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ORIGINAL PAPER

STUDY ON RELATIONSHIPS BETWEEN THE CONTENT OF CHEMICAL ELEMENTS AND POLYPHENOLS AND ANTIOXIDANT ACTIVITY IN *SAMBUCUS NIGRA*

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Abstract

Black elder is a popular medicinal plant. Its flowers contain flavonoids, phenolic acids, tannins, essential oil and other compounds. The fruits contain carbohydrates, anthocyanins, flavonoids, tannins, fatty acids, vitamins C and B_2, folic acid and organic acids. According to research results, its flower extracts show anti-inflammatory and antibacterial activity, and they control the blood glucose level, whereas fruit extracts have immunostimulatory, antiviral, antibacterial and antioxidative activity. Alcoholic extracts of fruits and leaves show anti-toxoplasma activity. The objective of the present study was to determine selected chemical elements (using ICP-OES), total polyphenolic compounds (using the Folin-Ciocalteu reagent) and antioxidative activity (using the radical DPPH and total activity of iron (III) ions reduction by FRAP) in aqueous extracts of black elder-based materials. The test material included the flowers, leaves and fruits of *Sambucus nigra* from eight locations. The samples were powdered and sieved prior to obtaining extracts. The findings underwent the Spearman's rank correlation analysis and cluster analysis. The tests confirmed the presence of very high and high correlations between certain chemical elements and the content of polyphenols versus antioxidative activity in the raw materials. The results of the cluster analysis revealed a series of correlations between antioxidative activity, total polyphenols and the chemical elements determined.

Keywords: *Sambucus nigra*, ICP-OES, polyphenolic compounds, Spearman's rank correlation, cluster analysis.

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INTRODUCTION

Sambucus nigra L. (black elder) is a plant in the family *Adoxaceae*. It is a deciduous shrub growing to 10 m high. Its leaves are ovate or ovate--elliptic or ovate-lanceolate. It has fine, creamish white flowers borne in corymbs. Its fruits are black, smooth and globose (drupes) (Atkinson, Atkinson 2002).

The flowers of *Sambucus nigra* contain flavonols (rutin, isoquercetin, astragalin, kaemferol, hyperoside, isorhamnetin 3-O-rutinoside, quercetin 3-O-6-acetylglucoside, isorhamnetin 3-O-glucoside, kaemferol 3-O-rutinoside), flavanones (naringenin), flavones (luteolin), phenolic acids (e.g., ferulic, caffeic, chlorogenic and p-coumaric acids), fatty acids, polysaccharides (mucilage), terpenes, tannins, essential oil (composed mainly of linalol) and phenylcarboxylic acids (Roschek et al. 2009, Laffita, Castillo 2011, Sidor, Gramza-Michałowska 2015, Porter, Bode 2017).

The fruits contain anthocyanins, (cyanidin 3-sambubioside, sambucyanin, chrysanthemin, cyanidin 3-sambubioside-5-glucoside, cyanidin-3,5-diglucoside, cyanidin 3-glucoside, antirrhinin (cyanidin 3-rutinoside), calistefin (pelargonidin 3-O-glucoside), tulipanin, peonidin sambubioside-3, peonidin glucoside-3, peonidin sambubioside-3, peonidin monoglucuronide, chrysanthemin monoglucuronide), flavonols (rutin, isoquercetin, kaemferol 3-O-rutinoside, isorhamnetin 3-O-rutinoside, quercetin, quercetin 3-rutinoside, quercetin 3-glucoside, astragalin, isorhamnetin 3-O-glucoside), phenolic acids, organic acids (shikimic, fumaric, citric, malic, tartaric, valeric), vitamins (C, K, retinol), sugars (mucilage), lectins, pectins, tannins (Veberic et al. 2009, Sidor, Gramza-Michałowska 2015, Porter, Bode 2017, Młynarczyk et al. 2018).

The fruits, leaves and flowers contain cyanogenic glycosides, for instance sambunigrin, from which toxic hydrogen cyanide is released on hydrolytic decomposition (Laffita, Castillo 2011).

Research has shown that anthocyanins from the black elder fruits are beneficial in treating diabetes because of their ability to stimulate insulin secretion and reduce oxidative stress. Polyphenols (mainly derivatives of cyanidin but also peonidin) separated from *S. nigra* modulate specific and non-specific immune systems, reduce inflammation (Badescu et al. 2015). In folk medicine, the fruits, which contain pectins and organic acid, are used as a laxative (Uncini et al. 2005). The leaves are externally applied for inflammations, burns, wounds, bruises, eczema or conjunctivitis (Uncini et al. 2005, Laffita, Castillo 2011). Owing to the content of mucilage, the flowers show antitussive activity and a slightly laxative effect. The presence of minerals as well as ursolic acid and oleanolic acid accounts for their diuretic effect. Moreover, the flowers show anti-inflammatory, antioxidative and immunostimulatory activity due to the presence of glycosides, flavonoids and organic acids. They are used for eye redness or gastro-intestinal disturbances, and are able to reduce the level of certain genotoxins; this makes

