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DETERMINATION OF THE CONTENT OF PHENOLIC COMPOUNDS IN RUBUS L. EXTRACT USING THE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD

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Abstract. In this study, the quantitative composition of phenolic compounds in the extract of Rubus L. was determined using the High-Performance Liquid Chromatography (HPLC) method. The Rubus L. plant is highly valued in traditional medicine as a medicinal raw material with immunostimulatory, antipyretic, and anti-inflammatory properties. The primary objective of this research was to determine the concentration of biologically active substances (phenols, flavonoids, and antioxidants) in the plant and to evaluate their pharmacognostic significance.
An extract was prepared from Rubus L. leaves using 96% ethanol and the ultrasonic extraction method. The analysis was conducted using a "Shimadzu LC-40 Nexera Lite" high-precision chromatograph. Standard solutions of gallic acid, salicylic acid, rutin, quercetin, apigenin, and kaempferol were employed for calibration. The results showed that the extract contained the highest concentrations of quercetin (1.235 mg/100 g) and rutin (0.785 mg/100 g). These phenolic compounds possess strong antioxidant and anti-inflammatory properties, thereby enhancing the plant's medicinal value.
The findings indicate that Rubus L. represents a significant natural source of bioactive compounds with potential applications in the pharmaceutical and food industries. Furthermore, the study demonstrates the practical effectiveness of using the HPLC method for determining the chemical composition of medicinal plants.

Keywords: Rubus L., phenolic compounds, High-Performance Liquid Chromatography (HPLC), flavonoids, antioxidant activity, extract, pharmacognosy, biologically active substances.

Relevance. In traditional medicine, the fruits of Rubus L. are used as general tonics and immunostimulants with antipyretic effects. The leaves are applied in the treatment of gastrointestinal and respiratory tract inflammations, while the roots serve as diuretic and astringent agents [1,3].
This unique plant contains amino acids, flavonoids, antioxidants, tannins, anthocyanins, and vitamins, all of which exert multifaceted biological effects on the human body [2].
Purpose of the study. To determine the amount of biologically active substances (phenols, flavonoids, antioxidants) contained in blackberries (Rubus L.) and evaluate their pharmacognostic significance.
Materials and Methods. To determine the amount of phenolic compounds in Rubus L. extract, the following reagents and instruments were employed: gallic acid ("Macklin", China), salicylic acid ("Rhydburg Pharmaceuticals", Germany), quercetin, apigenin, and kaempferol ("Regal", China). Rutin was isolated from natural sources using extraction and column chromatography. HPLC-grade water, acetonitrile, glacial acetic acid, and sodium hydroxide of analytical purity

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were used as solvents and reagents. The analysis was performed on a "Shimadzu" LC-40 Nexera Lite high-performance liquid chromatograph (Japan).

Results and Discussion. Before preparing the blackberry (Rubus L.) extract, standard solutions were prepared: gallic acid (5.2 mg), salicylic acid (5.2 mg), rutin (5 mg), quercetin (5 mg), apigenin (5 mg), and kaempferol (5 mg) were dissolved in 96% ethanol for 20 minutes in an ultrasonic bath, transferred to 50 mL flasks, and diluted to the mark with ethanol.

A 200 µl sample was taken from each solution, mixed, and then four different solutions were prepared using serial dilution. Each solution was placed in vials and used for analysis.

Preparation of the Plant Extract. To extract phenolic compounds, 1 g of the test sample was weighed to an accuracy of 0.01 g on an NV222 scale from "OHAUS" (USA), placed in a 50 ml conical flask, and 25 ml of 96% ethanol was added.

The mixture was extracted in an ultrasonic bath of the "GT SONIC-D3" brand (China) at a temperature of 60 ° C for 20 minutes. Then the mixture was cooled, filtered and made up to 25 ml with ethanol in a volumetric flask.

1.5 ml of the extract was centrifuged at 7000 rpm in a Mini-7 centrifuge ("BIOBASE", China) and filtered through a 0.45 µm syringe filter for analysis.

Determination of Phenolic Compounds. For analysis, standard solutions and extract samples were injected into the system using a Shim pack GIST C18 reversed-phase column (150 × 4.6 mm; 5 µm, Shimadzu, Japan) and a gradient mobile phase consisting of acetonitrile (A) and 0.5% acetic acid in water (B) (Table 1). The injection volume was set to 10 µl, the flow rate to 0.5 ml/min, and the column thermostat to 40oC. The analytical signal (peak area) of phenolic compounds was recorded at 300 nm (Figure 1).

Table 1. Gradient Elution Program of the Mobile Phase

Time (min)	Acetonitrile (A) %	0.5% Acetic Acid (B), %
0	5	95
5	5	95
17	40	60
22	40	60
22,1	5	95
40	Finish	

Figure 1. Chromatogram of standards at 300 nm.

