Peer-reviewed version available at *Molecules* **2020**, *25*, 876; doi:10.3390/molecules25040876

Article

The content of selected minerals, bioactive compounds and the antioxidant properties of the flowers and fruit of selected cultivars and wildly growing plants of *Sambucus nigra*

Karolina Młynarczyk[1], Dorota Walkowiak-Tomczak[1], Halina Staniek[2], Marcin Kidoń[1] and Grzegorz P. Łysiak[3,*]

[1] Department of Food Technology of Plant Origin, Poznan University of Life Sciences, Wojska Polskiego 28, 60-637 Poznań, Poland; mlynk@up.poznan.pl (K.M.); tomczak@up.poznan.pl (D.W-T.); kidon@up.poznan.pl (M.K.)

[2] Institute of Human Nutrition and Dietetics, Poznan University of Life Sciences, Wojska Polskiego 28, 60-637 Poznań, Poland; halina.staniek@up.poznan.pl

[3] Department of Dendrology, Pomology and Nursery Production, Poznan University of Life Sciences, Dąbrowskiego 159, 60-594 Poznań, Poland; glysiak@up.poznan.pl

* Correspondence: glysiak@up.poznan.pl; Tel.: +48-61-848-7946

Abstract: This study compared the mineral content and bioactive properties of flowers and fruit coming from wild elderberry plants with those of flowers and fruit harvested from elderberry cultivars grown in an orchard. Elderberry fruit and flowers were analysed for the content of selected minerals, phenolic compounds and anthocyanins and for antioxidant activity. Mineral content was determined by atomic absorption spectrometry method, while antioxidant activity and the content of polyphenols and anthocyanins were determined by spectrophotometric methods. Flowers were found to contain more total ash and to have much higher content of most of minerals, except magnesium, which was present in high concentrations in fruit. Fruit showed significantly higher antioxidant activity than flowers, whereas the total phenolic content varied depending on the growing location / cultivar. The material obtained from selected cultivars growing in an orchard had higher antioxidant activity and polyphenol and anthocyanin content than the material obtained from wild plants. Fruit of the 'Haschberg' cultivar and flowers of the 'Sampo' cultivar had the best bioactive properties of the studied samples.

Keywords: elderberry minerals; antioxidant activity; phenolic compounds; anthocyanins.

1. Introduction

Elderberry belongs to the *Adoxaceae* family, which is rather widespread in temperate regions of Europe and other continents of the northern hemisphere. There are many cultivars of this shrub, of which 'Sampo', 'Samyl', 'Alleso', 'Korsor', 'Haschberg' are the most popular. Compared with wildly growing plants they bear more abundant crops of larger and heavier berries [1]. Elderberry is grown mainly for its fruit, which can be used to produce juices, soft and alcoholic beverages, marmalades or colourants. Berries are rich in various bioactive compounds, the most important of which are polyphenolic pigments, including anthocyanins, notably cyanidin-3-sambubioside-5-glucoside, cyanidin-3,5-diglucoside, cyanidin-3-sambubioside, cyanidin-3-glucoside, cyanidin-3-rutinoside. Among other phenolic compounds present in elderberry fruit, chlorogenic acid and quercetin derivatives occur in highest concentrations [2]. Not only elderberry fruit but also elderberry flowers abound with polyphenolic compounds. White or creamish-white flowers do not contain anthocyanins but are rich in phenolic acids, and kaempferol, quercetin and isorhamnetin derivatives. Also cathechin, epicathechin and naringenin are found there [2-5]. Like elderberry fruit, flowers are used in food industry to make beverages, infusions and liqueurs. Elderberry fruit and

flowers are believed to have antiviral, anti-inflammatory and antipyretic properties, so they are often used in natural medicine to produce juices or infusions to treat common cold or upper respiratory tract infections [6]. Such properties have been confirmed by some scientific studies. For example, elderberry fruit extract was shown to block influenza virus glycoproteins and increase the expression of Interleukin-8 (IL-8), Interleukin-6 (IL-6), and TNF (Tumor Necrosis Factor) [7]. Treatment with elderberry fruit extract was also found to shorten and help ease cold symptoms [8]. Components present in elderberry have strong antioxidant properties, hence their potential to prevent diabetes, cardiovascular or even cancerous diseases [9,10]. Elderberry fruit also contains ascorbic acid (6-25 mg/100 g) [11] and elderberry seed flour is a good source of alpha-tocopherol (vitamin E) and gamma-tocopherol [12]. Elderberry fruit and flowers contain valuable mineral nutrients, which include relatively large amounts of potassium, phosphorus, calcium, sodium and magnesium, and various microelements such as iron, zinc, manganese and copper. Unfortunately, heavy metals such as lead or cadmium, were also identified in elderberry in some growing locations [2,5].

Owing to high levels of bioactive compounds and anthocyanin pigments, elderberry attracts increasing interest from consumers and food industry [13].

In many countries elderberry is grown in orchards, but raw material used in food processing industry comes mostly from wild plants. Little is known about differences in the content of bioactive compounds and minerals in elderberry fruit and flowers depending on their growing location. Therefore, this study was designed to compare the chemical composition and biochemical properties of fruit and flowers of wildly growing elderberry plants with those of fruit and flowers of several elderberry cultivars grown in an orchard.

