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Exploring the Feasibility of Solvent Recirculation in the Extraction Process of (Poly)Phenols from Elderflower (*Sambucus Nigra L.*)

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ABSTRACT

The traditional maceration technique with a two-step solid-liquid extraction was used in this paper. After the first extraction step, an extract was separated from the exhausted plant material (elderberry flower) and then a plant material was added to the extract. Experimental parameters included different ethanol concentrations (30 %, 60 %, and 90 %), solid-to-solvent ratios (1:15, 1:30, and 1:45 w/v), pH values (3, 7, and 10), and extraction times (15, 30, and 45 minutes), while one-factor-at-a-time (OFAT) was experimental design. The results indicated that the optimal ethanol concentration in the solvent was 60 vol. % for both extraction steps. Increasing the solid-to-solvent ratio from 1:15 w/v to 1:30 w/v resulted in higher (poly)phenol content, with a slight decrease observed at higher ratios. (Poly)phenols content remained consistent in acidic and neutral environments, but decreased at pH 10. While the content of (poly)phenolic compounds increased with longer extraction times, 30 minutes was considered optimal, as further extension did not significantly increase the content. Additionally, it was observed that the content of total (poly)phenols was lower in the second extraction step, suggesting saturation of the solvent after the first extraction..

1. Introduction

Black elderberry (*Sambucus nigra L.*) is a widespread shrub of the Caprifoliaceae family that grows across the majority of Europe, West Asia, North Africa, and the United States (Fazio et al., 2013). It is an evergreen shrub or low tree that can grow up to 10 meters tall and has a rounded, thin canopy. It is often found on the fringes of forests, in thickets and hedges, and along the sides of rivers and streams. It grows in warm, sunny, or semi-shady areas with humid and humus soils (Atkinson and Atkinson, 2002).

The applications of *Sambucus nigra* can be attributed to its characteristic chemical composition, which includes essential oils, free fatty acids, flavonoids, anthocyanins, phenolic acids, carotenoids, vitamins and minerals (Lee and Fin, 2007; Viapana and Wesolowski, 2017). Elderberry flowers have strong antioxidant activity due to their natural polyphenolic components, which include flavanols, phenolic acids, and anthocyanins. (Poly)phenolic compounds are present in the leaves, fruits and flowers.

These compounds are well known as free radical scavengers and can protect the human body from oxidative stress and lipid peroxidation (Sidor and Gramza-Michałowska, 2015).

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Elderberry flowers and fruits, apart from flavonols, contain large amounts of phenolic acids. Fruits contain chlorogenic, crypto-chlorogenic and neochlorogenic acids, as well as trace levels of ellagic acid (0.04 mg/100 g) (Fazio et al., 2013). Elder flowers also contain N-phenylpropenoyl-l-amino acid amides, which strongly stimulate mitochondrial activity and cell proliferation in human keratinocytes and liver cells and inhibit Helicobacter pylori adhesion to the human stomach without causing necrotic toxicity (Hensel et al., 2007; Vasiljević et al., 2023b). The predominant flavonols were quercetin, kaempferol and isorhamnetin. Flavonols derived from elderberry occur as glycosides of rutin and glucose; moreover, acylated quercetins were also present (Christensen et al., 2008). The predominant anthocyanins of *S. nigra* is cyanidin 3-glucoside, depending on the variety and fruition (204.6–481.4 mg cyanidin-3-glucoside equivalents (CGE)/100 g fruits) and cyanidin 3-sambubioside (122.2–269.1 mg CGE/100 g fruits) (Lee and Fin, 2007). The flowers of elderberry contain ten times more flavonols (214.25 mg/100 g) than fruits (20.18 mg/100 g) and several times more than the leaves (17.01 mg/100 g) (Dawidowicz et al., 2006). This paper aimed to investigate how process parameters (extraction time, solid-to-solvent ratio, pH value of solvent, and ethanol content) affected the yield of (poly)phenolic components in an ethanol extract of black elderberry flower. Also, the capacity of the solvent during initial extraction and recirculation will be examined.

2. Materials and methods

2.1. Plant materials and reagents

Dried elderflower blooms, sourced from Zvornik municipality in the eastern part of Bosnia and Herzegovina, were used for extraction. Ethanol served as the solvent for sample extraction, while characterization of the extract employed the following reagents: Folin-Ciocalteu reagent (Carlo Erba, Germany), sodium carbonate (Lach:ner, Czech Republic), gallic acid (Sigma Aldrich, USA), aluminum chloride (Lach:ner, Czech Republic), sodium hydroxide (Lach:ner, Czech Republic), sodium nitrite (Zorka Šabac, Serbia), catechin hydrate (Sigma Aldrich, USA), acetate buffer pH=4.5 (Lach:ner, Czech Republic), and potassium chloride buffer pH=1.0 (Lach:ner, Czech Republic).