them potentially useful in suppressing the growth of some tumors (Uncini et al. 2005, Dawidowicz et al. 2006, Porter, Bode 2017). In *in vitro* experiments, aqueous extracts of black elder flowers showed an effect on the blood glucose level (Gray et al. 2000), as well as anti-inflammatory (Harokopakis et al. 2006) and antibacterial activity (Izzo et al. 1995, Matte, Mata 2015). Studies on black elder fruit extracts showed their positive effect on the auto-immunological system (Barak et al. 2001), and anti-influenza (Torobian et al. 2019), antiviral (Zakay-Rones et al. 1995), antibacterial activity (Chatterjee et al. 2004) and antioxidative activity (Pool-Zobel et al. 1999). Alcoholic extracts of black elder fruits and leaves show anti-toxoplasma activity (Daryani et al. 2015).

The objective of the present study was to determine selected chemical elements, total polyphenolic compounds and antioxidative activity (DPPH and FRAP) in aqueous extracts of black elder-based materials. Based on the collected results, statistical analyses based on the Spearman's analysis and cluster analysis were performed.

MATERIAL AND METHOD

The test material included the flowers (SFL), leaves (SFO) and fruits (SFR) of *Sambucus nigra*, originating from eight locations (Table 1). Samples

Table 1

Characterization of the sampling sites

Sample number	Place of harvesting (province)	Description of the area
1	Chrzypsko (Wielkopolska)	agricultural land, off a rye field
2	Puszcza Notecka, near Kwiejce (Wielkopolska)	forest margin, rather far from homesteads, some 50 m off a field road
3	Września (Wielkopolska)	sewage plant area
4	Poznań (Wielkopolska)	roadside, heavy traffic (Lutycka Street)
5	Sokołowo (Wielkopolska)	near a field, 1 km off a national road (DK 15)
6	Tuczno (Wielkopolska)	near meadows, some 50 m off a field road
7	Witnica area, near Gorzów Wielkopolski (Lubuskie)	area with very low automobile traffic
8	Suchy Las (Wielkopolska)	area with very low automobile traffic, off a rye field and near the Selective Waste Collection Point

of elderberry leaves and flowers were collected in June, and fruit samples in September 2017 in the amount of about 1 kilogram. They were dried in a dark and airy place. The research was conducted in November 2017. The materials were powdered and sieved (mesh 0.2 mm) prior to obtaining their extracts. Approximately 1.00 g of the powdered material in 20 ml of distilled water was kept at a boiling temperature for 30 min, cooled down and filtered into a 100-ml measuring flask. The procedure was made in triplicate and water was added to the mark.

Determination of total polyphenolics using the Folin-Ciocalteu (FC) reagent

An aqueous stock solution of caffeic acid (200 μg ml^{-1}) was obtained and solutions containing 0.01, 0.03, 0.04, 0.06, 0.07, 0.08, 0.09, 0.10, 0.125 mg of caffeic acid in 1 ml were made using the serial dilution method. Each of the test tubes containing water (0.74 ml) and the standard solution or test solution (0.01 ml) was filled with 0.05 ml of the FC reagent and, after the lapse of 2 min, with 0.20 ml of a 20% sodium carbonate solution.

A reference solution was obtained by combining the above reagents at same time intervals, with the exception of the caffeic acid solution (0.75 ml of distilled water, 0.05 of ml FC reagent and, after the lapse of 2 min, with 0.20 ml of a 20% solution Na_2CO_3). The solution was mixed and, after 30 min, the absorbances of the resulting colored solutions were measured at the wavelength λ = 760 nm. Five assays were performed for each solution or nine for the test solution.

The standard curve A = a C + b was determined, where: A – absorbance, C – concentration, a, b – standard curve coefficients. The absorbance of the mixture of reagents was determined and, after substituting 0.01 ml of the standard with the test extract, the total content of polyphenols in the test sample was found.

Determination of antioxidative activity using the radical DPPH

To calculate the IC50 coefficient, serial dilutions were made from each extract. Six dilutions were made for each of the respective extracts.

To perform the assay, 2-ml Eppendorf test tubes were filled with 0.1 ml of the respective solutions of gallic acid or test extract and 0.7 ml of DPPH solution. This procedure was performed five times for each dilution. After adding DPPH, the solutions were closed in a light-protective cassette and placed on a shaker. After 30 min, a spectrophotometric measurement was performed at a wavelength λ = 515 nm. The reference sample was water and methanol (0.1 ml and 0.7 ml, respectively). Before proceeding to the absorbance measurements for the gallic acid solutions, absorbance of the DPPH solution (0.1 ml of deionized water and 0.7 ml of DPPH solution) was measured in five repetitions.

