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BIOACTIVE COMPOUNDS AND ANTIOXIDANT PROPERTIES OF BLACK ELDERBERRY (*Sambucus nigra* L.)

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ABSTRACT

Elderberry (*Sambucus nigra* L.) raw materials are a rich and valuable source of bioactive substances and popular herbal medicine. The research aimed to determine the content of polyphenolic compounds and pigments in leaves, flowers, and wild elderberry fruits and evaluate the antioxidant activity. The raw material was collected in various stages of development, dried in natural conditions and at a temperature of 45/60°C. The presence of polyphenolic compounds was demonstrated: phenolic acids, flavonoids, anthocyanins, and chlorophyll and carotenoids in all tested raw materials. The elderberry flower turned out to be the richest source of total polyphenols (10.04%) and phenolic acids (2.90%), leaf – flavonoids (1.48%), chlorophylls and carotenoids (931.7 and 133.77 mg · 100 g^{-1} DM, respectively), and the fruit – anthocyanins (0.29 g · 100 g^{-1} DM). Elderberry extracts were characterised by high antioxidant activity: *Sambuci flos* > *Sambuci fructus* > *Sambuci folium*. Fully ripe elderberry fruit dried at a temperature of 60°C contained finally less moisture and more flavonoids and chlorophyll than harvested at the beginning of ripening.

Key words: medicinal plant, plant development phase, drying conditions, polyphenols, chlorophyll, carotenoids

INTRODUCTION

Elderberry (*Sambucus nigra* L., Adoxaceae) is one of the most commonly used medicinal plants in the world [Bartak et al. 2020, Ran et al. 2020, Marțiș (Petruț) et al. 2021]. All parts of the plant (leaves, fruits, inflorescences, roots, shoots, bark) have a long history of herbal medicine and many culinary uses. In folk medicine, elderberry was used primarily as a diaphoretic, antipyretic and diuretic agent [Petruț et al. 2017]. The traditional use of *S. nigra* has been confirmed by scientific research [Torabian et al. 2019, Mahboubi 2021]. A much broader therapeutic effect of elderberry extracts has also been proven: antioxidant [Imenšek et al. 2021], anti-inflammatory [Mota et al. 2020], cytotoxic [Pereira et al. 2020], adaptogenic [Neekhra et al. 2021], antiallergic [Alrumaihi et al. 2020], immunomodulatory and antiviral [Schön et al. 2021], antibacterial, photoprotective, antidepressant, antidiabetic, antiatherosclerotic and hypoglycaemic, and lowering the concentration of fat and lipids [Marțiș (Petruț) et al. 2021]. Consumption of 100 g of elderberry fruit covers about 0.2–30% of the recommended intake of calcium, potassium, sodium, phosphorus, magnesium, manganese, iron, zinc, and copper for women and men [Diviš et al. 2015].

Elderberry raw materials are a valuable source of bioactive substances: primary metabolites (sugars,

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protein, organic acids, dyes), secondary metabolites (polyphenols, essential oil) and other components (vitamins, minerals, cytokinins) [Miraj 2016, Petruț et al. 2017, Młynarczyk et al. 2018, Youdim et al. 2000, Ferreira et al. 2020]. The chemical profile of elderberry flowers and fruits changes under the influence of the harvest date, drying temperature and other factors [Bajer et al. 2017, Sedláčková et al. 2018, Marțiș (Petruț) et al. 2021]. Thomas et al. [2008] emphasise the influence of ontogenesis on the level of rutin and chlorogenic acid in leaves and shoots of *S. nigra*. The studies by Csorba et al. [2020] prove that the chemical parameters of elderberry are primarily determined by the genotype, although climatic conditions also influence the changes in these contents. The antioxidant activity of elderberry flower and fruit extracts probably results from the presence of polyphenolic compounds, mainly flavonols, phenolic acids and anthocyanins [Duymus et al. 2014, Viapiana and Wesołowski 2017, Imenšek et al. 2021]. Güzelmeriç et al. [2021], emphasising the growing demand for herbal supplements containing elderberries, indicate the need to develop a monograph specifying the chemical composition and quality criteria of elderberry raw materials. Pliszka [2020] notes that to obtain valuable elderberry products, the selection of varieties should be considered, and the processing technology should be developed. Sedláčková et al. [2018] document that it is possible to select genotypes with the required economic characteristics for practical use in phytotherapy in natural elderberry populations. There is little information in the available literature on the changes in the content of active ingredients in individual elderberry organs during the ontogenesis and after harvesting the raw material. A relatively detailed assessment of the influence of the genotype and maturity stage on phenolic compounds, antioxidant capacity, and elderberry's mineral profile was carried out by Marțiș (Petruț) et al. [2021]. The authors pointed to the high concentration of quercetin-3-rutoside, defined as the critical compound in the research. This study aimed to determine the content of polyphenolic compounds and biologically active pigments in elderberry leaves, flowers and fruits, as well as to assess the antioxidant activity of individual raw materials under the influence of ontogenetic variability (type of raw material and harvest phase) and post-harvest variability (drying method).

