Journal of Chemistry of Goods and Traditional Medicine

J Chem Good Trad Med, Volume 5, Issue 3, 2026 Traditional Medicine

PHENOLIC COMPOUNDS IN WALNUT (JUGLANS REGIA L.) LEAF EXTRACT

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DOI: https://doi.org/10.55475/jcgtm/vol5.iss3.2026.642

Keywords: Juglans regia L, phenolic compounds, antioksidant faollik, YuSSX, HPLC

Annotation:
In this study, phenolic compounds in walnut (Juglans regia L.) leaves were identified and their quantitative content was evaluated using HPLC. The analysis revealed the presence of various phenolic acids and flavonoids. These compounds exhibit strong antioxidant properties and help protect the body against oxidative stress. The results confirm that walnut leaves are a rich source of biologically active substances and have potential applications in pharmaceuticals and other fields.

How to Cite
Khojimatov M.M., Abdulboqiyeva, N. DETERMINATION OF PHENOLIC COMPOUNDS IN WALNUT (Juglans regia L.) LEAF EXTRACT BY HPLC METHOD. Journal of Chemistry of Goods and Traditional Medicine, 5(3). https://doi.org/10.55475/jcgtm/vol5.iss3.2026.642

Introduction: The identification of biologically active compounds in medicinal plants and the study of their pharmacological properties have become one of the priority areas of modern science and industry. In particular, phenolic compounds attract considerable scientific interest as important secondary metabolites of plants. These compounds are distinguished by their strong antioxidant properties and play a significant role in neutralizing free radicals, reducing oxidative stress, and preventing cardiovascular, oncological, and other chronic diseases [1,2].

Walnut (*Juglans regia L.*) is one of the valuable medicinal plants widely used in both traditional and scientific medicine. Its leaves are especially rich in phenolic compounds, including flavonoids, phenolic acids, and other antioxidant components. These substances participate in the plant's defense mechanisms, ensuring resistance to environmental stress factors. At the same time, they have beneficial biological effects on the human body, including anti-inflammatory, antibacterial, and antioxidant properties [3].

Studies in this field confirm that walnut leaves are a rich natural source of biologically active compounds and have promising applications in pharmaceutical, food, and preventive medicine industries [4]. The aim of this study is to determine phenolic compounds in walnut (*Juglans regia L.*) leaf extract using HPLC (High-Performance Liquid Chromatography), evaluate their quantitative content, and scientifically substantiate their beneficial effects on human health [5].

Experimental part. In this study, reference standards of gallic acid, salicylic acid, quercetin, apigenin, kaempferol, and rutin, previously isolated from natural plant sources, were used. HPLC-grade water, acetonitrile, acetic acid, and sodium hydroxide were also used. To prepare the standard solutions, each compound was dissolved in 96% ethanol using an ultrasonic bath, and the volume of each solution was adjusted to 50 mL. The standard solutions were then mixed to prepare working solutions of different concentrations and transferred into HPLC vials for subsequent analysis.For the preparation of the plant extract, 1.0 g of the sample was weighed, and 25 mL of 96% ethanol was added. Extraction was performed in an ultrasonic bath at 60°C for 20 min, and the procedure was repeated three times. The extract was then

cooled, filtered, and adjusted to the required volume. The resulting extract was centrifuged, filtered through a 0.45 µm membrane filter and prepared for chromatographic analysis.

Chromatographic analysis was carried out using a **Shim-pack GIST C18** column and a gradient mobile phase consisting of acetonitrile and 0.5% aqueous acetic acid. The injection volume was 10 µL, the flow rate was 0.5 mL/min, and the column temperature was maintained at 40°C. Phenolic compounds were detected at a wavelength of 300 nm, and their quantitative contents were determined.

Results.

The extract obtained from 1.0 g of walnut leaves was subjected to high-performance liquid chromatography (HPLC), and the resulting chromatogram is presented in **Figure 1**. Based on the chromatographic results, the contents of phenolic compounds in 100 g of walnut leaves were calculated using the corresponding equation. The calculated values are presented in **Table 1**, which summarizes the quantitative contents of the identified phenolic acids and flavonoids.

$$X = \frac{C_{phen} \cdot V_{ekstrakt}}{m_{namuna}} \cdot 100\ g$$

Where:

X – amount of phenolic compounds in 100 g of sample, mg;

$\mathbf{C_{phen}}$ – concentration of phenolic compounds in the extract determined by HPLC, mg/L;

$\mathbf{V_{extra}c_t}$ – volume of the extract, L;

$\mathbf{m_{sample}}$ – mass of the sample used for extraction, g.