2. Results and Discussion

2.1. Content of ash and selected minerals in elderberry flowers and fruit

Ash is what is left after material has been completely burned and consists mainly of macro- and microelements. The analysed samples varied in terms of the content of ash and minerals (Table 1). There was, on average, about twice as much ash in elderberry flowers than in elderberry fruit. The average content of flower and fruit ash in the analysed samples was consistent with that reported in literature [5,14,15]. Flowers also contained more analysed elements, namely iron, copper, zinc and manganese. Also the content of calcium was higher in flowers than in fruit (except location W3). The only exception was magnesium, which was present in considerably larger amounts in elderberry fruit than in flowers. Calcium was the dominant mineral in flowers, whereas magnesium was the most abundant mineral in fruit.

Certain regularities were identified depending on whether the samples were taken from cultivars or wildly growing plants (Table 2). The fruit of cultivars contained more ash than the fruit of wild plants, whereas there was no such difference between flowers. Kołodziej et al. 2012 noted higher concentrations of the analysed mineral components in the flowers than in the fruit of elderberry plants growing in sixteen locations, with potassium, magnesium and calcium being the most abundant elements in both flowers and fruit [16]. Potassium was found to be the dominant mineral also in studies that analysed only elderberry fruit. Of all the minerals measured in the present study, copper was the least abundant in both fruit and flowers, which is consistent with earlier findings [17,14].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 January 2020 doi:10.20944/preprints202001.0141.v1

Peer-reviewed version available at Molecules 2020, 25, 876; doi:10.3390/molecules25040876

Table 1. The content of ash (%) and selected minerals (µg/g d.m.) in elderberry flowers and fruit, depending on the location (wild plants) / cultivar.

Raw material	Location / cultivar	Ash	Ca	Mg	Fe	Cu	Zn	Mn
Flowers	W1	1.5^{d}	2673.9^{b}	493.7^{c}	103.0^{b}	13.4^{a}	39.0^{ab}	19.7^{e}
	W2	2.1^{a}	3333.6^{a}	1097.6^{b}	87.4^{b}	6.9^{b}	35.2^{ab}	60.0^{a}
	W3	1.9^{bc}	2925.2^{ab}	567.2^{c}	92.1^{b}	13.8^{a}	41.1^{a}	27.9^{d}
	Sampo	1.7^{cd}	2868.7^{ab}	1525.7^{a}	54.5^{a}	6.5^{b}	31.8^{b}	37.2^{c}
	Haschberg	1.9^{b}	2724.7^{ab}	1555.6^{a}	46.8^{a}	8.0^{b}	38.1^{ab}	49.7^{b}
	Samyl	1.9^{bc}	3252.6^{ab}	1000.0^{b}	52.2^{a}	13.1^{a}	38.2^{ab}	23.1^{de}
Fruit	W1	0.8^{d}	2503.2^{b}	13555.1^{a}	41.3^{a}	5.2^{b}	12.4^{ab}	8.8^{de}
	W2	0.8^{cd}	2563.5^{b}	12565.1^{a}	35.3^{ab}	4.4^{b}	10.8^{bc}	26.0^{a}
	W3	0.6^{e}	3033.0^{a}	9774.3^{b}	28.7^{bc}	5.3^{b}	14.0^{a}	11.9^{c}
	Sampo	1.0^{ab}	662.1^{d}	9058.1^{b}	26.2^{c}	4.9^{b}	11.6^{abc}	9.8^{d}
	Haschberg	1.1^{a}	1508.2^{c}	9731.4^{b}	28.1^{c}	4.6^{b}	9.5^{c}	17.1^{b}
	Samyl	0.9^{bc}	1513.9^{c}	6909.2^{c}	25.7^{c}	6.7^{a}	9.8^{bc}	8.5^{e}

a,b,c,d – differences between flowers or fruit from different locations (wild plants) / cultivar, statistically significant at $p < 0.05$; d.m. – dry mass.

Table 2. The average content of ash (%) and selected minerals (µg/g d.m.) in elderberry flowers and fruit, depending on raw material origin.

Raw material	Origin	Ash	Ca	Mg	Fe	Cu	Zn	Mn
Flowers	Wild-growing	1.8^{a}	2977.6^{a}	719.5^{b}	94.2^{a}	11.3^{a}	38.4^{a}	35.9^{a}
	Orchard	1.8^{a}	2948.7^{a}	1360.4^{a}	51.1^{b}	9.2^{a}	36.1^{a}	36.7^{a}
Fruit	Wild-growing	0.7^{b}	2699.9^{a}	11964.8^{a}	35.1^{a}	5.0^{a}	12.4^{a}	15.6^{a}
	Orchard	1.0^{a}	1228.1^{b}	8566.2^{b}	26.6^{b}	5.4^{a}	10.3^{b}	11.8^{a}

a,b - differences between average values measured in raw material: wild-growing plants versus cultivars grown in orchards, statistically significant at $p < 0.05$.

2.2. Antioxidant capacity (AC) of elderberry flower and fruit extracts

Elderberry fruit and flowers are a very rich source of antioxidants [18,19]. The elderberry fruit analysed in this study had an equal or higher antioxidant capacity as compared with flowers of the same cultivars or wild plants from the same locations (Figure 1.). This stands in contrast to earlier studies: Dawidowicz et al. 2006 [20] and Kołodziej et al. 2011 [21], who used the DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) methods for their measurements, found higher radical scavenging activity in elderberry flowers. In addition, fruit and flowers of the analysed cultivars demonstrated significantly higher antioxidant activity that those harvested from wildly growing plants (Table 3). As for the cultivars themselves, the fruit of 'Haschberg' had the highest antioxidant capacity. Antioxidant capacity of flowers ranged from 304 to 444 µmol Trolox/g d.m. and, like in the case of fruit, it was higher in the flowers of cultivars than in those of wild plants.