MATERIAL AND METHODS

Research material

The research material consisted of leaves (*Sambuci folium*), flowers (*Sambuci flos*) and fruit (*Sambuci fructus*) of elderberry (*Sambucus nigra* L.) obtained from the natural state (Pilaszkowice, Lublin Province, 22°85'19"E, 50°99'75"N – the area away from the road, ecologically clean). The area from which the raw material was obtained is located within the boundaries of the Nałęczów – Lublin mesoclimatic unit. The main factor influencing the changes in the weather in this area are the atmospheric fronts. The thermal and humidity conditions in 2020 are presented in Table 1. The collection of raw materials was carried out from May to September 2020 in the following stages of development: leaves – before flowering, at the beginning of flowering (up to 10% of developed flowers in the inflorescence) during fruiting (more than 50% well-colored fruits in the infructescence); flowers – at the beginning of flowering (up to 10% of developed flowers in the inflorescence), in full bloom (more than 50% of the developed flowers in the inflorescence), and fruit – at the beginning of maturation (up to 10% of the well-colored fruit in the infructescence) and full maturity (all fruit completely ripe and black). Plant material was collected from 25 randomly selected plants, and 10 organs from each plant were used for research. Healthy, well-developed leaves were harvested from the center of the plant. The plant samples was dried in two ways: (1) in natural conditions, under a roof, out of sunlight, at a temperature of about 20–25°C (leaves and flowers); and (2) in the Concept SO-1063 fruit and mushroom dryer (Wrocław, Poland), at 45°C (leaves and flowers) and 60°C (fruit). The fruit has not been dried under natural conditions due to the relatively quick moulding and loss of quality.

Methods of chemical analyses and measurement of antioxidant activity

Moisture content. The moisture content was determined by weighing a sample of 1 g of the raw material in a moisture analyser (RADWAG MA 50.R, Radom, Poland) and then drying it at 105°C. The determination was repeated three times.

Samples of air-dried leaves, inflorescences and fruits were subjected to chemical analyses, determining

Table 1. Weather conditions in the research area in 2020

Month	Decade			Mean of I–III	Decade			∑ of I–III	Deade			∑ of I–III
	I	II	III		I	II	III		I	II	III	
	temperature (°C)				precipitation (mm)				insolation (h)			
I	– 9.0	–2.0	–9.7	–6.9	10.4	6.1	6.7	23.2	11.9	16.7	18.6	47.2
II	–5.6	–7.5	–5.4	–6.2	22.5	2.5	0	25.0	25.2	18.4	11.3	54.9
III	–1.9	1.8	4.5	1.5	0.6	4.4	1.6	6.6	18.6	51.7	66.7	137.0
IV	0.7	7.5	11.2	6.5	10.0	27.3	3.4	40.7	81.9	77.5	66.0	225.4
V	16.5	14.3	17.8	16.2	8.9	40.6	21.9	71.4	58.9	48.6	51.1	158.5
VI	19.1	17.0	16.1	17.4	2.6	15.3	21.7	39.6	58.6	54.8	50.3	163.7
VII	17.5	19.4	22.4	19.8	24.9	43.7	29.5	98.1	62.7	53.6	77.2	193.5
VIII	20.1	19.2	17.5	18.9	0.7	6.7	19.6	27.0	70.1	67.5	56.0	193.6
IX	12.3	13.9	14.2	13.5	2.7	14.1	12.2	29.0	43.7	73.0	48.4	165.2
X	9.8	5.3	1.1	5.4	26.0	11.7	12.4	50.1	33.1	8.3	17.0	58.4
XI	6.0	2.0	7.0	5.0	5.6	11.4	0	17.0	13.8	15.2	8.3	37.3
XII	1.2	0.7	–1.1	0.3	6.7	16.9	12.7	36.3	9.4	1.4	9.0	19.9

