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DETERMINATION OF THE QUANTITY OF PHENOLIC COMPOUNDS IN TRIBULUS TERRESTRIS (TEMIRTIKAN) EXTRACT USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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Abstract

This article focuses on determining the content of phenolic compounds in Tribulus terrestris (puncture vine) extract using high-performance liquid chromatography (HPLC). For the extraction of phenolic compounds, 96% ethanol was used, and the extraction was carried out in an ultrasonic bath. The analyses were performed on a Shimadzu LC-40 Nexera Lite high-performance liquid chromatograph using a reverse-phase C18 column. A gradient system consisting of acetonitrile and 0.5% aqueous acetic acid solution was used as the mobile phase. The results revealed the presence of rutin, salicylic acid, quercetin, and apigenin in the extract. The highest amount corresponded to rutin, with an average concentration of 19.687 ± 1.488 mg/L. The obtained results demonstrate that the Tribulus terrestris extract is rich in biologically active substances, and the HPLC method is effective in identifying phenolic compounds.

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INTRODUCTION

In recent years, interest in natural food supplements based on medicinal plants has significantly increased. The main reason for this is the certain negative effects of synthetic drugs and the relatively better absorption of preparations obtained from natural sources by the body. The healing properties of medicinal plants are primarily directly related to biologically active substances in their chemical composition, particularly phenolic compounds.

Tribulus terrestris L. (puncture vine) has long been used in traditional medicine to treat inflammatory processes, improve urination, and enhance the body's overall tone. Modern scientific research shows that the biological activity of this plant is related to flavonoids, phenolic acids, and other polyphenolic compounds in its composition. Nevertheless, the quantitative composition and relative proportions of certain phenolic compounds in puncture vine extract have not been sufficiently studied.

Taking the above into account, the main objective of this research is to determine phenolic compounds in Tribulus terrestris extract using high-performance liquid chromatography (HPLC) and to scientifically substantiate the phenolic composition of the extract. The obtained results will have important scientific and practical significance in the development of natural food supplements based on puncture vine.

EXPERIMENTAL SECTION

High-purity standard substances were used in the research to determine phenolic compounds. Specifically, gallic acid ("Macklin," China), salicylic acid ("Rhydburg Pharmaceuticals," Germany), quercetin, apigenin, and kaempferol ("Regal," China) were used as standards. Rutin was isolated from natural sources using extraction and column chromatography methods. HPLC-grade water and acetonitrile, as well as chemically pure acetic acid and sodium hydroxide reagents, were used in preparing the mobile phase. The determination of polyphenol content in samples was performed using a Shimadzu LC-40 Nexera Lite high-performance liquid chromatograph.

Preparation of Standard Solutions

To prepare standard solutions, gallic acid (5.2 mg), salicylic acid (5.2 mg), rutin (5 mg), quercetin (5 mg), apigenin (5 mg), and kaempferol (5 mg) were taken separately and completely dissolved in 96% ethanol in an ultrasonic bath for 20 minutes. The obtained solutions were transferred to 50 ml volumetric flasks and filled to the mark with ethanol. From each standard solution, 200 µl was taken, mixed, and four standard solutions of different concentrations were prepared using serial dilution method. The prepared solutions were transferred to vials and used for HPLC analysis.

Preparation of Sample Extract. To extract phenolic compounds, 1 g of the test sample was weighed on an OHAUS NV222 (USA) electronic balance with 0.01 g accuracy and placed in a 50 ml conical flask. 25 ml of 96% ethanol was added to the sample and extracted in a GT SONIC-D3 (China) ultrasonic bath at 60°C for 20 minutes. After the extraction process, the mixture was cooled, filtered, and brought to 25 ml volume with ethanol in a volumetric flask. From the obtained extract, 1.5 ml was centrifuged in a Mini-7 (BIOBASE, China) centrifuge at 7000 rpm, passed through a 0.45 µm syringe filter, and prepared for chromatographic analysis.

Chromatographic Conditions. Separation and determination of phenolic compounds were performed on a Shim-pack GIST C18 (150 × 4.6 mm; 5 µm, Shimadzu, Japan) reverse-phase column. A gradient system consisting of acetonitrile (A) and 0.5% aqueous solution of acetic acid (B) was used as the mobile phase. The injection volume was 10 µl, flow rate was 0.5 ml/min, and column temperature was set at 40°C. Analytical signals of phenolic compounds were recorded at a wavelength of 300 nm.

