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QUANTIFICATION OF FLAVONOID CONTENT IN CRATAEGUS TURKESTANICA (TURKESTAN HAWTHORN)

M. M. Mo‘minjonov
Doctor of Chemical Sciences, Associate Professor, Deputy Chairman of the Academy of Medicine of Uzbekistan, Tashkent, Uzbekistan
E-mail: mirjalolmominjonov0@gmail.com
ORCID: 0009-0002-8896-202X

M. N. Mirzaabdullayeva
Student of Chemistry at Andijan State University, Andijan, Uzbekistan

Abstract
This article presents the results of a phytochemical analysis conducted to identify flavonoid compounds present in Crataegus turkestanica (Turkestan hawthorn) using high-performance liquid chromatography (HPLC). The study aims to quantify key bioactive flavonoids, including rutin, salicylic acid, quercetin, apigenin, and kaempferol, which contribute to the plant’s medicinal potential. The findings provide insight into the chemical composition of C. turkestanica and support its traditional use in herbal medicine. The results may serve as a scientific basis for future applications in pharmaceutical and nutraceutical development.

Keywords: Crataegus turkestanica, flavonoids, traditional medicine, gallic acid, rutin, salicylic acid, quercetin, apigenin, kaempferol.

Introduction

Plants have been extensively used since ancient times for the maintenance and restoration of human health. Particularly, medicinal plants remain a cornerstone of both traditional and modern therapeutic practices. Their value lies in the presence of biologically active substances such as alkaloids, glycosides, flavonoids, saponins, and other secondary metabolites, many of which serve as the chemical basis for the development of modern pharmaceutical drugs [1]. One notable example is Turkestan hawthorn (*Crataegus turkestanica*), a species belonging to the Rosaceae family that is native to Uzbekistan and widely distributed across Central Asia [2].

Crataegus turkestanica is traditionally used as a natural remedy for cardiovascular diseases. Its medicinal properties are largely attributed to the presence of flavonoid compounds, which are known for their diverse pharmacological effects, including antioxidant, anti-inflammatory, vasodilatory, and cardioprotective actions [3]. These compounds play a crucial role in scavenging free radicals, thereby protecting cells from oxidative stress and related pathological damage [4].

Hawthorn is both a food and medicinal plant with a long history of use. Since antiquity, it has been employed in traditional medicine for a variety of health purposes. Classical medical texts describe

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the internal consumption of hawthorn fruits as highly nutritious and therapeutic, with the ability to act as a tonic, astringent, and choleretic. The fruits have been used to reduce high blood pressure, calm vomiting, strengthen the digestive system, and support liver function. They are also noted for their diuretic properties, and their effectiveness in treating chronic intestinal diseases and diarrhea. In folk medicine, hawthorn fruits are used to combat fatigue, enhance cognitive function, relieve headaches and dizziness, and are even employed in the treatment of bronchial asthma. Tea made from the dried fruits and leaves of hawthorn is traditionally consumed to relieve heart pain, shortness of breath, hypertension, and gastrointestinal disorders.

Modern scientific medicine has further validated many of these uses. Hawthorn bark infusions are applied as desensitizing agents for allergic conditions. The flowers, fruits, and leaves of the plant contain a high concentration of phenolic compounds, giving them strong antioxidant properties. Recent pharmacological studies have also identified its immunomodulatory, antiallergic, antimutagenic, and antitumor effects. These multifaceted properties have contributed to hawthorn's widespread adoption in cardiological practice, where it is used as a supportive treatment for heart failure, arrhythmias, and hypertension [5].

Given the broad therapeutic potential and rich ethnopharmacological history of *Crataegus turkestanica*, this study focuses on the quantitative identification of selected flavonoid compounds in its plant material using high-performance liquid chromatography (HPLC). The objective is to provide a scientific basis for its traditional use and explore its suitability for future development into natural therapeutic agents or functional food additives.

Materials and Methods

Reagents and Equipment. Gallic acid was obtained from Macklin (China), while salicylic acid was sourced from Rhydburg Pharmaceuticals (Germany). Quercetin, apigenin, and kaempferol were purchased from Regal (China). Rutin was isolated from natural plant materials using solvent extraction and column chromatography techniques. All solvents and reagents, including deionized water, acetonitrile, glacial acetic acid, and sodium hydroxide, were of HPLC-grade purity.

Quantitative determination of polyphenolic compounds in the plant extract was carried out using a high-performance liquid chromatography system LC-40 Nexera Lite (Shimadzu, Japan).

Preparation of Standard Solutions. Stock solutions were prepared by dissolving 5.2 mg of gallic acid, 5.2 mg of salicylic acid, 5 mg of rutin, 5 mg of quercetin, 5 mg of apigenin, and 5 mg of kaempferol in 96% ethanol. The compounds were sonicated in an ultrasonic bath for 20 minutes to ensure complete dissolution, then transferred to a 50 mL volumetric flask and brought to volume with ethanol. From each stock solution, 200 µL was taken, mixed, and further diluted to obtain four working standard solutions. These were transferred into individual vials for chromatographic analysis.

