

POLYPHENOL PROFILE OF FLOWERS, FRUITS AND FOOD SUPPLEMENTS OF *SAMBUCUS NIGRA*

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Abstract

Currently, a wide variety of food supplements derived from Sambucus nigra is available on the market, claiming to have powerful antioxidant effects, due to their high level of polyphenols. However, quantitative data for their polyphenolic content is sporadic and inconsistent. In the present study, water and methanol extracts of Sambucus nigra flower and fruits, as well as food supplements containing them, were analyzed for their Total Phenols and Total Flavonoid content, along with HPLC analysis for the levels of individual flavonoids (+)-Catechin, (-)-Epicatechin, and Rutin. The results show that the Total Phenols and Total Flavonoids are highest in infusions from the plant's flowers. Of the individual flavonoids, significant amounts were only found for Rutin. Conclusion: Sambucus nigra is a significant source of antioxidant polyphenolic compounds, with levels in its flowers exceeding those in its fruits. At the same time, the polyphenol levels in various Black Elderberry based food supplements can vary significantly, primarily depending on whether standardized extracts are used in the formulations.

Keywords: Polyphenols, Total Phenols, Total Flavonoids, *Sambucus nigra*

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Introduction

Black Elderberry (Sambucus nigra), also known as European Elder, is a small tree or shrub whose fruits and flowers are a valuable source of nutritional and pharmacological substances. It is found in natural habitats across Bulgaria, typically in open areas. The flowers and fruits of Elderberry are popular natural sources of bioactive compounds with high antioxidant activity (Schumacher, 2015). Black Elderberry contains a wide range of beneficial nutrients, including various minerals such as iron, potassium, phosphorus, and copper; vitamins A, B, and C, dietary fiber, and organic compounds (Ferreira et al., 2020). The flowers (*Sambucus Flos*) and fruits (*Sambucus Fructus*) are particularly rich in polyphenolic compounds, primarily flavonols,

phenolic acids, and anthocyanins, which are responsible for their antioxidant and anti-inflammatory properties.

The presence of phenolic compounds in plant extracts determines their antioxidant and pharmacological properties. Polyphenols can counteract the harmful effects of free radicals in the body through three different mechanisms: as preventive antioxidants at the initiation stage of lipid peroxidation by chelating transition metal ions, thereby inactivating their catalytic role in chain radical reactions; by quenching singlet oxygen; and through their potent radical-scavenging ability, acting as free radical scavengers and breaking the chain of peroxidation (Rudrapal, 2022). Polyphenolic compounds in Black Elderberry extracts can interact with reactive oxygen species (ROS) generated by chain radical reactions and deactivate them. Under normal homeostatic conditions, ROS play an important role in various physiological processes, regulating key cellular functions such as proliferation, differentiation, and apoptosis, and participating in the defense against pathogens, however at the same time express harmful oxidative effects *in vivo* towards biomolecules like proteins, lipids and DNA (Nakamura&Takada, 2021). Numerous studies confirm the high antioxidant capacity of *Sambucus nigra* fruits (Dawidowicz et al., 2006; Li et al, 2021; Olejnik et al., 2016).

Anti-inflammatory activity

In addition to their high antioxidant capacity, Elderberry extracts are associated with strong anti-inflammatory properties (Studzinska-Sroka et al., 2024). Current research shows that inflammation is among the primary factors contributing to various chronic diseases, including certain cancers, hypertension, cardiovascular diseases, dyslipidemia, metabolic syndrome, insulin resistance, allergies, inflammatory bowel disease, chronic asthma, neurodegenerative diseases, rheumatoid arthritis, multiple sclerosis, and more. Due to their high anthocyanin content, *Sambucus nigra* fruit extracts exhibit powerful anti-inflammatory effects by inhibiting prostaglandin activity (Mena et al., 2014). It has also been found that the anti-inflammatory activity of Elderberry extracts increases proportionally with their antioxidant activity and their polyphenol content (Stepien, et al., 2023).