Determination of total activity of iron(III) ions reduction by FRAP

A series of dilutions was prepared from the extracts of the raw materials obtained from *Sambucus nigra*. Five repetitions were made for each prepared solution. Eppendorf test tubes (2 ml) were filled each with 0.1 ml of the analyte and 1.5 ml of the FRAP mixture. They were incubated on a shaker for 30 min at a temp. of 37°C and absorbance was measured for the wavelength λ = 593 nm. The curve equation A = aC + b was determined, based on which the concentration was calculated for the absorbance A = 0.500.

Determination of chemical elements

Selected chemical elements were determined using ICP-OES. The test samples of the fruiting bodies were mineralized using a Mars 5 device from CEM Corporation, the USA, and 10 ml of concentrated HNO_3 Ultranal; deionized water was added to obtain a volume of 15 ml. The mineralization parameters were as follows: max. temp. 210°C, maintenance time 10 min; power 600 W, pressure 195 PSI, and temp. rise time 20 minutes.

The test samples were analyzed to detect aluminum, boron, barium, calcium, chromium, copper, iron, potassium, magnesium, manganese, molybdenum, sodium, nickel, cadmium, lead, vanadium, phosphorus, rubidium, silicon, strontium, titanium and zinc.

The concentrations given for the respective elements are mean values from six determinations.

Statistical analysis

The correlation coefficients are significant for $p<0.05$. The calculations were made using Statistica 10.

It was assumed that the variables: are not correlated $R=0$, weakly correlated $0<R<0.5$, highly correlated $0.5\leq R<0.7$, very highly correlated $0.7\leq R<0.9$, nearly fully correlated $0.9\leq R<1$, fully correlated $R=1$.

To illustrate the 'data structure' for the content of selected chemical elements, total polyphenols and antioxidative activity, all the results of determinations were transformed into standardized values according to the equation:

$(x_i - x_m)$ / SD, wherein: x_i – value of single result, x_m – mean value of a given parameter, SD – standard deviation of the parameter of interest.

Such transformations enabled us to obtain the results in a dimensionless form before proceeding to a cluster analysis.

The analysis comprises several different classification algorithms and may be used for detecting structures in data sets, without interpreting or explaining any relationships. The cluster analysis is not a statistical test: rather, it is a set of different algorithms which 'arrange objects in clusters'. Such grouping is used in many research areas. If some data have an explicit

'structure', i.e., there are clusters of subjects which are similar to one another, then, more often than not, the structure will be reflected in a hierarchical tree as separate branches. The method of agglomeration enables detection of clusters (branches) and their interpretation. To form clusters, the measures of discrepancy or distance between objects are used. The distance that is most often selected is the Euclidean distance, that is, the metric distance in a multidimensional space. It is calculated from the formula:

$$\text{distance}(x,y) = \{\sum_i (x_i - y_i)^2\}^{\frac{1}{2}},$$

The calculations were performed using Statistica ver. 10.

RESULTS AND DISCUSSION

Tables 2, 3 and 4 show the results of determinations of light metals, heavy metals, and non-metals and metalloids, respectively, with standard deviation. Table 5 shows the results of determinations of total polyphenols

Table 2

Results of determinations of light metals in flowers, leaves and fruits of *S. nigra*

Average value	K		Na		Ca		Mg		Al		Ba		Rb		Sr	
	ppm	SD	ppm	SD	ppm	SD	ppm	SD	ppm	SD	ppm	SD	ppm	SD	ppm	SD
SFL	25167	2808	27.1	13.4	4995	1443	4290	519	26.8	13.7	5.95	2.75	18.3	7.2	9.64	9.16
SFO	28850	4638	22.1	27.0	8377	2060	4899	1123	56.3	15.9	12.8	7.1	11.0	4.9	16.6	10.9
SFR	13532	3196	18.6	5.4	3615	1352	2167	278	8.65	2.97	4.93	2.59	8.92	5.06	7.84	5.99

ppm – parts per million (concentration), SD – Standard Deviation

Table 3

Results of determinations of heavy metals in flowers, leaves and fruits of *S. nigra*

Average value	Cr		Cu		Fe		Mn		Mo		Ni		Ti		Zn	
	ppm	SD	ppm	SD	ppm	SD	ppm	SD	ppm	SD	ppm	SD	ppm	SD	ppm	SD
SFL	0.24	0.12	10.45	3.31	64.36	29.19	52.4	29.4	0.90	0.31	1.65	0.82	1.17	1.29	33.2	5.9
SFO	0.30	0.14	5.38	1.89	106.93	25.48	104.4	79.3	1.13	0.61	1.87	1.03	1.75	0.63	24.4	8.7
SFR	0.13	0.04	4.42	1.75	37.10	6.20	< 1.0		0.49	0.17	0.57	0.32	0.20	0.05	10.7	3.5

