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PHENOL COMPOUNDS IN THE BIOLOGICALLY ACTIVE SUPPLEMENT "PROSTAFITO"

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Annotation
Phenolic compounds are beneficial substances found in plants. They have a strong antioxidant, anti-inflammatory and positive effect on blood circulation. In this study, the amount and types of phenolic substances in the biologically active supplement "Prostafito" were determined. The analysis was carried out using the HPLC (high-performance liquid chromatography) method. 96% ethanol and ultrasound were used to obtain the extract. As a result, a total of 1.78 mg of phenolic substances were detected per 100 grams of sample. The most common were rutin (58%), apigenin (30%), salicylic acid (7%) and quercetin (5%). The presence of these substances indicates that the drug "Prostafito" has antioxidant and blood-thinning effects.

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Introduction. Phenolic compounds are the most abundant natural compounds in plants, and their structure, production, and biological activity have been extensively studied. Recent studies have shown that these compounds can be used as antioxidant and antimicrobial agents, as well as as natural preservatives and drug additives [1]. According to a meta-analysis of 281 clinical trials conducted in 2024, polyphenol supplements help reduce blood pressure and LDL-cholesterol levels [2]. Flavonoids (rutin, quercetin, apigenin) block the NF-κB pathway and have anti-inflammatory effects [3]. Modern UHPLC-HRMS technology allows for rapid and accurate determination of phenolic compounds [4]. However, the response to polyphenols in each individual may vary. This depends on metabolism, genetic background, and intestinal microflora, and requires an individual approach [5]. Polyphenol-rich foods, especially those from seeds, have been shown to reduce inflammation and improve blood lipid profiles in patients with coronary heart disease [6].

Experimental section. Reagents and equipment used. Gallic acid was purchased from Macklin (China), Salicylic acid from Rhydburg Pharmaceuticals (Germany), quercetin, apigenin, and kaempferols from Regal (China), and rutin were extracted from natural sources by extraction and column chromatography. HPLC-grade water, acetonitrile, chemically pure acetic acid, and sodium hydroxide were used as reagents.

The content of polyphenols in plant extracts was determined using an LC-40 Nexera Lite high-performance liquid chromatograph manufactured by Shimadzu, Japan.

Preparation of standard solutions. Gallic acid (5.2 mg), salicylic acid (5.2 mg), rutin (5 mg), quercetin (5 mg), apigenin (5 mg), kaempferol (5 mg) were dissolved in 96% ethanol for 20 minutes in an ultrasonic bath and transferred to a 50 ml flask and made up to the mark with ethanol. 200 µl of

each solution was taken and mixed and a total of 4 different solutions were prepared by diluting them. Each solution was poured into a vial and used for analysis.

Preparation of plant extract. For the extraction of phenolic compounds, 1 g of the test sample was weighed with an accuracy of 0.01 g on an NV222 balance manufactured by 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 GT SONIC-D_3 (China) at 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 and used for analysis.

Chromatographic conditions. Determination of phenolic compounds. Standard solution, sample extract 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) were used. The injection volume was 10 µl, the flow rate was 0.5 ml/min and the column thermostat was set to 40°C. The analytical signal (peak area) of phenolic compounds was recorded at 300 nm (Figure 1).

Figure 1. Mobile phase gradient program.