Antibacterial activity

The antibacterial activity of *S. nigra* extracts is linked to their phenolic acid and flavonoid content. The compounds that have the greatest impact on antibacterial activity include kaempferol, apigenin, ferulic acid, and p-coumaric acid. Studies show that bacteria most sensitive to *S. nigra* phenolic compound extracts include *Micrococcus luteus*, *Proteus mirabilis*, *Pseudomonas fragi*, and *Escherichia coli* (Przybylska-Balcerek et al., 2021). For instance, a study by Hearst and colleagues demonstrated that lyophilized Elderberry extracts have a strong antibacterial effect against most of the 13 nosocomial pathogens tested, including methicillin-resistant *Staphylococcus aureus* (MRSA), as well as *Staphylococcus sp.*, *Bacillus cereus* (gram-positive), *Salmonella poona*, and *Pseudomonas aeruginosa* (gram-negative) (Hearst et al., 2010). Additionally, Chatterjee and colleagues showed that Elderberry inhibits *Helicobacter pylori* with approximately 30% effectiveness (Chatterjee et al., 2004).

Antiviral properties

Numerous studies reveal that compounds such as kaempferol-3-Rutinoside, isorhamnetin-3-Rutinoside, isorhamnetin-3-glucoside, and cyanidin derivatives exhibit antiviral properties (Ren et al, 2024). Scientific literature describes that *Sambucus nigra* extracts bind phenolic compounds to the H1N1 virus, rendering it incapable of infecting host cells (Roschek et al, 2009). Experimental data from various *in vitro* and *in vivo* studies demonstrate the ability of Elderberry extracts to inhibit the replication of influenza A and B viruses (Tiralomgo et al.,

2016; Zakay-Rones et al., 1995). Additionally, some studies show that Elderberry stimulates the immune response and effectively suppresses viral replication (Torabian, 2019). These findings support the health claims of various food supplements made from *Sambucus nigra* and *Sambucus ebulus* for use in flu treatments.

Antidiabetic properties

Over the past decade, scientific publications have increasingly shown that anthocyanins in *Sambucus nigra* extracts, particularly cyanidin-3-glucoside, procyanidins, and their metabolites, enhance glucose uptake in human skeletal muscle cells. Black Elderberry inhibits fat and sugar absorption in the gastrointestinal tract, positively influencing obesity and demonstrating antidiabetic effects in metabolic dysfunctions (Ho et al., 2017). Elderberry extracts are rich in anthocyanins, which alleviate systemic inflammation and insulin resistance (Zima et al., 2024). Recent studies emphasize the beneficial effects of flavonoids in improving glucose metabolism, preventing, and managing diabetes and its complications. Research on cell lines and animal models suggests that all flavonoid classes possess antidiabetic activity, improve β -cell function and insulin action (Yi et al., 2023), enhance glucose utilization, and reduce cholesterol and triglyceride synthesis (Thompson et al., 2024).

Currently, a rich palette of nutritional supplements from *Sambucus nigra*, with claims of powerful antioxidant action, is available on the global and Bulgarian markets. However, quantitative data for their evaluation is sporadic and inconsistent. The aim of the present study is to characterize the quantitative content of Total Phenols compounds, Total Flavonoids, and individual flavonoids representatives – (+)-Catechin, (-)-Epicatechi, and the flavonol Rutin in Bulgarian food supplements as well as flowers and fruits from *Sambucus nigra*, which are widely used for the preparation of herbal infusions and decocts.

Methodology

In the present study, the flowers and fruits of Bulgarian *Sambucus nigra*, purchased from retail shops in Sofia, were examined alongside two types of food supplements: syrup containing both standardized and non-standardized extracts from *Sambucus nigra* fruits. Three individual dry samples (100g each) were purchased from herbal pharmacies, while three packages of each selected food supplement were acquired from the pharmacy network.

