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DETERMINATION OF VITAMIN CONTENT IN CRATAEGUS TURKESTANICA (TURKESTAN HAWTHORN)

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Abstract
This study presents the results of a chromatographic analysis aimed at identifying and quantifying the vitamin content in the fruit of *Crataegus turkestanica* (Turkestan hawthorn). The research was conducted using high-performance liquid chromatography (HPLC) to determine the presence and concentration of both water-soluble and fat-soluble vitamins, including vitamin C, B1 (thiamine), B2 (riboflavin), B3 (niacin), B6 (pyridoxine), B9 (folic acid), and PP (niacinamide). The study confirmed the presence of several essential vitamins in varying concentrations, contributing to the understanding of the plant's nutritional and therapeutic potential. The findings support the traditional medicinal use of *C. turkestanica* and provide a scientific basis for its incorporation into functional foods and phytopharmaceutical preparations. The methodological approach, based on reversed-phase HPLC with UV detection, demonstrated high sensitivity and accuracy for multivitamin profiling in plant matrices.

Keywords: *Crataegus turkestanica*, vitamin C, vitamin B1, B2, B3, PP, B6, B9, vitamins, chromatography, HPLC.

Introduction

In traditional medicine, the flowers, fruits, leaves, and bark of the hawthorn plant (*Crataegus* spp.) have been widely used in the treatment of various ailments. Decoctions and infusions prepared from these plant parts are traditionally employed to alleviate symptoms such as palpitations, high blood pressure, dizziness, shortness of breath, colds, insomnia, and cardiac neurosis. Fresh fruits,

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in particular, have been used as a mild laxative. According to experimental studies by A.T. Turova et al. (1987), hawthorn increases cardiac output and reduces nervous excitability [1].

Natural sources, particularly medicinal plants, play a pivotal role in modern pharmacology. They are not only applied in traditional remedies but are also increasingly utilized in clinical settings. Among these, plants of the *Crataegus* genus have long been recognized for their cardiovascular benefits. Hawthorn contains a diverse range of biologically active compounds, including antioxidants, flavonoids, phenolic acids, sterols, triterpenes, and vitamins, which collectively contribute to its cardiotonic effects [2].

In Uzbekistan and other regions of Central Asia, *Crataegus turkestanica* (Turkestan hawthorn) is a native and endemic species. It is widely used in folk medicine to treat heart palpitations, nervous disorders, and hypertension [3]. In recent years, there has been growing interest in the pharmacochemical profiling of this plant using advanced analytical techniques, particularly high-performance liquid chromatography (HPLC) [4].

The pharmacological effects of hawthorn, especially its impact on cardiac function, are closely associated with its content of flavonoids and B-group vitamins. Vitamins such as thiamine (B1), riboflavin (B2), pyridoxine (B6), folate (B9), and niacin (PP) are known to support cardiac metabolism, regulate energy production in myocardial cells, improve circulation, and facilitate neurotransmission [5,6]. Niacin, in particular, has been acknowledged for its effectiveness in reducing cholesterol levels and controlling arterial blood pressure [7].

Laboratory-based vitamin analysis using high-precision technologies provides valuable insights into the medicinal value of this plant. In this study, HPLC analysis conducted with Shimadzu's LabSolutions software confirmed the presence of key B-group vitamins in the fruit extract of *Crataegus turkestanica* [8]. These findings underscore the plant's potential as a natural therapeutic agent for cardiovascular diseases and validate its application as a scientifically supported raw material for pharmaceutical formulation.

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This article presents the experimental results of vitamin profiling in *Crataegus turkestanica* fruits.

Materials and Methods

Reagents and Equipment. Vitamin standards were obtained from the following manufacturers: Vitamin B12 from Rhydburg Pharmaceuticals (Germany), Vitamin C from Carl Roth GmbH (Germany), Vitamin B9 from DSM Nutritional Products GmbX (Germany), and Vitamins B1, B2, B3, B6, and PP from BLDPharm (China). HPLC-grade water, acetonitrile, glacial acetic acid, and sodium hydroxide were used as reagents in the analysis.