Figure 1. Chromatogram of polyphenols in the sample extract

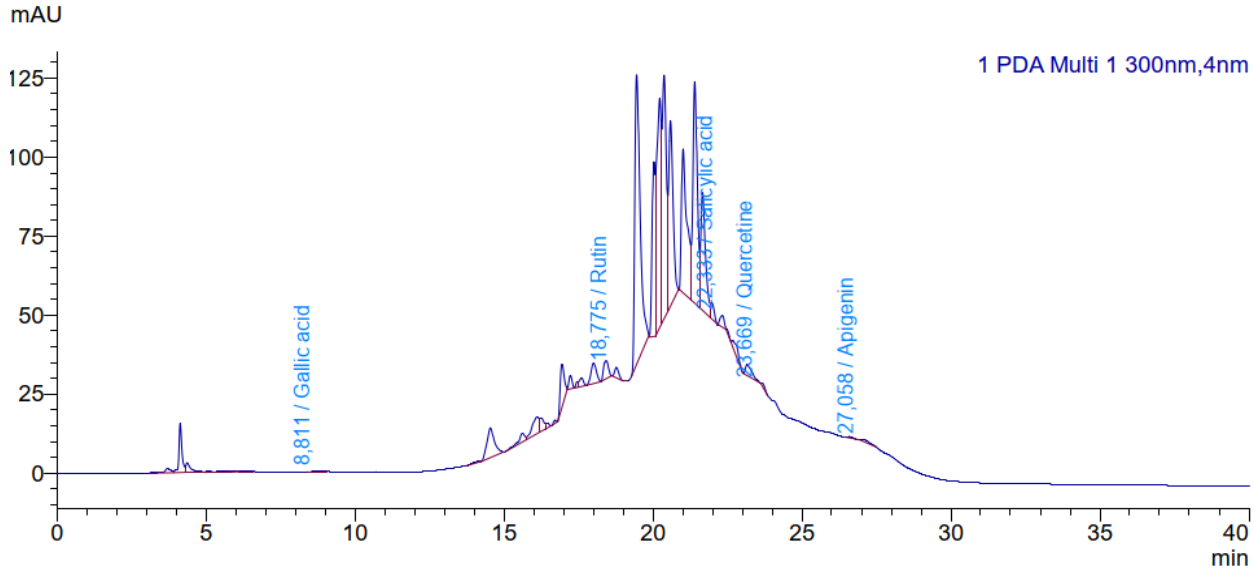


Table 1. Content of polyphenols in walnut leaf extract and their retention times

Phenolic compound	Retention time (min)	Concentration (mg/L)	Content in 100 g sample (mg)
Gallic acid	8.811	0.111	0.278
Rutin	18.775	2.355	5.888
Salicylic acid	22.333	1.256	3.140
Quercetin	23.669	0.236	0.590
Apigenin	27.058	0.249	0.623

Kaempferol	Not detected	0	0.000

The main phenolic compounds in walnut (*Juglans regia L.*) leaf extract were qualitatively and quantitatively determined using the HPLC method. Based on chromatographic results, retention times, concentrations, and quantitative content in 100 g of the sample were evaluated.

The analysis showed that phenolic compounds are unevenly distributed in the extract. The highest amount corresponds to the flavonoid rutin (5.888 mg/100 g), indicating that it is one of the dominant components. Salicylic acid (3.140 mg/100 g) was also found in significant amounts, occupying an important place among phenolic acids.

Apigenin (0.623 mg/100 g) and quercetin (0.590 mg/100 g) were identified in moderate amounts, confirming that the extract is rich in biologically active compounds. Gallic acid was detected in relatively low quantity (0.278 mg/100 g).

The absence of kaempferol may be explained by its very low concentration in the sample or its level being below the detection limit of the analytical method used.

It should be noted that although the composition of phenolic compounds was determined in this study, their antioxidant activity was not directly evaluated using experimental methods (such as DPPH, FRAP, ABTS). Therefore, conclusions can only be drawn about the potential biological activity of these compounds.

Conclusion. The obtained results indicate that walnut leaf extract is a rich source of phenolic compounds and justify the need for further research as a raw material for pharmaceutical applications and as a source of biologically active substances. Due to its ecological purity and the presence of diverse natural compounds, walnut leaves are recommended for use in various fields. These products may also contribute to significant economic efficiency.

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