Table 4

Results of determinations of metalloids and non-metals in flowers, leaves and fruits of *S. nigra*

Average value	B		P		Si	
	ppm	SD	ppm	SD	ppm	SD
SFL	26.9	4.8	5713	590	54.4	14.6
SFO	30.1	10.7	3455	750	86.3	28.1
SFR	16.8	1.2	2998	697	65.6	19.6

Table 5

Results of determinations of total polyphenols and antioxidative activity

Average value	Total of polyphenols (%)	IC50 (DPPH)	IC0.5 (FRAP)
SFL	5.33±1.28	0.69±0.17	1.04±0.19
SFO	5.75±0.96	0.59±0.10	0.91±0.15
SFR	3.13±1.68	0.95±0.72	3.15±2.63

and antioxidative activity as expressed by coefficient IC50 for determinations with DPPH radical and coefficient IC0.5 for determinations with FRAP.

The mean content of all the chemical elements, with the exception of silicon, was the lowest in the fruits of *Sambucus nigra*. The lowest silicon content was found in the flowers SLF(Si)=54.4 ppm and the highest was found in the leaves SFO(Si)=86.3 ppm. The highest difference in concentrations (nearly 9-fold) was recorded for titanium SFO(Ti)=1.75 ppm, SFR(Ti)=0.20 ppm, 6.5-fold for aluminum SFO(Al)=56.0 ppm, SFR(Al)=8.65 ppm, 3.3-fold for nickel SFO(Ni)=1.87 ppm, SFR(Ni)=0.57 ppm, 3.1-fold for zinc SFL(Zn)=33.2 ppm; SFR(Zn)=10.7 ppm, 2.9-fold for iron SFO(Fe)=106.93 ppm, SFR(Fe)=37.10 ppm, more than 2-fold for chromium, copper, molybdenum, potassium, calcium, magnesium, barium, rubidium, strontium, and nearly 2-fold for sodium, boron, and phosphorus.

Mean levels of total polyphenols in the leaves and flowers were comparable, 5.75 and 5.33%, respectively, in terms of caffeic acid. The fruits had a lower content of total polyphenols – 3.13%. The highest antioxidative activity was observed in the leaves IC50(DPPH)=0.59 mg ml^{-1}, IC0.5(FRAP)=0,91mg ml^{-1} and the lowest in the fruits IC50(DPPH)=0.95 mg ml^{-1}, IC0.5(FRAP)=3.15 mg ml^{-1}. The largest difference between total polyphenols and antioxidative activity was recorded for the fruits: coefficient of variation being 53.7, 75.9 (DPPH), 83.5 (FRAP), respectively. For the leaves and flowers, the coefficient of variation was in the range 16.1-24.1.

Results of the Spearman's rank correlation analysis applied to total polyphenolic compounds and antioxidative activity, as expressed by IC coefficients with selected chemical elements, are shown in Tables 6, 7, 8 for the flowers, leaves and fruits, respectively.

The Spearman's rank correlation analysis has confirmed the correlation between the content of polyphenolic compounds and antioxidative activity as determined by the radical DPPH and reagent FRAP. The correlation coefficients were in the range of very high or nearly full correlation (R from –0.7619 to –0.9762). Moreover, an analysis of the results of determinations for the flowers showed a high correlation between total polyphenols and antioxidative activity (DPPH and FRAP) with phosphorus, and antioxidative activity (DPPH and FRAP) with aluminum, silicon and magnesium. For the leaves, the authors found a high or very high correlation between total polyphenolic compounds and antioxidative activity (DPPH and FRAP)

Table 6

The Spearman's rank correlation results for the flowers of *S. nigra* (p<0.0500)

