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DETERMINATION OF WATER-SOLUBLE VITAMINS IN MACLURA POMIFERA FRUIT

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Annotation
In this article, the amount of water-soluble vitamins contained in the Maclura pomifera fruit growing in Uzbekistan was determined and analyzed using the HPLC method. While 7 different vitamins have been studied in this, Maclura pomifera fruit extract contains 100 grams of B_1>PP>B_3>B_6>B_2>C>B_9 in this order. In this case, the effect of water-soluble vitamins on human health was analyzed and studied on the basis of scientific sources. Water-soluble vitamins are essential for the body's metabolism, immune system, eye and skin health, bone and circulatory systems. Water-soluble vitamins Group B vitamins are of great importance in carrying out important tasks in the body. Water-soluble vitamins should regularly enter the body through food, since their excess amounts are not stored in the body. Their deficiency can lead to various health problems. The level of study of the resulting plant was calculated, and the studies came to the conclusion that it was not yet enough

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Introduction. Water-soluble vitamins are those that enter the body through food, dissolve in water, and are primarily associated with the water content in the blood [1]. They are involved in various biochemical processes in the body, including metabolism, immune system function, and overall health [2,3].

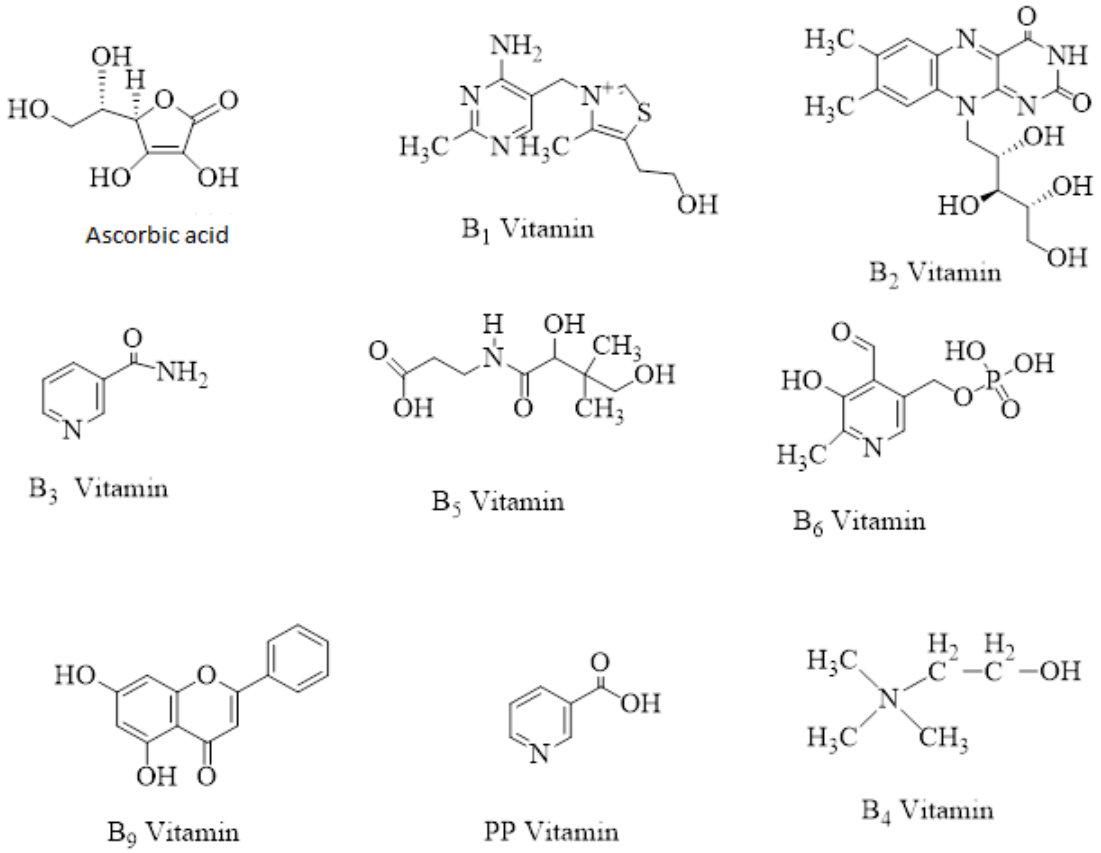


Figure 1. Water-Soluble Vitamins

These vitamins are not stored in the body for long periods, so they must be regularly obtained through diet. Water-soluble vitamins are excreted from the body via urine, which means excess amounts are not stored as reserves [5,6]. It is recommended to consume them with water to enhance absorption by the body. Water-soluble vitamins are essential for health because they participate in various biochemical processes and help protect the body. Regular intake of these vitamins is crucial, as their deficiency can lead to various health issues. Obtaining the required amounts of these vitamins is easy and effective through the consumption of a wide variety of foods [7].