Preparation of Plant Extract. For the extraction of phenolic compounds, 1.0 g of dried and finely ground *Crataegus turkestanica* plant material was accurately weighed using an NV222 analytical balance (OHAUS, USA) with a precision of 0.01 g. The sample was placed into a 50 mL conical

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flask, and 25 mL of 96% ethanol was added. The mixture was subjected to ultrasonic-assisted extraction using a GT SONIC-D3 ultrasonic bath (China) at 60 °C for 20 minutes.

After cooling to room temperature, the extract was filtered and the final volume was adjusted to 25 mL with ethanol in a volumetric flask. A 1.5 mL aliquot of the filtrate was centrifuged at 7000 rpm using a Mini-7 centrifuge (BIOBASE, China), and the resulting supernatant was passed through a 0.45 µm syringe filter prior to HPLC analysis.

Chromatographic Conditions

The identification of phenolic compounds in the extract of *Crataegus turkestanica* was performed using reversed-phase high-performance liquid chromatography (RP-HPLC). A Shim-pack GIST C18 column (150 × 4.6 mm, 5 µm; Shimadzu, Japan) was employed for the separation. The mobile phase consisted of a binary solvent system: Solvent A – acetonitrile, and Solvent B – 0.5% aqueous acetic acid, operated under a gradient elution program as detailed in Table 1.

The injection volume was 10 µL, the flow rate was set to 0.5 mL/min, and the column was maintained at a constant temperature of 40 °C using a column oven. Detection of phenolic compounds was carried out at a wavelength of 300 nm, where their characteristic absorbance peaks (analytical signals) were recorded.

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	Finish	

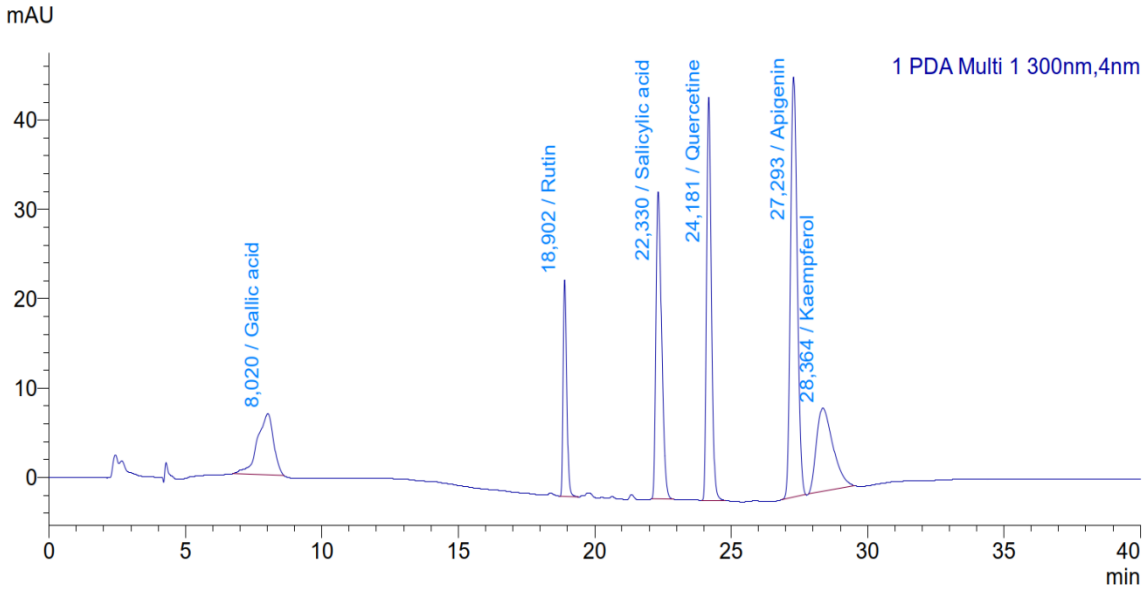

Figure 1. Chromatogram of standards at 300 nm.

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Results and Discussion

The chromatogram of the plant extract obtained from a 1-gram sample was recorded (see Figure 2). Based on the retention times and peak areas of the detected compounds, the concentration of phenolic compounds was calculated and expressed per 100 grams of plant material.

Quantification was carried out using the following formula:

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

Here, X – 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.

