Investigation of the possibilities for obtaining antioxidants from elderflower (*Sambucus nigra* L.) using different extraction methods

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Abstract: In recent years, the medicinal properties of elderberry have been researched due to its high content of polyphenolic compounds, especially flavonols, phenolic acids, and anthocyanins. These compounds are known for effectively neutralizing free radicals and protecting the body from oxidative stress and lipid peroxidation. The aim of this study is to examine the influence of process parameters on various elderflower extraction methods on the content of phenols, flavonoids, and anthocyanins. The extraction techniques used include maceration and mixing in a shaker, as examples of classical extraction methods. The study results indicate that higher concentrations of total phenols, flavonoids, and anthocyanins are achieved using the shaking extraction technique, which is a more advanced method compared to maceration.

Keywords: Elderflowers; Extraction; Phenols; Flavonoids; Anthocyanins

1. Introduction

The Elderberry (*Sambucus nigra* L.) is a perennial shrub or small tree with a shallow root system propagated by seeds. This tree can grow to a height of 8 to 10 m, with a trunk diameter of 20 to 30 cm. The flowering period lasts from May to June, depending on altitude. The flowers are white or cream-colored, sometimes greenish-yellow, arranged in flat, compound inflorescences with a diameter between 10 and 20 cm, containing 3 to 5 small seeds. The inflorescences have a fresh, intense, fruity-sweet aroma.

In Europe, elders have been used for centuries in the food industry for the production of pies, jellies, jams, ice cream, yogurt, and various alcoholic beverages [1]. Additionally, it is used in folk medicine for treating many diseases and ailments due to its antioxidant, anti-carcinogenic, immune-stimulating, anti-allergic, antiviral, and antibacterial properties [2].

Recently, increasing attention has been paid to the antioxidant capacity of elder, which is used as a functional ingredient in food products, such as natural preservatives or dietary supplements [3]. Preventing oxidative stress is crucial for human health, as reactive oxygen species, which are generated by the uncontrolled production of free radicals, can cause oxidative damage to biomacromolecules, amino acids, DNA, lipids, and proteins. Under

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normal physiological conditions, the endogenous defense system can remove reactive oxygen species, but in pathological conditions, it is more susceptible to oxidative damage [4].

Elderberry's medicinal properties derive from its high content of polyphenolic compounds, primarily flavonols, phenolic acids, and anthocyanins. These compounds effectively neutralize free radicals and protect the body from oxidative stress and lipid peroxidation [5].

Among the polyphenolic compounds, elder contains flavonols, phenolic acids, proanthocyanidins, and anthocyanins, which give the fruit its red-purple color. The anthocyanins in elder are derivatives of cyanidin, with the most abundant being cyanidin-3-glucoside (204.6-481.4 mg CGE/100 g of fruit) and cyanidin-3-sambubioside (122.2-269.1 mg CGE/100 g of fruit) [6].

The dominant flavonols are quercetin, kaempferol, and isorhamnetin, as well as rutin, which consists of quercetin and glucose [7]. Among the phenolic acids present are chlorogenic acid, derivatives of caffeic and p-coumaric acids, and small concentrations of ellagic acid [6].

The demand for elder-based products is growing alongside new information about the plant's pharmacological attributes provided by scientific research. However, in addition to the mentioned metabolites, elder contains cyanogenic glycosides, which can cause nausea and vomiting primarily due to the release of the toxic component hydrogen cyanide (HCN) [8]. However, thermal processing of the fruits and all parts of the plant used as food disrupts the chemical structure of cyanogenic glycosides, rendering them incapable of causing adverse health effects.

The study examined the impact of processing parameters on the content of (poly)phenols during different elderflower (*Sambucus nigra* L.) extraction procedures. Extraction was performed by maceration and mixing on a shaker at different extraction times, ratios of plant material-to-solvent, and ethanol concentrations in the solvent.