Experimental Section

Reagents and Equipment Used. Vitamin B12 was purchased 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). Water, acetonitrile, acetic acid, and sodium hydroxide of analytical grade and HPLC purity were used. The analysis of water-soluble vitamins in the plant material was performed using the LC-40 Nexera Lite high-performance liquid chromatograph manufactured by Shimadzu (Japan).

Preparation of Standard Solutions. Solutions 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) at a concentration of 100 mg/L were prepared by dissolving 5 mg of each vitamin in 50 mL of 0.1 N HCl. 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. Next, 200 µL of each of the B1, B6, B3, B12, and PP vitamin stock solutions were mixed to obtain a solution with 14.286 mg/L of each vitamin. Diluted solutions with concentrations of 7.143, 3.571, and 1.786 mg/L were also prepared.

Standard solutions of vitamin C were prepared at concentrations of 286, 143, 71.5, and 57.2 mg/L. Distilled water was used as the 0 mg/L blank for calibration curve preparation.

Sample Extract Preparation. To extract water-soluble vitamins, 1 g of the test sample was weighed, placed in a 50 mL conical flask, and mixed with 25 mL of 0.1 N HCl. The mixture was sonicated using a GT SONIC-D3 ultrasonic bath (China) at 60 °C for 20 minutes. After cooling, the mixture was filtered and the volume was adjusted to 25 mL with water in a volumetric flask. Then, 1.5 mL of the extract was filtered through a 0.22 μm syringe filter, transferred to a vial, and used for analysis.

Chromatographic Conditions.

Vitamin Determination. Analysis of standard solutions and sample extracts was carried out using an LC-40 Nexera Lite HPLC system consisting of an LC-40D pump, SIL-40 autosampler, and SPD-M40 photodiode array (PDA) detector, with LabSolutions software version 6.92. A reverse-phase GIST C18 column (150 × 4.6 mm; 5 μm, Shimadzu, Japan) was used. The mobile phase was a gradient of acetonitrile (A) and 0.25% aqueous acetic acid (B). Injection volume was 10 μL, flow rate 0.6 mL/min, and the column temperature was maintained at 40 °C. Analytical signals (peak areas) for each vitamin were recorded at wavelengths of 265, 291, and 550 nm. For vitamin C determination, a 15-minute gradient program was used, and the analytical signal was recorded at 265 nm.

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

Time in minutes	Acetonitrile (A), %	0,5 % acetic acid (B), %
0	0	100
3	0	100
14	20	80
17	50	50
18	0	100
25	Termination	

Table 2. Mobile phase gradient program for the determination of vitamin C content.

Time in minutes	Acetonitrile (A), %	0,5 % acetic acid (B), %
0	0	100
2	0	100
6	50	50
6,01	0	100
15	Termination	

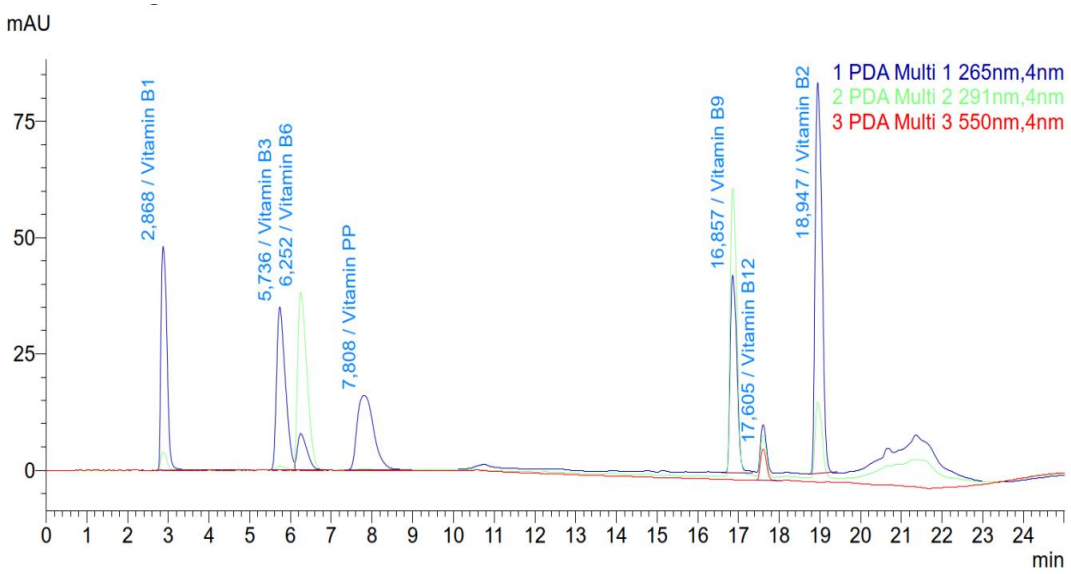


Figure 2. Chromatogram of the standard vitamin solution.