the content polyphenols, pigments and the antioxidant activity of the extracts. All reagents and solvents were analytical grade chemicals from POCH (Gliwice, Poland). All tested bioactive substances were determined in triplicate.

Polyphenols. The total content of polyphenolic compounds was determined spectrophotometrically using the Folin-Ciocalteu reagent according to the method of Singleton and Rossi [1965]. A 0.5 g sample of raw material was extracted with 50 ml of reflux distilled water in a boiling water bath for 1 hour. Four ml of distilled water, 1 ml of the obtained extract and 0.5 ml of Folin-Ciocalteu's reagent were added to 10 ml volumetric flasks. After 1 min, the mixture was supplemented with 2 ml of sodium carbonate and distilled water. The solution was left for 30 min without light. Then, the absorbance at $\lambda = 760$ nm was measured against the reference sample (reagents without the tested extract). Total of polyphenols content was calculated as gallic acid equivalents (mg GAE/100 g). All the procedures were performed in triplicate. The following equation was used to calculate the total sum of polyphenols (mg GAE/100 g): $0.0843 \times X + 0.0592$.

According to the Polish Pharmacopoeia V [1999], the phenolic acid content was determined by the Arnov method. After a reflux condenser, one gram of the ground raw material was extracted with 25 ml of methanol in a water bath. The operation was repeated three times. The extracts were filtered off and combined. The solvent was then distilled off under reduced pressure. Twenty ml of hot distilled water was added to the flask. The resulting solution was left in the refrigerator for 12 hours. The extracts were filtered into 100 ml volumetric flasks and made up to date with water. One ml of distilled water, 1 ml of the tested extract, 1 ml of 0.5 M HCl and 1 ml of Arnov reagent were placed in 10 ml measuring tubes. After 6 min, 1 ml of 1M NaOH was added and made up with distilled water. A reagent mixture without extract was used as a reference. The wavelength for measuring absorbance was 490 nm. Cafeic acid was used as a reference standard. To calculate the content of phenolic acids (%), the following formula was adopted:

$$X = \frac{0.35087 \times A}{m},$$

where: A – absorbance, m – sample weight in grams.

The content of flavonoids was determined according to the Polish Pharmacopoeia XI [2017]. 0.5 g of powdered raw material, 2 ml of 25% HCl and 1 ml of an aqueous solution of methenamine (5 g/l) were placed in a round bottom flask. The mixture was kept for 30 min in a boiling water bath under reflux. The

hydrolysate was then filtered through cotton wool into a 100 ml volumetric flask. The sediment and cotton wool were placed in the flask, treated with 20 ml of acetone, then refluxed again for 10 min. The extraction was repeated once more, filtering the successive extracts into a volumetric flask. The extracts were then made up to 100 ml with acetone. Twenty ml of the test solution were measured and placed in a separating funnel. Forty ml of distilled water was added. Then, it was extracted twice with 20 ml of ethyl acetate. The successive organic layers were filtered into a 50 ml volumetric flask through cotton wool and completed with ethyl acetate. After that, 10 ml of the solution obtained during the analytical procedure were treated with 2 ml of aluminium chloride methanol solution (20 g/l) and made up with a mixture of acetic acid (1.02 kg/l) with methanol in the proportion of 1 : 19 to 25 ml. Ten millilitres of a stock solution made up of a mixture of acetic acid with methanol to a volume of 25 ml was used as the reference solution. After 45 minutes, the absorbance of the solutions was measured at 425 nm, and the reference solution was used as a reference. The content of flavonoids (%) (as isoquinitroside) was calculated according to the formula:

$$X = \frac{A \times 1.25}{m},$$

where: A – absorbance, m – sample weight in grams.