Antioxidant potential of flowers can be used in infusions [22], which are characterized by higher DPPH and FRAP values than infusions made of dried fruit. The antioxidant capacity of infusions will certainly be significantly affected by the method of their preparation, the method of drying of flowers and fruit [20-22], the growing location and conditions and individual variation in plants. This can be exemplified by the ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic

acid)) value obtained for the cultivars 'Sampo' and 'Samyl'. Our study suggests that the fruit of 'Sampo' has higher antioxidant capacity than the fruit of 'Samyl'. But this difference may be due to other factors, especially that other studies suggest the opposite [23].

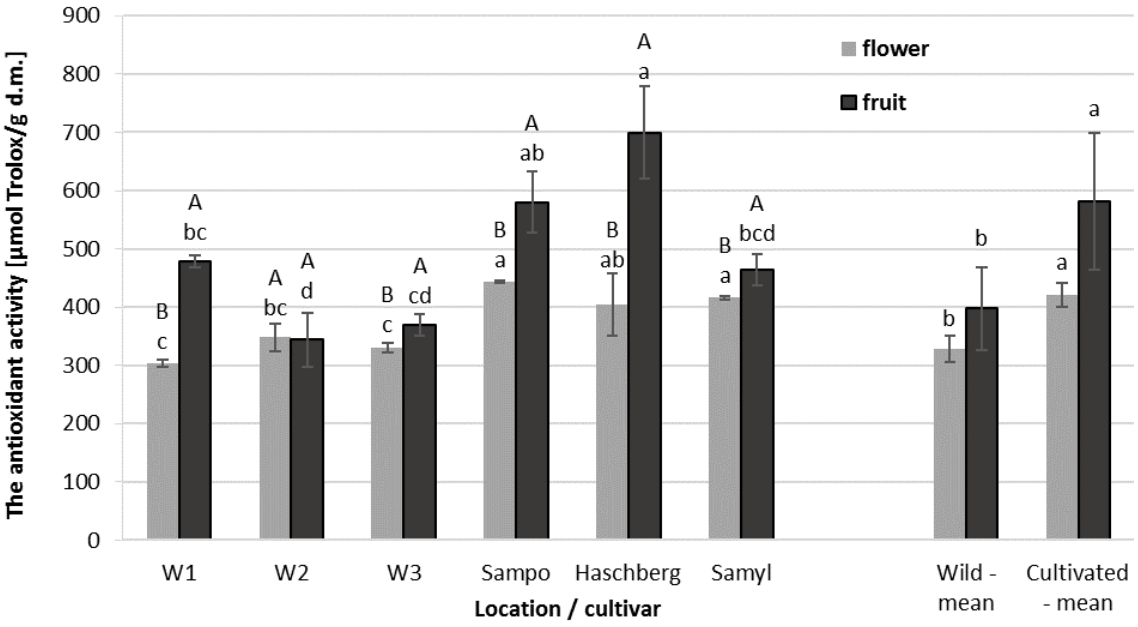

Figure 1. Antioxidant activity in elderberry flowers and fruit, depending on location (wild plants) / cultivar; A,B – differences between flowers and fruit per location (wild plants) / cultivar, statistically significant at $p < 0.05$; a,b,c,d – differences between flowers or fruit from different locations (wild plants) / cultivar, statistically significant at $p < 0.05$; d.m. – dry mass.

2.3. Total phenolic content (TPC) of elderberry flower and fruit extracts

The total phenolic content in elderberry flowers and fruit varied (Figure 2). The fruit of the 'Haschberg' cultivar and the fruit of plants growing naturally in W1 had significantly higher content of polyphenols than the flowers from the same locations. However, this was not a general rule because flowers were richer in total polyphenols than fruit in the rest of the samples. Nevertheless, the growing environment and the cultivar do significantly affect the total phenolic content since both flowers and fruit of cultivars grown in an orchard contained significantly higher levels of polyphenols than those obtained from wild plants (Table 3). The highest total phenolic content was found in the fruit of 'Haschberg' (8405 mg/100 g d.m.), 'Sampo' and in the fruit of wild plants from W1 (7233-7329 mg/100 g d.m.). Fruit collected from wild plants growing in the other two locations (W2 and W3) contained the lowest amounts of polyphenolic compounds, even about 40% less than the fruit of 'Haschberg'. Total phenolic content varied less in flowers. The richest in polyphenols were the flowers of the 'Sampo' cultivar (8156 mg/100 g d.m.), followed by the flowers of 'Samyl' and then by those of 'Haschberg'. Also the study conducted in Denmark by Christensen et al. 2008 suggests that polyphenol content depends on the cultivar – like in our study, the total phenolic content was higher in the flowers of 'Sampo' than in those of 'Haschberg' [24].

