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ORIGINAL PAPER

CONTENT AND CORRELATION OF POLYPHENOLIC COMPOUNDS, BIOELEMENTS AND ANTIRADICAL ACTIVITY IN BLACK ELDER BERRIES (*SAMBUCUS NIGRA* L.)*

Barbara Pliszka

Chair of Chemistry
University of Warmia and Mazury in Olsztyn, Poland

Abstract

The black elder berries are a valuable source of polyphenolic compounds and minerals. Owing to their antioxidant activity and anticancerogenic properties, fruit of the black elder shrubs are used in medicine and dietary supplementation. The aim of this study has been to determine the content of polyphenolic compounds, bioelements (Ca, Mg and Zn) and antiradical activity as well as correlation between polyphenols and antiradical activity, also between polyphenols and bioelements in the fruit. The material consisted of berries from several cultivars of the black elder (Alleso, Korsor, Sampo, Samyl). A spectrophotometric method was employed to determine the content of polyphenols and the antiradical activity. The content of bioelements was determined with the flame atomic absorption spectrophotometric method (FAAS). The results implicate that the highest content of total phenols and anthocyanins was in fruit of cv. Samyl. The content of flavonols was higher in fruit of the cultivars Samyl and Korsor than in Sampo and Alleso. The content of phenolic acids was higher in fruit produced by cv. Korsor, Sampo and Samyl than by Alleso. Fruit from cv. Samyl and Korsor had higher antiradical activity (ABTS) than that from Alleso and Sampo, although results of DPPH show that these values were high and comparable in all fruit. The antiradical activity is positively correlated with the content of polyphenols. Berries of cv. Samyl were characterized by the highest content of calcium and magnesium, but had less zinc. The polyphenolic compounds were positively correlated with calcium, whereas magnesium was correlated with total phenols, while zinc correlated with flavonols and phenolic acids.

Keywords: antiradical activity, bioelements, correlation, elderberry, fruit, polyphenols.

Barbara Pliszka, Ph.D., Department of Environment and Agriculture, Chair of Chemistry, University of Warmia and Mazury in Olsztyn, Pl. Łódzki 4, 10-957 Olsztyn, Poland, phone: +48 89 523 44 47, Fax: 48 89 523 48 01, e-mail: barbara.pliszka@uwm.edu.pl
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INTRODUCTION

Black elderberry (*Sambucus nigra* L.) belongs to the honeysuckle family *Caprifoliaceae*. The black elder shrubs grow wild in several countries in Europe and are also cultivated on a small scale in some European countries (Charlebois et al. 2010). In Poland, Danish cultivars of black elderberry are distinguished by higher yields and superior morphological traits of fruit compared to wild forms (Waźbińska, Puczel 2002). Elderberries present antibacterial, antiallergic, antifungal, anti-inflammatory, antiviral and immunological properties. Owing to their antioxidant and anticancerogenic characteristics, fruit of the black elder are used in medicine and as a source of dietary supplementation (Netzel et al. 2005, Dawidowicz et al. 2006). Moreover, elderberries are a potential source of bioactive compounds for formulations to be used as co-adjuvants in diabetes management (Salvador et al. 2017). The elderberries are a valuable source of polyphenolic compounds, such as: flavonols (quercetin, kaempferol, rutin), anthocyanins (cyanidin glycosides), phenolic acids (caffeic acid, ferulic acid, *p*-coumaric acid) (Lee, Finn 2007, Kaack et al. 2008, Veberic et al. 2009). Phenolic acids with flavonols, along with anthocyanins, constitute the principal secondary metabolites of elderberry. They also enter in positive, synergistic relations with other bioactive compounds present in food (Dawidowicz et al. 2006). Anthocyanins are the largest group of polyphenolic compounds and, like flavonoids, they are capable of complexing with metal ions and the resulting compounds stabilize the colour of anthocyanins. In the process of chelation of polyphenols with metal ions, they can act as carriers of bioelements and toxic metals (Smyk et al. 2008, borowska et al. 2018). Other compounds occurring in black elder berries are organic acids (e.g. malic, citric, fumaric and malonic acids), sugars, pectines, carotenoids and vitamins C and B (Dawidowicz et al. 2006, Veberic et al. 2009). Berries of black elder shrubs also contain such minerals as calcium, magnesium, potassium, sodium, phosphorus, zinc, manganese, iron (Szymański et al. 2013, Diviš et al. 2015).

