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IDENTIFICATION AND ANALYSIS OF WATER-SOLUBLE VITAMINS IN CHELIDONIUM MAJUS EXTRACT BASED ON HPLC ANALYSIS

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Abstract. In this study, water-soluble vitamins of the B group (B_1, B_2, B_3, B6, B9) and vitamin C in the aqueous extract of Chelidonium majus L. were simultaneously identified using High-Performance Liquid Chromatography (HPLC).

The results showed that the extract contained high amounts of vitamin B3 (957.150 mg/100 g) and vitamin B_9 (24.230 mg/100 g). These findings indicate that the aqueous extract of Chelidonium majus L. possesses high metabolic activity and nutraceutical potential. The study scientifically substantiates the use of this plant's raw material as a promising source for the development of functional phytopreparations and biologically active food supplements.

Keywords. Chelidonium majus, HPLC, B-group vitamins, ascorbic acid, phytochemical analysis, nutritional value.

Introduction. The scientific evaluation of the composition of phytopreparations and biologically active supplements derived from medicinal plants is one of the key tasks in modern phytopharmaceutical research. The effectiveness of such products depends not only on alkaloids and phenolic compounds but also on the content of water-soluble bioactive substances, such as vitamins. In particular, B-group vitamins and ascorbic acid play a crucial role in cellular metabolism, antioxidant protection, and enzymatic processes [1].

The bloodroot plant (Chelidonium majus), widely used in phytotherapy, is known for its anti-inflammatory, antioxidant, and immunomodulatory properties. Although its alkaloid and phenolic content have been extensively studied, the quantity of water-soluble vitamins in this plant has not been sufficiently investigated. Meanwhile, the presence of B-group vitamins (B_1, B_2, B_3, B_6, B_9, B_{12}) and ascorbic acid is of significant importance in determining the biological activity and nutraceutical value of plant extracts [2,3].

In the literature, water-soluble vitamins are typically analyzed separately or using different methods, and studies aimed at the simultaneous comprehensive assessment of B-group vitamins and ascorbic acid in plant extracts using HPLC are limited. This highlights the need to develop a universal analytical approach to simplify phytochemical analysis and standardize phytopharmaceuticals [4].

From this perspective, the aim of the present study is to simultaneously identify B-group vitamins and ascorbic acid in the aqueous extract of Chelidonium majus using HPLC and to scientifically evaluate the vitamin content of this plant extract based on the obtained results.

Experimental Section. Determination of water-soluble vitamins in the extract using HPLC. Standards of vitamin B_{12} were obtained from Rhydburg Pharmaceuticals (Germany), vitamin C from Carl Roth GmbH (Germany), vitamin B9 from DSM Nutritional Products GmbH (Germany), and vitamins B_1, B2, B3, and B6 from BLDPharm (China). HPLC-grade reagents, including water, acetonitrile, chemically pure acetic acid, and sodium hydroxide, were used.

The content of water-soluble vitamins in the plant was analyzed using a Shimadzu LC-40 Nexera Lite high-performance liquid chromatograph (Japan) [5].

Preparation of Standard Solutions. Solutions of vitamins C (CAS 50-81-7), B1 (CAS 59-43-8), B_6 (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 HCl to obtain 100 mg/L solutions. Standard solutions of vitamins B2 (CAS 83-88-5) and B9 (CAS 59-30-3) were prepared by dissolving 5 mg of each vitamin in 50 mL of 0.025% NaOH solution.

Next, 200 µL of the initial B_1, B_6, B_3, B_{12}, and PP solutions were mixed to produce a combined solution with a concentration of 14.286 mg/L for each vitamin. Using the same procedure, standard solutions with concentrations of 7.143, 3.571, and 1.786 mg/L were prepared. For vitamin C, standard solutions with concentrations of 286, 143, 71.5, and 57.2 mg/L were prepared. Distilled water was used to prepare a 0 mg/L solution for calibration purposes.

Preparation of Sample Extract. To isolate water-soluble vitamins, 1 g of the sample was weighed and placed into a 50 mL conical flask, and 25 mL of 0.1 N HCl was added. The mixture was subjected to extraction in a GT SONIC-D3 ultrasonic bath (China) at 60 °C for 20 minutes. The resulting solution was then cooled, filtered, and brought up to 25 mL with water in a volumetric flask. From this extract, 1.5 mL was filtered through a 0.22 µm syringe filter into a vial for analysis.

Chromatographic Conditions. Vitamin Analysis. Standard solutions and the sample extract were analyzed using a Shimadzu LC-40 Nexera Lite high-performance liquid chromatograph equipped with an LC-40D pump, SIL-40 autosampler, and SPD-M40 photodiode array detector (PDA), with data acquisition performed using LabSolutions software, version. 6.92. A Shim-pack GIST C18 reverse-phase column (150 × 4.6 mm, 5 µm, Shimadzu, Japan) was employed.

The mobile phase consisted of a gradient system of acetonitrile (A) and 0.25% acetic acid solution in water (B) (Table 1). The injection volume was set at 10 µL, the flow rate at 0.6 mL/min, and the column oven temperature at 40 °C. The analytical signal (peak area) for each vitamin was recorded at wavelengths of 265, 291, and 550 nm (Figures 1-3).