The content of polyphenols was strongly correlated with antioxidant capacity, which is in line with the findings of numerous studies [25,26]. The correlation coefficient was 0.93 for fruit and 0.97 for flowers (Table 4). Kołodziej et al. 2011 showed that elderberry flowers contained more phenolic compounds than elderberry fruit collected from the same locations. A similar observation was made for infusions from dried elderberry flowers and dried elderberry fruit – flowers were always found to be richer in various phenolic compounds, especially flavonoids, than fruit [21, 22].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 January 2020 doi:10.20944/preprints202001.0141.v1

Peer-reviewed version available at Molecules 2020, 25, 876; doi:10.3390/molecules25040876

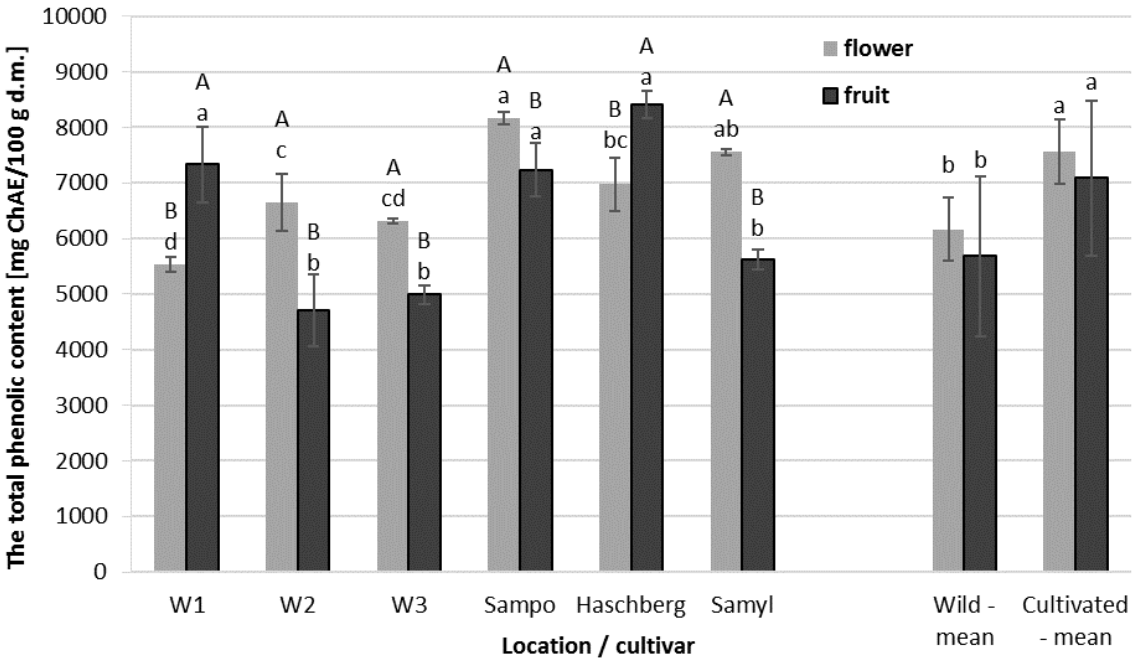

Figure 2. Total phenolic content in elderberry flowers and fruit, depending on location (wild plants) / cultivar; A,B – differences between flowers and fruit per location (wild plants) / cultivar, statistically significant at $p < 0.05$; a,b,c,d – differences between flowers or fruit from different locations (wild plants) / cultivar, statistically significant at $p < 0.05$; ChAE – chlorogenic acid equivalent.

2.4. Total anthocyanin content (TAC) in elderberry fruit extracts

Anthocyanins are pigments responsible for the dark colour of elderberry fruit [27]. The content of anthocyanins in elderberry fruit was found to depend on cultivar and location (Figure 3). The largest amounts of anthocyanins were extracted from 'Haschberg' fruit. Fruit collected from wild plants growing in two locations (W2 and W3) contained less than half of that amount. 'Samyl' had the smallest amount of anthocyanins of all the analysed cultivars. Fruit harvested from the cultivars varied in terms of total anthocyanin content, just like fruit of wild plants from three different locations, but fruit of the cultivars had significantly more anthocyanins than the wild fruit (Table 3).

Moreover, as could be expected [27,28], there was also a high correlation (0.98) between total anthocyanin content and antioxidant capacity and total anthocyanin content and total phenolic compounds in fruit (Table 4). The study by Kaack et al. 1998 analysed the level of anthocyanins in the fruit of 13 different elderberry cultivars, including 'Sampo'', 'Haschberg' and 'Samyl'. According to that study, the content of anthocyanins ranged between 664 and 1816 mg/100 g of fresh mass, whereas in our study their content was lower – anthocyanins accounted for 390 to 970 mg /100 g of fresh mass. Kaack et al. also pointed to 'Sampo' and 'Samyl' as the cultivars whose fruit was the richest in anthocyanin pigments [11]. But it seems that other factors were in play that affected the level of anthocyanins in elderberry fruit. The difference could have been due to the growing location, weather conditions [29,30] or availability of nutrients because, contrary to the results obtained in this study, Kaack et al. found that 'Haschberg' fruit contained the lowest concentration of anthocyanins [11]. What is more, according to the earlier work of the same author [31], the fruit of 'Haschberg' had less anthocyanins than the fruit of 'Sampo' and 'Samyl'. Pliszka 2017 [23] showed recently that the fruit of 'Samyl' contained more anthocyanins that the fruit of 'Sampo'. There must be some additional factors involved here since our study shows that 'Sampo' is richer in anthocyanins than 'Samyl'. This may suggest that the influence of environmental aspects on the content of anthocyanin pigments in elderberry fruit has been so far underestimated.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 January 2020 doi:10.20944/preprints202001.0141.v1

Peer-reviewed version available at Molecules 2020, 25, 876; doi:10.3390/molecules25040876

