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DETERMINATION OF THE FLAVONOID COMPOSITION OF A CHOLERETIC HERBAL MIXTURE BY HPLC METHOD

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Annotation.
As a result of the HPLC analysis, the presence of biologically active flavonoids and phenolic acids was identified in the choleretic herbal mixture sample. The obtained results indicate that the studied sample is rich in flavonoid compounds. According to the quantitative analysis, the flavonoid rutin was detected in the highest amount and was identified as the main flavonoid component. The results demonstrate the antioxidant activity and pharmacological potential of the choleretic herbal mixture.

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Introduction. It is known that, worldwide, diseases of the liver, biliary tract, and gallbladder are highly prevalent, and their treatment remains one of the pressing problems of modern medicine. The development of these diseases is associated with dysfunction of the central nervous system, various disorders of internal organs, environmental factors, infectious diseases, improper nutrition, as well as the long-term use of certain medications [1].

In recent years, the demand for herbal preparations for the treatment of liver and biliary tract diseases has increased markedly. This is primarily explained by the lower toxicity of plant-derived medicines compared with synthetic drugs, their milder effects on the body, and the possibility of long-term use. Scientific studies have confirmed the hepatoprotective, antioxidant, and choleretic properties of flavonoids, phenolic acids, and other biologically active compounds found in medicinal plants [2].

In this regard, the present study, based on scientific and practical evidence, prepared a choleretic herbal mixture from four medicinal plants, and its flavonoid composition was investigated using high-performance liquid chromatography (HPLC).

Literature review and methods. Medicinal plants and their biologically active compounds play an important role in pharmacognosy and phytotherapy. In particular, marigold (Calendula offi-cinalis L.), rosehip (Rosa spp.), dastarbosh, and chamomile (Matricaria chamomilla L.) are widely used in traditional medicine and modern clinical practice [3].

The literature reports that marigold flowers are rich in flavonoids, triterpenoids, and carotenoids and exhibit anti-inflammatory, choleretic, and antioxidant properties. Rosehip fruits are a rich source of ascorbic acid, flavonoids, and phenolic compounds and exert a supportive effect on liver function [3].

Flavonoids and essential oils contained in chamomile flowers have antispasmodic and anti-inflammatory effects and improve biliary tract function. The dastarbosh plant is also rich in biologically active substances and is used in traditional medicine as a choleretic and sedative agent [3-4].

Sample preparation and analytical methods. In recent years, HPLC has been recognized as one of the most reliable and highly accurate methods for the qualitative and quantitative determination of flavonoids in medicinal plants and their mixtures. This method enables precise assessment of the composition and content of biologically active substances.

The study object was a choleretic herbal mixture composed of marigold flowers, rosehip fruits, dastarbosh, and chamomile flowers. The mixture was prepared in the following ratio.

Composition of the choleretic herbal mixture:

Marigold flowers - 1 part

Rosehip fruits - 2 parts

Dastarbosh flowers - 1 part

Chamomile flowers - 1 part

The aim of the study was to investigate the flavonoid composition of extracts obtained from the choleretic herbal mixture using high-performance liquid chromatography.

To study flavonoids, the dried plant materials of the mixture were ground to a particle size of 0.1-1.5 mm. A precisely weighed 1 g sample of the choleretic mixture was taken and extracted with 99 mL of 70% ethanol for 2 h at 50–60 °C in a flat-bottom flask equipped with a reflux condenser under intensive stirring. The resulting solution was placed in an ultrasonic bath, and extraction was performed twice at 35 °C for 15 min with a 10-min interval. The extract was cooled to room temperature. The solution was first filtered; then 2 mL of the filtrate was passed through a membrane filter (0.2 µm). An aliquot of 100 µL was taken from this solution and diluted with the eluent to a final volume of 1 mL [5].

Preparation of the standard solution. Standard compounds (rabinin, isorhamnetin, hyperoside, rutin, apigenin, luteolin, hypolaetin, and gallic acid) were weighed in an amount of 10 mg, transferred to a 100 mL volumetric flask, and 80 mL of methanol was added. To ensure complete dissolution and obtain a true solution, the mixture was placed in an ultrasonic bath and dissolved until a homogeneous solution was formed. The volume was then brought to the mark with methanol to obtain a stock solution with a mass concentration of 0.1 mg/mL. Into a 50 mL volumetric flask, 5 mL of each stock standard solution was transferred, and the volume was brought to the mark with methanol. In this way, working standard solutions were prepared with a mass concentration of 0.01 mg/mL. These solutions were filtered through a 0.45 µm pore-size filter and injected into the chromatograph.

Recording of Results and Calculations

Quantitative determination of the samples was performed by comparison with the peak of the external standard on the chromatogram.

The mass fraction of flavonoids was calculated according to the following equation:

$$X = (S_2 \cdot C_1 \cdot V) / (S_1 \cdot m) \cdot 1000$$

where S_2 is the peak area corresponding to the flavonoid in the sample solution; C_1 is the mass concentration of the flavonoid in the working standard solution (0.01 mg/mL);

V is the volume of the analyzed sample solution, mL;

S_1 is the peak area corresponding to the flavonoid in the working standard solution;

m is the sample mass, g;

1000 is the conversion factor.

For each sample, the analysis was performed in triplicate. The final result was determined as the arithmetic mean of the three replicate measurements.