The aim of this study has been to determine the content of polyphenolic compounds, bioelements (Ca, Mg and Zn) and antiradical activity as well as correlation between polyphenols and antiradical activity, also between polyphenols and bioelements in the fruit.

MATERIAL AND METHODS

Materials

The plant material consisted of fruits from Danish cultivars (Alleso, Korsor, Sampo, Samyl) of elderberry (*Sambucus nigra* L.) originating from a field experiment at the Research Station Garden, which belongs to the University of Warmia and Mazury in Olsztyn (53°44′48,49″ N, 20°26′39,80″ E). Elderberries were harvested at full maturity. All chemicals used in the experiment were of highest grades and were purchased from Merck.

Climatic and soil conditions

The identification of the climatic conditions during the study (air temperature, rainfall sunshine) was based on the measurements of the weather elements taken by the Institute of Meteorology and Water Management in Olsztyn. During the plant growing season, the average air temperature in the locality was 18.3°C. The average of rainfall during that period in the experiment was 347 mm, and the average number of hours with insolation reached 473. The site lay on deluvial soil formed from light silt loam underlain by loamy sand. The soil was classified as representing the R-IVa in the Polish soil valuation classification, with the pH of 6.5 and the content of organic matter circa 2.5%. The soil was very rich in available elements (P, K, Mg).

Sample preparations

For each analysis, 20 g portions of elderberries in three replications were weighed. Frozen fruit were stored at –25°C. Prior to analyses, samples of fruit were defrosted by keeping them at room temperature for 2 h, after which they were crushed in a mortar. A solution of citric acid in the amount of 300 cm^3 and concentration of 0.1 mol dm^3 was poured to each portion of the homogenized material. Samples were left at a temp. of 2°C, in the dark, for 2 hours. Afterwards, beakers holding the samples were shaken in water bath at 37°C for 15 minutes. Next, the samples were centrifuged for 15 min at RCF equal 1790 g in order to separate solid parts of fruits from the extract. The subsequent shaking and centrifugation of the samples were done with portions of the solvent (100 cm^3). Extraction continued until the red colour disappeared, thus ensuring complete leaching of the pigment from the fruits. Volumes of the resulting extracts were aggregated into single samples and submitted to analyses.

Determinations of the content of polyphenolic compound

The content of total phenols (TP) in extracts from elderberries was determined with the Folin-Ciocalteu method (Singleton et al. 1999). Namely, the fruit extract (40 μL) was mixed with 200 μL of the Folin-Ciocalteu reagent and 3.2 cm^3 H_2O, and incubated at room temperature for 6 minutes. Following the addition of 600 μL 20% sodium carbonate to the mixture, the solution was left at room temperature for 2 hours. Absorbance was measured at $\lambda = 765$ nm. The TP was assessed according to the calibration curve. Gallic acid served as an equivalent (mg GAE kg^{-1} FW; FW – fresh weight). The content of total anthocyanins (TA) in the extracts was assessed by the Giusti and Wrolstad method (2001). The extract (1 cm^3) was mixed with 4 cm^3 buffer of pH 1 and buffer of pH 4.5. The quantitative determination of TA consisted in measuring the difference in the absorbances of extracts in buffer solutions of pH 1 and pH 4.5 at λ = 508 nm and λ = 700 nm. The TA was reported as the cyanidin 3 glucoside equivalent (mg CGE kg^{-1} FW). The content of flavonols (FV) in extracts was determined according to the Christ-Müller method (Farmakopea Polska VI 2002). The FV determination consisted in measuring the absorbance of a sample after 45 min for λ = 425 nm. The results were given as quercetin equivalent (mg QE kg^{-1} FW). The content of total phenolic acids (PA) in extracts was determined with the Arnova reagent in line with the methodology described in relevant literature (Farmakopea Polska V 1999). The PA determinations involved measurements of the absorbance of a sample at λ = 490 nm. The results were expressed as caffeic acid equivalent (mg CAE kg^{-1} FW). Absorbance of extracts was measured using a Shimadzu UV1800 spectrophotometer.

