidant capacity of the currant leaf extracts was also tested against the DPPH free radicals. A significant decrease in antioxidant properties was demonstrated in almost all currant leaf extracts obtained between May and the turn of June and July. The exception was the extracts of the black and white currant leaves collected in 2019. In these cases, the antioxidant capacity of the DPPH free radical increased in samples from the July harvest. However, the value of this parameter increased significantly in all extracts from the August harvest, except for those made from the white and black currant leaves from 2018 and 2019, respectively. The significantly highest ability to extinguish the DPPH free radicals was found in the blackcurrant leaf extracts collected at the turn of June and July and in

August 2019, while the whitecurrant leaves from the same harvests of 2018 had the lowest antioxidant activity.

## 4. Discussion

### 4.1. Basic Chemical Composition

It is worthy to note that there is no literature data concerning the basic chemical composition of currant leaves harvested in various time periods. Currently, researchers are more focused on the concentration of bioactives and antioxidant activity in blackcurrant leaves [19,20]. However, our study was focused on three types of currant leaf harvested in different months during the two-year period. It was found that the leaves of the redcurrant had the highest content of dry matter out of the examined currants. The exception was the May harvests in both years, when the blackcurrant leaves had a dry matter content the same as or higher than the redcurrant leaves. It was also found that the dry matter content in all varieties of currant leaf increased at subsequent harvest times. Although there is no available data in the literature on the dry matter content in currant leaves there are studies carried out on other plants, which confirmed our findings. Tichá et al. [21] have indicated an increase in the dry matter content in plant leaves during their life cycle. A similar tendency was also observed by Athokpam et al. [22] in plant leaves growing in Northeast India. In our study, the highest protein content was found in whitecurrant leaves. Only in the second and third harvest from 2019 was a similar amount of protein detected in the dry matter of the leaves of other currant species. Once again, no literature information was found on the protein content of currant leaves, however, in blackcurrant fruit, protein concentration was 1.4 g/100 g of fresh matter (7.77 g/100 g DM) [1]. Our results indicated that leaves of various types of currant have significantly higher content of fat than in the fruit (0.77 g/100 g dm) as reported earlier by Lim [1]. Dobson [9] has shown that fat in blackcurrant leaves is rich in fatty acids such as $\alpha$-linolenic, cis7,10,13-hexadecatrienoic, stearidine and $\gamma$-linolenic, as well as phosphatidylcholine and phosphatidylglycerol. It is also known that blackcurrant seeds are rich in unsaturated fatty acids in the range of 14% to 23% per dry matter [23]. The ash content in all studied currants increased during the shrub's growing period in both 2018 and 2019 (Table 1). Many researchers have reported that the leaves of currant shrubs are a rich source of calcium, potassium and magnesium, but their content depends on the various factors including the time of harvesting of the leaves [7,24,25]. In addition, Nour et al. [7] have reported that currant leaves in mid June had the highest content of calcium, magnesium, iron, manganese, aluminum, chromium and boron. A similar relationship between the harvest time and the content of nitrogen, magnesium, calcium, manganese and copper was demonstrated by Domagała-Świątkiewicz and Kolarski [26] in blueberry leaves. According to Rumasz-Rudnicka et al. [27] and Bednarek et al. [25], meteorological conditions and the intensity of soil fertilization affect the content of minerals in the plant. However, they can affect their amount in various ways, depending on the minerals. It is noteworthy to indicate that currant shrubs from which the research material was collected were not fertilized. Therefore, a significantly higher amount of ash in the dry matter of the currant leaves collected in 2019 could result from weather conditions. Based on information found in the Bulletin of the State Hydrological and Meteorological Service in Poland [28], the year 2019 was warmer and drier than the previous year [29].

### 4.2. Total Phenolic Content and Antioxidant Activity

The content of polyphenols was generally affected by the time of harvest. Whitecurrant leaves collected in August 2018 were characterized by the lowest polyphenol content among all the types of currant. Blackcurrant leaves collected in 2019 showed the highest content of polyphenols regardless of harvest time (Table 2). Our findings are in line with data published by Kendir and Köroğlu [30]. These authors also showed a higher polyphenol content in blackcurrant leaves (26,000 mg/100 g DM) than in redcurrant leaves (18,031 mg/100 g DM). A similar relationship can be noticed between the fruits of these two varieties of currant shrub in studies by Gryszczyńska et al. [31], which have shown that blackcurrant fruit (688.55 mg/100 g) had a higher content of polyphenols than redcurrant fruit (417.57 mg/100 g). Similar trends, confirming our findings, have been reported by Vagiri and coworkers [32] who show that the content of polyphenols in blackcurrant leaves increased between the first and the last harvest period. In addition, Tabart et al. [3] have proven that blackcurrant leaves contain the highest level of phenolic compounds after reaching full maturity. Furthermore, other studies on leaves of various shrubs and trees have indicated that the concentration of polyphenolic compounds in leaves depends on maturity, variety and climate conditions. Venskutonis et al. [33] reported that the concentration of polyphenols in raspberry leaves depends on the growing location of the plants and may vary. Dziadek et al. [34] have reported that the concentration of phenolics in cherry leaves varied substantially from 5076 to 12,844 mg/100 g DM. The results of the research carried out by Cieślik et al. [35] indicate that the content of polyphenols in fruits and vegetables, which is widely recognized as a good source of these substances, was significantly lower than in currant leaves as reported by our data. According to the aforementioned authors, pink grapes contained only 793 mg, Hungarian plums 1599 mg and broccoli 1924 mg of phenolic compounds per 100 g of DM. That is about 16 to 40 times lower than the level of phenolics found in currant leaves (Table 2).

