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DETERMINATION OF THE QUANTITY OF WATER-SOLUBLE VITAMINS IN ST. JOHN'S WORT FLOWERS

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Annotation
Modern medicine carries out a lot of practical work on the treatment and prevention of vitiligo. The article provides brief information on the determination of some vitamins in the plant St. John's wort (*Hypericum perforatum L*.) using high-performance liquid chromatography and their importance in medicine, especially in the treatment of skin diseases.

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Introduction. In many countries, the use of traditional medicine methods in providing medical care to the population and maintaining its health is becoming increasingly popular. In this area, the government of our country is implementing a number of measures.

We know that the use of plants rich in natural biologically active substances gives effective results in maintaining public health and treating diseases. One of the plants that has such beneficial properties and is widely used in modern medicine and folk medicine is St. John's wort (*Hypericum perforatum L* .)[1].

Reagents and equipment used. Vitamin B12 was obtained from Rhydburg Pharmaceuticals (Germany), vitamin C from Carl Roth GmbH (Germany), vitamin B9 from DSM Nutritional Products GmbX (Germany), vitamins B1, B2, B3, B6, PP from BLDPharm (China). HPLC grade water, acetonitrile, chemically pure acetic acid and sodium hydroxide were used as reagents.

The amount of water-soluble vitamins in the plant was determined using a high-performance liquid chromatograph LC-40 Nexera Lite (YUSSX) manufactured by Shimadzu, Japan.

Preparation of standard solutions. 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 vitamin PP (CAS 98-92-0) solutions (100 mg/l) were prepared by dissolving 5 mg of each vitamin in 50 ml of 0.1 N HCl solution. Standard solutions of vitamins B2 (CAS 83-88-5) and B9 (CAS 59-30-3) were prepared by dissolving 5 mg of these vitamins in 50 ml of 0.025% sodium hydroxide solution. Then 200 µl of each of the original vitamins B1, B6, B3, B12 and PP were mixed and a solution with a concentration of 14.286 mg/l of each vitamin was prepared. In this way, standard solutions of 7.143, 3.571 and 1.786 mg/l were prepared. Standard solutions of vitamin C with concentrations of 286, 143, 71.5 and 57.2 mg/l were also prepared. Pure water with a concentration of 0 mg/l was used to plot the calibration graph[5].

Preparation of sample extract. To extract water-soluble vitamins, 1 g of the test samples were weighed, placed in a 50 ml conical flask, and 25 ml of 0.1 N HCl solution were added. The mixture was extracted in a GT SONIC-D3 ultrasonic bath (China) at 60 °C for 20 minutes. The mixture was then cooled, filtered, and the volume was brought to 25 ml with water in a measuring flask. 1.5 ml of the extract was filtered through a syringe filter with a pore size of 0.22 μm, placed in a vial, and used for analysis.

Chromatographic conditions.

Vitamin Determinations. Standard Solutions and Sample Extraction An LC-40 Nexera Lite high performance liquid chromatograph consisting of an LC-40D pump, a SIL-40 autosampler, an SPD-M40 photodiode array detector (PDA) and LabSolutions ver. 6.92 software was run. A Shim pack GIST C18 reversed-phase column (150 × 4.6 mm; 5 μm, Shimadzu, Japan) and a gradient mobile phase consisting of acetonitrile (A) and 0.25% acetic acid in water (B) were used (Table 1).) was used. The injection volume was set at 10 μL, the flow rate was set at 0.6 mL/min and the column oven temperature was set at 40 °C. The analytical signal (peak area) of each vitamin was recorded at three wavelengths: 265, 291, 550 nm. A 15-minute gradient was used to determine vitamin C (Table 2), and the analytical signal was measured at a wavelength of 265 nm.

Table 1. Program of the mobile phase gradient for the determination of vitamins.

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

Table 2. Application of mobile phase gradient for quantitative determination of vitamin C.

Time, minute	Acetonitrile (A), %	0.5% acetic acid (B), %
0	0	100
2	0	100
6	50	50
6.01	0	100
15	End	

Results obtained.

Determination of vitamins in sample extract. The chromatogram of the sample extract was obtained, and based on the results, the amount of vitamins in 100 g of sample was calculated using the following formula, presented in Tables 3-4.

$$X = \frac{C_{\text{вит}} \cdot V_{\text{экстракт}}}{m_{\text{образец}}} \cdot 100\text{г}$$

Table 3. Amount of vitamins and retention time in sample 1.

Vitamin	Exposure time, sec	Concentration, mg/l	Quantity in 100 g sample, mg

Vitamin B 1	2,944	1,581	3,953
Vitamin B 3	5,592	10,057	25,143
Vitamin PP	7,545	0.281	0.703
Vitamin B 9	16,858	4,607	11,518
Vitamin B 2	18,965	0.373	0.933
Vitamin B 6	5,806	1.16	2,900
Vitamin B 12	Aniqlanmadi	0	0,000
Vitamin C	4,321	0.863	2,158

Table 4. Amount of vitamins and retention time in sample 2.

Vitamin	Exposure time, sec	Concentration, mg/l	Quantity in 100 g sample, mg
Vitamin B 1	3,009	1,887	4,718
Vitamin B 3	5,578	5,814	14,535
Vitamin PP	8.07	1,117	2,793
Vitamin B 9	16,986	1,655	4,138
Vitamin B 2	19,296	0.307	0.768
Vitamin B 6	5,785	0.551	1,378
Vitamin B 12	Aniqlanmadi	0	0,000
Vitamin C	4,063	0.539	1,348

Conclusion. To sum up, we can say that the dalakoya flower contains water-soluble vitamins B_1, B_2, PP, B_3, B_6 and C. The presence of vitamins determines its healing properties, since vitamins are actively involved in improving skin regeneration, the functioning of the circulatory, nervous and endocrine systems, and metabolism.

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