Quantification of water-soluble vitamins in the plant extract was performed using a LC-40 Nexera Lite high-performance liquid chromatograph (Shimadzu, Japan) equipped with gradient pumps and a photodiode array detector [9].

Preparation of Standard Solutions. Stock solutions (100 mg/L) of the following vitamins were prepared:

- Vitamin C (CAS 50-81-7), B1 (CAS 59-43-8), B6 (CAS 58-56-0), B3 (CAS 59-67-6), B12 (CAS 68-19-9), and PP (CAS 98-92-0) were each dissolved in 50 mL of 0.1 N HCl.
- Vitamins B2 (CAS 83-88-5) and B9 (CAS 59-30-3) were each dissolved in 50 mL of 0.025% sodium hydroxide solution.

From these, working solutions were prepared by mixing 200 µL of each vitamin solution and diluting to obtain final concentrations of 14.286, 7.143, 3.571, and 1.786 mg/L for multi-vitamin analysis. For vitamin C, separate standard solutions were prepared at concentrations of 286, 143, 71.5, and 57.2 mg/L. Pure water was used as a blank (0 mg/L) for the calibration curve.

Preparation of Sample Extract. To extract water-soluble vitamins, 1.0 g of dried plant material was weighed using an analytical balance and placed in a 50 mL conical flask. Then, 25 mL of 0.1 N HCl was added. The mixture was subjected to ultrasonic-assisted extraction using a GT SONIC-D3 (China) ultrasonic bath at 60 °C for 20 minutes. After cooling, the extract was filtered and the final

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volume adjusted to 25 mL with distilled water. A 1.5 mL aliquot was filtered through a 0.22 µm syringe filter and transferred into HPLC vials for analysis.

Chromatographic Conditions. Vitamin analysis was performed using a Shimadzu LC-40 Nexera Lite HPLC system equipped with:

- LC-40D dual pump system
- SIL-40 autosampler
- SPD-M40 photodiode array (PDA) detector
- LabSolutions software (ver. 6.92)

A Shim-pack GIST C18 column (150 × 4.6 mm; 5 µm, Shimadzu, Japan) was used. The mobile phase consisted of Solvent A: acetonitrile and Solvent B: 0.25% aqueous acetic acid, operated under a gradient elution program as shown in Table 1.

Injection volume was 10 µL, flow rate was set to 0.6 mL/min, and column oven temperature maintained at 40 °C. Analytical detection of each vitamin was carried out using specific wavelengths: 265 nm, 291 nm, and 550 nm, according to their optimal absorbance properties (Figures 1–3).

For vitamin C, a 15-minute gradient elution program was applied separately, with detection at 265 nm (Table 2).

Table 1. Mobile phase gradient program in the determination of vitamins.

Time, min	Acetonitrile (A), %	0.5% acetic acid (B), %
0	0	100
3	0	100
14	20	80
17	50	50
18	0	100
25	Finish	

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Table 2. Mobile phase gradient program for vitamin C quantification.

Time, min	Acetonitrile (A), %	0.5% acetic acid (B), %
0	0	100
2	0	100
6	50	50
6,01	0	100
15	Finish	