2.2. Methods

The determination of total (poly)phenol content relies on oxidation-reduction reactions involving phenol hydroxyl groups and the Folin-Ciocalteu reagent, along with molybdenum and tungsten polymer complex ions. Sodium carbonate is added to the reaction mixture to create a basic environment. Spectrophotometric measurement at 765 nm using gallic acid as the standard was conducted with a Shimadzu 1800 spectrophotometer. Results are expressed in milligrams of gallic acid equivalent per gram of plant material (mg GAE/g) (ISO 14502-1, 2005). Flavonoid content is determined spectrophotometrically with aluminum chloride, forming stable complexes with flavones and flavonols. Spectrophotometric measurement at 510 nm with catechin hydrate as the standard was performed. Results are given in milligrams of catechin hydrate equivalents per gram of plant material (mg CTH/g) (Smolinski-Savi et al., 2017).

Total anthocyanin content, including non-degraded monomers and degradation products, is quantified using the "pH differential" method, involving absorbance measurements at pH=1 and pH=4.5 (Giusti and Wrolstad, 2001). Results are expressed as cyanidin-3-glucoside equivalents (mg Cy3G/g) using a specific formula (Vasiljević et al., 2023a).

$$C_{tot} = (A \cdot M \cdot F \cdot 10^3)/(\varepsilon \cdot l \cdot R)$$

where are:

C_{tot} - total anthocyanins content (mg/g),
A - $(A_{520nm} - A_{700nm})_{pH=1.0}$ - $(A_{520nm} - A_{700nm})_{pH=4.5}$,
M - molar mass (for Cy3G it is 449,2 g/mol),
F - dilution factor ((3+3)/3=2),
10^3- factor for converting grams to milligrams,
ε - molar absorption extinction coefficient (for Cy3G it is 26900 L / (mol·cm)),
l - cuvette thickness (1 cm) and
R - factor for recalculating the value of anthocyanins per gram of drug - mass of drug per volume of solvent (g/L).

A Shimadzu 1800 spectrophotometer was used to determine anthocyanins, the same as it was for total (poly)phenols and flavonoids.

3. Results and discussion

Table 1 presents the results of an investigation into the effects of various parameters on the extraction of total (poly)phenols, flavonoids, and anthocyanins from a selected sample. The variations in experimental conditions include ethanol content, solvent-to-solid ratio, pH value, extraction time, and the number of extraction steps. Each of these parameters significantly influences the number of extracted compounds, which can be observed through changes in their concentrations expressed in mg/g. The results indicate that a 60 % ethanol concentration consistently results in higher yields compared to lower (30 %) and higher (90 %) ethanol levels. Higher solvent-to-solid ratios, such as 1:30 and 1:45, are also more effective, likely due to improved interaction between the solvent and the compounds being

extracted. Additionally, neutral (pH 7) and slightly acidic (pH 3) conditions enhance extraction efficiency, suggesting that these pH levels optimize the solubility and stability of the target compounds. Longer extraction times, particularly up to 45 minutes, further increase yields, especially for flavonoids and anthocyanins, as extended durations allow more thorough diffusion of these compounds into the solvent.

The first extraction step is the most productive, yielding the majority of the extractable compounds, while subsequent steps contribute less significantly.

These observations suggest that the initial extraction phase captures most of the available compounds, with diminishing returns in later stages. The impact of each parameter will be discussed in more detail in the following sections.

Table 1
Process parameters and results of (poly)phenolic compound content in the extract

Ethanol content [vol. %]	Solvent-to-solid ratio [w/v]	pH	Extraction time [min]	Extraction step	Content [mg/g]		
					Total (poly)phenols	Flavonoids	Anthocyanins
30					35.06	27.78	0.048
60				1	41.42	38.78	0.474
90					25.97	23.82	0.204
	1:30	7	30				
30					23.35	21.81	0.039
60				2	39.83	26.49	0.417
90					17.95	18.98	0.206
	1:15				42.14	30.16	0.374
	1:30			1	56.73	38.73	0.448
	1:45				54.43	48.02	0.391
60		7	30				
	1:15				30.10	19.80	0.242
	1:30			2	45.33	29.90	0.341
	1:45				45.88	31.91	0.361
		3			54.07	37.61	0.364
		7		1	53.25	35.46	0.380
		10			38.32	26.42	0.362
60	1:30		30				
		3			29.42	25.78	0.298
		7		2	39.20	25.69	0.383
		10			34.93	24.57	0.289
			15		43.05	31.97	0.258
			30	1	53.86	35.88	0.453
			45		56.50	37.71	0.549
60	1:30	7					
			15		31.79	21.83	0.245
			30	2	37.41	27.74	0.344
			45		43.21	27.99	0.384

3.1. The influence of ethanol content in the solvent on the total (poly)phenol, flavonoid, and anthocyanin content in the extract

Figure 1 depicts the dependency diagram of (poly)phenols, flavonoids, and anthocyanins content on the ethanol content in the solvent, with constant other process parameters (solvent-to-solid ratio = 1:30 w/v, pH = 7, and time of 30 minutes).

The first observation from Figure 1a is that there is an optimal ethanol content in the solvent, at which the highest (poly)phenol content is achieved. Specifically, at a 60 % ethanol concentration, the histogram shows the highest peak. In the first extraction step, the (poly)phenol content increased by 15.35% with an increase in ethanol content from 30% to 60% (rising from 35.06 mg (GAE) / g to 41.42 mg (GAE) / g).

