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DETERMINATION OF VITAMIN CONTENT IN MULBERRY (MORUS ALBA) LEAF EXTRACT

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Abstract:
The present study investigates the chemical composition of Morus alba (white mulberry) leaves, focusing on the concentration of water-soluble vitamins extracted from dried leaf samples. Given the increasing interest in plant-based bioactive compounds, mulberry leaves are recognized for their potential health-promoting properties, including antioxidant, anti-inflammatory, and metabolic regulatory effects. Using High-Performance Liquid Chromatography (HPLC), the quantitative analysis revealed the presence of key B-group vitamins (B1, B2, and B3) and vitamin C. The results indicate a progressive increase in vitamin concentration in the order of B1 < B2 < B3, highlighting the rich nutritional profile of the extract. This finding underscores the potential of mulberry leaf extract as a natural source of essential micronutrients and supports its application in nutraceutical and functional food industries. Additionally, the study discusses the relevance of glycosides, alkaloids, and organic acids identified in the extract, as well as their synergistic role in promoting human health.

Keywords: Mulberry leaf, vitamin C, vitamin B1, vitamin B2, vitamin B3, glycoside, acid, compounds, arctium, alkaloids, extract, minerals, HPLC.

Introduction

Recent advancements in chemical sciences have significantly accelerated the development of the pharmaceutical industry, leading to the synthesis of novel and fast-acting therapeutic agents. A considerable number of pharmaceutical compounds used in modern medicine are either derived from or synthesized using plant-based raw materials. Plants serve as essential sources of bioactive constituents such as alkaloids, glycosides, flavonoids, coumarins, saponins, essential oils, and various vitamins—many of which possess considerable therapeutic potential [1, 2].

One such medicinally valuable plant is *Morus alba*, commonly known as white mulberry. Belonging to the Moraceae family, the genus *Morus* is native to regions including Azerbaijan, Dagestan, Turkmenistan, and Iran, where wild varieties are referred to as *shotut*. Historical references, such as Mahmud al-Kashgari's "Compendium of the Turkic Dialects," mention the fruit as *tut*. Mulberry leaves are rich in a diverse array of nutrients, including provitamin A, vitamins C, E, and PP, B-complex vitamins, and a variety of macro- and microelements. The leaves also contain notable amounts of potassium, sodium, malic acid, citric acid, tartaric acid, pectin, and carbohydrates—primarily fructose.

The pharmacological value of mulberry leaves is largely attributed to their complex of vitamins, monosaccharides, and biologically active compounds. These constituents provide various health benefits, including treatment of ocular diseases, sclerosis, hypertension, and sore throat. Moreover, the leaf extract exhibits emollient, anti-inflammatory, regenerating, and antiseptic properties [3, 4].

Considering the therapeutic and nutritional relevance of mulberry leaves, this study was conducted in the scientific laboratory of the "Department of Commodity Chemistry and Folk Medicine" at Andijan State University. The primary objective was to quantitatively determine the concentration of water-soluble vitamins present in the extract of *Morus alba* leaves using the High-Performance Liquid Chromatography (HPLC) method.

2.1. Quantitative Determination of Water-Soluble Vitamins in Mulberry Leaf Extract Using HPLC

Reagents and Instruments.

Vitamin B12 was obtained from *Rhydburg Pharmaceuticals* (Germany), vitamin C from *Carl Roth GmbH* (Germany), and vitamin B9 from *DSM Nutritional Products GmbH* (Germany). Vitamins B1, B2, B3, B6, and PP were purchased from *BLDPharm* (China). Analytical-grade reagents included HPLC-grade water, acetonitrile, glacial acetic acid, and sodium hydroxide.

The quantitative analysis of water-soluble vitamins in plant material was carried out using a Shimadzu LC-40 Nexera Lite high-performance liquid chromatography (HPLC) system manufactured in Japan.