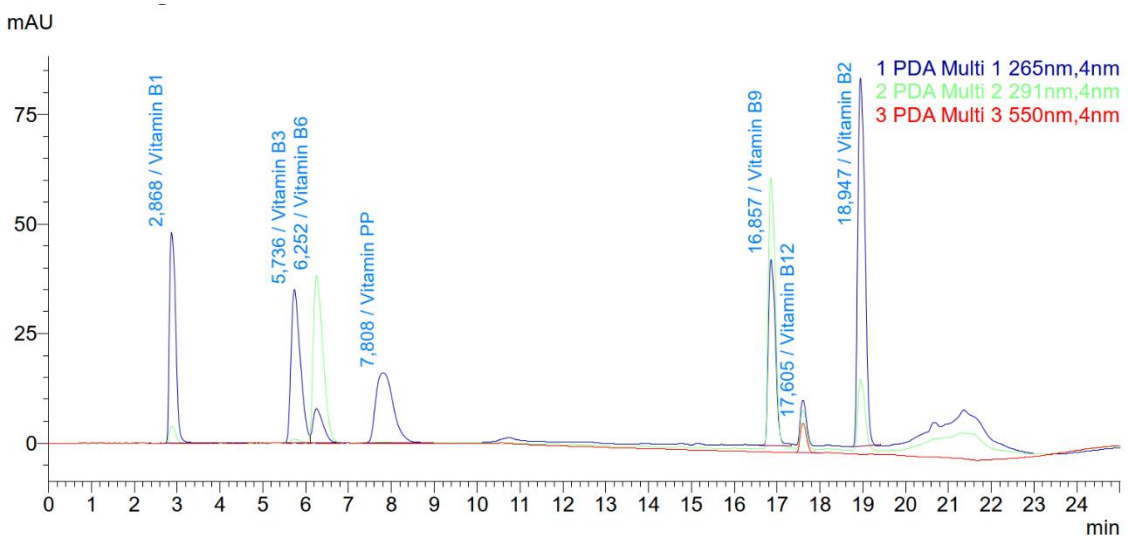

Figure 1. Chromatogram of a standard solution of vitamins.

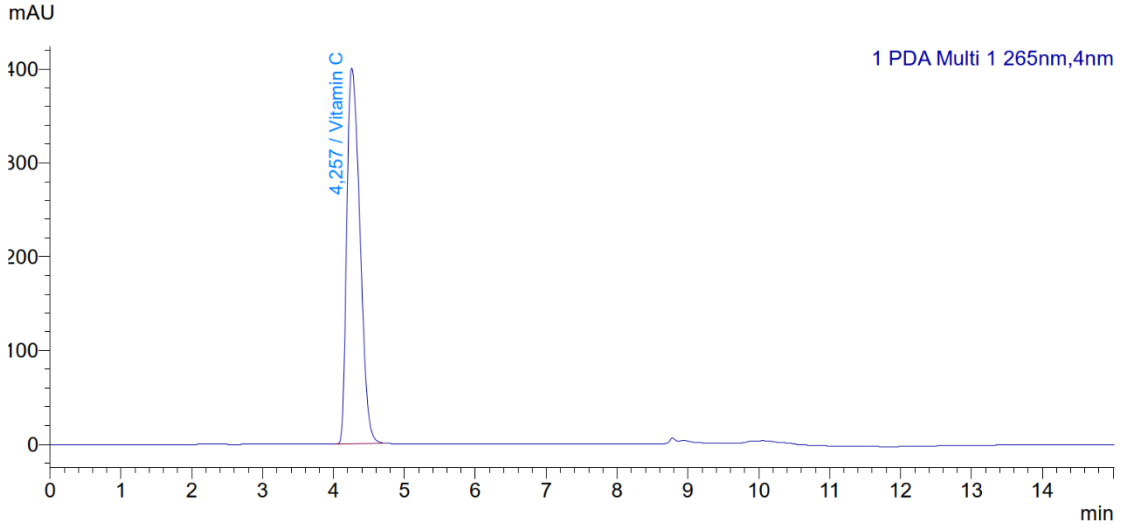

Figure 2. Chromatogram of a vitamin C standard solution.

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Results and Discussion

The chromatograms obtained from the sample extract (Figures 3–4) displayed well-resolved peaks corresponding to the retention times of water-soluble vitamin standards. These peaks confirmed the presence of multiple essential vitamins in the ethanolic extract of *Crataegus turkestanica* fruit.

Based on the calibration curves constructed for each vitamin and their respective absorbance at optimal wavelengths (265, 291, and 550 nm), the concentrations of vitamins in the extract were calculated using the following formula:

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

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

C_{vit} – concentration of vitamin 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.