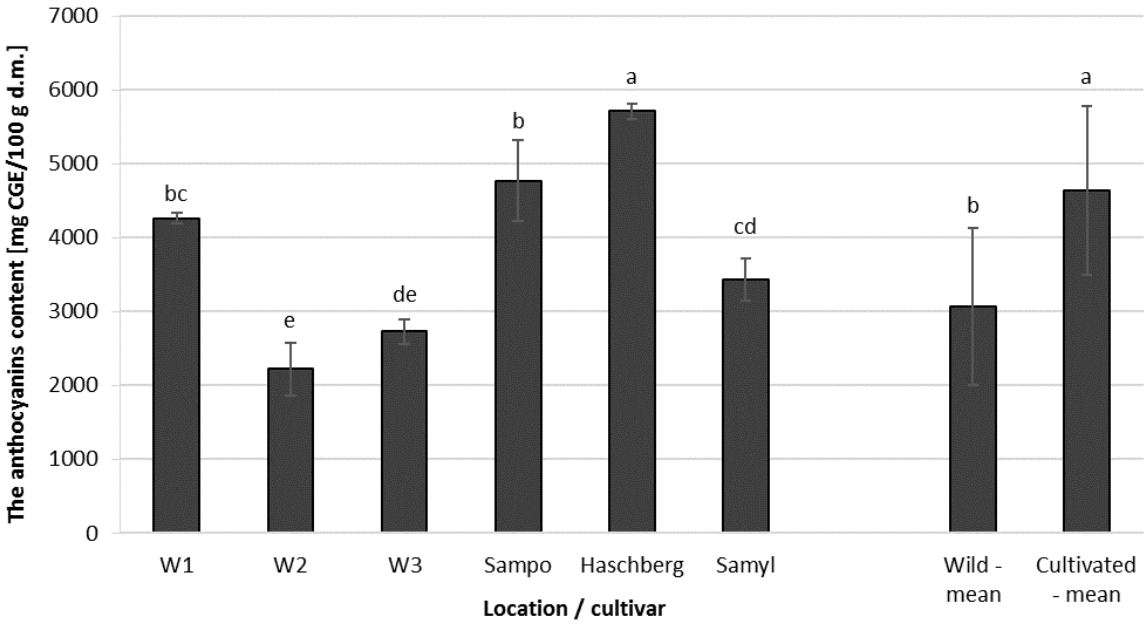

Figure 3. Total anthocyanin content in elderberry fruit, depending on location (wild plants) / cultivar; a,b,c,d – differences between average values, statistically significant at $p < 0.05$; CGE – cyanidin-3-glucoside equivalent.

Table 3. Average values of bioactive properties of elderberry flowers and fruit, depending on raw material origin.

Raw material	Origin	Antioxidant activity [µmol Trolox/g d.m.]	Total phenolic content [mg ChAE/100g d.m.]	Total anthocyanin content [mg CGE/100 g d.m.]
Flowers	Wild-growing	327.7[b]	6164.4[b]	-
	Orchard	421.5[a]	7561.8[a]	-
Fruit	Wild-growing	397.5[b]	5678.8[b]	3071.0[b]
	Orchard	581.3[a]	7087.3[a]	4638.2[a]

ChAE – chlorogenic acid equivalent; CGE – cyanidin-3-glucoside equivalent; a,b – differences between average values measured in raw material: wild-growing plants versus cultivars grown in orchards, statistically significant at $p < 0.05$.

Table 4. Pearson correlation coefficient between antioxidant activity (AC) and total phenolic content (TPC) and total anthocyanin content (TAC) in elderberry flowers and fruit.

Raw material	AC / TPC	AC / TAC	TPC / TAC
Flowers	0.97	-	-
Fruit	0.93	0.98	0.98

3. Materials and Methods

3.1. Plant material

3.1.1. Flowers

Elderberry flowers were collected at the full flowering stage in May 2015 by cutting the whole corymbs from wild shrubs growing in three different locations of central and western Poland (W1:

52°25'26.798"N, 16°54'34.437"E; W2: 52°27'34.274"N, 16°50'2.572"E; W3: 52°25'18.472"N, 16°55'47.887"E).

Elderberry flowers were also collected at the full flowering stage from plants grown in an orchard near the growing location of the wild elderberry shrubs (52°39'24.451"N; 16°57'10.719"E). The raw material was taken from three cultivars: 'Sampo', 'Haschberg' and 'Samyl'. Corymbs were harvested in the morning and transported to a laboratory where they were cut, while discarding the thickest twigs and underdeveloped or too dry flowers.

3.1.2. Fruit

Elderberry fruit was harvested when fully ripe (ripeness was judged based on colour being one of the most reliable ripeness indicators [32] from the same locations as those for flowers at the turn of August and September 2015. The whole clusters were collected and transported to a laboratory where berries were separated from pedicels and unripe, overripe and dry berries were discarded.

3.2. Preparation of samples

The plant material – flowers and fruit respectively – was divided into two parts. One part (flowers and fruit separately) was used to produce extracts. The other part was dried (flowers) or frozen at -50°C (fruit). Flowers were dried in a laboratory drier (POL-EKO apparatus, type SLW 115 STD) using convective drying at 45°C with an airflow speed of 2.5 m/s and variable air flow direction in the forced-air system until they were reduced to 19-21 % of their initial mass.