2. Materials and Methods

After the extraction, the content of total (poly)phenols, flavonoids, and anthocyanins in the obtained extracts was spectrophotometrically determined using the Shimadzu UV-1800 spectrophotometer (Cole Parmer, USA). The measurement of total (poly)phenols was performed at a wavelength of 765 nm, using gallic acid (Sigma Aldrich, USA) as the standard [9]. The following reagents were used for the spectrophotometric measurement: Folin-Ciocalteu reagent (Carlo Erba, Germany) and sodium carbonate (Lach, Czech Republic). The method is based on redox processes between the hydroxyl groups of phenols and the Folin-Ciocalteu reagent, a polymeric complex ion of molybdenum and tungsten.

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The content of flavonoids in the sample was determined using the colorimetric method of aluminum chloride. Aluminum from aluminum chloride forms stable complexes in an acidic medium with the C-4 keto group or the C-3 and C-5 hydroxyl groups of present flavones and flavonols and unstable complexes with ortho-dihydroxyl groups in the A or B ring of flavonoids. The measurement was carried out at a wavelength of 510 nm, with catechin hydrate (Sigma Aldrich, USA) as the standard [10]. For color development, aluminum chloride (Lach, Czech Republic), sodium hydroxide (Lach, Czech Republic), and sodium nitrite (Zorka Šabac, Serbia) were used.

The content of anthocyanins was determined by measuring the absorbance of potassium chloride buffer at pH = 1 and acetate buffer at pH = 4.5 at wavelengths of 520 nm and 700 nm [11.12]. The quantitative determination of total anthocyanins (non-degraded monomers and their degradation products) is based on the property of anthocyanins to reversibly change their structure with changes in pH, which also leads to changes in the absorption spectrum. Over time, and under the influence of various factors (temperature, oxygen, vitamin C, etc.), monomer anthocyanins degrade, associating with each other or with other present compounds, forming degradation products. Although the quantity of monomer anthocyanins decreases, the intensity of the color does not change, as condensation reactions produce colored condensation products that are even more stable than monomer (free) anthocyanins.

The content of total (poly)phenols, flavonoids, and anthocyanins in the extract is expressed as an equivalent of gallic acid [mg GAE/g], catechin hydrate equivalent [mg KaH/g], and cyanidin-3-glucoside equivalent [Cy3G/g], respectively.

3. Results and Discussion

In this study, the extraction parameters (reaction time, plant material-to-solvent ratio, ethanol concentration in the solvent) of elderflower (*Sambucus nigra* L.) were examined using different extraction techniques (maceration, mixing on a shaker) to obtain total phenols, flavonoids, and anthocyanins, and the efficiency of obtaining these antioxidant compounds was evaluated.

3.1. Maceration

Table 1 presents the qualitative and quantitative composition of the extract obtained by maceration depending on the extraction time, the plant material-to-solvent ratio, and the ethanol concentration in the solvent. The extraction was performed at 10, 15, 25, 35, 60, and 90 min, with plant material-to-solvent ratios of 1:15, 1:30, and 1:45 m/v, and ethanol concentrations in the solvent of 25%, 50%, and 75% v/v.

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Table 1. The qualitative and quantitative composition of the extract obtained by maceration depends on the extraction time, the plant material-to-solvent ratio, and the ethanol concentration in the solvent.

Reaction Time [min]	Plant Material-to-solvent Ratio [m/v]	Ethanol Concentration in Solvent [%]	Total Phenol Content [mg GAE/g]	Total Flavonoid Content [mg KaH/g]	Total Anthocyanin Concentration [mg Cy3G/g]
10	1:30	50	16.52	4.78	0.1003
15	1:30	50	16.76	6.51	0.1365
25	1:30	50	18.37	8.13	0.1996
35	1:30	50	20.13	8.34	0.1965
60	1:30	50	20.93	10.05	0.2959
90	1:30	50	22.42	12.53	0.2628
20	1:15	50	12.87	6.79	0.1499
20	1:30	50	18.62	7.25	0.1374
20	1:45	50	24.11	8.22	0.1429
20	1:15	25	9.42	4.19	0.0191
20	1:30	25	16.78	6.95	0.0190
20	1:45	25	22.50	6.54	0.0150
20	1:15	75	10.93	5.64	0.1309
20	1:30	75	17.78	7.14	0.1053
20	1:45	75	22.50	8.94	0.0737

First, the kinetics of extraction (samples 1-6) at a constant plant material-to-solvent ratio (1:30 m/v) and ethanol concentration in the solvent (50 %v/v) were examined, and it can be observed that with an increase in extraction time, the concentration of total phenols and flavonoids increases. The concentration of total anthocyanins increases up to 35 min. From Table 1, it can be observed that the optimal time for performing maceration is 35 min.