However, further increasing the ethanol content in the solvent from 60 % to 90 % resulted in a decrease in (poly)phenol content by as much as 37.3 % (dropping from 41.42 mg (GAE) / g to 25.97 mg (GAE) / g). Examining the influence of the second extraction step on the total (poly)phenol content, a similar effect as in the first step is noticed, where the (poly)phenol content initially increased and then decreased with an increase in ethanol content in the solvent. Increasing the ethanol content from 30% to 60% led to a remarkable 70.58% increase in (poly)phenol content in the extract (rising from 23.35 mg (GAE) / g to 39.83 mg (GAE) / g). However, increasing the ethanol content from 60% to 90% resulted in a decrease in (poly)phenol content from 39.83 mg (GAE) / g to 17.95 mg (GAE) / g, representing a decrease of 54.94%. Another noticeable aspect is that the extraction yield in the second step is lower than in the first step for all three ethanol content values in the solvent. The reduction in extraction yield is more pronounced when using 30 % and 90 % ethanol compared to 60 % ethanol.

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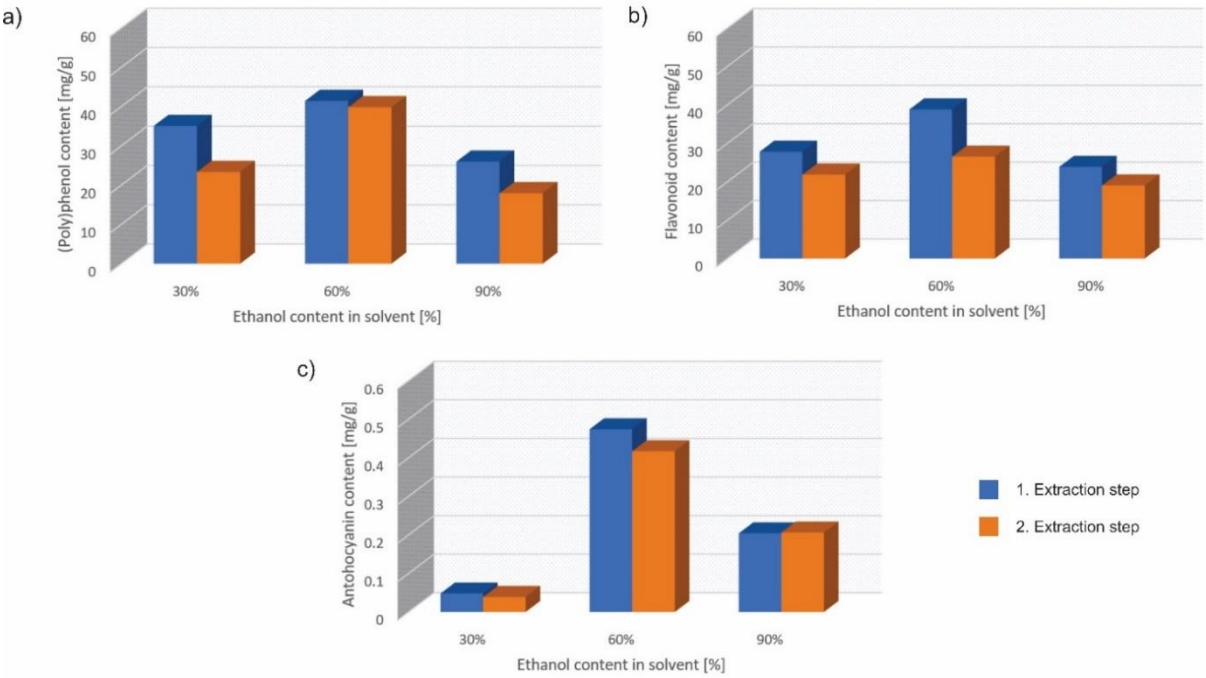

Figure 1. Influence of ethanol content in the solvent on the content of a) (poly)phenols, b) flavonoids, and c) anthocyanins in the extract

Similar to the total (poly)phenol content, there is an optimal ethanol content in the solvent at which the flavonoid content is highest (Figure 1b). In the first extraction step, the flavonoid content increased by 39.60 % with an increase in ethanol content from 30 % to 60 % (rising from 27.78 mg (CTH) / g to 38.78 mg (CTH) / g). However, further increasing the ethanol content in the solvent from 60 % to 90 % resulted in a decrease in flavonoid content by 38.57 % (dropping from 38.78 mg (CTH) / g to 23.82 mg (CTH) / g). Looking at the second extraction step's influence on the total flavonoid content, it is observed that the flavonoid content initially increases from 30 % to 60 % (from 21.81 mg (CTH) / g to 26.49 mg (CTH) / g), and then decreases from 60 % to 90 % (from 26.49 mg (CTH) / g to 18.98 mg (CTH) / g). Comparing the first and second extraction steps, it is noticed that with the use of 30 % ethanol, the flavonoid content in the second extraction step is reduced by 21.49 % compared to the first step (from 27.78 mg (CTH) / g to 21.81 mg (CTH) / g), with 60 % ethanol, this reduction is 31.69 % (from 38.78 mg (CTH) / g to 26.49 mg (CTH) / g), while with 90 % ethanol, this reduction is 20.32 % (from 23.82 mg (CTH) / g to 18.98 mg (CTH) / g).