3.3. Determination of mineral content by atomic absorption spectrometry method (AAS)

3 g of dried elderberry flowers and 5 g of thawed elderberry fruit were weighed into quartz crucibles and subjected to mineralization by ashing at 550°C in a muffle furnace PP330 (Nobertherm, Germany). The samples were then burned over the burner using a nitric acid solution (Merck) diluted 1:1 with deionized water. The samples were placed in a muffle furnace and reheated at 550°C. The ash obtained in this process was weighed again and the percentage ash content in the plant material tested was calculated. The ash was then taken up in 1N nitric acid (Merck) and transferred quantitatively into volumetric flasks made of polypropylene. The content of minerals such as calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) in the obtained samples was determined by atomic absorption spectrometry with flame atomization (F -AAS) [33] using an AAS-3 spectrometer with background correction (Carl-Zeiss AAS-3 with BC, Germany). The studied elements were determined at the following wavelength and slit width, respectively: Ca (λ = 422.7 nm; slit = 0.50 nm), Mg (λ = 285.2 nm; slit = 0.20 nm), Fe (λ = 248.3 nm; slit = 0.15 nm), Cu (λ = 324.8 nm; slit = 0.30 nm), Zn (λ = 213.9 nm; slit = 0.20 nm), Mn (λ = 279.5 nm; slit = 0.20 nm). The concentrations of elements obtained in the analysed samples were converted into content in the tested plant material and expressed in μg/g dry by simultaneous analysis mass (d.m.). The accuracy of Ca, Mg, Fe, Cu, Zn and Mn measurements was ensured of certified reference material (Virginia Tobacco Leaves CTA-VTL-2, Poland). The average recoveries of certified levels (expressed as the average percentage of certified values) were as follows: Ca - 103%, Mg - 104%, Fe - 97%, Cu - 103%, Zn - 101% and Mn - 102%.

3.4. Preparation of extracts

Flower extracts were prepared by mixing 5 g of fresh flowers with 100 g of 80% methanol (Sigma-Aldrich). The mixture was left for 24 h at 4°C in a completely dark room and afterwards the extract was filtered using a vacuum pump (KNF LAB, type: N816.3KT.18). Extracts were prepared in triplicate and kept at -18°C until the start of the analyses. Fruit extracts were prepared by submerging 25 g of fruit mash obtained using a manual homogenizer in 50 g of 80% methanol and then filtering it according to the procedure applied to flower extracts.

3.5. Measuring antioxidant capacity (AC) using ABTS radical cation

The antioxidant capacity of extracts was determined spectrophotometrically using the ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) radical cation (Sigma-Aldrich), which was diluted with PBS (phosphate-buffered saline) buffer (pH=7.4) until reaching an absorbance of about 0.730. Extracts were diluted with the same buffer to obtain four different concentrations. The resulting extract samples of 50 µl each were mixed with 5 ml of the ABTS radical solution and after 6 minutes of reaction at 30°C the sample absorbance was measured at a wavelength of 734 nm, and compared with the control sample (which was mixed with PBS buffer instead of an extract sample) using a spectrophotometer (Helios Alpha, Thermo Elektron Corporation). The results were expressed in µmol Trolox / 1 g of dry mass [34].

3.6. Measurement of total phenolic content (TPC)

Total phenolic content of the analysed elderberry flower and fruit extracts was measured by the spectrophotometric method, using Folin-Ciocalteau reagent (POCH). Distilled water was added to 100 µl of flower extract / 200 µl of diluted fruit extract (2 ml of extract in 25 ml of distilled water) to obtain 1 ml of solution. Next, 5 ml of 0.2 N Folin-Ciocalteau reagent and after 3 minutes 4 ml of sodium carbonate solution (75 g/l) were added to the samples. The sample ingredients were blended and the samples were left in a dark place for 2 hours. After that time, the absorbance value of the samples was measured at a wavelength of 765 nm and compared with the reagent blank sample in a spectrophotometer (Helios Epsilon, Thermo Fisher Scientific). The results were expressed in mg of chlorogenic acid / 100 g of dry mass [35].

3.7. Measurement of total anthocyanin content (TAC)

Total anthocyanin content of elderberry fruit extracts was measured spectrophotometrically, by the differential method. 50 µl of extract was mixed with 10 ml of buffer (pH=1 and pH=4.5) and the obtained samples were left in a dark place for 1 hour. Afterwards the absorbance value of the samples was measured at wavelengths of 515 nm and 700 nm and compared with a blank sample (buffer, pH=1) using a spectrophotometer (Helios Epsilon, Thermo Fisher Scientific). The results were expressed in mg of cyanidin-3-glucoside / 100 g of dry mass [36,37].

3.8. Statistical analysis

The results are the average of three repetitions (three extract samples) with standard deviations. The results were subjected to one-factor ANOVA and a post-hoc HSD Tukey test using software Statistica 13.3 (StatSoft, Poland). The correlation between each pair of the variables: antioxidant capacity, total phenolic content and total anthocyanin content in the samples was calculated in MS Excel 2010 using the Pearson correlation coefficient.

4. Conclusions

Elderberry flowers and fruit are above all a rich source of bioactive compounds with antioxidant properties, but they also contain important minerals, such as calcium or magnesium. Both flowers and fruit were shown to have high concentrations of phenolic compounds, but fruit additionally contained high amounts of anthocyanins, and thus could show higher antioxidant capacity than flowers. Elderberry flowers and fruit harvested from cultivars grown in an orchard were characterized by higher content of antioxidants than flowers and fruit collected from wildly growing plants. At the same time, however, the differences between the studied cultivars were rather large in some cases but very small in others and depended on the parameter and raw material under analysis. Elderberry flowers, on the other hand, contained higher amounts of most of the analysed minerals than fruit, with no clear relationship between the origin of flowers and the mineral content.

Author Contributions: Conceptualization, K.M. and D.W-T.; Methodology, K.M., D.W-T. and H.S.; Software, M.K., H.S. and K.M.; Validation, D.W-T., M.K. and H.S.; Formal Analysis, K.M., D.W-T., H.S. and M.K.; Investigation, K.M. and H.S.; Resources, K.M., D.W-T. and H.S.; Data Curation, K.M. and H.S.; Writing – Original Draft Preparation, K.M., M.K. and G.P.Ł.; Writing – Review & Editing, K.M., G.P.Ł., D.W-T., M.K. and H.S.; Visualization, K.M., G.P.Ł. and M.K.; Supervision, K.M., G.P.Ł., D.W-T. and M.K.; Project Administration, D.W-T., K.M. and G.P.Ł.; Funding Acquisition, D.W-T.