Redcurrant leaf extracts had the greatest ability to extinguish the ABTS free radicals within a given harvest in 2018. The dependence of the value of this parameter on the species of currant was impossible to determine in 2019. The tendency of changes in antioxidant activity of the currant was also difficult to determine and was dependent on the date of collection. Antioxidant properties of black and red currant leaf extracts increased between May and June in 2019, reaching their peak in June. This could have been due to weather conditions in June that year. According to the Bulletin of the State Hydrological and Meteorological Service, this month was much drier and hotter than May and August [28]. Research conducted on plants of *Secondatia floribunda* by Ribeiro et al. [36] showed that the extracts of these plants obtained in the dry season were also characterized by a higher antioxidant activity than extracts made from material collected in wet conditions. The antioxidant activity, as determined by the ABTS assay against free radicals, decreased in the whitecurrant leaves in the second harvest in both years, however, activity in the leaves harvested in 2018 remained unchanged. In August of 2019, antioxidant value decreased in black and red currant leaf extracts but increased in the same varieties in 2018 and in whitecurrant leaves harvested in 2019, while it did not change in the whitecurrant leaves from 2018. Blackcurrant leaf extracts from the second and third harvest in 2019 had the strongest antioxidant properties as determined by the DPPH free radical method. According to Katsube et al. [37] the free radical quenching properties of the extracts against DPPH was 238.5, 208.0, 287.9 µmol Trolox/g for blackberry, raspberry and blueberry, respectively.

Although these fruits are considered to be one of the best sources of antioxidants [38,39], aforementioned values are much lower as compared with antioxidant activity of currant leaves at the lowest value (1018.0 µmol Trolox/g) from our own research. According to our results, blackcurrant leaves showed the strongest antioxidant properties in relation to the DPPH radicals in each harvest. On the contrary, Kendir and Köroğlu [30] have shown that the leaves of the redcurrant shrub exhibited a greater ability to extinguish the DPPH radicals than the leaves of the blackcurrant shrub. This could be due to differences in extraction times. There were significant differences between time of extraction in the preparation of our samples (2 h) and extraction of up to 8 h by Kendir and Köroğlu [30]. As pointed out by Zych et al. [40] extending the extraction time increases the antioxidant activity against the DPPH free radicals in redcurrant leaves but decreases in blackcurrant leaf extracts. In the second harvest of 2018, this ability decreased in all extracts and in the redcurrant leaf extract harvested a year later. In the third harvest, the antioxidant activity against the DPPH free radicals decreased in all extracts but remained unchanged in the whitecurrant extract. It was a different case for extracts prepared from white and black currants obtained in the second harvest of 2019. In both extracts, there was a noticeable increase in antioxidant activity. However, there was a further decrease in activity in the blackcurrant extracts and a further increase in the whitecurrant extracts. Our data based on the FRAP (ferric-reducing antioxidant power) assay indicate that the ability to reduce iron (III) ions by leaf extracts of all tested currant species decreased between May and the turn of June. Black and red currant leaf extracts harvested in August 2018 and 2019, respectively, showed an increase in this value. In the remaining harvest time, the antioxidant activity decreased in all leaf extracts. Whitecurrant leaf extracts collected in August 2018 had the lowest ability to reduce trivalent iron ions (3517.63 µmol Trolox/g DM). However, this value was much higher than reported by researchers using leaves of other plants. Teleszko [41] has reported that the antioxidant properties of cranberry leaf extracts based on the reduction power of iron (III) ions were only at the levels of 650 to 930 µmol Trolox/g DM. In addition, Dziadek et al. [34] have shown similar values in cherry leaves (460–1713 µmol Trolox/g DM). It is worthy to note that currant leaves are rich in antioxidants but their basic composition and concentration in plant tissues depend on many factors. Not all of them have been taken into account in this study. Changes in meteorological conditions, as well as the place and time of collecting the material, may be the reason for differences between our results and data reported by other authors. Our studies were limited to two years but extending this project for one more year may give more consistent results. Furthermore, evaluation of total phenolics content instead of the profile of these compounds is the additional limitation of this study. However, this work is planned to be conducted in the future.

## 5. Conclusions

Proximate constituents comprising dry mass, crude fat and ash increased in all types of currant leaf depending on time of harvest. The concentration of polyphenolic compounds and antioxidant activity, measured by the ABTS and DPPH methods, were highest in the blackcurrant leaves collected in July at the time of full ripeness of the fruit. The high concentration of phenolic compounds, as well as substantial antioxidant activity in all current leaf extracts, makes currant leaves a rich source of bioactive compounds and potential functional nutraceuticals. These promising results can be used for the design of in vitro studies, for example, cell culture and in vivo studies. The strong antioxidant activity especially can be evaluated for the immune system response caused by various factors, i.e., oxidative stress and various types of infections.

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