Preparation of Methanol Extracts

The finely ground plant material or crushed fruits, herba or food supplements (0.2500 g) were weighed into a 25.00 ml volumetric flask and extracted with 80% methanol/water (v/v) in an ultrasonic bath for 30 minutes at 30°C. The solutions were ultracentrifuged at 10,000 rpm for 5 minutes and filtered through a membrane filter (0.45 μ m) prior to analysis.

Preparation of Aqueous Extracts: Infusions and Decocts

For the preparation of high-quality infusions and decocts, specific technological protocols and basic rules for water extraction must be followed. Infusions were steeped for 60 minutes, while decocts were prepared over 40 minutes.

Infusion Preparation

Ratio: 1 gram of herbal material per 100 g of distilled water. The infusions were prepared in special containers called "infundirs," made of materials with low thermal conductivity — most commonly porcelain. The infundir was preheated for 15 minutes in a boiling water bath, after

which the herbal material and water were added. The heating process lasted 15 minutes, followed by cooling at room temperature for 45 minutes. The obtained extract was filtered and stored in a cool place.

Decocts Preparation

Ratio: 5 grams of fruit per 100 grams of distilled water. The fruits were boiled in boiling distilled water for 5–10 minutes according to the manufacturer's instructions. They were steeped for 30 minutes, cooled, and filtered. The resulting samples were stored at 4°C for analysis, without preservatives, and were analyzed no later than 24 hours after preparation.

Determination of Total Phenols

The Folin-Ciocalteu reagent was used to determine total phenols by oxidizing phenolates into a blue complex, which was measured spectrophotometrically at a wavelength of $\lambda = 750$ nm. The determination followed the method described by Marinova et al., 2005. Briefly, 1 ml of appropriately diluted aqueous extracts, methanol extracts, or standard gallic acid solutions (GA) was added to a 25 ml volumetric flask containing 9 ml of distilled water. Then, 1 ml of Folin-Ciocalteu reagent was added, and after 5 minutes, 10 ml of 7% Na₂CO₃ was added. The solution was topped up to the mark with distilled water, mixed, and left for 90 minutes at room temperature. Absorption was measured against a blank at $\lambda = 750$ nm. The Total Phenols were quantified using an external calibration method with Gallic acid as the standard. Results were expressed as Gallic acid equivalents (mg GAE/100 ml) for aqueous extracts (infusions and decocts) and in mg GAE/g for methanol extracts, allowing comparative analysis via cluster analysis.

Determination of Total Flavonoids

Total flavonoids were determined according to Marinova et al., 2005. An aliquot of 1 ml of extract or standard Rutin solutions was placed in a 10 ml volumetric flask containing 4 ml of distilled water. Next, 0.3 ml of 5% NaNO₂ was added, followed by 0.3 ml of a 10% aqueous AlCl₃ solution after 5 minutes. After 6 minutes, 2 ml of 1M NaOH was added, and the solution was diluted with distilled water to the mark, mixed, and left for 30 minutes. Absorption of the pink complex was measured against a blank at $\lambda = 510$ nm. Total flavonoid content was calculated using an external calibration method. Results were expressed as Rutin equivalents (mg RE/100 ml) for aqueous extracts and as mg RE/g for methanol extracts, enabling comparative statistical analysis.

Spectrophotometric absorption measurements for total phenols and flavonoids were performed with a Lambda 25 spectrophotometer (Perkin Elmer).

Determination of (+)-Catechin, (-)-Epicatechin, and Rutin Using High-Performance Liquid Chromatography (HPLC)

HPLC Apparatus and Conditions

The HPLC system consisted of a Perkin-Elmer (Norwalk, CT) Flexar LC pump, Flexar Photo Diode Array Plus detector (PDA), autosampler, thermostat, and integrated degasser. Chromatographic data were processed with Chromera HPLC PDA software, version 4.1.1.6396.