For the determination of vitamin C, a 15-minute gradient was applied (Table 2), and the analytical signal was measured at 265 nm.

Gradient Program of the Mobile Phase for Vitamin Determination (Table 1)

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	Completion	

Gradient Program of the Mobile Phase for Vitamin C Determination (Table 2)

Time, min	Acetonitrile (A), %	0.5% Acetic Acid (B), %
0	0	100
2	0	100
6	50	50
6,01	0	100
15	Completion	

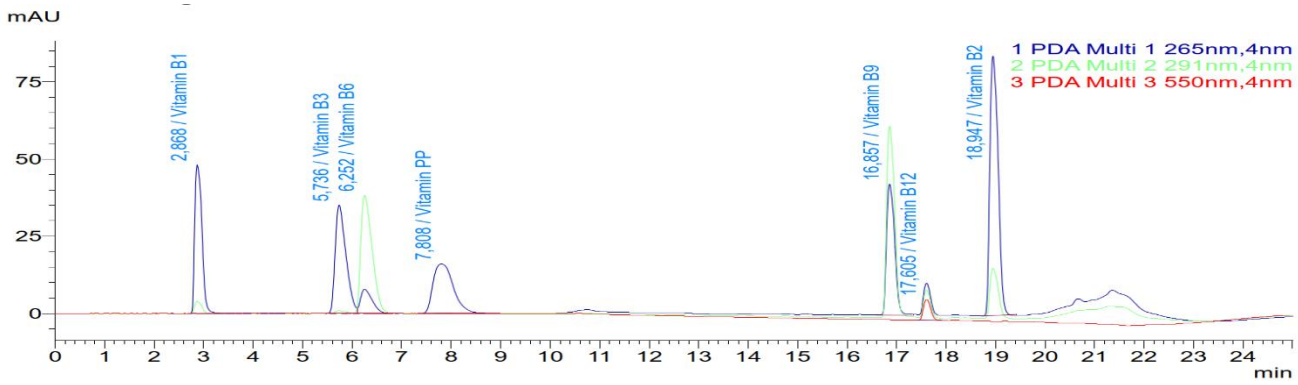


Figure 1. Chromatogram of the standard vitamin solution.

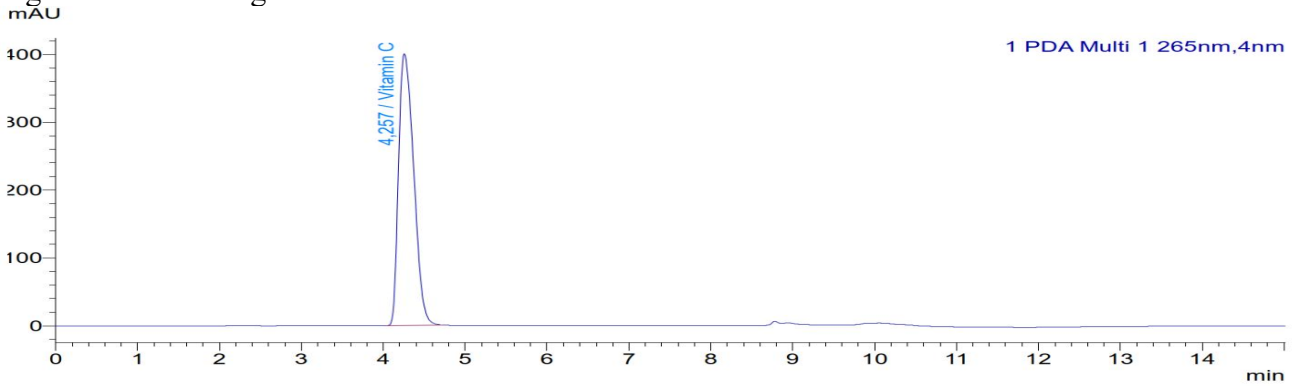


Figure 2. Chromatogram of the standard vitamin C solution.

Results. Determination of Vitamins in the Sample Extract. The chromatogram of the sample extract was obtained (Figures 3–4), and based on the results, the content of vitamins per 100 g of the sample was calculated using the following formula and presented in Table 3.

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

Here,

X – content of the vitamin in 100 g of the sample, mg;

C_vit – concentration of the vitamin in the extract determined by HPLC, mg/L;

V_extract – volume of the sample extract, L;

m_sample – mass of the sample taken for extract preparation.