From Figure 1c, it is evident that the highest anthocyanin content is achieved with the use of 60 % ethanol. In the first extraction step, increasing the ethanol content from 30 % to 60 % resulted in an increase in anthocyanin content from 0.048 mg (Cy3G) / g to 0.474 mg (Cy3G) / g, representing a remarkable 8.875-fold increase. However, further increasing the ethanol content in the solvent from 60 % to 90 % led to a decrease in anthocyanin content (from 0.474 mg (Cy3G) / g to 0.204 mg (Cy3G) / g). Examining the influence of the extraction step, it is observed that in the second extraction step, the anthocyanin content is slightly changed compared to the first step. Once again, there is a significant increase in anthocyanin content with an increase in ethanol content from 30 % to 60 % (from 0.039 mg (Cy3G) / g to 0.417 mg (Cy3G) / g). However, with further increase in ethanol content in the solvent from 60 % to 90 %, the anthocyanin content decreased by 50.60 % (from 0.417 mg (Cy3G) / g to 0.206 mg (Cy3G) / g).

The moderate ethanol content in the solvent (60 vol. %) has a favorable impact on the extraction of total (poly)phenols, flavonoids, and anthocyanins. Solvents with lower ethanol content more easily penetrate plant cells, facilitating the extraction of phenolic compound (Yang et al., 2010). Solvents with higher ethanol concentrations may induce protein denaturation, inhibit the breakdown of phenols from the matrix, and reduce the synthesis of (poly)phenolic compounds (Gunathilake et al., 2019).

3.2. The influence of the solid-to-solvent ratio on the content of total (poly)phenols, flavonoids, and anthocyanins in the extract

The dependency diagram of the content of total (poly)phenols, flavonoids, and anthocyanins in the extract on the solid-to-solvent ratio, with constant other process parameters (ethanol content in the solvent = 60 %, pH = 7, and time of 30 min), is shown in Figure 2.

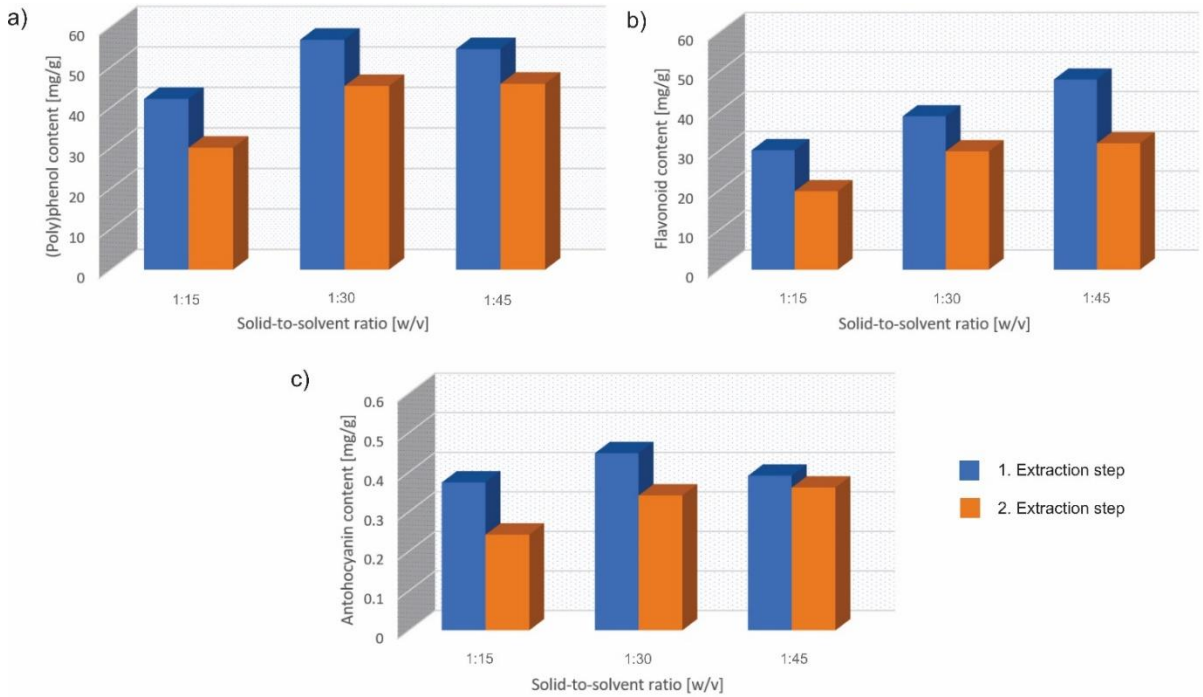

Figure 2. Influence of the solid-to-solvent ratio on the content of a) total (poly)phenols, b) flavonoids, and c) anthocyanins in the extract.