Free radical scavenging ability

The antiradical activity (ABTS) was determined with the Miller et al. method (1993) with certain modifications. The ABTS method involved the generation of an ABTS$^{®\cdot+}$ cation radical, the formation of which was inhibited by adding an antioxidant. The extract (20 µL) was mixed with 1 cm^3 reagent (ABTS – 610 µmol L^{-1}, metmyglobin – 6.1 µmol L^{-1} and buffer – 5 mmol L^{-1}) and the reaction was initiated by the addition 200 µL of hydrogen peroxide (250 µmol L^{-1}). The absorbance was measured at 37°C, after 6-minute incubation, at λ = 734 nm. The determinations were performed in reference to the control reagent (deionized water) and Trolox standard (1.65 mmol L^{-1}). The results were converted to Trolox equivalent (mmol TE kg^{-1} FW). The antiradical activity (DPPH) was determined by Yen, Hung (2000) with slight modification. The extract (0.5 cm^3) in 4 cm^3 of distilled water/methanol (1:3, v/v) was added to 1 cm^3 solution of DPPH (2.5 mmol dm^3) in methanol. The mixture was shaken and left to stand in the dark at room temperature. Absorbance was measured after 30 min at λ = 517 nm. The results were expressed as an inhibition percentage. Inhibition of free radical DPPH in percent (I%) was calculated as follows: $I\% = [(A_{control} – A_{sample})/A_{control}] \cdot 100\%$. Absorbance of fruit extracts was measured using a Shimadzu UV1800 spectrophotometer.

Determinations of the content of bioelements

Extracts were digested in teflon bombs by applying microwave energy. 1 cm^3 of extract and 2 cm^3 of concentrated HNO_3 made up a sample for digestion. Digested samples were quantitatively transferred to 50 cm^3 volumetric flasks and replenished with deionised water. The content of mineral elements (Ca, Mg and Zn) was determined with a technique of flame atomic absorption spectrometry (FAAS). Determinations were run on a Unicam 939 Solar atomic absorption spectrometer coupled with an Optimus data station, background correction and appropriate cathode lamps. The bioelements Ca, Mg, Zn were measured at the respective wavelengths of 422.67 nm, 285.21 nm, and 213.85 nm. The standards defined in the PN-EN ISO 11885:2009 norm were applied to the analysed elements. The results were expressed in mg kg^{1} FW.

Statistical analysis

The results were verified in three replications. The data regarding the content of polyphenolic compounds, bioelements and the antiradical activity of fruit were submitted to statistical analysis of variance for univariate experiments (the Duncan test). Evaluation of correlations between selected traits was performed using the Pearson's analysis of correlation. The correlation coefficients achieved were converted to determination coefficients (r^2 100%), indicating the degree of reciprocal effects. Statistica 12 PL (StatSoft PL) software (p<0.05, p<0.01) was employed for calculations.