Preparation of Standard Solutions

Stock solutions (100 mg/L) of vitamins 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 prepared by dissolving 5 mg of each vitamin in 50 mL of 0.1 N hydrochloric acid. Standard solutions of vitamins B2 (CAS 83-88-5) and B9 (CAS 59-30-3) were prepared by dissolving 5 mg of each in 50 mL of 0.025% sodium hydroxide solution. A mixed standard solution was then prepared by mixing 200 µL of the B1, B6, B3, B12, and PP stock solutions, resulting in a final concentration of 14.286 mg/L per vitamin. Further dilutions were prepared to obtain concentrations of 7.143, 3.571, and 1.786 mg/L.

Vitamin C calibration solutions were prepared at concentrations of 286, 143, 71.5, and 57.2 mg/L. Distilled water was used as the 0 mg/L concentration for calibration curve plotting.

Sample Preparation

For extraction of water-soluble vitamins, 1.0 g of the dried mulberry leaf sample was weighed and transferred into a 50 mL conical flask. Then, 25 mL of 0.1 N hydrochloric acid was added. The mixture was subjected to ultrasonic extraction in a GT SONIC-D3 ultrasonic bath (China) at 60°C for 20 minutes. After cooling, the mixture was filtered and the filtrate was diluted to 25 mL with distilled water in a volumetric flask.

Subsequently, 1.5 mL of the extract was filtered through a 0.22 µm syringe filter, transferred to a vial, and used for HPLC analysis.

2.2. Chromatographic Conditions

Vitamin Detection. Standard solutions and sample extracts were analyzed using the LC-40 Nexera Lite high-performance liquid chromatography (HPLC) system, which included an LC-40D pump, SIL-40 autosampler, and SPD-M40 photodiode array detector (PDA), supported by LabSolutions software (version 6.92). The separation was performed on a reversed-phase Shim-pack GIST C18 column (150 × 4.6 mm, 5 µm; Shimadzu, Japan). The mobile phase consisted of a gradient system composed of acetonitrile (A) and 0.25% aqueous acetic acid (B), as outlined in Table 1. Injection volume was set at 10 µL, with a flow rate of 0.6 mL/min, and the column temperature was maintained at 40°C. Each vitamin's analytical signal (peak area) was monitored at three different wavelengths: 265 nm, 291 nm, and 550 nm (Figures 1 and 2).

For vitamin C, a separate 15-minute gradient elution program was applied (Table 2), and the detection wavelength was fixed at 265 nm.

Table 1. Gradient Program for the Determination of B-Group Vitamins

Time (min)	Acetonitrile (A), %	0.25% Acetic Acid (B), %
0	0	100
3	0	100
14	20	80
17	50	50
18	0	100
25	Finish	

Table 2. Gradient Program for the Determination of Vitamin C

Time (min)	Acetonitrile (A), %	0.25% Acetic Acid (B), %
0	0	100
2	0	100
6	50	50
6,01	0	100
15	Finish	

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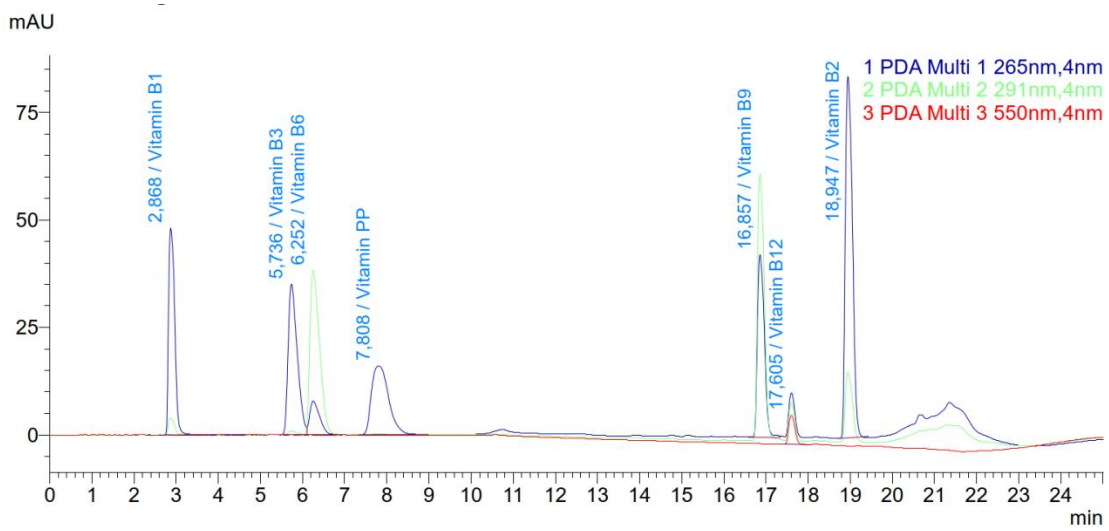