From Figure 2a, it is observed that in the first extraction step, the content of total (poly)phenols in the extract increased by 34.62 % with an increase in the solid-to-solvent ratio from 1:15 w/v to 1:30 w/v (from 42.14 mg (GAE) / g to 56.73 mg (GAE) / g). However, with further increase in this parameter (solid-to-solvent ratio = 1:45 w/v), there is a slight decrease in the total (poly)phenol content in the extract (from 56.73 mg (GAE) / g to 54.43 mg (GAE) / g, representing a decrease of 4.05 %). On the other hand, the influence on the total (poly)phenol content in the extract in the second extraction step is somewhat different. When using the medium (1:30 w/v) and higher ratios (1:45 w/v), similar values of total (poly)phenol content in the extract are observed (45.33 mg (GAE) / g and 45.88 mg (GAE) / g). However, with a solid-to-solvent ratio of 1:15 w/v, the lowest total (poly)phenol content in the extract is observed (30.1 mg (GAE) / g). Similar to the influence of ethanol content in the solvent on the (poly)phenol content, a decrease in (poly)phenol content in the extract is evident in the second extraction step.

From Figure 2b, it is observed that in the first extraction step, the flavonoid content in the extract increases with an increase in the solid-to-solvent ratio. At a solid-to-solvent ratio of 1:15 w/v, the flavonoid content in the extract is 30.16 mg (CTH) / g, at 1:30 w/v it is 38.73 mg (CTH) / g, and at 1:45 w/v it is 48.02 mg (CTH) / g. In the second extraction step, with the use of medium (1:30 w/v) and higher ratios (1:45 w/v), similar values of flavonoid content in the extract are observed (29.9 mg (CTH) / g and 31.91 mg (CTH) / g). However, at a solid-to-solvent ratio of 1:15 w/v, the lowest flavonoid content in the extract is observed (19.8 mg (CTH) / g). Regarding the decrease in flavonoid content in the second step compared to the first step, it is 34.35 % for a solid-to-solvent ratio of 1:15 w/v, 22.80 % for a solid-to-solvent ratio of 1:30 w/v, and 33.55 % for a solid-to-solvent ratio of 1:45 w/v. From Figure 2c, it is observed that in the first extraction step, the anthocyanin content in the extract increased by 19.79 % with an increase in the solid-to-solvent ratio from 1:15 w/v to 1:30 w/v (0.374 mg (Cy3G) / g to 0.448 mg (Cy3G) / g). With further increase in the solid-to-solvent ratio to 1:45 w/v, there is a decrease in the anthocyanin content in the extract by 12.72 % (0.391 mg (Cy3G) / g). In the second extraction step, with the use of medium (1:30 w/v) and higher ratios (1:45 w/v), similar values of anthocyanin content in the extract are observed (0.341 mg (Cy3G) / g and 0.361 mg (Cy3G) / g). However, at a solid-to-solvent ratio of 1:15 w/v, approximately 30% lower anthocyanin content in the extract is observed (0.242 mg (Cy3G) / g). The decrease in anthocyanin content in the second step is least pronounced when using a solid-to-solvent ratio of 1:45 w/v.

When considering the influence of the solid-to-solvent ratio on flavonoid content, it is clear that the highest degree of extraction is achieved at the highest solid-to-solvent ratio. This can be attributed to the larger contact surface area between the sample and the solvent, allowing for more efficient mass transfer of flavonoids from the solid matrix to the liquid phase. A higher ratio can lead to faster mass transfer, resulting in higher yields due to the amount of solvent available for dissolving (poly)phenolic compounds. A higher concentration of solvent in the extraction system often increases extraction efficiency as more solid material is available for interaction with the solvent (Hamdan et al., 2008; Sai-Ut et al., 2023; Vasiljević et al., 2024). On the other hand, the highest content of total (poly)phenols and anthocyanins is extracted at the medium solid-to-solvent ratio. The most likely reason for this phenomenon is that a very high ratio of solid material to liquid may lead to the dissolution of impurities, reducing the solubility of total (poly)phenols and anthocyanins (Liu et al., 2021).

3.3. The influence of pH value on the content of total (poly)phenols, flavonoids, and anthocyanins in the extract

The influence of pH value on the content of total (poly)phenols, flavonoids, and anthocyanins in the extract, with constant other process parameters (ethanol content in the solvent = 60 %, solid-to-solvent ratio = 1:30 w/v, and time of 30 min), is depicted in Figure 3.

From Figure 3a, it can be observed that in the first extraction step, the content of total (poly)phenols in the extract slightly decreased with the increase in pH value from 3 to 7 (from 54.07 mg (GAE) / g to 53.25 mg (GAE) / g). However, the content of total (poly)phenols significantly decreased with further increase in pH value from 7 to 10 (from 53.25 mg (GAE) / g to 38.32 mg (GAE) / g, i.e., by 28.04 %). On the other hand, there is a different effect on the content of total (poly)phenols in the extract in the second extraction step. Increasing the pH value from pH = 3 to pH = 7 resulted in an increase in the content of total (poly)phenols in the extract (29.42 mg (GAE) / g and 39.2 mg (GAE) / g, respectively). However, further increase in pH value to 10 led to a decrease in the content of total (poly)phenols in the extract (34.93 mg (GAE) / g). It is also noticeable that the highest content of total (poly)phenols in the extract (39.2 mg (GAE) / g) was achieved in a neutral environment (pH = 7).

