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Issue Article

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CHROMATOGRAPHIC STUDY OF PHENOLIC COMPOUNDS IN DRIED FRUITS OF SAMBUCUS NIGRA

Abstract: *The aim of the present study was to determine the qualitative and quantitative composition of flavonoid glycosides and anthocyanins in the fruits of Sambucus nigra using high-performance liquid chromatography (HPLC). Sambucus nigra L. is a medicinal plant widely distributed in Georgia and many other regions of the world. Its fruits are rich in biologically active compounds, including flavonoids, anthocyanins, vitamins, and phenolic acids. Qualitative analysis was performed using a Waters gradient chromatograph (USA). Chromatographic analysis confirmed the presence of at least 10 flavonoid glycosides in elderberry fruits. The results demonstrate that Sambucus nigra fruits are rich in phenolic compounds, especially anthocyanins and polyphenols, which remain relatively stable after drying. These findings support the potential use of elderberry fruits as a source of biologically active compounds for the development of herbal medicinal products.*

Key words: *Sambucus nigra, HPLC, anthocyanins, flavonoids, chlorogenic acid, phenolic compounds.*

Language: *English*

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Introduction

Sambucus nigra L. - black elderberry is a shrub or small tree belonging to the Caprifoliaceae family. In Georgia, the plant is widely distributed and occurs in regions such as Svaneti, Guria, Adjara, Kartli, and Kakheti. Elderberry generally grows in moderately fertile, moist, and well-drained soils rich in mineral compounds, including phosphates, nitrogen, and potassium.

Among approximately 40 species distributed worldwide, two are represented in Georgia: black elderberry and dwarf elder. The black berries of *Sambucus nigra* are traditionally used for medicinal purposes, whereas the red berries are considered toxic.

In many countries, elderberry raw materials are collected from wild-growing plants. During harvesting, contamination with flowers of *Sambucus ebulus* and other related species may occur. Since some of these species contain compounds toxic to humans, commercial cultivation of black elderberry began experimentally in Europe, the United States, and Canada in the early 1980s.

The chemical composition of *Sambucus nigra* depends on factors such as cultivar, geographical location, ripening stage, and climatic conditions. Elderberry fruits contain carbohydrates, dietary fibers, pectin, proteins, vitamins, essential oils, organic acids, and trace elements. Previous studies have reported the presence of ascorbic acid and vitamin E, although quantitative findings vary [1, 2, 3].

All parts of the elderberry plant contain cyanogenic glycosides, particularly sambunigrin and prunasin, which may release hydrocyanic acid upon hydrolysis. These compounds are mainly present in unripe berries and are largely degraded during thermal processing.

Elderberry has long been used in traditional medicine because of its immunostimulatory, antioxidant, and anti-inflammatory properties. Both fresh and dried fruits are utilized in folk medicine for the treatment and prevention of various disorders.

Materials and Methods: Dried fruits of *Sambucus nigra* were used for the study. For sample preparation, 5–10 g of dried fruits were mixed with 100 mL ethanol acidified with 3% hydrochloric acid or another organic acid.

Extraction Procedure: For optimal extraction of anthocyanins, the extractant and sample were maintained at temperatures of 15–18°C.

HPLC Analysis: Qualitative analysis was carried out using a Waters (USA) gradient chromatograph equipped with: UV/Visible Detector 2489; Binary HPLC Pump 1525; Symmetry C18 chromatographic column; The mobile phase consisted of: Solvent A: 5% formic acid, Solvent B: methanol; A linear gradient elution system was applied with a solvent flow rate of 1 mL/min. The injection volume was 20 µL [4, 5].

Detection wavelengths were:
- 370 nm for flavonoid glycosides;
- 510 nm for anthocyanins;
- 310 nm for chlorogenic acid.

Results and Discussion:

Flavonoid Glycosides: Chromatographic analysis confirmed the presence of at least 10 flavonoid glycosides in elderberry fruits detected at 370 nm. A peak tentatively attributed to sambunigrin was observed in the chromatogram based on retention characteristics.

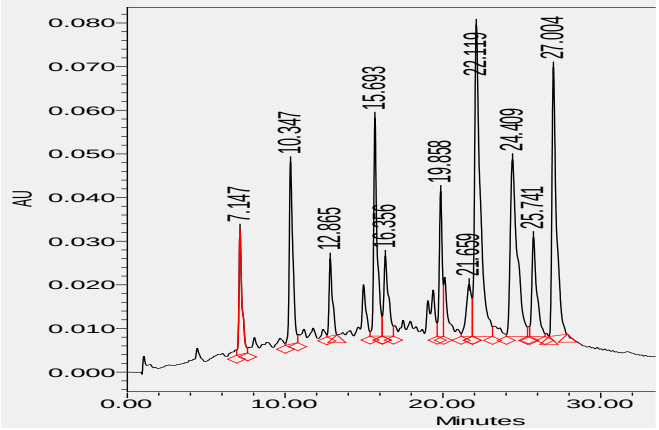

Figure 1. HPLC chromatogram of flavonoid glycosides from elderberry fruits detected at 370 nm.
- **Rutin**
- **Quercetin-3-O-glucoside**
- **Quercetin-3-O-rutinoside**
- **Sambunigrin**

Rutin, also known as vitamin P, possesses significant pharmacological properties, including reduction of capillary permeability, improvement of blood circulation, and anti-inflammatory activity.