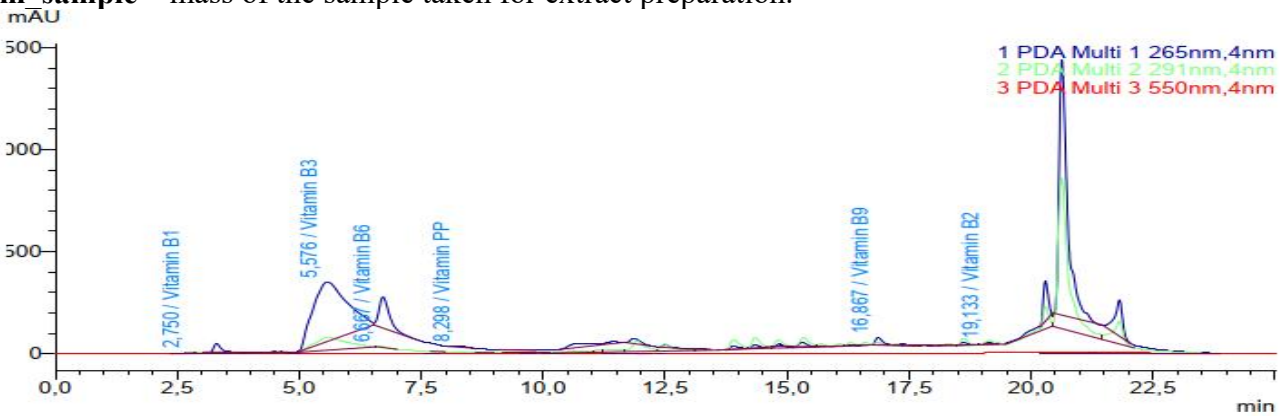


Figure 3. Chromatogram for the determination of vitamins in the sample extract.

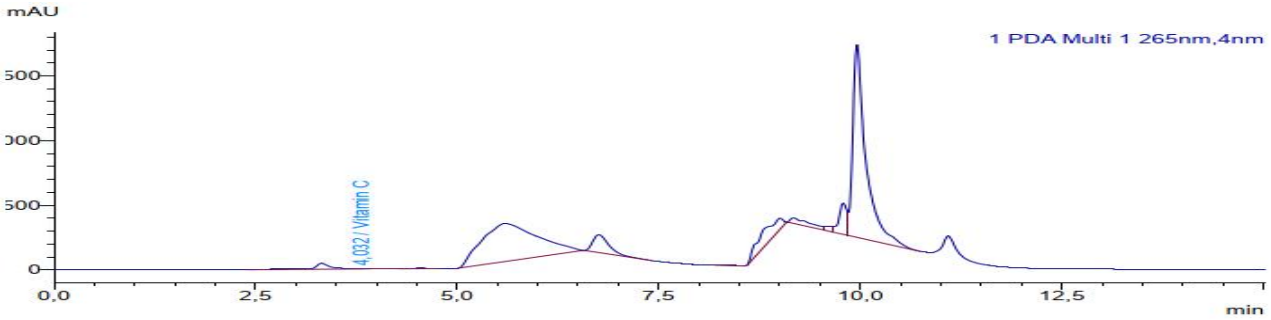


Figure 4. Chromatogram for the determination of vitamin C in the sample extract.

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

Vitamin	Retention Time, s	Concentration, mg/L	Content per 100 g of Sample, mg
Vitamin B_1	2,75	0,839	2,098
Vitamin B_3	5,576	382,86	957,150
Vitamin PP	8,298	3,271	8,178
Vitamin B_9	16,867	9,692	24,230
Vitamin B_2	19,133	0,928	2,320
Vitamin B_6	6,667	1,202	3,005
Vitamin B_{12}	Not detected	0	0,000
Vitamin C	4,032	0,98	2,450

Discussion and Analysis. The HPLC results clearly demonstrated the possibility of simultaneous reliable identification and quantitative determination of water-soluble vitamins in Chelidonium majus extract. The well-resolved peaks in the chromatograms confirm that the selected eluent composition and gradient program are optimal for this group of compounds. Quantitative analysis revealed the predominance of vitamin B_3 (niacin) at 957.150 mg/100 g, which indicates the extract's potential to support metabolic processes, as niacin participates in the formation of NAD^+/$NADP^+$ coenzymes [6]. The detection of folate (B9) at 24.230 mg/100 g highlights its biological significance in nucleic acid synthesis and cell division.

Nicotinamide (PP) at 8.178 mg/100 g and vitamin B6 at 3.005 mg/100 g indicate the presence of components that act as coenzymes in enzymatic reactions. Although the contents of vitamins B1 (2.098 mg/100 g) and B2 (2.320 mg/100 g) were comparatively lower, they still play crucial roles in energy metabolism and redox processes [8]. Ascorbic acid (2.450 mg/100 g) was observed as a factor supporting the antioxidant potential of the extract. Vitamin C may also enhance antiradical protection in combination with phenolic compounds. The absence of detectable vitamin B_{12} is characteristic of plant sources and further confirms the reliability of the results.

The identified vitamins reveal not only the phytochemical but also the nutraceutical value of Chelidonium majus extract. In particular, the high content of vitamins B_3 and B_9 provides a scientific basis for the inclusion of this plant in functional phytopreparations and biologically active supplements. The applied HPLC–PDA conditions (C18 column, 0.6 mL/min flow rate, 40 °C, multi-wavelength detection) allowed for reproducible and accurate simultaneous determination of the vitamins. This supports the use of the method as a universal analytical approach for assessing water-soluble vitamins in Chelidonium majus extract. Consequently, an analytical profile based on vitamin markers enables the

phytopharmaceutical standardization of the extract, implementation of quality control, and scientific evaluation of the product's biological value.

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