From Figure 3a, it can be observed that in the first extraction step, the content of total (poly)phenols in the extract slightly decreased with the increase in pH value from 3 to 7 (from 54.07 mg (GAE) / g to 53.25 mg (GAE) / g). However, the content of total (poly)phenols significantly decreased with further increase in pH value from 7 to 10 (from 53.25 mg (GAE) / g to 38.32 mg (GAE) / g, i.e., by 28.04 %). On the other hand, there is a different effect on the content of total (poly)phenols in the extract in the second extraction step. Increasing the pH value from pH = 3 to pH = 7 resulted in an increase

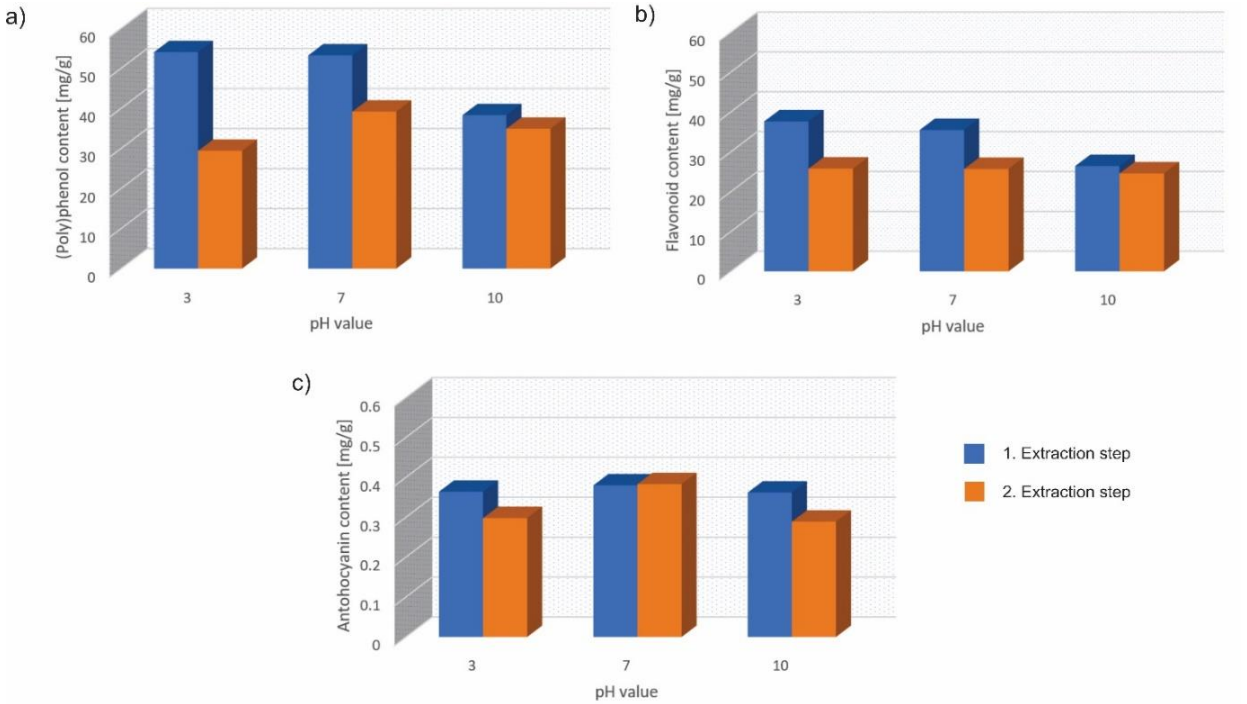

Figure 3. Influence of pH value on the content of a) total (poly)phenols, b) flavonoids, and c) anthocyanins in the extract

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From Figure 3b, it can be observed that in the first extraction step, the content of total flavonoids in the extract was highest at pH = 3 (37.61 mg (CTH) / g). The content of flavonoids in the extract slightly decreased in a neutral pH environment (35.46 mg (CTH) / g), while it significantly decreased in a basic environment (26.42 mg (CTH) / g). On the other hand, in the second extraction step, there were no drastic differences in the content of flavonoids in the extract. Thus, at pH = 3, the content of flavonoids in the extract was 25.78 mg (CTH) / g, at pH = 7 it was 25.69 mg (CTH) / g, and at pH = 10, the content of flavonoids in the extract was 24.57 mg (CTH) / g. It is noticeable here that the highest content of total flavonoids in the extract (25.78 mg (CTH) / g) was in an acidic environment.

From Figure 3c, it can be observed that in the first extraction step, the content of anthocyanins in the extract was highest at pH = 7 (0.38 mg (Cy3G) / g). At pH values of 3 and 10, the content of anthocyanins was slightly lower, at 0.364 mg (Cy3G) / g and 0.362 mg (Cy3G) / g, respectively. In the second extraction step, it is noticeable that at pH = 7, the content of anthocyanins remained almost unchanged (0.383 mg (Cy3G) / g) compared to the first step. On the other hand, when pH = 3 and pH = 10 were used, a decrease in the content of anthocyanins in the extract by 18-20 % was observed (0.298 mg (Cy3G) / g and 0.289 mg (Cy3G) / g, respectively).