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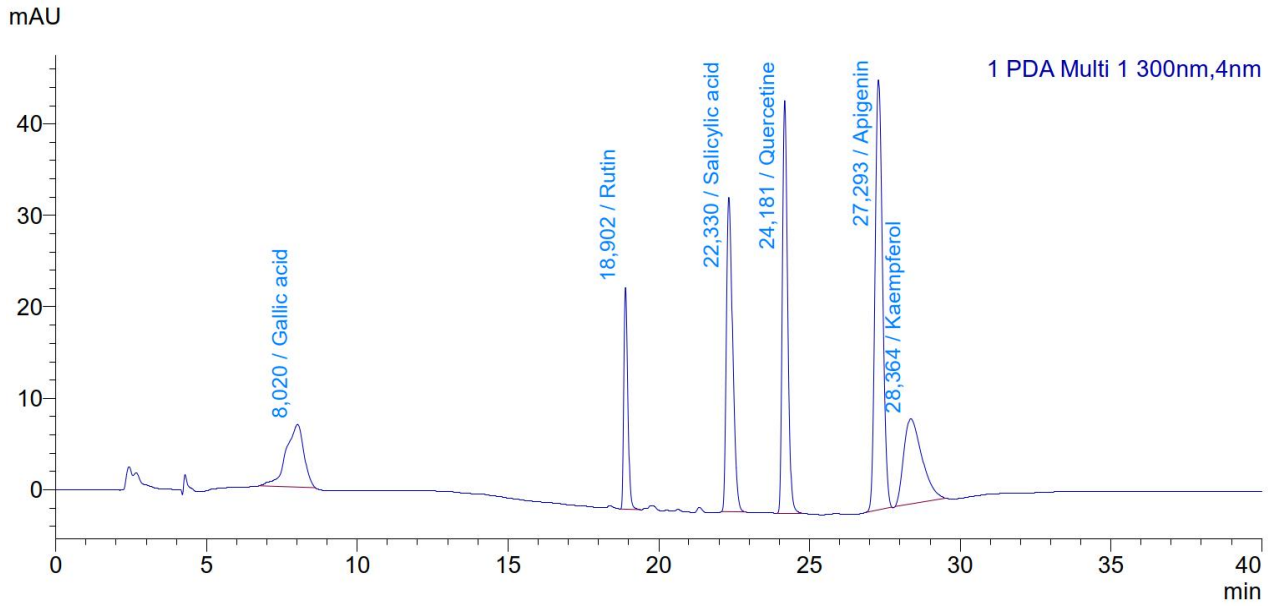

Determination of the amount of phenolic compounds in a sample extract. A chromatogram of a 1-gram extract was obtained (Figure 2). Based on these results, the amount of phenolic compounds contained in 100 g of the test sample was calculated using the following formula, and the data are presented in Table 2.

$$X = \frac{C_{\text{phenolic}} \cdot V_{\text{extract}}}{m_{\text{sample}}} \cdot 100\text{g}$$

where:

X — the amount of phenolic compounds in 100 g of plant material, mg;

C_{phenolic} — the concentration of the phenolic compound in the extract determined by the HPLC method, mg/L;

V_{extract} — the volume of the sample extract, L;

m_{sample} — the mass of the sample taken for extract preparation.

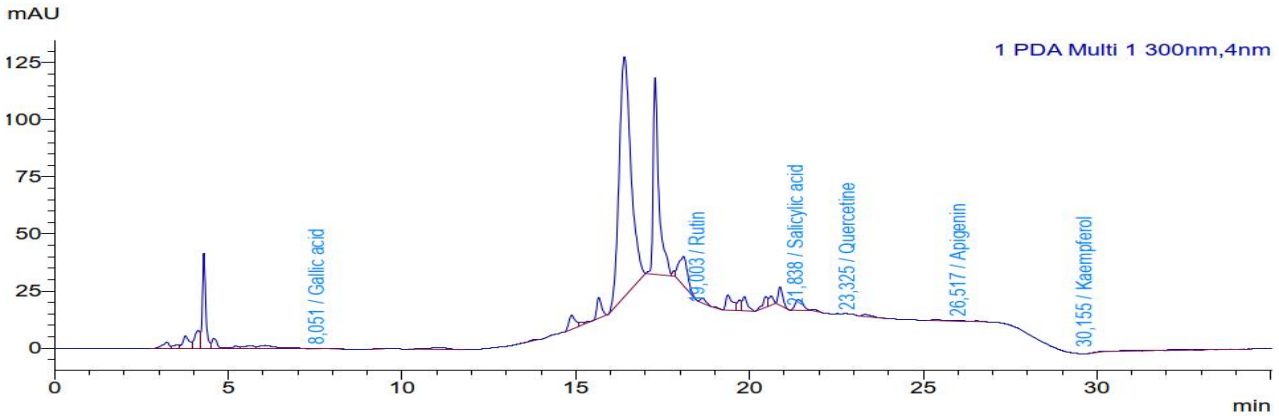

Figure 2. Chromatogram for the determination of polyphenols in the sample extract.

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Table 2. The amount of polyphenols and their retention time in the extract.

Name of phenolic compound	**Retention time, sec.**	**Concentration, mg/l**	**Content in 100 g of sample, mg**
Gallic acid	8,051	0,059	0,148
Rutin	19,003	0,314	0,785
Salicylic acid	21,838	0,308	0,770
Quercetine	23,325	0,494	1,235
Apigenin	26,517	0,097	0,243
Kaempferol	30,155	0,196	0,490

Conclusion. Recent pharmacognostic studies are again considering the blackberry plant (Rubus L.) as a natural source of biologically active substances. This plant contains various flavonoids, including anthocyanins, proanthocyanidins, and flavonols [4]. These polyphenolic compounds are largely responsible for the wide range of beneficial effects of Rubus L., such as anti-inflammatory and antioxidant activity. The findings of this study demonstrate the potential of Rubus L. as an important source of natural bioactive substances and confirm the efficacy of HPLC as a precise analytical method for the qualitative and quantitative determination of plant-derived phenolic compounds.

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