Total of polyphenols (%)	Total of polyphenols (%)	DPPH	FRAP	Al.	B	Ba	Cr	Cu	Fe	K	Na	P	Rb	Si	Sr	Zn	Ca	Mg
	1.000	-0.976	-0.786	-0.476	0.024	0.048	-0.238	-0.381	-0.214	0.119	-0.262	-0.524	0.333	-0.476	-0.310	0.024	-0.119	-0.452
DPPH	-0.976	1.000	0.857	0.500	0.048	-0.024	0.214	0.452	0.238	-0.214	0.238	0.548	-0.190	0.500	0.333	0.071	0.190	0.571
FRAP	-0.786	0.857	1.000	0.786	0.405	0.119	0.500	0.524	0.571	-0.143	0.500	0.690	-0.167	0.786	0.548	0.381	0.500	0.643
Al.	-0.476	0.500	0.786	1.000	0.667	0.333	0.810	0.619	0.833	0.214	0.881	0.810	-0.071	1.000	0.690	0.429	0.595	0.500
B	0.024	0.048	0.405	0.667	1.000	0.119	0.786	0.690	0.857	0.238	0.667	0.810	0.190	0.667	0.905	0.905	0.810	0.548
Ba	0.048	-0.024	0.119	0.333	0.119	1.000	0.048	0.381	0.071	0.643	0.595	0.095	0.333	0.333	-0.119	-0.024	-0.214	0.452
Cr	-0.238	0.214	0.500	0.810	0.786	0.048	1.000	0.429	0.976	0.405	0.786	0.762	-0.238	0.810	0.833	0.643	0.833	0.262
Cu	-0.381	0.452	0.524	0.619	0.690	0.381	0.429	1.000	0.500	0.190	0.643	0.857	0.452	0.619	0.690	0.548	0.357	0.881
Fe	-0.214	0.238	0.571	0.833	0.857	0.071	0.976	0.500	1.000	0.310	0.762	0.786	-0.095	0.833	0.857	0.738	0.905	0.381
K	0.119	-0.214	-0.143	0.214	0.238	0.643	0.405	0.190	0.310	1.000	0.619	0.167	-0.095	0.214	0.143	0.167	0.048	0.143
Na	-0.262	0.238	0.500	0.881	0.667	0.595	0.786	0.643	0.762	0.619	1.000	0.738	-0.024	0.881	0.619	0.405	0.429	0.476
P	-0.524	0.548	0.690	0.810	0.810	0.095	0.762	0.857	0.786	0.167	0.738	1.000	0.024	0.810	0.929	0.667	0.643	0.690
Rb	0.333	-0.190	-0.167	-0.071	0.190	0.333	-0.238	0.452	-0.095	-0.095	-0.024	0.024	1.000	-0.071	-0.071	0.119	-0.024	0.429
Si	-0.476	0.500	0.786	1.000	0.667	0.333	0.810	0.619	0.833	0.214	0.881	0.810	-0.071	1.000	0.690	0.429	0.595	0.500
Sr	-0.310	0.333	0.548	0.690	0.905	-0.119	0.833	0.690	0.857	0.143	0.619	0.929	-0.071	0.690	1.000	0.833	0.810	0.524
Zn	0.024	0.071	0.381	0.429	0.905	-0.024	0.643	0.548	0.738	0.167	0.405	0.667	0.119	0.429	0.833	1.000	0.833	0.571
Ca	-0.119	0.190	0.500	0.595	0.810	-0.214	0.833	0.357	0.905	0.048	0.429	0.643	-0.024	0.595	0.810	0.833	1.000	0.333
Mg	-0.452	0.571	0.643	0.500	0.548	0.452	0.262	0.881	0.381	0.143	0.476	0.690	0.429	0.500	0.524	0.571	0.333	1.000

Table 7

The Spearman's rank correlation results for the leaves of *S. nigra* (p<0.0500)