The reason for better extraction of (poly)phenols in acidic and neutral environments is that they are slightly acidic compounds, which means they can dissociate into phenolate ions in alkaline solutions. At lower pH values, phenols are in the unionized form, while at higher pH values, they dissociate into phenolate ions.

3.4. The influence of time on the content of total (poly)phenols, flavonoids, and anthocyanins in the extract

Figure 4 illustrates the dependency of the content of total (poly)phenols, flavonoids, and anthocyanins in the extract on time, under constant other process parameters (ethanol content in the solvent = 60 %, pH = 7, and solid-to-solvent ratio = 1:30 w/v).

From Figure 4a, it is observed that the content of total (poly)phenols in the extract is lowest after 15 minutes of extraction (30.16 mg (GAE) / g). With further extraction times of 30 minutes and 45 minutes, there is an increase in the content of total (poly)phenols in the extract (38.73 mg (GAE) / g and 48.02 mg (GAE) / g, respectively). Therefore, by extending the time from 15 minutes to 45 minutes, there is an increase in the content of total (poly)phenols by 59.22 %. The effect in the second

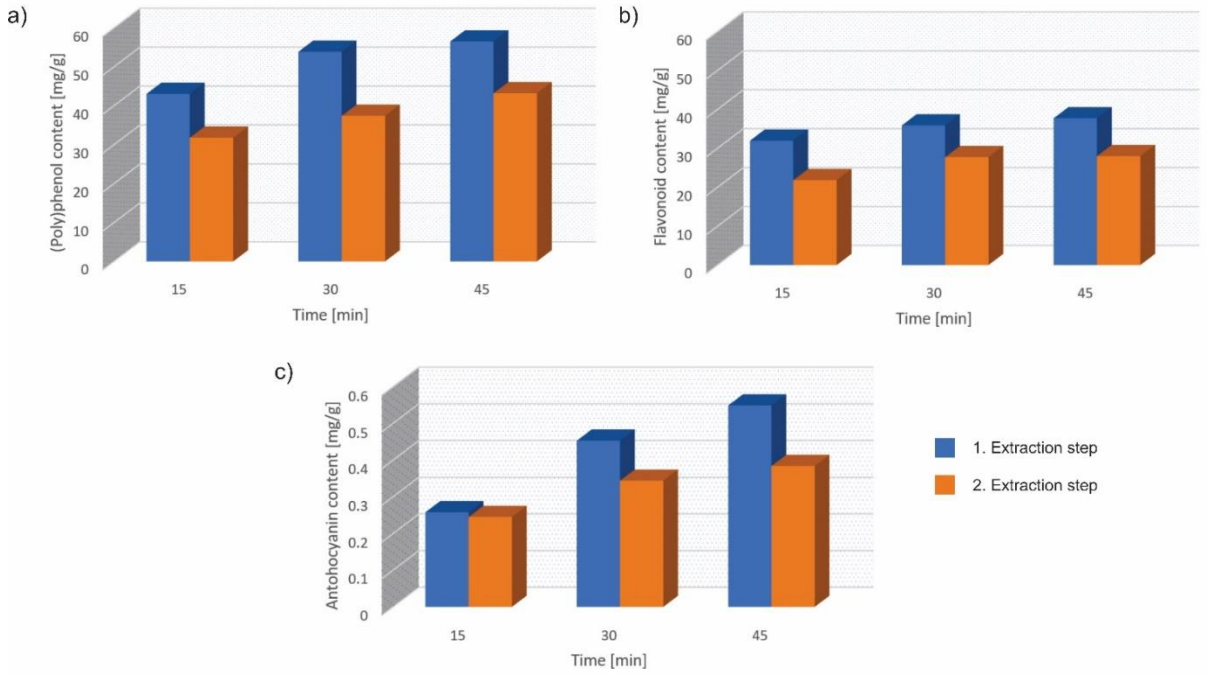

Figure 4. The influence of time on the content of a) total (poly)phenols, b) flavonoids, and c) anthocyanins in the extract

extraction step is similar to the first extraction step. After 15 minutes, the lowest content of total (poly)phenols in the extract is observed (19.8 mg (GAE) / g), while increasing this parameter to 30 minutes increases the content of total (poly)phenols in the extract by 51.01 % (29.9 mg (GAE) / 100 g). With further time extension to 45 minutes, the content of total (poly)phenols slightly increases (31.91 mg (GAE) / g), thus indicating that 30 minutes is the optimal time for the second extraction step.

Looking at the first extraction step from Figure 4b, it is observed that the content of flavonoids in the extract is highest after 15 minutes of extraction (31.97 mg (CTH) / g). With further extension of time to 30 minutes and 45 minutes, there is a slight increase in the content of flavonoids in the extract (35.88 mg (CTH) / g and 37.71 mg (CTH) / g, respectively). Hence, 15 minutes is optimal for flavonoid extraction, as further extension of time does not significantly change its content. In the second extraction step, the lowest content of flavonoids in the extract is observed after 15 minutes (21.83 mg CTH / g). Similar to the first extraction step, further extension of this parameter increases the content of flavonoids in the extract (27.74 mg (CTH) / g after 30 minutes and 27.99 mg (CTH) / g after 45 minutes). Since the flavonoid content does not significantly change after 30 minutes, 30 minutes is considered optimal.