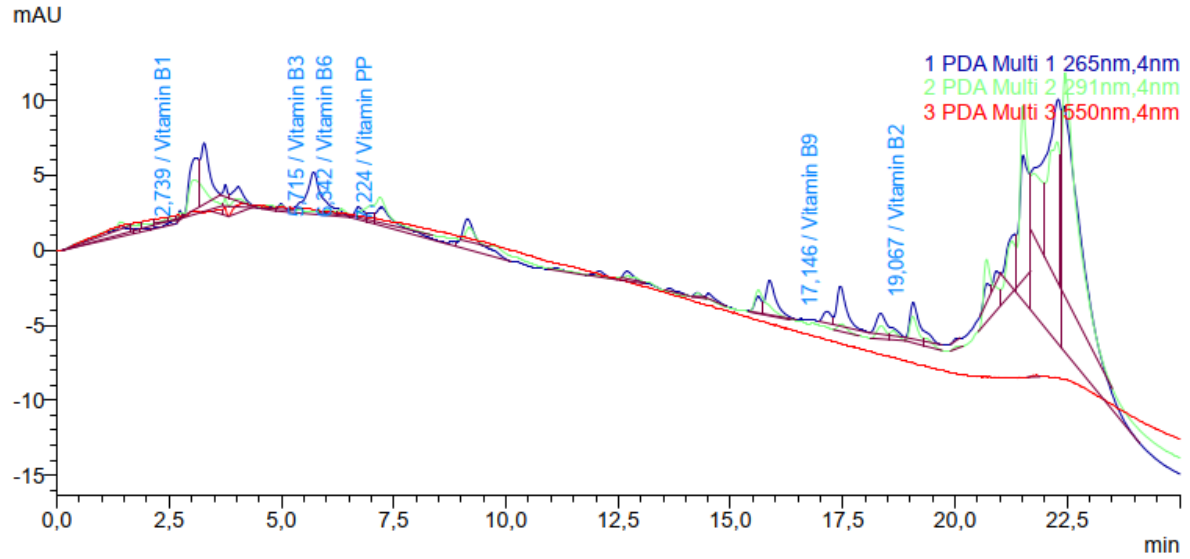

Figure 3. Chromatogram of the determination of vitamins in the sample extract.

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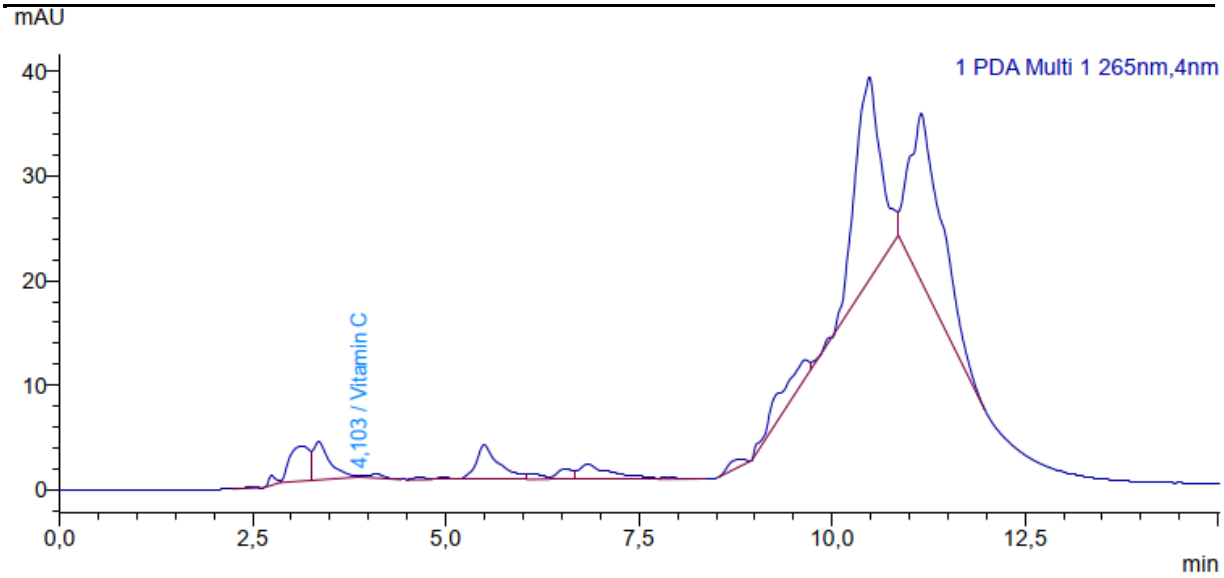