Chromatographic separation of phenolic compounds ((+)-Catechin, (-)-Epicatechin, and Rutin) was achieved at a constant temperature of 30°C using a Luna C18 column (3 μ m, 150 mm x 4.6 mm, Phenomenex, USA) with a guard column of the same stationary phase. Elution was performed at a flow rate of 0.9 ml/min with a mobile phase consisting of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). A linear gradient elution

program was applied as follows: 0–2 minutes: 15% B; 2–4 minutes: 20% B; 4–6 minutes: 25% B; 6–8 minutes: 30% B; 8–10 minutes: 35% B; 10–12 minutes: 35% B. The column was re-equilibrated for 5 minutes with 15% B. Sample and standard solution injection volumes were 20 µl. Detection wavelengths for (+)-Catechin and (-)-Epicatechin were set at 280 nm, and for Rutin, at 355 nm, with a reference wavelength of 620 nm and a fixed bandwidth of 19 nm. Chromatographic peaks were identified by comparing retention times and peak shapes across two wavelengths (280 and 355 nm). Quantification of catechin, epicatechin, and Rutin was performed using the external standard method with calibration curves obtained by injecting a series of standard solutions.

Research Results

The results for total phenolic and flavonoid content in methanol extracts and aqueous extracts (infusions and decocts) of the selected medicinal plants (herbs and fruits), along with data from the HPLC analysis of (+)-Catechin, (-)-Epicatechin, and Rutin, are presented in Table 1.

The data in Table 1 are expressed as mean values from triplicate analyses, along with the standard deviation of the mean.

At the industrial level, Elderberry fruits are primarily used as colorants due to their high pigment content (especially anthocyanins). Their health benefits have been proven in numerous studies. In traditional medicine, infusions and decocts of Elderberry flowers and fruits are widely applied.

Table 1

Total Phenols, Total Flavonoids, (+)-Catechin and Rutin content in *Sambucus nigra* medicinal plant and food supplements

Sample	Total Phenols mg GAE/g	Total Flavonoids mg RE/g	(+)-Catechin mg/g	Rutin mg/g
Medicinal plant				
Methanolic extracts				
<i>Sambucus nigra</i> , flower	43.07 ± 0.24	34.61 ± 0.63	-	18.58 ± 0.38
<i>Sambucus nigra</i> , fruit	16.88 ± 0.44	16.24 ± 0.44	0.15 ± 0.03	2.82 ± 0.07
Infusions				
<i>Sambucus nigra</i> , flower	55.90 ± 0.69	53.53 ± 0.52	-	20.64 ± 0.57
Decocts				
<i>Sambucus nigra</i> , fruit	16.63 ± 0.40	14.17 ± 0.37	-	2.15 ± 0.05
Food Supplements – syrup containing extract of <i>Sambucus nigra</i> fruits				
Methanolic extracts				
<i>Sambucus nigra</i> – non standardized extract (1)	5.16 ± 0.03	3.60 ± 0.01	-	0.05 ± 0.002
<i>Sambucus nigra</i> – standardized extract (2)	20.76 ± 0.21	18.76 ± 0.11	0.10 ± 0.01	0.26 ± 0.01
(-)-non detected				

The analysis results show that among the individual flavonoids, the flavonol Rutin was detected in all examined samples. However, (-)-Epicatechin was not found in any of the samples and is therefore not included in the table. The content of (+)-Catechin was identified in the methanol extracts of Elderberry fruits and one food supplement containing a standardized extract of Elderberry fruits. (+)-Catechin was not detected in the aqueous extracts.

Based on the results for *Sambucus nigra* flowers and fruits, it was observed that the level of polyphenols was higher in the flowers of the medicinal plants compared to their fruits, except for (+)-Catechin, which was detected in the methanol extracts of Black Elderberry fruits. The Rutin content in Elderberry flower extracts was approximately six times higher than in the fruit extracts, suggesting that flavonoid levels decrease during the ripening and maturation process of the fruit. Literature data also indicate that flavonoid accumulation in fruits is significantly influenced by the ripening stage. Numerous studies confirm the general trend that flavonoids are typically present in higher quantities in unripe fruits and plant tissues (Wang & Lin, 2000).