Table 1

Mobile Phase Gradient Program

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	End	

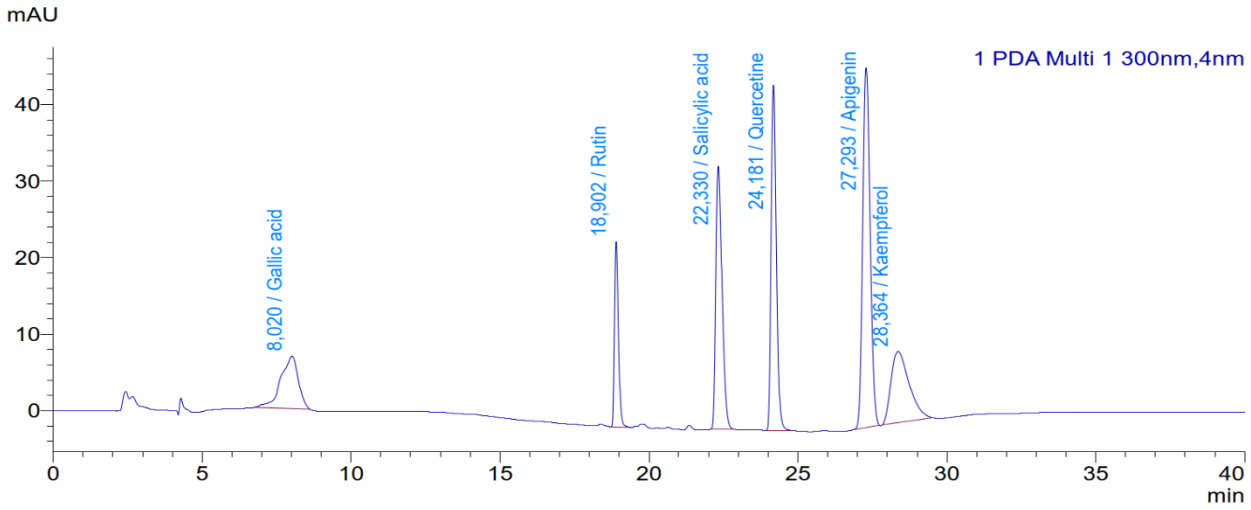


Figure 1. Chromatogram of standards at 300 nm

RESULTS AND DISCUSSION

Analysis conducted using high-performance liquid chromatography (HPLC) determined the presence of several biologically active phenolic compounds in the PULMOCLIN extract prepared based on Tribulus terrestris L. During the chromatographic analysis process, rutin, salicylic acid, quercetin, and apigenin were reliably identified by comparing retention times and UV-spectral characteristics of standard samples. At the same time, gallic acid and kaempferol were not found in this extract because their amounts were below the detection limit.

Studies were conducted based on three replicate experiments, and results were expressed as mean value ± standard deviation (SD). According to the obtained data, the highest amount in the extract belonged to rutin compound, with a concentration of 19.687±1.488 mg/L. The amount of salicylic acid was 11.415 ± 0.552 mg/L, which also had a significant proportion in the extract. The amounts of quercetin and apigenin, belonging to the flavonoid group, were relatively low, constituting 0.061±0.002 mg/L and 0.259±0.023 mg/L, respectively.

Statistical analysis of the obtained results showed that relative standard deviation (%RSD) values for the determined phenolic compounds ranged from 3.8-8.9%. This confirms the high level of accuracy and reproducibility of the HPLC method. These indicators are within the framework of international analytical requirements and demonstrate the reliability of the method used in quantitatively determining phenolic compounds in Tribulus terrestris extracts (Table 2).

Determination of Phenolic Compound Content in Sample Extract

A chromatogram of the extract from a 1 g sample was obtained (Figure 2), and based on the results, the amounts of phenolic compounds in 100 g of sample were calculated using the following formula and presented in Table 3:

Formula:

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

Where:

X – amount of phenolic compounds in 100 grams of sample, mg

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

$V_{extract}$ – volume of sample extract, L

m_{sample} – mass of sample weighed for extract preparation

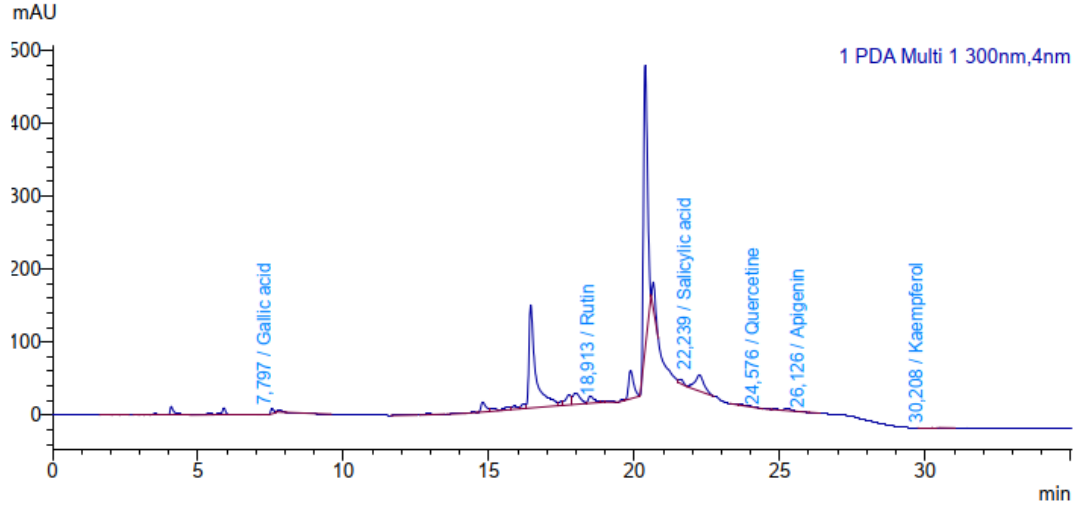


Figure 2. Chromatogram for determining polyphenols in sample extract

Table 2.

Amounts and Retention Times of Polyphenols in Tribulus terrestris L. Extract

Sample Name	Gallic acid	Rutin	Salicylic Acid	Quercetin	Apigenin	Kaempferol
Experiment 1	-	21,014	10,963	0,062	0,241	-
Experiment 2	-	18,078	12,030	0,058	0,285	-
Experiment 3	-	19,971	11,252	0,062	0,251	-
Average	-	19,687	11,415	0,061	0,259	-
%RSD	-	7,561	4,836	3,819	8,932	-
Maximum	-	21,014	12,030	0,062	0,285	-
Minimum	-	18,078	10,963	0,058	0,241	-
Std. Dev.	-	1,488	0,552	0,002	0,023	-
Average (mg/L)	-	**19,687±1,488**	**11,415±0,552**	**0,061±0,002**	**0,259 ± 0,023**	-

The results obtained during this research showed that the extract prepared based on Tribulus terrestris L. is rich in phenolic compounds. Specifically, the detection of relatively high amounts of rutin and salicylic acid is one of the main factors determining the biological and pharmacological activity of the extract. According to scientific sources, rutin has strong antioxidant properties and plays an important role in neutralizing free radicals, strengthening blood vessel walls, and supporting the cardiovascular system. From this point of view, the presence of rutin in high concentration in Pulmoclin extract scientifically substantiates its therapeutic effectiveness.

The detection of quercetin and apigenin in relatively small amounts shows that the extract has a complex and multi-component composition. These flavonoids can show synergistic effects even at low concentrations and increase the overall biological activity of the extract. Specifically, quercetin is significant for its antioxidant and anti-inflammatory properties, while apigenin is important for its antioxidant effect and activation of cellular protection mechanisms.

The absence of gallic acid and kaempferol can be explained by several factors. These include the possibility that these compounds are present in very small amounts in the plant raw material, are not completely extracted during the extraction process, or are below the detection limit under the applied chromatographic conditions. This indicates the need to further improve extraction conditions or apply more sensitive analysis methods.

CONCLUSION

As a result of research conducted using high-performance liquid chromatography (HPLC) method, the presence of biologically active phenolic compounds was determined in the extract prepared based on Tribulus terrestris L. The main identified phenolic compounds were rutin, salicylic acid, quercetin, and apigenin, while gallic acid and kaempferol were not found in the extract composition. Average values were determined as follows:

Rutin - 19.687 ± 1.488 mg/L
Salicylic acid - 11.415 ± 0.552 mg/L
Quercetin - 0.061 ± 0.002 mg/L
Apigenin - 0.259 ± 0.023 mg/L

The relative standard deviation values of the results (%RSD 3.8-8.9) confirm the high accuracy and reproducibility of the HPLC method and enable reliable determination of phenolic compounds in the extract composition.

The presence of rutin and salicylic acid in high concentrations scientifically substantiates the extract's antioxidant, anti-inflammatory, and capillary-strengthening properties. These properties are closely related to the extract's long-standing use in traditional medicine. The presence of quercetin and apigenin enhances the complex biological activity of the extract, and together they show synergistic effects in supporting the body's defense mechanisms.

The absence of gallic acid and kaempferol may be due to their very small amounts in the raw material or dependence on extraction conditions. By further improving extraction and analysis conditions, the possibility of determining these components may arise.

The obtained results are of great importance in scientifically substantiating the chemical composition of natural medicines based on Tribulus terrestris, implementing a quality control system, and applying traditional medicine experience to modern phytotherapy practice. At the same time, quantitative assessment of the extract's phenolic composition serves to standardize the effectiveness and quality of traditional medicine remedies.

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