RESULTS AND DISCUSSION

The content of polyphenolic compounds and antiradical activity in black elder berries are presented in Table 1. This study has demonstrated that the content of polyphenolic compounds in elderberries depended on a cultivar of the black elder. The highest total phenols (TP) and anthocyanins (TA) were determined in fruit of cv. Samyl, while the lowest content of the above compounds were in fruit of cv. Alleso. Analyzing the effect of a cultivar on the content of flavonols (FV) in elderberries, it was concluded that cv. Samyl and Korsor fruit with a higher content of FV than cv. Sampo and Alleso. A significantly higher content of phenolic acids (PA) was determined

Table 1

Content of polyphenolic compounds and antiradical activity in black elder berries

Cultivar	Polyphenolic compounds (mg kg^{1} FW)				Antiradical activity	
	TP	TA	FV	PA	ABTS (mmol TE kg^{1} FW)	DPPH (%)
Alleso	5984^{d}	3047^{d}	418.5^{b}	2190^{b}	30.85^{b}	87.96^{a}
Korsor	9661^{b}	5795^{b}	428.7^{a}	2274^{a}	33.56^{a}	88.15^{a}
Sampo	6313^{c}	3849^{c}	419.1^{b}	2255^{a}	30.57^{b}	87.51^{a}
Samyl	10 388^{a}	6974^{a}	426.8^{a}	2276^{a}	33.81^{a}	87.89^{a}

a,b,c,d – values denoted by same letters in the column do not differ significantly at p<0.05, FW – fresh weight, TP – total polyphenols, TA – total anthocyanins, FV – flavonols, PA – phenolic acids, TE – Trolox equivalent in fruit of cv. Korsor, Sampo and Samyl, while a significantly lower one was in cv. Alleso. In the natural environment of Denmark, the cultivar Sampo could be distinguished as having a high content of anthocyanins (over 800 mg 100 g^{-1} of fruit), whereas cv. Alleso had the least anthocyanins (Kaack, Austed 1998). According Jakobek et al. (2007*b*), the content of total phenols and anthocyanins determined in fruit of *Sambucus nigra* was smaller, although the content of flavonols was higher than in this experiment. Differences in the content of polyphenols in berries can be attributed to various factors, e.g. a geontype (Kaack et al. 2008), or environmental factors (air temperature, sunshine, moisture) (Jackson, Lombard 1993). In this study, the course of the weather (temperature, rainfall, sunshine) during the year of the experiment is illustrated in Figure 1. This year was

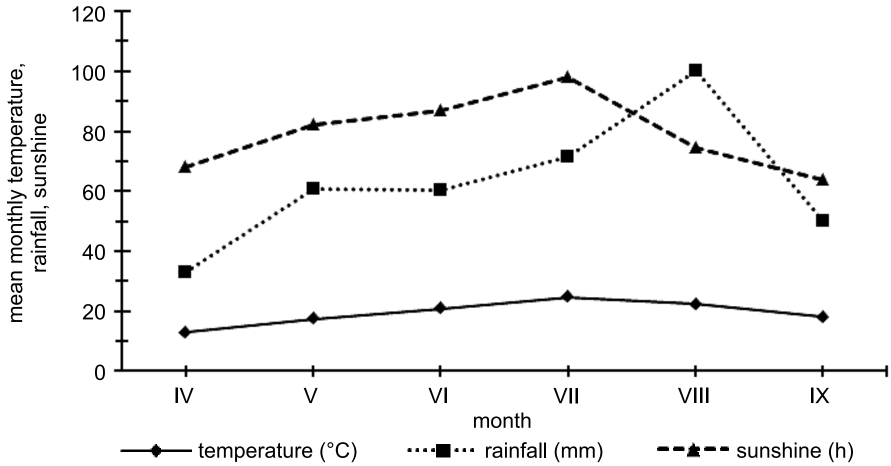

Fig. 1. Temperature, rainfall and sunshine during cultivation of *Sambucus nigra* (data from the Institute of Meteorology and Water Management in Olsztyn)

warm, sunny and less wet. High temperature and sunshine in July enabled the fruits to ripe and color uniformly, but abundant rainfall in August delayed the maturation of fruits, which occurred in the first ten days of September. In this research, the cultivation site might be a factor that had a considerable effect on the content of polyphenols in berries. Pliszka et al. (2007) found a large content of total phenols and anthocyanins in fruits of wild elder growing on R-IVa class soil. It needs to be emphasized that fruits of black elder growing on soil classified as R-IVa produced high and good quality yields (Waźbińska, Puczel 2002).