Pigments (anthocyanins, chlorophyll, carotenoids)

Anthocyanins content. The determination of the content of anthocyanins was performed according to the Polish Pharmacopoeia IX [2011]. The fruits were crushed in a fine grinding mill (IKA MF 10, Shanghai, China) at 3000 rpm, leaves and flowers were ground in a mortar. Ninety-five ml of methanol was added to 5 g of the raw material, followed by mechanical stirring for 30 min. The solution was filtered into a 100 ml volumetric flask, and the filter was washed and made up to 100 ml with methanol. Next, a 50-fold dilution of this extract in a solution of 0.1% (v/v) hydrochloric acid in methanol was prepared. A solution of 0.1% (v/v) hydrochloric acid in methanol was used as a reference. A wavelength of 528 nm was used for the measurement of absorbance. The percentage of anthocyanins, expressed as cyanidinium 3-O-glucoside chloride, was calculated using the following formula:

$$X = \frac{A \times 5000}{718 \times m}$$

718 – specific absorption of cyanidinium 3-O-glucoside at wavelength λ = 528 nm, A – absorbance of the tested solution at the wavelength λ = 528 nm, m – mass of the test plant substance in grams.

Total chlorophyll and carotenoid content. Chlorophyll a (Cha), chlorophyll b (Chb), total chlorophylls (TCh) and total carotenoids (TCA) were determined according to the method described by Holm [1954] and Wettstein [1957]. For the extraction of pigments from leaves, flowers and fruits elderberry 0.2 g ±0.01 of the air dry plant material was weighed, and a total of 15 mL of acetone (p.a.) was added three times. After each addition of acetone, the samples were homogenized using a laboratory homogenizer (IKA, UltraTurrax T-18, Staufen, Germany). The final solution was filtered through Whatman filter paper and transferred to a 25 mL volumetric flask. The absorbance was measured spectrophotometrically (Spectrophotometr UV-Vis Hitachi U-2900, Kyoto, Japan) at three wavelengths, 662, 644 and 440 nm, using acetone as a blank. Holm–Wettstein equations were used to quantify each pigment (1), and the final content was expressed in mg/g.

$$Cha = 9.784 \times A_{662} - 0.990 \times A_{644}\ [mg/L];$$

$$Chb = 21.426 \times A_{644} - 4.65 \times A_{662}\ [mg/L];$$

$$TCh = 5.134 \times A_{662} + 20.436 \times A_{644}\ [mg/L]\ (1);$$

$$TCA = 4.695 \times A_{440} - 0.268 \times TCh\ [mg/L];$$

where: $A_{663.}$ indicates the absorbance value at 662 nm; A_{645} indicates the absorbance value at 644 nm; A_{470} indicates the absorbance value at 440 nm.

Antioxidant activity (DPPH•)

The antioxidant activity, expressed as % inhibition of DPPH radicals, was measured using the method of Yen and Chen [1995]. A reagent was prepared to contain a solution of DPPH radicals (2,2-diphenyl-1--picrylhydrazyl). The blank test was performed by placing 1 ml of distilled water, 3 ml of methanol and 1 ml of DPPH solution in a test tube. The test sample was obtained: 1 ml of diluted raw material sample (2 g of ground raw material per 25 ml of methanol),

3 ml of methanol and 1 ml of DPPH solution were mixed and measured. After 10 min, the absorbance was read at wavelength λ = 517, and methanol was used as a reference. Inhibition of free radical by DPPH in percent (%) was calculated using following formula: [Rossi et al. 2003]:

$$\text{DPPH•}\ (\%) = 100 - \frac{A}{B} \times 100$$

where: A – absorbance of the test sample, B – absorbance of the blank.

Statistical analysis

The results of the morphological and chemical analyzes were statistically processed using the analysis of variance method. Tukey's test was used to evaluate the differences, the confidence intervals were calculated at the significance level of 0.05. All calculations and analyses were performed using the Statistica 13 program (TIBCO, Palo Alto, California, United States).