Time	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	

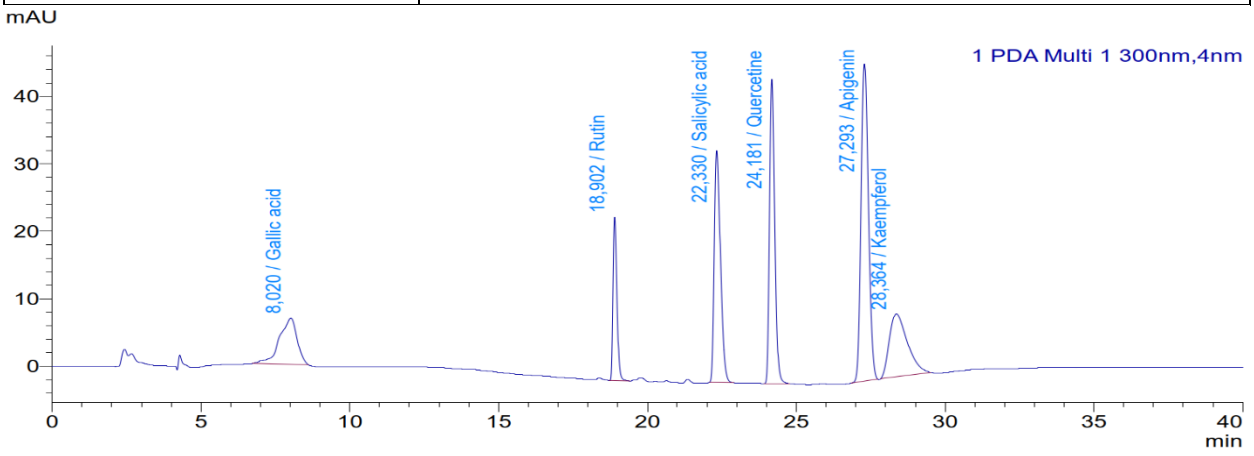


Figure 1. Chromatogram of standards at 300 nm.

Results obtained. Determination of the amount of phenolic compounds in the sample extract. A chromatogram of a sample extract weighing 1 g was obtained (Figure 2) and based on the results, the amount of phenolic compounds in 100 g of the sample was calculated using the following formula and presented in Table 3.

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

Here, X is the amount of phenolic compounds in 100 grams of fruit, mg;

C_{phen} – concentration of phenolic compounds in the extract determined by the HPLC method, mg/l;

$V_{extract}$ – volume of sample extract, l;

M_{sample} – mass of sample taken for extract preparation.

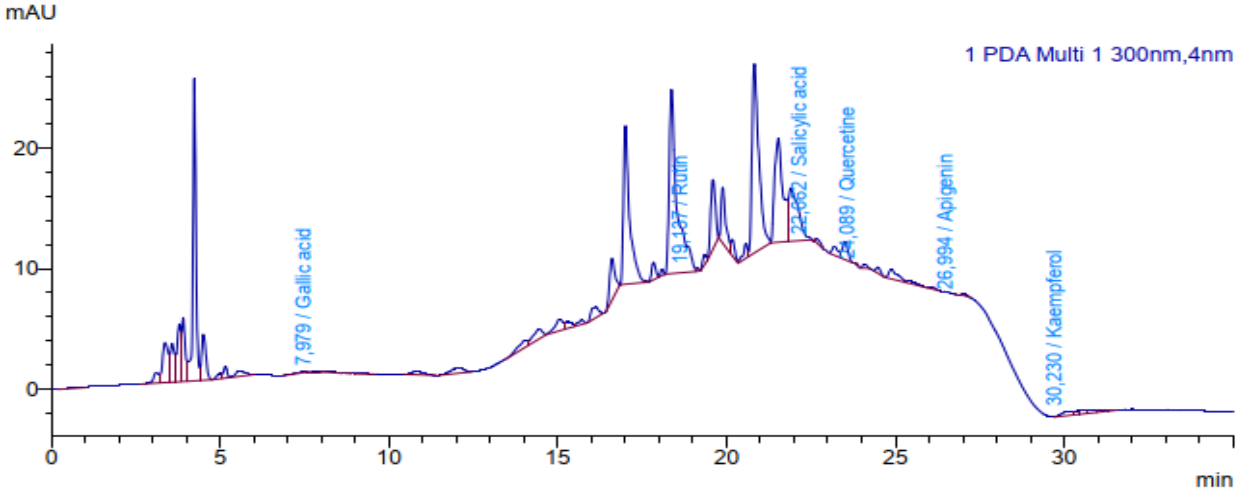


Figure 2. Chromatogram of polyphenols in the sample extract.

Table 2. Amount of polyphenols in the extract and retention times.

Phenol compound name	Holding time, sec	Concentration, mg/l	Amount in 100 g of sample, mg
Gallic acid	7,979	0,013	0,033
Rutin	19,137	0,105	0,263
Salicylic acid	22,662	0,123	0,308
Quercetine	24,089	0,09	0,225
Apigenin	26,994	0,029	0,073
Kaempferol	30,23	0,349	0,873

Conclusion. The results of the study showed that the composition of the drug "Prostafito" contains a total of 6 phenolic compounds. Among them, kaempferol has the highest share, accounting for about half of the total phenolic substances. Also, salicylic acid, rutin, quercetin and apigenin are present in significant amounts. This composition of phenolic compounds confirms the richness of the drug in flavones and flavonols. These substances are characterized by antioxidant, anti-inflammatory and blood circulation-improving properties. The high content of kaempferol and other flavonoids scientifically substantiates the biological activity of "Prostafito". The obtained data expand the possibilities of using the drug for therapeutic and prophylactic purposes. At the same time, the development of new food products based on biologically active additives rich in phenolic compounds is one of the promising directions.

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