The results of this paper has demonstrated that fruit of cv. Samyl and Korsor had greater antiradical activity than those of cv. Alleso and Sampo (in ABTS), which agrees with previous research results, although the ABTS were lower (Pliszka 2017). In this study, the results suggest that the antiradical activity of fruit from the analyzed cultivars was the closest to the results (36.50 mmol TE kg^{-1} FW) obtained by Mikulic-Petkovsek et al. (2016).

The antiradical activity of elderberries in DPPH was generally high and on comparable levels. The results of the DPPH assays were similar to the ones achieved by Pliszka (2017). Lower antiradical activity in elderberries in DPPH (63.26%) was noted Anton et al. (2013).

The content of bioelements in elderberries determined in the current study is shown in Table 2. The highest calcium content was found in fruit

Table 2

Content of bioelements (mg kg^{-1} FW) in black elder berries

Cultivar	Bioelements		
	Ca	Mg	Zn
Alleso	94.5^{c}	190.1^{c}	6.401^{b}
Korsor	109.6^{a}	175.2^{d}	7.710^{a}
Sampo	100.2^{b}	226.3^{b}	5.453^{c}
Samyl	110.5^{a}	248.7^{a}	3.582^{d}

a,b,c,d – values denoted by same letters in the column do not differ significantly at $p<0.05$

of the cv. Korsor and Samyl, while the lowest one was in cv. Alleso. An analysis of the effect of a cultivar on the magnesium content showed that the highest content of this element was in fruit of cv. Samyl, while the least Mg was in berries of cv. Korsor. In contrast, cv. Korsor elderberries had the highest content of Zn, whereas berries from cv. Samyl were the poorest in this element. According Diviš et al. (2015), fruit of *Sambucus nigra* were determined to contain more Ca, Mg, Zn than found in this study.

The calculated correlation coefficients between the analyzed properties in the black elder berries are presented in Table 3. The statistical analysis proved that the content of total phenols, anthocyanins, flavonols and phenolic acids was mutually positively correlated. A highly significant correlation was determined between TP and TA (r=0.878). The determination coefficient for this dependence was around 77%. The correlation between TP and FV (r=0.520) was also highly significant, and the determination coefficient was 27%. The coefficients of the correlation between TA and FV and PA were much lower, and the determination coefficients for these characteristics reached values of about 15%. The content of anthocyanins in elderberry can be as high as 66% of the content of polyphenols, and can therefore affect the mutual dependences to the highest degree (Jakobek et al. 2007*a*). At present, the literature lacks data on the subject of mutual relationships between total phenols and anthocyanins versus flavonols and phenolic acids in the elderberries. This study shows that polyphenolic compounds were positively correlated with the antiradical activity in the elderberries. Both the ABTS and DPPH values resulted in the correlation coefficients for the antiradical activity and TP and TA being higher than between the antiradical activity and FV and PA (Table 3). Converting the correlation coefficients to coefficients of determination, it was found that TP and TA conditioned

Table 3

Correlation coefficients between analyzed properties in black elder berries

Property	TP	TA	FV	PA	ABTS	DPPH
TP	1					
TA	0.878**	1				
FV	0.520**	0.381**	1			
PA	0.287*	0.397**	0.467**	1		
ABTS	0.842**	0.806**	0.409**	0.202*	1	
DPPH	0.349**	0.380**	0.246*	0.250*	0.512**	1