RESULTS AND DISCUSSION

Bioactive compounds of elderberry raw materials

The presence of polyphenolic compounds: phenolic acids, flavonoids, and anthocyanins were demonstrated in all examined elderberry organs, the flower turned out to be the richest source of total polyphenols and phenolic acids, the leaf contained the most flavonoids, and the anthocyanin fruit (Tab. 2). El-Hawary et al. [2020] argue that elderberry leaves and flowers contain significant amounts of phenolic compounds and flavonoids, while shoots are much less abundant in these components. The authors attribute the cytotoxic potential of the flowers, leaves, and stems of *Sambucus nigra* to the synergistic action of phenolic compounds. The research by Kołodziej and Drożdżal [2011] shows that elderberry flowers contain more polyphenolic compounds than fruits from the same places of natural occurrence.

Elderberry fruits are rich in active compounds and show high antioxidant activity [Jabłońska-Ryś et al. 2009, Duymuş et al. 2014, Csorba et al. 2020]. Imenšek et al. [2021] showed an increase in the antioxidant activity of elderberries during ripening, strongly contributed by phenolic compounds. The tested extracts were characterised by high antioxidant activity (DPPH•): *Sambuci flos* > *Sambuci fructus* > *Sambuci folium*. The high DPPH• of flower extracts was probably related to high levels of total polyphenols and phenolic acids. Palomino et al. [2021] proved the high level of flavonoids and hydroxycinnamic acids and the antioxidant activity of *S. flos*. The authors showed that *S. flos* aqueous extract promotes neuroprotection, partly due to its antioxidant capacity. The research by Dawidowicz et al. [2006] shows that elderberry flower extracts (considered the richest source of flavonols) show more potent properties that neutralise the activity of free radicals than extracts from leaves; there is no direct correlation between the level of flavonoids in the extracts and their antioxidant activity. Similarly, Dżugan et al. [2019] indicate that elderberries are more affluent in anthocyanins and flowers in polyphenols. The dye obtained from the fruit has a lower antioxidant activity than the dye prepared from flowers.

Table 2. Polyphenol compounds of elderberry and antioxidant activity (DPPH•). The results are the averages of the given samples dried at 45°C; average values of the results are given in air-dry weight of the raw material

Raw material	Final moisture (%)	Total polyphenols mg (GAE · 100 g^{-1})	Phenolic acids (%)	Flavonoids (%)	Anthocyanins (g · 100 g^{-1})	DPPH• (%)
Sambuci folium	5.20^{b}	8.62^{b}	2.32^{b}	1.48^{a}	0.25^{b}	46.57^{c}
Sambuci flos	5.78^{a}	10.04^{a}	2.90^{a}	1.05^{b}	0.20^{c}	85.50^{a}
Sambuci fructus	2.73^{c}	4.45^{c}	0.57^{c}	0.32^{c}	0.29^{a}	76.01^{b}

Values marked with the same letter do not differ significantly for α = 0.05

Table 3. Biologically active pigments of elderberry raw material. The results are the averages of the given samples dried at 45°C; average values of the results are given in air-dry weight of the raw material

Raw material	Chlorophyll a	Chlorophyll b	Chlorophyll a + b	Carotenoids
	mg · 100 g^{-1}			
Sambuci folium	691.34[a]	240.35[a]	931.70[a]	133.77[a]
Sambuci flos	48.12[c]	56.89[c]	105.01[c]	5.60[c]
Sambuci fructus	77.22[b]	88.22[b]	165.42[b]	23.93[b]

Values marked with the same letter do not differ significantly for α = 0.05

The presence of biologically active pigments characterised the analysis of elderberry raw materials: chlorophylls and carotenoids, with most of these compounds found in leaves and the least in flowers (Tab. 3). Chlorophyll, a powerful antioxidant, increases blood clotting activity, supports hormonal balance, is essential for detoxification, improves digestion. In fruits, this compound is often degraded during the ripening process and is transformed into other pigments, including anthocyanins [Pareek et al. 2018]. Elderberry seems to be a more stable anthocyanin raw material regarding the level of chlorophyll. Carotenoids have a significant impact on many human diseases, and their consumption reduces the risk of certain forms of cancer, cardiovascular disease, and macular degeneration. The consumption of carotenoids can significantly reduce the risk of developing liver diseases such as non-alcoholic fatty liver disease [Elvira-Torales et al. 2019]. Recently, attempts have been made to increase the content of these components in plants for pro-health benefits [Simkin 2021]. The high content of these pigments in elderberry leaf extracts may be essential for their potential use.