Regarding the first extraction step, Figure 4c shows that the content of anthocyanins in the extract increases with longer extraction times. After 15 minutes of extraction, the anthocyanin content is 0.258 mg (Cy3G) / g, while further extension of time to 30 minutes and 45 minutes results in an increase in the content of anthocyanins in the extract to 0.453 mg (Cy3G) / g and 0.549 mg (Cy3G) / g, respectively. Since increasing the time from 15 minutes to 30 minutes results in a substantial increase in anthocyanin content by 43.04 %, while further extension results in only a 21.19 % increase, 30 minutes is considered optimal for anthocyanin extraction in the first step. In the second extraction step, using a lower parameter value (15 minutes), it is observed that the content of anthocyanins in the extract slightly decreases (0.258 mg (Cy3G) / g in the first step and 0.245 mg (Cy3G) / g in the second step) compared to the first step. At the parameter value of 30 minutes and 45 minutes, there is a significant decrease in the content of anthocyanins in the second step compared to the first step - for 30 minutes, the anthocyanin content is 0.453 mg (Cy3G) / g in the first step and 0.344 mg (Cy3G) / g in the second step, which is a decrease of 24.06 %, while for 45 minutes, the anthocyanin content is 0.549 mg (Cy3G) / g in the first step and 0.384 mg (Cy3G) / g in the second step, which is a decrease of 30.05 %.

4. Conclusion

The moderate ethanol content in the solvent (60 vol. %) has a favorable effect on the extraction of total (poly)phenols, flavonoids, and anthocyanins, as water acts as a swelling agent, while ethanol breaks the bonds between dissolved matter and the matrix. A high concentration of ethanol in the solvent leads to lower yield of (poly)phenolic compounds due to possible protein denaturation, inhibition of phenol breakdown from the matrix, and reduced synthesis of (poly)phenolic compounds. The highest degree of flavonoid extraction occurs at the highest solid-to-solvent ratio due to the

greater contact surface area between the sample and the solvent, enabling the more efficient mass transfer of flavonoids from the solid matrix to the liquid phase. A higher ratio can lead to faster mass transfer, resulting in higher yields due to the amount of solvent available for dissolving (poly)phenolic compounds. The highest content of total (poly)phenols and anthocyanins is extracted at a moderate solid-to-solvent ratio because very high ratios lead to impurity dissolution, reducing the solubility of total (poly)phenols and anthocyanins. (Poly)phenol extraction is more efficient in acidic and neutral environments, as they are weakly acidic compounds, which means that they can dissociate into phenolate ions in alkaline solutions. The optimal extraction time for most (poly)phenolic compounds is 15 minutes, as further extension of the time does not significantly increase their content in the extract. Finally, it was found that extraction in the first step is more efficient because the (poly)phenolic compounds diffuse more slowly during solvent recirculation, or the driving force is lower due to solvent saturation.

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Ispitivanje izvodljivosti recirkulacije rastvarača u procesu ekstrakcije (poli)fenola iz cveta zove (*Sambucus nigra L.*)

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I Z V O D

U ovom radu izvedena je dvostepena ekstrakcija čvrsto – tečno tradicionalnom tehnikom maceracije. Nakon prvog koraka ekstrakcije, ekstrakt je odvojeno istrošenog biljnog materijala (cvet zove), nakon čega je sveži cvet zove dodat ekstraktu. Eksperimentalni parametri uključivali su različite koncentracije etanola (30 %, 60 % i 90 %), odnose čvrsto-tečno (1:15, 1:30 i 1:45 m/v), pH vrednosti (3, 7 i 10) i vremena ekstrakcije (15, 30 i 45 minuta), dok je jedan faktor u vremenu (One-factor-at-a-time (OFAT)) korišćen kao eksperimentalni dizajn. Rezultati su pokazali da je optimalna koncentracija etanola u rastvaraču 60 vol.% za oba koraka ekstrakcije. Povećanje odnosa čvrsto-tečno sa 1:15 m/v na 1:30 m/v rezultuje većim sadržajem (poli)fenola, dok je blagi pad sadržaja (poli)fenola primećen kod većih odnosa čvrsto-tečno. Sadržaj (poli)fenola je približno isti u kiseloj i neutralnoj sredini, dok je u baznoj sredini manji u odnosu na kiselu i neutralnu sredinu. Iako sadržaj (poli)fenolnih jedinjenja raste sa produženjem vremena ekstrakcije, 30 minuta je optimalno vreme budući da se daljim produženjem ne povećava značajno njihov sadržaj. Dodatno, primećeno je da je ukupan sadržaj (poli)fenola bio niži u drugom koraku ekstrakcije, što sugeriše na zasićenje rastvarača nakon prvog ekstrakcionog koraka.