Samples 7-9 were extracted for 20 min at an ethanol concentration in the solvent of 50 %v/v, with the plant material-to-solvent ratio being varied (1:15, 1:30, and 1:45 m/v). From Table 1, it can be observed that the highest content of total phenols and flavonoids is obtained using the highest plant material-to-solvent ratio (1:45 m/v), yielding 24.11 mg GAE/g of total phenols and 8.22 mg KaH/g of total flavonoids. The concentration of total anthocyanins (0.1499 and 0.1429 mg Cy3G/g) is highest when using plant material-to-solvent ratios of 1:15 and 1:45 m/v, respectively. From this, it can be concluded that the optimal plant material-to-solvent ratio for maceration is 1:45 m/v.

In samples 10-15, in addition to the plant material-to-solvent ratio (1:15, 1:30, and 1:45 m/v), the effect of ethanol concentration in the solvent was also examined. For samples 10-12, an ethanol concentration of 25 %v/v in the solvent was used, while for samples 13-15, an ethanol concentration of 75 %v/v was used. The highest content of total phenols (22.50 mg GAE/g) is obtained in samples 12 and 15 at a reaction time of 20 min, a plant

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material-to-solvent ratio of 1:45 m/v, and ethanol concentrations in the solvent of 25 and 75 %v/v, respectively. The highest content of total flavonoids (8.94 mg KaH/g) is obtained in sample 15 (extraction time 20 min, plant material-to-solvent ratio 1:45 m/v, and ethanol concentration in the solvent of 75 %). The highest concentration of total anthocyanins (0.1309 mg Cy3G/g) is obtained during maceration for 20 min, with a plant material-to-solvent ratio of 1:15 and ethanol concentration in the solvent of 75 %v/v (sample 13). From this, it can be observed that a higher ethanol concentration in the solvent and a lower plant material-to-solvent ratio yield higher concentrations of total anthocyanins during maceration.

From Table 1, the optimal conditions for performing maceration can be observed as follows:

- Reaction time: 35 min,
- Plant material-to-solvent ratio: 1:45 m/v,
- Ethanol concentration: 50 %v/v.

3.2. Mixing on a Shaker

In Table 2, the qualitative and quantitative composition of the extract obtained by mixing on a shaker, depending on the extraction time, the plant material-to-solvent ratio, and the ethanol concentration in the solvent, is given. The extraction was carried out at 10, 15, 25, 35, 60, and 90 min, with plant material-to-solvent ratios of 1:15, 1:30, and 1:45 m/v, ethanol concentrations in the solvent of 25, 50, and 75 %v/v, and a shaking frequency of 50 oscillations per minute.

The kinetics of extraction (samples 16-21) at a constant plant material-to-solvent ratio (1:30 m/v) and ethanol concentration in the solvent (50 %v/v) were examined, and it can be observed that with an increase in extraction time, the concentration of total phenols and flavonoids increases. The concentration of total anthocyanins increases up to 35 min, after which equilibrium is established. From Table 2, it can be observed that the optimal time for performing mixing on a shaker is 35 min.

Samples 22-24 were extracted for 20 min at an ethanol concentration in the solvent of 50 %v/v, with the plant material-to-solvent ratio being varied (1:15, 1:30, and 1:45 m/v). From Table 2, it can be observed that the highest content of total phenols and flavonoids is obtained using the highest plant material-to-solvent ratio (1:45 m/v), yielding 36.51 mg GAE/g of total phenols and 15.28 mg KaH/g of total flavonoids. The concentration of total anthocyanins (0.4423 mg Cy3G/g) is highest when using a plant material-to-solvent ratio of 1:45 m/v. From this, it can be concluded that the optimal plant material-to-solvent ratio for mixing on a shaker is 1:45 m/v.