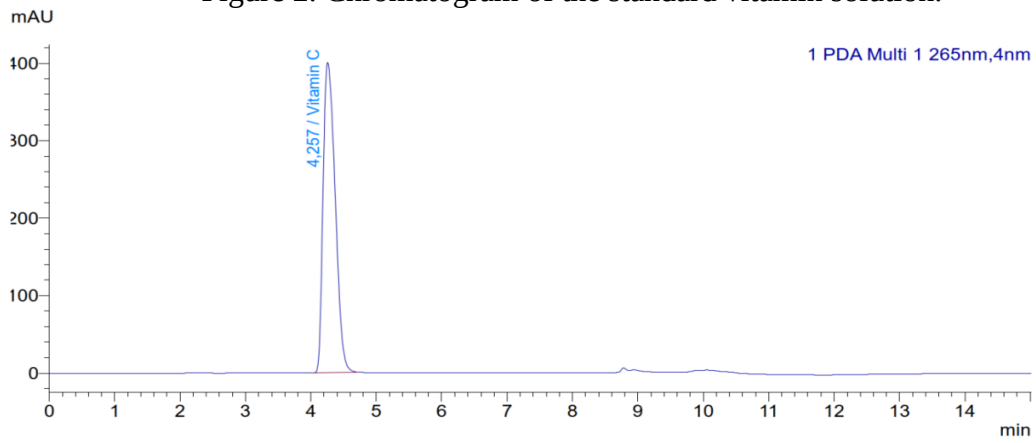


Figure 3. Chromatogram of the standard vitamin C solution.

Results and discussion

Chromatograms of the *Maclura pomifera* fruit extract were obtained (Figures 4–5), and based on the results, the vitamin content in 100 grams of fruit was calculated using the following formula and is presented in Table 1:

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

Here:

X – vitamin content in 100 grams of fruit, mg;

C_{vit}– concentration of the vitamin in the extract, determined by HPLC, mg/L;

$V_{extract}$ – volume of the extract, L;

m_{sample} – mass of the sample used to prepare the extract.

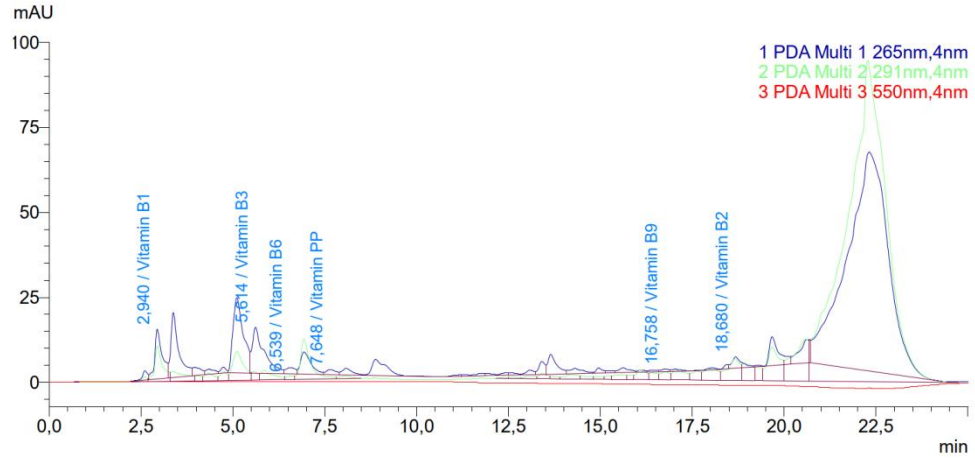


Figure 4. Chromatogram for the determination of vitamins in the *Maclura pomifera* fruit extract.