The influence of the harvest phase and drying conditions on the level of active substances

The harvest time and drying method had a significant effect on the chemical composition of *Sambuci folium* (Tab. 4). *S. folium* harvested before flowering turned out to be the richest in phenolic acids, flavonoids, and anthocyanins; in the case of the latter, similar to those harvested at the beginning of flowering. The highest amounts of total polyphenols, chlorophyll a + b and carotenoids were found in the raw material collected at the beginning of flowering, similar to fruiting in the case of carotenoids. In the research by Marțiș (Petruț) et al. [2021], it appears that the elderberry growth phase has a more significant effect on the chemical composition of plants than the cultivar. The authors showed that the total phenolics content and antioxidant activity are highest in the early stage of development until flowering.

Elderberry leaf (*Sambuci folium*) dried at 45°C contained less moisture and more total polyphenols, phenolic acids, flavonoids, and carotenoids than dried in natural conditions (Tab. 4). Orphanides et al. [2013] found that convection oven-dried mint has a lower phenolic content and antioxidant power than air-dried mint. The authors explain that heat-sensitive phenolic compounds are degraded or biotransformed at high temperatures. It is confirmed by the results of Garcìa et al. [2021], indicating a temperature of 35°C as the best for maintaining a high level of phenolic compounds in the nettle leaf. In turn, Saifullah et al. [2019] found that the temperature of drying lemon myrtle leaves with hot air in the range of 50–90°C significantly affects retention. The authors explain this phenomenon with a longer drying time of the raw material at a lower than higher temperature, resulting in the degradation of phenols and flavonoids, compounds more unstable than proanthocyanidins.

Elderflower (*Sambuci flos*) harvested in full flowering after drying contained more moisture and chlorophyll a + b than harvested at the beginning of flowering. The naturally dried raw material finally contained more moisture and less bioactive substances than the one dried at 45°C, which in turn was richer in total polyphenols, phenolic acids, flavonoids, and chloro-

Table 4. Chemical components of *Sambuci folium* and antioxidant activity (DPPH•) according to the harvest period and drying method. Average values of the results are given in air-dry weight of the raw material

Harvest period	Drying method		Mean for harvest period
	dryer	natural conditions	
Final moisture (%)			
Before flowering	5.33^{c}	6.58^{b}	5.96^{A}
Beginning of flowering	4.98^{c}	7.06^{ab}	6.02^{A}
Fruiting	5.29^{c}	7.42^{a}	6.36^{A}
Mean for drying method	5.20^{B}	7.02^{A}	
Total polyphenols (mg GAE · 100 g^{-1})			
Before flowering	8.21^{b}	3.18^{c}	5.70^{B}
Beginning of flowering	10.18^{a}	4.06^{c}	7.12^{A}
Fruiting	7.46^{b}	3.77^{c}	5.62^{B}
Mean for drying method	8.62^{A}	3.67^{B}	
Phenolic acids (%)			
Before flowering	2.74^{a}	0.84^{c}	1.79^{A}
Beginning of flowering	2.48^{a}	0.59^{cd}	1.54^{B}
Fruiting	1.75^{b}	0.47^{d}	1.11^{C}
Mean for drying method	2.32^{A}	0.63^{B}	
Flavonoids (%)			
Before flowering	2.07^{a}	1.45^{b}	1.76^{A}
Beginning of flowering	1.34^{bc}	1.20^{c}	1.27^{B}
Fruiting	1.04^{d}	0.93^{d}	0.99^{B}
Mean for drying method	1.48^{A}	1.19^{B}	
Anthocyanins (%)			
Before flowering	0.26^{a}	0.24^{bc}	0.25^{A}
Beginning of flowering	0.25^{ab}	0.24^{bc}	0.25^{A}
Fruiting	0.23^{c}	0.23^{c}	0.23^{B}
Mean for drying method	0.25^{A}	0.24^{A}	
Chlorophyll a + b (mg · 100 g^{-1})			
Before flowering	736.30^{d}	715.65^{d}	725.98^{C}
Beginning of flowering	1207.75^{b}	1425.90^{a}	1316.83^{A}
Fruiting	922.37^{c}	1069.52^{c}	995.95^{B}
Mean for drying method	955.47^{A}	1070.36^{A}	
Carotenoids (mg · 100 g^{-1})			
Before flowering	83.23^{de}	67.45^{e}	75.34^{B}
Beginning of flowering	168.50^{a}	116.10^{c}	142.30^{A}
Fruiting	149.57^{b}	96.52^{cd}	123.05^{A}
Mean for drying method	133.77^{A}	93.36^{B}	
DPPH• (%)			
Before flowering	44.59^{c}	45.50^{c}	45.05^{A}
Beginning of flowering	48.01^{b}	43.22^{c}	45.62^{A}
Fruiting	50.10^{a}	42.74^{c}	46.42^{A}
Mean for drying method	47.57^{A}	43.82^{A}	