Total of polyphenols (%)	Total of polyphenols (%)	DPPH	FRAP	Al.	B	Ba	Cr	Cu	Fe	K	Mn	Na	P	Rb	Si	Sr	Ti	Zn	Ca	Mg
	1.000	-0.833	-0.810	-0.119	-0.143	-0.190	-0.500	-0.667	-0.548	-0.619	-0.238	-0.071	-0.381	0.000	0.452	0.000	-0.405	-0.452	-0.310	-0.619
DPPH	-0.833	1.000	0.929	0.357	0.024	0.214	0.762	0.667	0.667	0.548	0.095	0.143	0.667	0.095	-0.357	0.262	0.786	0.667	0.571	0.881
FRAP	-0.810	0.929	1.000	0.357	0.048	0.190	0.786	0.667	0.833	0.333	-0.143	0.262	0.429	0.190	-0.595	0.429	0.643	0.595	0.738	0.905
Al.	-0.119	0.357	0.357	1.000	0.595	0.429	0.595	0.000	0.595	0.119	0.071	-0.595	0.452	0.500	0.214	0.619	0.714	0.238	0.333	0.310
B	-0.143	0.024	0.048	0.595	1.000	-0.048	0.119	-0.190	0.167	-0.190	0.095	-0.738	0.048	0.333	0.310	0.286	0.167	0.262	-0.119	-0.048
Ba	-0.190	0.214	0.190	0.429	-0.048	1.000	0.071	0.571	0.500	0.429	0.619	-0.024	0.548	0.667	0.238	0.119	0.429	-0.381	0.286	0.238
Cr	-0.500	0.762	0.786	0.595	0.119	0.071	1.000	0.214	0.619	0.357	-0.357	-0.119	0.381	-0.071	-0.381	0.714	0.690	0.714	0.690	0.690
Cu	-0.667	0.667	0.667	0.000	-0.190	0.571	0.214	1.000	0.595	0.357	0.381	0.524	0.595	0.476	-0.214	0.048	0.452	0.167	0.571	0.762
Fe	-0.548	0.667	0.833	0.595	0.167	0.500	0.619	0.595	1.000	0.119	-0.071	0.167	0.333	0.595	-0.476	0.524	0.595	0.190	0.738	0.738
K	-0.619	0.548	0.333	0.119	-0.190	0.429	0.357	0.357	0.119	1.000	0.548	-0.190	0.524	-0.262	0.000	-0.214	0.405	0.119	-0.048	0.190
Mn	-0.238	0.095	-0.143	0.071	0.095	0.619	-0.357	0.381	-0.071	0.548	1.000	-0.238	0.571	0.333	0.571	-0.548	0.238	-0.333	-0.405	-0.095
Na	-0.071	0.143	0.262	-0.595	-0.738	-0.024	-0.119	0.524	0.167	-0.190	-0.238	1.000	-0.071	0.048	-0.524	-0.119	-0.143	-0.071	0.429	0.405
P	-0.381	0.667	0.429	0.452	0.048	0.548	0.381	0.595	0.333	0.524	0.571	-0.071	1.000	0.381	0.405	0.119	0.881	0.381	0.310	0.619
Rb	0.000	0.095	0.190	0.500	0.333	0.667	-0.071	0.476	0.595	-0.262	0.333	0.048	0.381	1.000	0.238	0.262	0.381	-0.238	0.357	0.333
Si	0.452	-0.357	-0.595	0.214	0.310	0.238	-0.381	-0.214	-0.476	0.000	0.571	-0.524	0.405	0.238	1.000	-0.143	0.143	-0.143	-0.429	-0.357
Sr	0.000	0.262	0.429	0.619	0.286	0.119	0.714	0.048	0.524	-0.214	-0.548	-0.119	0.119	0.262	-0.143	1.000	0.405	0.476	0.786	0.476
Ti	-0.405	0.786	0.643	0.714	0.167	0.429	0.690	0.452	0.595	0.405	0.238	-0.143	0.881	0.381	0.143	0.405	1.000	0.524	0.500	0.738
Zn	-0.452	0.667	0.595	0.238	0.262	-0.381	0.714	0.167	0.190	0.119	-0.333	-0.071	0.381	-0.238	-0.143	0.476	0.524	1.000	0.452	0.643
Ca	-0.310	0.571	0.738	0.333	-0.119	0.286	0.690	0.571	0.738	-0.048	-0.405	0.429	0.310	0.357	-0.429	0.786	0.500	0.452	1.000	0.833
Mg	-0.619	0.881	0.905	0.310	-0.048	0.238	0.690	0.762	0.738	0.190	-0.095	0.405	0.619	0.333	-0.357	0.476	0.738	0.643	0.833	1.000

Table 8

The Spearman's rank correlation results for the fruits of *S. nigra* (p<0.0500)