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Table 2. Qualitative and quantitative composition of the extract obtained by mixing on a shaker depending on extraction time, plant material-to-solvent ratio, and ethanol concentration in the solvent (Shaking Frequency is 50 oscillations/min).

	Reaction Time [min]	Plant Material-to-solvent Ratio [m/v]	Ethanol Concentration in Solvent [%]	Total Phenol Content [mg GAE/g]	Total Flavonoid Content [mg KaH/g]	Total Anthocyanin Concentration [mg Cy3G/g]
16	10	1:30	50	18.47	8.52	0.2056
17	15	1:30	50	21.64	9.00	0.2377
18	25	1:30	50	22.29	9.05	0.2557
19	35	1:30	50	25.22	10.89	0.3641
20	60	1:30	50	26.69	11.52	0.3289
21	90	1:30	50	27.25	14.17	0.3299
22	20	1:15	50	16.47	8.23	0.2322
23	20	1:30	50	27.24	14.75	0.3380
24	20	1:45	50	36.51	15.28	0.4423
25	20	1:15	25	15.31	7.34	0.0888
26	20	1:30	25	23.74	10.72	0.1434
27	20	1:45	25	30.08	11.70	0.1264
28	20	1:15	75	15.75	8.96	0.2537
29	20	1:30	75	22.68	11.83	0.2938
30	20	1:45	75	27.72	11.44	0.4287

Samples 25-30 were examined to assess the plant material-to-solvent ratio (1:15, 1:30, and 1:45 w/v) and the impact of ethanol concentration in the solvent. Samples 25-27 used 25% v/v ethanol, while samples 28-30 used 75% v/v ethanol. The highest total phenol content (30.08 mg GAE/g) was observed with sample 27, using a 20-min extraction time, a plant material-to-solvent ratio of 1:45 w/v, a shaking frequency of 50 oscillations per minute, and 25% v/v ethanol concentration in the solvent. Sample 29 yielded the highest total flavonoid content (11.83 mg KaH/g) under similar conditions but with 75% v/v ethanol. The maximum concentration of total anthocyanins (0.4287 mg Cy3G/g) was achieved with sample 30, using a 20-min maceration time, a plant material-to-solvent ratio of 1:45 w/v, a shaking frequency of 50 oscillations per min, and 75% v/v ethanol concentration in the solvent.

From Table 2, the optimal conditions for the shaking extraction method are identified as:

- Extraction time: 35 min,
- Plant material-to-solvent ratio: 1:45 w/v,
- Ethanol concentration in the solvent: 50% v/v.

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The study results indicate that higher concentrations of total phenols, flavonoids, and anthocyanins are achieved using the shaking extraction technique, which is a more advanced method compared to maceration.

In their study, Vasiljević and others used ultrasound-assisted extraction of elderflower to obtain polyphenolic compounds [13]. They found that rapid extraction occurs within the first 30 min and that increasing the ethanol concentration in the solvent enhances the concentration of anthocyanins in the extract. Additionally, they noted that approximately 60% of flavonoids in the extract comprise total polyphenolic compounds. Compared to the results presented in this paper, it is evident that ultrasound-assisted extraction, as a modern method, achieves higher yields of total phenols, flavonoids, and anthocyanins than classical extraction techniques such as maceration and mixing on a shaker.

4. Conclusions

Classical extraction techniques such as maceration and mixing on a shaker can produce extracts rich in polyphenolic compounds, which are known for their ability to effectively neutralize free radicals and protect the body from oxidative stress and lipid peroxidation, acting as antioxidants. Mixing on a shaker results in higher yields of total phenols, flavonoids, and anthocyanins compared to maceration.

More modern methods, such as ultrasound-assisted extraction, offer several advantages, including higher yields of polyphenolic compounds, reduced extraction time, and a lower plant material-to-solvent ratio, enhancing the efficiency of the process. Additionally, the use of solvents with lower ethanol concentrations makes this method more environmentally friendly.

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