Values marked with the same upper or lower letters do not differ significantly for $\alpha = 0.05$

Table 5. Chemical components of *Sambuci flos* and antioxidant activity (DPPH•) according to the harvest period and drying method. Average values of the results are given in air-dry weight of the raw material

Harvest period	Drying method		Mean for harvest period
	dryer	natural conditions	
Final moisture (%)			
Beginning of flowering	5.93^{bc}	6.13^{b}	6.03^{B}
Full flowering	5.62^{c}	10.32^{a}	7.97^{A}
Mean for drying method	5.78^{B}	8.23^{A}	
Total polyphenols (mg GAE · 100 g^{-1})			
Beginning of flowering	9.91^{ab}	7.50^{c}	8.71^{A}
Full flowering	10.17^{a}	8.71^{bc}	9.44^{A}
Mean	10.04^{A}	8.10^{B}	
Phenolic acids (%)			
Beginning of flowering	2.74^{ab}	2.43^{bc}	2.59^{A}
Full flowering	3.06^{a}	1.92^{c}	2.49^{A}
Mean	2.90^{A}	2.18^{B}	
Flavonoids (%)			
Beginning of flowering	1.17^{a}	0.98^{ab}	1.08^{A}
Full flowering	0.92^{bc}	0.78^{c}	0.85^{A}
Mean	1.05^{A}	0.88^{A}	
Anthocyanins (%)			
Beginning of flowering	0.19^{a}	0.20^{a}	0.20^{A}
Full flowering	0.20^{a}	0.20^{a}	0.20^{A}
Mean	0.20^{A}	0.20^{A}	
Chlorophyll a + b (mg · 100 g^{-1})			
Beginning of flowering	90.30^{b}	96.40^{b}	93.35^{B}
Full flowering	131.63^{a}	95.10^{b}	113.37^{A}
Mean	125.82^{A}	95.75^{B}	
Carotenoids (mg · 100 g^{-1})			
Beginning of flowering	6.23^{a}	4.17^{b}	5.20^{A}
Full flowering	4.97^{b}	4.60^{b}	4.79^{A}
Mean	5.60^{A}	4.39^{B}	
DPPH• (%)			
Beginning of flowering	86.19^{a}	86.33^{a}	86.26^{A}
Full flowering	84.81^{a}	86.14^{a}	85.48^{A}
Mean	85.50^{A}	86.24^{A}	

Values marked with the same upper or lower letters do not differ significantly for $\alpha = 0.05$

Table 6. Chemical components of *Sambuci fructus* and antioxidant activity (DPPH•) according to the harvest period and drying method. Average values of the results are given in air-dry weight of the raw material