Total of polyphenols (%)	Total of polyphenols (%)	DPPH	FRAP	Al.	B	Ba	Cr	Cu	Fe	K	Na	P	Rb	Si	Sr	Zn	Ca	Mg
	1.000	-0.762	-0.810	0.143	0.167	-0.095	0.500	-0.452	-0.214	0.571	0.548	0.071	0.357	0.476	0.095	-0.119	0.119	0.190
DPPH	-0.762	1.000	0.952	-0.190	0.167	0.190	-0.214	0.381	0.214	-0.690	-0.643	0.190	-0.452	-0.452	0.024	0.143	0.071	0.071
FRAP	-0.810	0.952	1.000	-0.143	-0.024	0.333	-0.119	0.405	0.262	-0.524	-0.452	0.262	-0.405	-0.571	-0.048	0.071	-0.119	0.000
Al.	0.143	-0.190	-0.143	1.000	0.548	0.429	0.452	0.405	0.452	-0.143	0.500	0.357	0.071	0.762	-0.143	0.357	-0.405	0.190
B	0.167	0.167	-0.024	0.548	1.000	0.071	0.167	0.048	0.238	-0.619	-0.167	0.048	0.095	0.714	-0.071	0.238	0.000	0.167
Ba	-0.095	0.190	0.333	0.429	0.071	1.000	0.667	0.024	0.643	0.119	0.524	0.667	-0.429	0.048	-0.095	0.048	-0.167	0.286
Cr	0.500	-0.214	-0.119	0.452	0.167	0.667	1.000	0.024	0.595	0.286	0.619	0.810	0.000	0.286	0.333	0.286	0.000	0.595
Cu	-0.452	0.381	0.405	0.405	0.048	0.024	0.024	1.000	0.405	-0.452	-0.214	0.476	-0.310	0.095	0.405	0.762	0.048	0.452
Fe	-0.214	0.214	0.262	0.452	0.238	0.643	0.595	0.405	1.000	-0.357	0.071	0.738	-0.286	0.262	0.524	0.690	0.190	0.667
K	0.571	-0.690	-0.524	-0.143	-0.619	0.119	0.286	-0.452	-0.357	1.000	0.762	0.000	0.143	-0.167	-0.190	-0.476	-0.167	-0.167
Na	0.548	-0.643	-0.452	0.500	-0.167	0.524	0.619	-0.214	0.071	0.762	1.000	0.286	0.143	0.286	-0.286	-0.238	-0.429	-0.024
P	0.071	0.190	0.262	0.357	0.048	0.667	0.810	0.476	0.738	0.000	0.286	1.000	-0.452	0.095	0.571	0.619	0.262	0.833
Rb	0.357	-0.452	-0.405	0.071	0.095	-0.429	0.000	-0.310	-0.286	0.143	0.143	-0.452	1.000	0.119	-0.214	-0.310	-0.548	-0.476
Si	0.476	-0.452	-0.571	0.762	0.714	0.048	0.286	0.095	0.262	-0.167	0.286	0.095	0.119	1.000	0.048	0.381	0.048	0.286
Sr	0.095	0.024	-0.048	-0.143	-0.071	-0.095	0.333	0.405	0.524	-0.190	-0.286	0.571	-0.214	0.048	1.000	0.810	0.738	0.857
Zn	-0.119	0.143	0.071	0.357	0.238	0.048	0.286	0.762	0.690	-0.476	-0.238	0.619	-0.310	0.381	0.810	1.000	0.500	0.833
Ca	0.119	0.071	-0.119	-0.405	0.000	-0.167	0.000	0.048	0.190	-0.167	-0.429	0.262	-0.548	0.048	0.738	0.500	1.000	0.690
Mg	0.190	0.071	0.000	0.190	0.167	0.286	0.595	0.452	0.667	-0.167	-0.024	0.833	-0.476	0.286	0.857	0.833	0.690	1.000

with chromium, copper, iron and magnesium, and high or very high antioxidative activity (DPPH and FRAP) with titanium, zinc, and calcium. In the case of fruits, the authors found a high correlation between the total polyphenols and antioxidative activity (DPPH and FRAP) with potassium.

The results of the cluster analysis are shown in Figures 1, 2, 3 for the flowers, leaves and fruits, respectively, of *S. nigra*.

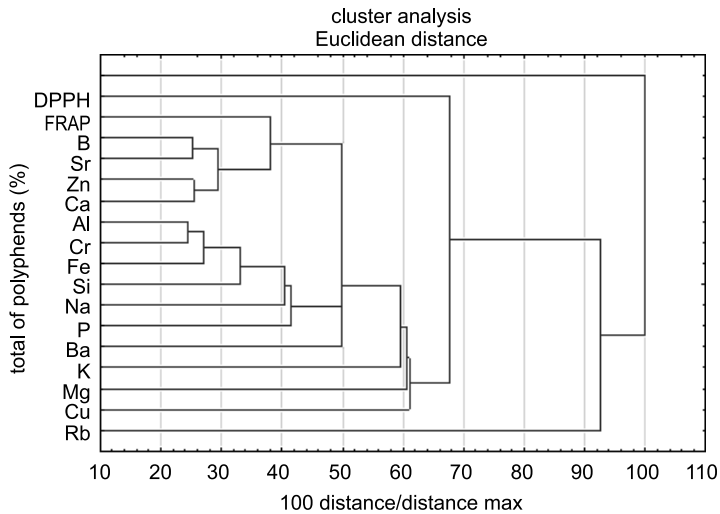

Fig. 1. Cluster analysis of the determined parameters for the flowers of *S. nigra*

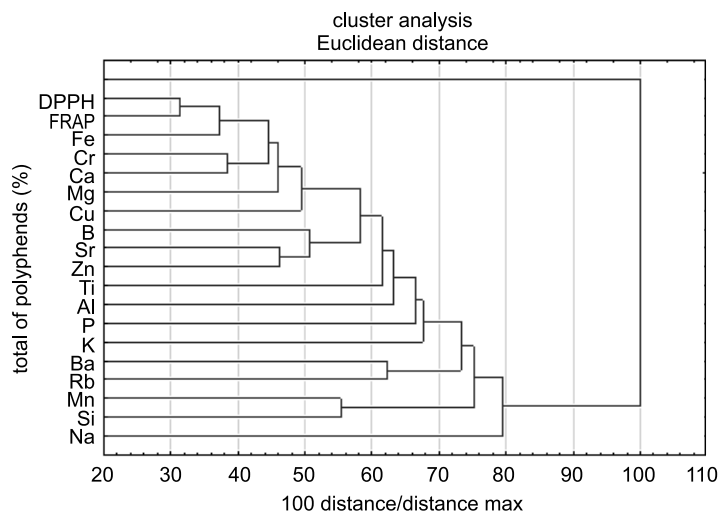

Fig. 2. Cluster analysis of the determined parameters for the leaves of *S. nigra*