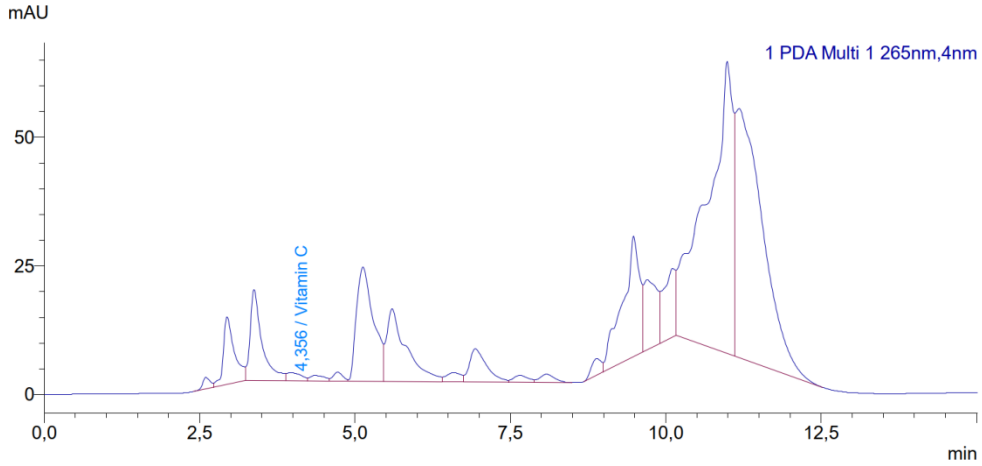


Figure 5. Chromatogram for the determination of vitamin C content in the *Maclura pomifera* fruit extract.

Table 1. Vitamin content and retention times in the *Maclura pomifera* fruit extract.

Vitamin	Maclura pomifera fruit	Maclura pomifera fruit growing in Turkey.
	Quantitity in a 100 g sample, mg	
Vitamin B_1	53.24	26.59
Vitamin B_3	20,46	15.26
Vitamin PP	22,253	19.12
Vitamin B_9	0.69	0.48
Vitamin B_2	2,885	1.89
Vitamin B_6	14.66	10.23
Vitamin C	1,503	0.98

As shown in the data, the vitamin content in *Maclura pomifera* fruit decreases in the following order: B1 > PP > B3 > B6 > B2 > C > B9. The most abundant vitamin is B1, which plays a crucial role in supporting the functions of the nervous system, heart, and muscles [1,8]. Additionally, vitamin B1 has significant effects on inflammation and immune response, and is recognized as an effective agent against inflammatory processes [3,4].

Vitamin B3, also known as niacin or niacinamide, is a water-soluble vitamin essential for the human body. It participates in numerous metabolic processes and is important for energy metabolism, cellular regeneration, and maintaining nervous system function. Moreover, vitamin B3 plays a notable

role in modulating inflammation and supporting the immune system, exhibiting strong anti-inflammatory activity [5].

Niacin and its metabolite niacinamide act as potent antioxidants, protecting cells from oxidative stress and free radicals, thereby contributing to the reduction of inflammation [6].

Vitamin B3 enhances the activity of key immune cells such as T-cells and macrophages. T-cells are involved in defending the body against infections, while macrophages regulate inflammatory responses and help eliminate harmful pathogens. Niacin also promotes the process of phagocytosis — the engulfment of harmful substances and microorganisms by immune cells — which is vital for protecting the body from infections [9,10].

Vitamin C also plays an important role in strengthening the immune system. It supports the function of white blood cells (leukocytes), the main components of the immune system, enhancing their ability to fight pathogens and detect infections more rapidly. In particular, vitamin C boosts the activity of phagocytes and T-cells. It also aids in the production of interferons — molecules that support antiviral immune responses and help the body fight infections. Furthermore, vitamin C supports collagen synthesis, aiding in skin repair and faster wound healing. It also contributes to cell growth and renewal, ensuring a rapid and effective immune response [11].

Conclusion. This article studied and analyzed the content of 7 water-soluble vitamins in the fruits of Maclura pomifera growing in Uzbekistan using the HPLC method. The analysis results were compared with the results of a study on the vitamin content in Maclura pomifera fruit growing in Turkey, conducted by Rifat Battaloglu, a scientist at Niğde Ömer Halisdemir University. It was found that the fruits of Maclura pomifera growing in Uzbekistan contain 2 times more vitamin B1, 1.34 times more vitamin B3, and 1.53 times more vitamin C than the vitamins in Maclura pomifera fruit growing in Turkey. In addition, based on scientific sources, the effect of water-soluble vitamins on human health and how their deficiency can lead to various health problems were analyzed and studied. The degree of study of the obtained plant was assessed, and it was concluded that the research conducted is not yet sufficient.It was concluded that Maclura pomifera, a growing fruit in Uzbekistan, has a high content of water-soluble vitamins, which can be used as a means of preventing and helping to treat many diseases. Further research is intended to be increased.

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