Figure 1. Chromatogram of the mixed vitamin standard solution.

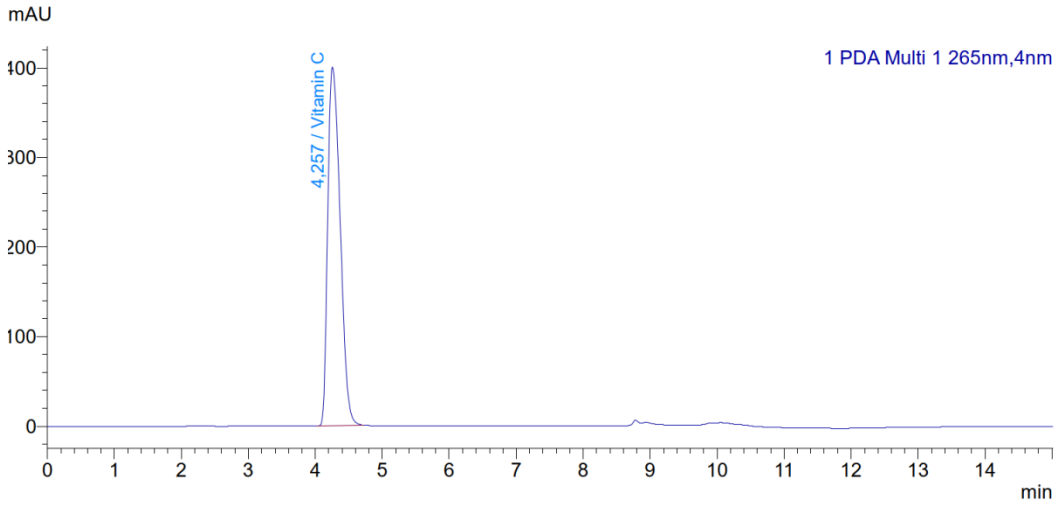

Figure 2. Chromatogram of the vitamin C standard solution.

3. Results and Discussion

A group of Russian scientists led by L.A. Bagdanova developed a methodology for the determination of water-soluble vitamins in premixes (nutritional supplements), which ensures the analytical accuracy required for reliable vitamin quantification. Their method included optimized extraction, chromatographic separation, and detection conditions, with a reported relative error of approximately 10% [5,6,7].

Additionally, researchers from Moscow State University under the leadership of A.V. Pirogov proposed a procedure for the simultaneous quantification of 14 different water-soluble vitamins using high-performance liquid chromatography (HPLC) [8].

Building upon these foundational methods, we successfully determined the content of water-soluble vitamins in the leaf extract of *Morus alba* (white mulberry) cultivated under the agro-climatic conditions of the Andijan region using HPLC techniques.

3.1. Determination of Vitamins in Mulberry Leaf Extract

The chromatogram of the sample extract was obtained (Figures 3 and 4), and based on the peak data, the concentration of vitamins in the plant material was calculated using the following formula:

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

Where:

X – Content of the vitamin in 100 g of plant material, in mg;

C_{vit} – Concentration of the vitamin in the extract as determined by HPLC, mg/l;

$V_{extract}$ – Volume of the extract, in liters;

m_{sample} – Mass of the sample used for extraction, in grams.