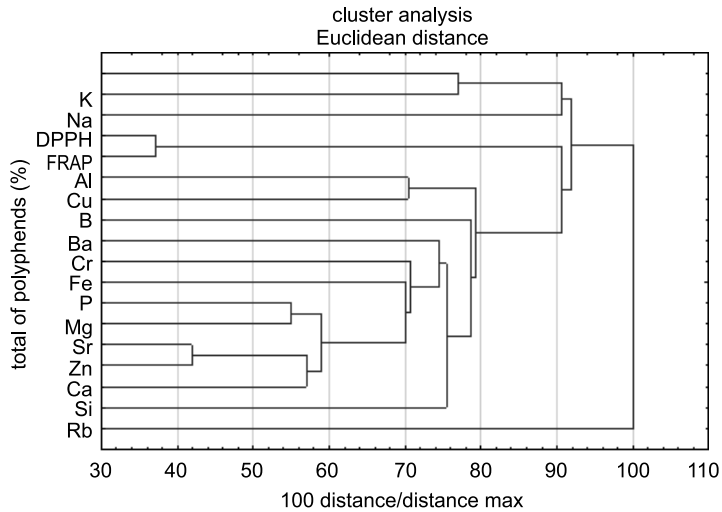

Fig. 3. Cluster analysis of the determined parameters for the fruits of *S. nigra*

For the flowers, the cluster analysis showed that antioxidative activity (FRAP) had the closest correlation with boron, strontium, zinc, and calcium. For the leaves, antioxidative activity had a direct and least distant correlation with iron, followed by chromium, calcium, and then with magnesium and the other chemical elements. The total content of polyphenols was correlated with all the parameters. For the fruits, the authors found a direct correlation of the total content of polyphenols with potassium, and then with sodium, whereas antioxidative activity (DPPH and FRAP) was most closely correlated with aluminum and copper.

Earlier studies on the correlation of elements, antioxidant activity, sum of flavonoids and sum of phenolic acids in raw materials obtained from elderberry showed that the sum of flavonoids was linearly correlated with aluminum, barium, calcium, magnesium and iron, while the sum of phenolic acids – with aluminum, calcium, and magnesium, iron, manganese and lead. The cluster analysis showed that the sum of polyphenols and the sum of phenolic acids had direct and the least distant correlations with manganese, while flavonoids – with magnesium, and then with other elements (Szymański et al. 2013).

A similar study carried out on yarrow herb samples showed that polyphenolic compounds were strongly linearly correlated with aluminum, boron, chromium, iron and molybdenum, and the content of phenolic acids – with boron, barium, iron and titanium. The cluster analysis showed that the sum of phenolic acids was most closely related to the sum of polyphenolic compounds. The sum of polyphenols, including phenolic acids, had direct and the least distant correlations with barium, and only then with other elements (Szymański et al. 2014).

A study on fruiting bodies of polyporoid fungi obtained from birch (*Inonotus obliquus*, *Fomes fomentarius*, *Piptoporus betulinus*, *Trametes versicolor*, *Pycnoporus cinnabarinus* and *Daedaleopsis confragosa*) involved an attempt of making a rank correlation between the Spearman's correlations and the research results, which showed a linear correlation of the ABTS) and the presence of such elements as copper and potassium. The IC50 coefficient turned out to be correlated with the content of potassium, aluminum, rubidium, sulfur and iron. The cluster analysis showed that the sum of polyphenols was most closely related to rubidium and potassium, and then to manganese. On the other hand, the IC50 coefficient was related to aluminum, iron and sulfur, and only then to the sum of polyphenols (Szymański et al. 2019).

CONCLUSION

These results have shown that the lowest mean content of all the chemical elements determined in this study, with the exception of silicon, was found in the fruits of *S. nigra*. The lowest silicon content was found in the flowers and the highest – in the leaves. The leaves and the flowers had comparable mean content of total polyphenols and the total polyphenol value in the fruits was lower. The Spearman's rank correlation analysis confirmed a correlation between the content of polyphenolic compounds and antioxidative activity. An analysis of the results of determinations in the flowers showed a high correlation between the total polyphenols, and antioxidative activity with phosphorus, and antioxidative activity with aluminum, silicon, and magnesium. In the leaves, the correlation of total polyphenolic compounds and antioxidative activity was high or very high with chromium, copper, iron and magnesium, and antioxidative activity was high or very high with titanium, zinc and calcium. In the case of fruits, a high correlation between total polyphenols and antioxidative activity was found with potassium.

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