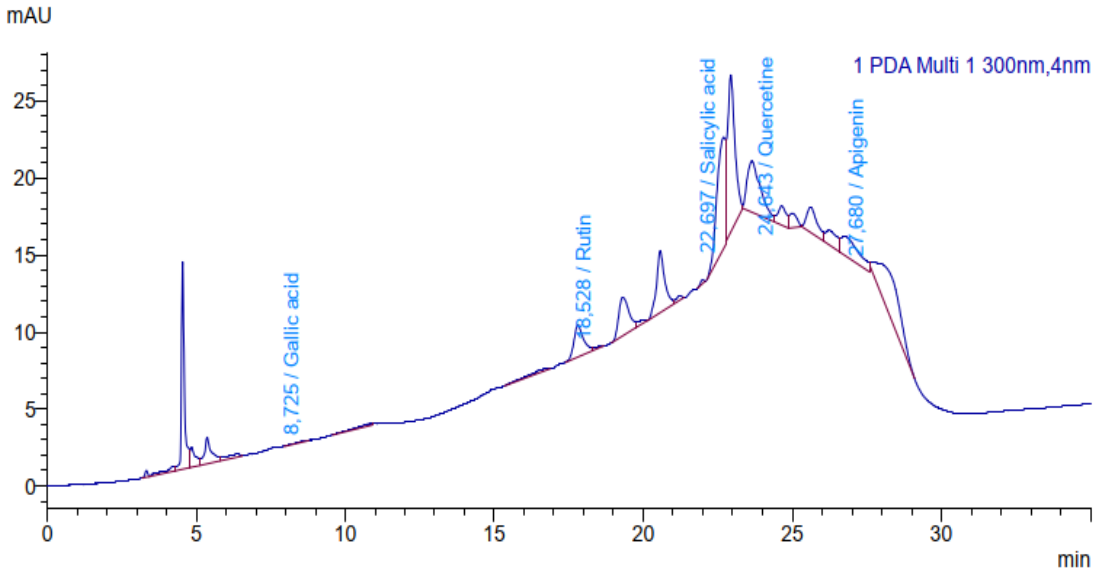

Figure 2. Chromatogram of polyphenols in the sample extract.

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

Phenol compound name	Holding time, sec	Concentration, mg/l	Amount in 100 ml of sample, mg
Gallic acid	8,725	0,094	0,235
Routine	18,528	0,184	0,460
Salicylic acid	22,697	5,219	13,048
Quercetin	24,643	0,75	1,875
Apigenin	27,68	2,929	7,323
Kaempferol	Not specified	0	0,000

Upon analysis of the chromatogram obtained from the 96% ethanolic extract of *Crataegus turkestanica* (see Figure 2), six flavonoid compounds were targeted for identification. Among these, the chromatographic peaks corresponding to salicylic acid and apigenin were the most prominent, indicating their relatively high concentration in the extract. In contrast, the

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chromatogram did not display any detectable peak for kaempferol, suggesting that this compound is absent in the analyzed plant material.

Quantitative analysis of the 100 g sample-based extract revealed that salicylic acid and apigenin were the most abundant phenolic compounds among the six flavonoids studied. The absence of kaempferol was consistent across multiple runs, supporting the conclusion that it is not a constituent of *Crataegus turkestanica* under the examined extraction conditions.

From a pharmacological perspective, salicylic acid is a well-known compound with anti-inflammatory and analgesic properties. Naturally occurring in plants, it is often found in association with flavonoids and contributes to the plant's medicinal effects. Its high concentration in *C. turkestanica* suggests that it may play a significant role in the cardiovascular support attributed to this species.

Apigenin is a flavonoid with sedative, antitumor, and antioxidant activities. Due to its broad biological effects, it is frequently included in phytopharmaceutical formulations. Its presence in considerable quantity enhances the therapeutic value of this plant.

Quercetin, another major flavonoid detected in this study, is widely regarded as one of the most bioactive polyphenols. It has been shown to support cardiovascular health, reduce inflammation, and stimulate the immune system. In the context of hawthorn, quercetin is recognized as one of its principal active constituents.

Rutin, also detected in moderate concentration, is a vascular-protective flavonoid known for its ability to strengthen capillary walls and improve microcirculation, making it a common component in cardiovascular medicines.

Gallic acid, though present in smaller amounts, contributes strong antioxidant and anti-inflammatory effects. Its presence supports the overall bioactivity profile of the extract, even in low concentrations.

No peak corresponding to kaempferol was detected in the chromatogram, indicating its absence from the tested sample under the applied extraction and analytical conditions.

Conclusion

In conclusion, the ethanolic extract of *Crataegus turkestanica* contains a diverse array of water-soluble phenolic compounds, including gallic acid, rutin, salicylic acid, quercetin, and apigenin. Among these, salicylic acid was identified in the highest concentration, highlighting its potential role as a cardioprotective and antioxidant agent. Furthermore, apigenin and quercetin were also detected in notable quantities, supporting their contribution to cardiovascular health.

The results of this study suggest that *Crataegus turkestanica* may serve as an effective natural source for the prevention and management of cardiovascular conditions, particularly for reducing blood pressure, enhancing vascular function, and providing antioxidant protection. These findings reinforce the traditional use of this plant in herbal medicine and support its further development as a natural therapeutic agent or functional food additive targeting cardiovascular health.

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