Figure 4. Chromatogram of vitamin C in the sample extract.

Table 3. Amount of vitamins in the extract and retention times.

Vitamin	Holding time, sec	Concentration, mg/l	Amount in 100 ml of sample, mg
Vitamin B_1	2,739	0,175	0,438
Vitamin B_3	5,715	1,978	4,945
Vitamin PP	7,224	1,194	2,985
Vitamin B_9	17,146	0,311	0,778
Vitamin B_2	19,067	0,831	2,078
Vitamin B_6	6,342	0,129	0,323
Vitamin B_{12}	Not specified	0	0,000
Vitamin C	4,103	0,049	0,123

The chromatographic analysis of the aqueous extract of *Crataegus turkestanica* revealed eight major water-soluble vitamins. Among these, the chromatogram (Figures 3–4) showed a distinct and pronounced peak for vitamin B3, indicating its relatively high concentration in the sample. Conversely, vitamin B12 was not detected, as evidenced by the absence of a corresponding peak in the chromatographic output.

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Quantitative analysis based on HPLC data for 100 g of dried sample demonstrated that vitamin B3 (niacin) was the most abundant among the examined vitamins. Other detected vitamins included PP (niacinamide), B2 (riboflavin), B9 (folic acid), B1 (thiamine), B6 (pyridoxine), and vitamin C, each contributing to the plant's known bioactivity. The absence of vitamin B12 is consistent with literature reports that it is rarely present in significant concentrations in plant-based sources.

The predominance of B-group vitamins in the extract supports the traditional medicinal use of *Crataegus turkestanica* and highlights its potential as a cardioprotective and neuroprotective agent. These vitamins are critically involved in various physiological processes such as circulatory health, nerve conduction, metabolic regulation, and antioxidant defense. Notably, vitamin B3 and PP—both involved in lipid metabolism and vascular integrity—were found at significantly higher levels than other compounds.

Chromatographic peaks and retention times provided conclusive identification and quantification. The reliability of these results was ensured by comparison with calibration standards and retention time matching using PDA detection.

Conclusion

In conclusion, the vitamin profiling of *Crataegus turkestanica* fruit extract demonstrated that vitamin B3 (niacin) and PP (niacinamide) are present in the highest concentrations, which may explain the plant's traditional effectiveness in improving circulation, reducing blood cholesterol, and mitigating cardiovascular risks. Vitamin B9 (folate), known for its role in cellular division and hematopoiesis, also contributes to the cardiovascular support offered by this plant.

Other detected vitamins, including B1, B2, B6, and C, further enhance the therapeutic value of *C. turkestanica*, particularly for energy metabolism, nervous system health, and immune function.

These scientifically grounded findings position *Crataegus turkestanica* as a promising natural candidate for developing cardiotonic and prophylactic herbal formulations. Moreover, the extract may be considered for preventive use,

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especially in populations at risk of cardiovascular disorders. Future research should focus on the isolation of additional bioactive constituents, in vivo pharmacological assessments, and clinical trials to validate and expand its therapeutic applications. Such efforts will bridge traditional knowledge with modern pharmaceutical development, offering an evidence-based pathway for integrating *C. turkestanica* into contemporary medicine.

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