Quercetin is a natural flavonoid with strong antioxidant effects. It protects tissues against oxidative stress and may reduce the risk of cardiovascular diseases.

Sambunigrin is a cyanogenic glycoside characteristic of elderberry fruits. Although elderberry possesses valuable medicinal properties, the potential toxicity of sambunigrin should also be considered [6, 7, 8].

Anthocyanins: Among anthocyanins detected at 510 nm, cyanidin glycosides were dominant, particularly cyanidin-3-glucoside with a retention time of 10.338 min and a peak area of 78.42%.

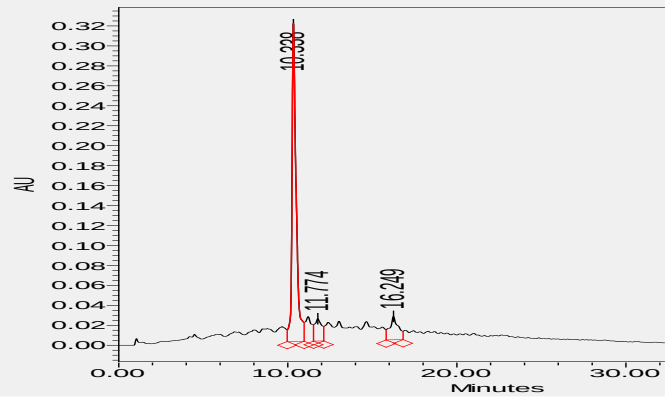

Figure 2. HPLC chromatogram of anthocyanins from elderberry fruits detected at 510 nm
- **Cyanidin-3-glucoside**
- **Cyanidin-3-glucoside-5-sambubioside**

Cyanidin-3-glucoside is a polyphenolic pigment responsible for the intense coloration of many plants. Previous studies have demonstrated its antioxidant and photoprotective properties.

Chlorogenic Acid: Chromatographic analysis demonstrated the presence of significant amounts of chlorogenic acid in elderberry fruits. Chlorogenic acid is an important natural antioxidant involved in plant respiration and growth. Pharmacological studies have suggested hypoglycemic, hepatoprotective, and antitumor effects.

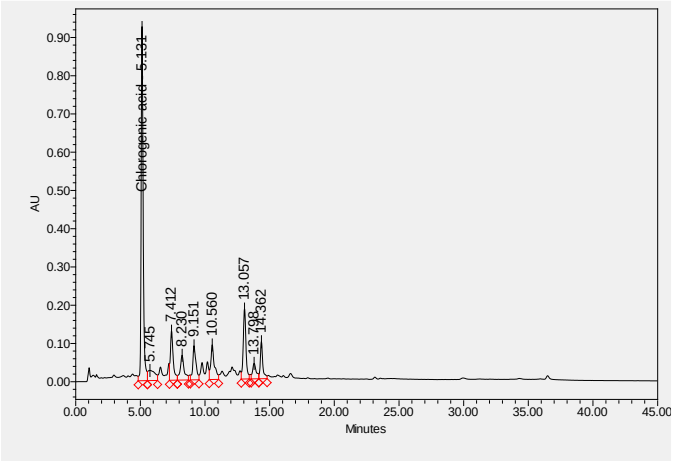

Figure 3. HPLC chromatogram of chlorogenic acid from elderberry fruits detected at 310 nm

Chromatographic Characteristics: Table 1 summarizes the chromatographic characteristics of selected biologically active compounds identified in elderberry fruits by HPLC analysis.

Table 1. Chromatographic characteristics of flavonoid glycosides and phenolic compounds identified in *Sambucus nigra* dried fruits

	Name	Retention Time	Area	% Area	Height	Int Type	Peak Codes
1	Delphinidin 3-galactoside	11.581	468500	3.15	29455	VV	Q20
2	Peak2	11.977	330133	2.22	24245	VV	Q20
3	Delphinidin 3 -glucoside	12.442				Missing	
4	Peak4	12.802	580919	3.90	35090	VV	Q20

5	Cyanidin 3-galactoside	13.033	117723	0.79	17902	VV	I06 Q20
6	Delphinidin 3-arabinoside	13.813	3410947	22.90	224873	VV	Q20
7	Peak7	14.608	404394	2.71	25088	VV	Q20
8	Petunidin 3-galactoside	15.014	239592	1.61	13892	VV	Q20
9	Peonidin 3-glucoside	18.158				Missing	
10	Quercetine	32.878				Missing	
11	Chlorogenic acid	4.655	7401885	20.58	581920	VV	
12	Quercetin 3-glucoside	19.480	94156	1.81	5518	VV	
13	Quercetin 3-rutinoside	17.906	112363	2.15	5783	VV	

Conclusion

HPLC analysis demonstrated that *Sambucus nigra* fruits are rich in phenolic compounds, particularly anthocyanins, with concentrations exceeding 1110.54 mg/kg. Anthocyanins represented the dominant class of phenolic compounds and remained relatively stable after drying.

The high content of natural polyphenols in elderberry fruits suggests considerable potential for the development of herbal medicinal products following appropriate pharmacological and toxicological investigations [9, 10].

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