*, ** – significant at $p<0.05$ and $p<0.01$, respectively, TP – total phenols,
TA – anthocyanins, FV – flavonols, PA – phenolic acid, ABTS, DPPH – antiradical activity

the antiradical activity of fruit in about 70%, FV – in 17%, and PA – in just 4% (ABTS test). According to the DPPH determinations, these dependences were much weaker. The correlation between antiradical activity (DPPH, ABTS) and total phenols and anthocyanins in elderberries has been determined by many authors (Pliszka et al. 2005, Waźbińska et al. 2006, Mikulic-Petkovsek et al. 2016). On the other hand, the dependence between the antiradical activity and flavonols and phenolic acids in elderberries is less often explored. For comparison, Viapiana, Wesołowski (2017) indicate that correlation coefficients between antioxidant activity (DPPH) and flavonoids and phenolic acids in the elder tea infusions were below 0.5. Jakobek et al. (2007*b*) noted a significant correlation in red fruit between antiradical activity and flavonols (r=0.79** and r=0.73** for DPPH and ABTS, respectively) and hydroxycinnamic acid (r=0.66* and r=0.64* for DPPH and ABTS, respectively). Good linear correlations between the mentioned polyphenolic groups indicate a possible influence of these polyphenols on the antioxidant activity of fruits (Jakobek et al. 2007*b*). In this study, the results pertaining to the antiradical activity in elderberries, determined with two methods (ABTS and DPPH), were mutually correlated in a statistically significant way (Table 3), which indicates that antioxidants contained in these berries had an ability to scavenge free radicals.

The correlation coefficients calculated between polyphenolic compounds and bioelements (Ca, Mg, Zn) in berries of the black elder are collated in Table 4. The total phenols, anthocyanins, flavonols and phenolic acids were positively correlated with calcium (r from 0.469 to 0.661). The determination coefficients indicate that PA values were more dependent on Ca (at 44%), but were least dependent on TA (at 22%). The TP results were correlated positively only with Mg, while FV and PA showed positive correlations only with Zn. The determination coefficients reveal that the TP in the black elder fruit depended in about 25% on Mg, while the PA and FV were slightly less dependent on Zn. The available literature provides relatively little information about the correlation between polyphenols and minerals in the black elder fruit. Szymański et al. (2013) found a positive correlation

Table 4

Correlation coefficients between polyphenolic compounds and bioelements in black elder berries

Compounds	Bioelements		
	Ca	Mg	Zn
TP	0.535**	0.496**	n.s.
TA	0.469**	n.s.	n.s.
FV	0.514**	n.s.	0.446**
PA	0.661**	n.s.	0.492**

** – significant at $p<0.01$, n.s. – statistically not significant, TP – total phenols, TA – anthocyanins, FV – flavonols, PA – phenolic acid

between the flavonols and calcium (r=0.867), and magnesium (r=0.767) in *Sambucus nigra* (inflorescences, leaves and fruit). The cited authors show that correlation between phenolic acids and Ca or Mg was determined at r=0.773 and 0.700, respectively. However, lower coefficients were determined for Zn (r from 0.367 to 0.550). Szymański et al. (2013) have not obtained any correlation between total phenols and Ca or Mg, which is contrary to the current study. The current results indicate that polyphenols in black elder fruits have a considerable effect on the creation of complexes with ions of the metals Ca, Mg, Zn. Bratu et al. (2018) report on the impact of polyphenols of the formation of chelates with Cu and Mn ions. Polyphenolic compounds capable of binding bioelmetals occur in fruit, including elderberries, and it seems reasonable to investigate relationships between the content of polyphenols and minerals and possible implications of these for health in the future.

CONCLUSION

The results show that elderberries can be good source of polyphenolic compounds and bioelements. Berries of the black elder cv. Samyl are distinguished by their highest content of total phenols and anthocyanins as well as calcium and magnesium. Fruit of the black elder cultivars tested in this study demonstrated high antiradical activity. The antiradical activity of elderberries was positively correlated with polyphenolic compounds, which suggests that mainly polyphenols are responsible for oxidation and reduction processes. Polyphenolic compounds influence calcium, while magnesium and zinc are affected only by some polyphenolic groups. The results can contribute to a selection of cultivars and development of processing technologies, in order to produce more valuable products used in dietary supplementation.

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