Using high-performance liquid chromatography (HPLC), the qualitative and quantitative composition of flavonoids in the choleretic herbal mixture was determined. The analytical results are presented in the table.

Chromatographic conditions: detector-diode-array detector (DAD) under gradient elution; spectral range-UV 200–400 nm; mobile phase (gradient mode)-acetonitrile - buffer solution, pH = 2.92: (4%:96%) 0 - 6 min, (10%:90%) 6-9 min, (20%:80%) 9-15 min, (4%:96%) 15-20 min; injection volume-10 µL; flow rate-0.75 mL/min; stationary phase (column) - Zorbax Eclipse XDB C18, 5.0 µm, 4.6 × 250 mm; DAD monitoring wavelengths - 254, 272, 292, 297, and 360 nm [6].

Results and Discussion

In the analysis of the flavonoid composition of the investigated choleretic herbal mixture, standard flavonoid compounds and their corresponding chromatographic parameters were used. The following standards were employed: rabinin, isorhamnetin, hyperoside, rutin, apigenin, luteolin, hypolaetin, and gallic acid.

The flavonoid profile of the choleretic mixture, as obtained from the HPLC chromatograms, is shown in the figure below. The analysis of the obtained results is presented in the table.

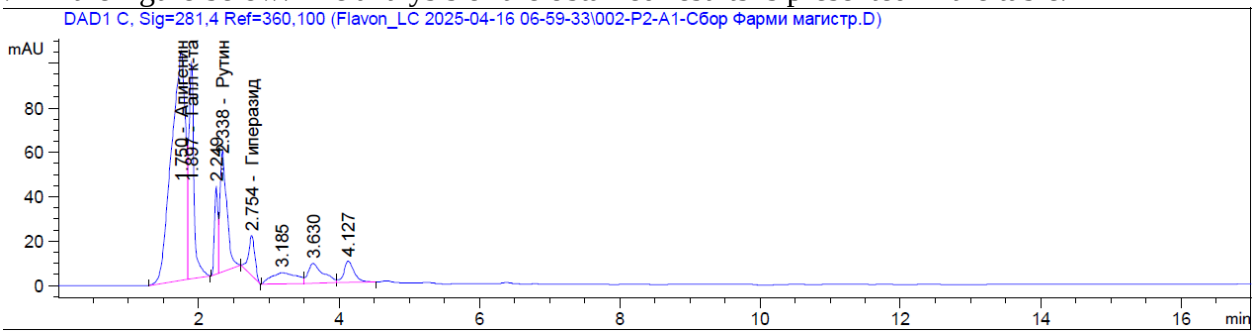


Figure 1. HPLC chromatogram of flavonoids in the choleretic herbal mixture.

Table 1. Results of flavonoid determination in the choleretic herbal mixture obtained by high-performance liquid chromatography (HPLC).

No.	Flavonoid name	Amount (mg/100 mL)	Sample name
1	Apigenin	2.142	Mixture - 2
2	Rabinin	—	
3	Rutin	119.189	
4	Hypolaetin	0.258	
5	Isorhamnetin	1.254	
6	Gallic acid	45.995	
7	Hyperoside	10.214	
8	Luteolin	0.866	

According to the analysis of the obtained HPLC chromatograms, the presence of several biologically active flavonoids and phenolic acids was confirmed in the “Mixture–2” sample. Based on the analytical results, rutin was the predominant flavonoid component, with a concentration of 119.189 mg/100 mL. This finding indicates that rutin is one of the major flavonoids in the investigated sample.

Gallic acid was also detected at a considerable level (45.995 mg/100 mL). Gallic acid is known for its strong antioxidant properties and plays an important role in protecting cells from oxidative stress.

Hyperoside was present at 10.214 mg/100 mL and can be classified among moderately abundant bioactive compounds. Although apigenin (2.142 mg/100 mL) and isorhamnetin (1.254 mg/100 mL) were detected in relatively lower amounts, their anti-inflammatory and antioxidant activities have been widely reported in the scientific literature.

In addition, luteolin (0.866 mg/100 mL) and hypolaetin (0.258 mg/100 mL) were recorded at low concentrations. Despite their comparatively low levels, these flavonoids are considered important due to their biological activity.

Rabinin was not detected in the sample according to the analytical results, which may indicate its absence in the mixture or a concentration below the detection limit of the method.

Overall, the sample is rich in flavonoids and phenolic acids. In particular, the high levels of rutin and gallic acid allow the “Mixture–2” sample to be regarded as a promising plant-based raw material from pharmacological and biological perspectives. These compounds may contribute to improved blood circulation, strengthening of the immune system, and enhancement of protective mechanisms against bacterial and viral agents in the human body.

Conclusion. Thus, the analyses confirmed the presence of biologically active flavonoids and phenolic acids in the choleretic herbal mixture sample. The results indicate that the investigated sample is rich in flavonoid constituents.

According to the quantitative analysis, rutin was identified as the most abundant flavonoid, confirming its role as the main flavonoid component of this sample.

In general, the findings suggest that the choleretic herbal mixture has pharmacological potential, particularly with possible antioxidant activity, immune-supporting effects, and the ability to improve blood circulation. From a practical standpoint, the results indicate that this sample is promising for application in the development of biologically active supplements and medicinal products.

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