Harvest period	FM (%)	TP (mg GAE · 100 g^{-1})	PA (%)	F (%)	AC (g · 100 g^{-1})	Ch+ (mg · 100 g^{-1})	C (mg · 100 g^{-1})	DPPH• (%)
Beginning of fruiting	3.77[a]	4.27[a]	0.56[a]	0.29[b]	0.30[a]	155.77[b]	21.83[a]	74.88[a]
Full fruiting	1.68[b]	4.63[a]	0.58[a]	0.35[a]	0.27[a]	175.07[a]	26.03[a]	77.14[a]

FM – final moisture; TP – total polyphenols; PA – phenolic acids; F – flavonoids; AC – anthocyanins; Ch+ – chlorophyll a + b; C – carotenoids

phyll and carotenoids (Tab. 5). Dried flowers are an excellent source of polyphenols and carotenoids, and they are also distinguished by high antioxidant activity [Fernandes et al. 2019, Stefaniak and Grzeszczuk 2020]. Drying is the most straightforward herb preservation and stabilisation method, with different responses to drying time and conditions [Fernandes et al. 2019]. Shi et al. [2021] indicate the combination of microwave-assisted drying and air-drying as a suitable method of drying tea flowers with the advantages of reduced processing time, retention of active compounds and high product quality.

Elderberry fruit (*Sambuci fructus*) harvested at full maturity contained finally less moisture and more flavonoids and chlorophyll than harvested at the beginning of ripening. Fruit extracts showed high and comparable antioxidant activity, regardless of the date of harvesting the raw material (Tab. 6). Bratu et al. [2012] proved that *Sambucus nigra* fruit powder has a very high antioxidant activity in vitro and is not mutagenic at low concentrations, making it recommended for food industry applications. The elderberry fruit and flowers components may show an inflammatory modulating effect, which increases their nutritional value [Ho et al. 2017].

Csorba et al. [2020] showed that the chemical composition of elderberry fruit is genetically determined, but it is also influenced by climatic factors. Elderberry growth phases represent an irreversible process involving a series of biochemical changes that significantly impact nutritional properties. According to the research of Marțiș (Petruț) et al. [2021], the flowering stage ends the period of increased accumulation of phenolic compounds. The next stage of plant development is associated with a decrease in the content of bioactive compounds in the fruit, persisting in the green and red fruit phases, followed by a slight increase towards full maturity, which is partially confirmed by our results (flavonoid level). Imenšek et al. [2021] report that early harvest of elderberry fruit does not significantly reduce their quality. On the other hand, the authors point to fully ripe fruit as a valuable source of nutrients and natural food pigments. The antioxidant activity of extracts from ripe elderberries is significantly correlated with the total content of phenols [Jabłońska-Ryś et al. 2009, Imenšek et al. 2021a].

CONCLUSIONS

Sambuci folium, *S. flos* and *S. fructus* can be considered valuable polyphenolic and dye materials with significant antioxidant activity. The antioxidant activity of extracts from the above-mentioned raw materials is as follows: *Sambuci flos* > *Sambuci fructus* > *Sambuci folium*. The elderberry flower turned out to be the richest source of total polyphenols and phenolic acids, leaf – flavonoids, chlorophylls and carotenoids, and the fruit – anthocyanins. The harvest time differentiates the chemical composition of *S. folium* to a greater extent than that of *S. flos* (the changes concerned only the content of chlorophyll) and *S. fructus* (the changes concerned only the content of flavonoids and chlorophyll). The leaf collected before flowering contained the most phenolic acids and flavonoids, while the leaf harvested at the beginning of flowering – total polyphenols and a + b chlorophyll. The level of anthocyanin in *S. folium* was higher before and at the beginning of flowering than during the fruiting phase. Elderberry

fruit harvested at full maturity contained less moisture and more flavonoids and chlorophyll than harvested at the beginning of ripening.

Our results show that drying elderberry leaves and flowers at 45°C allow for rapid water drainage and a high content of total polyphenols, phenolic acids, flavonoids (leaf), chlorophyll a + b (flower) and carotenoids. In the light of the obtained results and current scientific information, it is necessary to further research comparing the chemical composition and biological activity of various organs of elderberry depending on the genotype, development phase, place of cultivation/occurrence, method of preservation (drying, freezing) and methods of extraction, to make more efficient use of this extremely valuable plant.

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