The analysis of food supplements containing *Sambucus nigra* also yielded important findings. Currently, the Bulgarian market offers a variety of Elderberry-based food supplements aimed at enhancing the body's antioxidant protection, supporting immune defense, and improving cardiovascular function. The health benefits of *Sambucus nigra* products are largely attributed to their high content of phenolic compounds. The biological activity of polyphenols — secondary metabolites in higher plants is associated with their antioxidant, immunostimulatory, anti-inflammatory, antiallergic, anticancer, antibacterial, and antiviral properties.

The results of this study show that the differences in polyphenol content among various types of food supplements vary significantly. The total phenolic content in food supplement 2 is approximately four times higher than in food supplement 1.

Discussion

At the industrial level, Elderberry fruits are primarily used as dyes due to the high amounts of pigments they contain (especially anthocyanins). Their health benefits have been confirmed by numerous studies. In traditional medicine, infusions and decocts of elderflowers and fruits are widely used. Considering the results for the flowers and fruits of black Elderberry, it was established that the level of polyphenols is higher in the flowers of the medicinal plant compared to its fruits, except for (+)-Catechin, which was detected in the methanol extracts of *Sambucus nigra* fruits. The Rutin content in black elderflower extracts is approximately six times higher than in the fruits, suggesting that flavonoid levels decrease during the development and ripening of the fruit. Literature data also show that flavonoid accumulation in fruits is strongly influenced by the degree of fruit ripeness. Numerous studies confirm the general trend that flavonoids are usually present in higher quantities in unripe fruits and plant tissues (Wang and Lin, 2000).

The analysis of dietary supplements containing *Sambucus nigra* fruit extracts is also noteworthy. Currently, the Bulgarian market offers a range of dietary supplements from black and herbaceous Elderberries aimed at boosting the body's antioxidant defenses, enhancing immune protection, and improving cardiovascular system functions. The health benefits of *Sambucus nigra* products are largely attributed to their high content of phenolic compounds. The biological activity of polyphenols, secondary metabolites in higher plants, is associated with their antioxidant, immunostimulatory, anti-inflammatory, antiallergic, anticancer, antibacterial, and antiviral properties. The results of the study show that the polyphenol content in different types of dietary supplements varies significantly. The total phenol content in dietary supplement 2 is approximately four times higher than in dietary supplement 1. It should also be

noted that while the levels of total phenols and flavonoids in a dietary supplement containing standardized Black Elderberry extract slightly exceed those in aqueous decocts and methanol extracts of the plant's fruits, the Rutin content in dietary supplements is about ten times lower (2.82 mg/g, 2.15 mg/g, and 0.26 mg/g, respectively).

An important highlight of the results is that Black Elderflowers, in both aqueous and methanol extracts, contain a significantly higher level of polyphenols compared to the fruits. Similar results were found for Rutin. This indicates that, in addition to the widely used dietary supplements from Black Elderberry fruits, elderflowers hold great potential for developing antioxidant formulations and dietary supplements that can help reduce oxidative stress in the body. Although the flowers of the plant do not contain anthocyanins, which are the main group of red polyphenol pigments in the fruits, the presence of other groups of phenolic compounds, such as phenolic acids, appears to account for the very high polyphenol levels in the flowers.

The results of studies on the phytochemical composition of medicinal plants and the identification of components relevant to antioxidant activity and/or the anti-inflammatory action of herbs provide opportunities for further exploration of their biological effects in living systems, including cell cultures, experimental animals, and humans. Additional in-depth studies of individual components obtained through various extraction methods and investigations into the antioxidant potential of each individual fraction are necessary for their use as active substances in the pharmaceutical industry.

Conclusions

In the present study new data for polyphenolic content in *Sambucus nigra* medicinal plant – flowers, fruits and food supplements are presented. The content of Total Phenols, Total Flavonoids and individual representatives of catechins and flavonols in methanolic extracts, infusions and decocts have been assessed in order to provide new data for development of effective antioxidant medicinal plant compositions. Data show that *Sambucus nigra* flower extracts are among the richest sources of polyphenolic antioxidants among Bulgarian medicinal plants.

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