The calculated results are presented in Table 3, which summarizes the vitamin content in 100 g of the mulberry leaf sample.

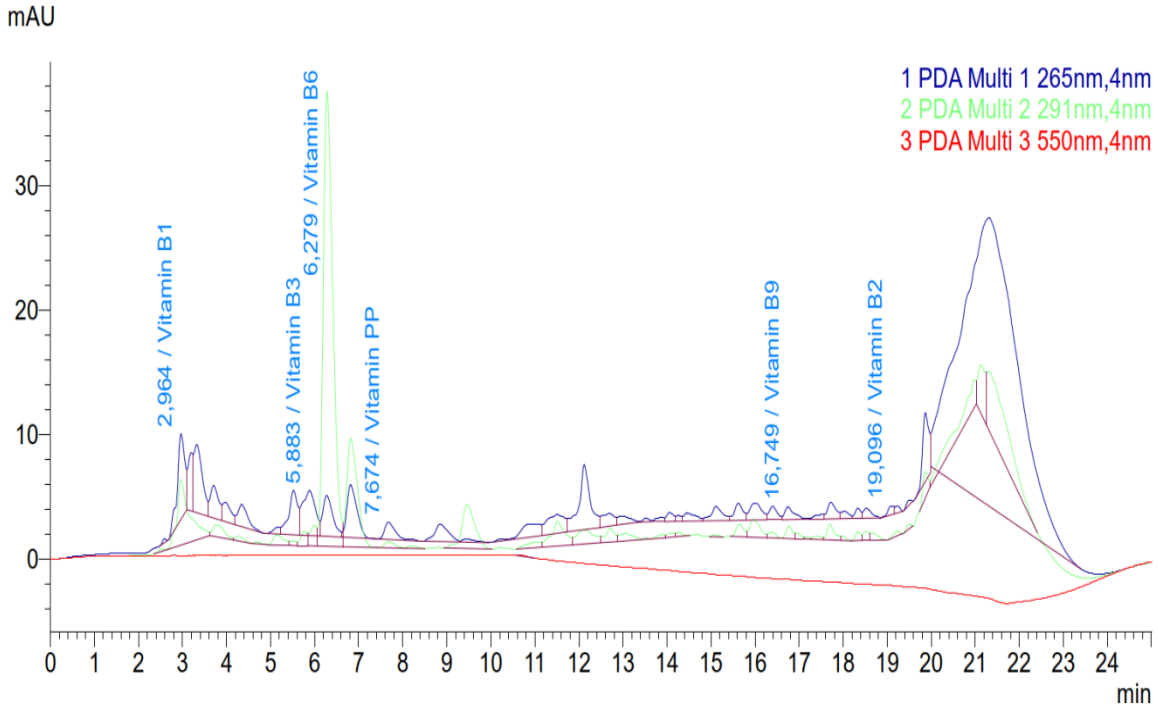

Figure 3. Chromatogram for determining vitamins in buckwheat leaf extract.