Funding: This research received no external funding.

Acknowledgments: We would like to thank "Euro-Sad" Gospodarstwo Sadownicze Joanna i Rafał Bak (Pacholewo, Poland) for providing us with material for research purposes.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Waźbińska, J. Sambucus for growers (in Polish). *Szkółkarstwo* **2002,** *6,* 29-30.
2. Młynarczyk, K.; Walkowiak-Tomczak, D.; Łysiak, G.P. Bioactive properties of *Sambucus nigra* L. as a functional ingredient for food and pharmaceutical industry. *J. Funct. Foods* **2018,** *40,* 377-390. https://doi.org/10.1016/j.jff.2017.11.025
3. da Silva, R.F.R.; Barreira, J.C.M.; Heleno, S.A.; Barros, L.; Calhelha, R.C.; Ferreira, I.C.F.R. Anthocyanin profile of elderberry juice: a natural-based bioactive colouring ingredient with potential food application. *Molecules* **2019,** *24(13),* 2359 (published). doi: 10.3390/molecules24132359
4. Senica, M.; Stampar, F.; Veberic, R.; Mikulic-Petkovsek, M. Processed elderberry (*Sambucus nigra* L.) products: A beneficial or harmful food alternative? *LWT – Food Sci. Technol.* **2016,** *72,* 182-188. http://dx.doi.org/10.1016/j.lwt.2016.04.056
5. Sidor, A.; Gramza-Michałowska, A. Advanced research on the antioxidant and health benefit of elderberry (*Sambucus nigra*) in food – a review. *J. Funct. Foods* **2015,** *18,* 941-958. http://dx.doi.org/10.1016/j.jff.2014.07.012
6. Assessment report on *Sambucus nigra* L., fructus. European Medicine Agency, Committee on Herbal Medicinal Products (HMPC), 2013, EMA/HMPC/44208/2012.
7. Torabian, G.; Valtchev, P.; Adil, Q.; Dehghani, F. Anti-influenza activity of elderberry (*Sambucus nigra*). *J. Funct. Foods* **2019,** *54,* 353-360. https://doi.org/10.1016/j.jff.2019.01.031
8. Tiralongo, E.; Wee, S.S.; Lea, R.A. Elderberry supplementation reduces cold duration and symptoms in air-travellers: a randomized, double-blind placebo-controlled clinical trial. *Nutrients* **2016**, *8,* 182. doi: 10.3390/nu8040182
9. Ferreira, S.S.; Silva, P.; Silva, A.M.; Nunes, F.M. Effect of harvesting year and elderberry cultivar on the chemical composition and potential bioactivity: A three-year study. *Food Chem.* **2020,** *302,* 125366 (accepted). doi: 10.1016/j.foodchem.2019.125366
10. Duymuş, H.G.; Göger, F.; Başer, K.H. In vitro antioxidant properties and anthocyanin compositions of elderberry extracts. *Food Chem.* **2014,** *155,* 112-119. 10.1016/j.foodchem.2014.01.028
11. Kaack, K.; Austed, T. Interaction of vitamin C and flavonoids in elderberry (*Sambucus nigra* L.) during juice processing. *Plant Foods Hum. Nutr.* **1998,** *52,* 187-198. doi:10.1023/A:1008069422202
12. Fazio, A,; Plastina, P.; Meijerink, J.; Witkamp, R.F.; Gabriele, B. Comparative analyses of seeds of wild fruits of *Rubus* and *Sambucus* species from Southern Italy: Fatty acid composition of the oil, total phenolic content, antioxidant and anti-inflammatory properties of the methanolic extracts. *Food Chem.* **2013,** *140,* 817-824. doi: 10.1016/j.foodchem.2012.11.010
13. Gold, M.; Cernusca, M.M.; Godsey, L.D. A Competitive Market Analysis of the US Elderberry Industry. In *HortScience: a publication of the American Society for Horticultural Science*, Proceedings of the 2010 ASHS Annual Conference, Palm Desert, 31.07-05.08.2010; American Society for Horticultural Science; 45(8).
14. Vulić, J.J.; Vračar, L.O.; Šumić, Z.M. Chemical characteristics of cultivated elderberry fruit. *APTEFF* **2008,** *39,* 85-90. DOI: 10.2298/APT0839085V
15. Mratinić, E.; Fotirić, M. Selection of black elderberry (*Sambucus nigra* L.) and evaluation of its fruits usability as biologically valuable food. *Genetika* **2007,** *39(3),* 305-314.
16. Kołodziej, B.; Maksymiec, N.; Drożdżal, K.; Antonkiewicz, J. Effect of traffic pollution on chemical composition of raw elderberry (*Sambucus nigra* L.). *J. Elem.* **2012,** *17(1),* 67-78. DOI: 10.5601/jelem.2012.17.1.06