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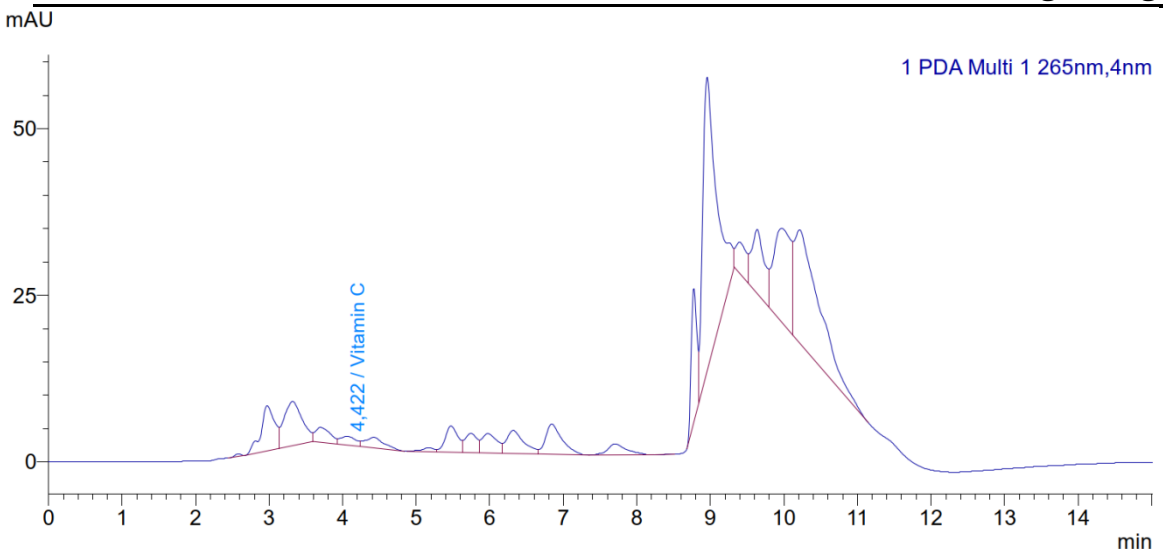

Figure 4. Chromatogram for determining the amount of vitamin C in the extract of rosehip leaves.

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

Vitamin	Holding time, sec	Concentration, mg/l	Amount in 100 g sample, mg
Vitamin B1	2.745	1.932	4.830
Vitamin B3	5.603	24.325	60.813
Vitamin PP	7.974	1.239	3.098
Vitamin B9	17.063	1.472	3.680
Vitamin B2	19.067	2.148	5.370
Vitamin B6	6.457	0.193	0.483
Vitamin B12	Not specified	0	0.000
Vitamin C	4.096	0.888	2.220

Upon analyzing the chromatograms of *Morus alba* (white mulberry) leaf extract (Figures 3 and 4), seven distinct vitamin peaks were identified. Among these, the peaks corresponding to vitamins B1, B2, and B3 were the most prominent, indicating their higher concentration in the extract. The absence of a distinct peak for vitamin B12 suggests that this compound was either not present or was below the detection limit under the specified chromatographic conditions.

The predominance of B-group vitamins in the mulberry leaf extract supports previous findings regarding the nutritional value of this plant. Specifically, the high content of:

- Vitamin B1 (thiamine) plays a vital role in carbohydrate, protein, and fat metabolism, as well as in the transmission of nerve impulses, making it essential for nervous system function.
- Vitamin B2 (riboflavin) contributes to the proper functioning of the nervous system, supports visual performance, and helps prevent symptoms of fatigue and reduced energy metabolism.

- Vitamin B3 (niacin) protects skin cells from ultraviolet radiation, may help in preventing certain types of skin cancer, and serves as a primary treatment for pellagra, a disease caused by niacin deficiency.

These findings underline the potential of mulberry leaf extract as a natural dietary supplement rich in essential water-soluble vitamins, particularly for applications in functional foods, nutraceuticals, and traditional medicine.

4. Conclusions

The quantitative analysis of water-soluble vitamins in mulberry (Morus alba) leaf extract using the HPLC method revealed that 100 grams of the sample contained 60.813 mg of vitamin B3, 5.370 mg of vitamin B2, and 4.830 mg of vitamin B1.

Based on the high content of vitamin B3 in the aqueous extract of mulberry leaves, it can be concluded that infusions made from these leaves may help protect skin cells from ultraviolet (UV) radiation, reduce the risk of certain types of skin cancer, and serve as a primary treatment agent for pellagra, a disease caused by niacin deficiency.

Given the confirmed chemical composition of mulberry leaves, including significant levels of B-group vitamins, it is recommended that the extract be used in the formulation of herbal teas, nutraceuticals, and traditional medicinal products. Such applications may contribute to dietary supplementation and preventive healthcare within the context of traditional medicine.

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