17. Diviš, P.; Pořízka, J.; Vespalcová, M.; Matějíček, A.; Kaplan, J. Elemental composition of fruits from different Black elder (*Sambucus nigra* L.) cultivars grown in the Czech Republik. *J. Elem.* **2015,** *20(3),* 549-557. DOI: 10.5601/jelem.2015.20.1.758
18. Zheng, W.; Wang, S.Y. Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries and lingoberries. *J. Agric. Food Chem.* **2003,** *51(2),* 502-509. DOI: 10.1021/jf020728u
19. Stoilova, I.; Wilker, M.; Stoyanova, A.; Krastanov, A.; Stanchev, V. Antioxidant activity of extract from elder flower (*Sambucus nigra* L.). *Herba Polonica* **2007,** *53(1),* 45-54.
20. Dawidowicz, A.L.; Wianowska, D.; Baraniak, B. The antioxidant properties of alkoholic extracts from *Sambucus nigra* L. (antioxidant properties of extracts). *LWT* **2006,** *39,* 308-315. doi:10.1016/j.lwt.2005.01.005
21. Kołodziej, B.; Drożdżal, K. Antioxidant properties of black elder flowers and berries harvested from the wild. *Żywność. Nauka. Technologia. Jakość* **2011,** *4(77),* 36-44.
22. Viapiana, A.; Wesolowski, M. The phenolic contents and antioxidant activities of infusions of *Sambucus nigra* L. *Plant Foods Hum. Nutr.* **2017,** *72,* 82-87. DOI 10.1007/s11130-016-0594-x
23. Pliszka, B. Polyphenolic content, antiradical activity, stability and microbiological quality of elderberry (*Sambucus nigra* L.) extracts. *Acta Sci. Pol. Technol. Aliment.* **2017,** *16(4),* 393-401. http://dx.doi.org/10.17306/J.AFS.2017.0523
24. Christensen, L.P.; Kaack, K.; Fretté, X.C. Selection of elderberry (*Sambucus nigra* L.) genotypes best suited for the preparation of elderflower extracts rich in flavonoids and phenolic acids. *Eur. Food Res. Technol.* **2008,** *227,* 293-305. DOI 10.1007/s00217-007-0723-8
25. Mikulic-Petkovsek, M.; Ivancic, A.; Schmitzer, V.; Veberic, R.; Stampar, F. Comparison of major taste compounds and antioxidative properties of fruits and flowers of different *Sambucus* species and interspecific hybrids. *Food Chem.* **2016,** *200,* 134-140. http://dx.doi.org/10.1016/j.foodchem.2016.01.044
26. Ruiz-Rodriguez, B.M.; de Ancos, B.; Sanchez-Moreno, C.; Fernandez-Ruiz, V.; de Cortes Sanchez-Mata, M.; Camara, M.; Tardio, J. Wild blackthorn (*Prunus spinosa* L.) and hawthorn (*Crataegus monogyna* Jacq.) fruits as valuable sources of antioxidants. *Fruits* **2014,** *69(1),* 61–73. https://doi.org/10.1051/fruits/2013102
27. Espín, J.C.; Soler-Rivas, C.; Wichers, H.J.; García-Viguera, C. Anthocyanin-based natural colorants: A new source of antiradical activity for foodstuff. *J. Agric. Food Chem.* **2000,** *48,* 1588-1592. Doi: 10.1021/jf9911390
28. Schmitzer, V.; Veberic, R.; Slatnar, A.; Stampar, F. Elderberry (*Sambucus nigra* L.) wine: a product rich in health promoting compounds. *J. Agric. Food Chem.* **2010,** *58,* 10143-10146. DOI:10.1021/jf102083s
29. Senica, M.; Stampar, F.; Veberic, R.; Mikulic-Petkovsek, M. The higher the better? Differences in phenolics and cyanogenic glycosides in *Sambucus nigra* leaves, flowers and berries from different altitudes. *J. Sci. Food Agric.* **2017,** *97(8),* 2623-2632. DOI 10.1002/jsfa.8085
30. Lee, J.; Finn, C.E. Anthocyanins and other polyphenolics in American elderberry (*Sambucus canadensis*) and European elderberry (*S. nigra*) cultivars. *J. Sci. Food Agric.* **2007,** *87,* 2665-2675. DOI: 10.1002/jsfa.3029
31. Kaack, K. 'Sampo' and 'Samdal', elderberry cultivars for juice concentrates. *Fruit Varieties J.* **1997,** *51(1),* 28-31.
32. Łysiak, G.; Kurlus, R.; Zydlik, Z.; Walkowiak-Tomczak, D. Apple skin colour changes during harvest as an indicator of maturity. *Acta Sci. Pol., Hortorum Cultus* **2014,** *13(3),* 71-83.
33. De la Guardia, M.; Garrigues, S. *Handbook of Mineral Elements in Food;* Wiley Blackwell, 2015.
34. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999,** *26(9–10),* 1231–1237. DOI: 10.1016/s0891-5849(98)00315-3

35. Fang, Z.; Zhang, M.; Sun, Y.; Sun, J. How to improve bayberry (Myrica rubra Sieb. Et Zucc.) juice color quality: effect of juice processing on bayberry anthocyanins and polyphenolics. *J. Agric. Food Chem.* **2006,** *54(1),* 99-106. DOI: 10.1021/jf051943o
36. Fuleki, T.; Francis, F.J. Quantitative methods for anthocyanins. 2. Determination of total anthocyanin and degradation index for cranberry juice. *J. Food Sci.* **1968,** *33*, 78-83. https://doi.org/10.1111/j.1365-2621.1968.tb00888.x
37. Giusti, M.M.; Wrolstad, R.E. Characterization and measurement of anthocyanins by UV- -visible spectroscopy. In *Current protocols in food analytical chemistry*, R.E. Wrolstad; Wiley, New York, 2001; pp. F1.2.1.-F1.2.13.

Sample Availability: The samples can be requested from the authors.