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DETERMINATION OF WATER-SOLUBLE VITAMINS IN THE EXTRACT OF HYPERICUM PERFORATUM L. BY THE YUSSX METHOD

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Abstract. This review provides a comprehensive overview of the chemical constituents and biological effects of Hypericum perforatum L. (Hypericaceae). Extracts from Hypericum perforatum L. have been found to have a wide range of biological and pharmacological effects, including antidepressant, antibacterial, wound healing, and antimicrobial activity. The antidepressant activity of the raw material extracts may be related to the ancient use of these plants as medicinal plants. The only antidepressant principle isolated to date is vitamin B6, which is responsible for the antidepressant activity of this medicinal plant. Based on the chemical and pharmacological properties of H. perforatum, we conclude that this species has beneficial therapeutic properties and has the potential to be used as an effective adaptogenic herbal remedy.

Keywords: Vitamin, antidepressant, neurasthenia, depression.

Introduction: Hypericum perforatum L. (St. John's wort, Hypericaceae) is a genus of about 400 species worldwide. It is native to Europe, Western Asia, North Africa, Madeira, and the Azores, and naturalized in many parts of the world, particularly North America and Australia. In recent years, the consumption of products derived from H. perforatum has increased dramatically, and it is now one of the most widely consumed medicinal plants in the world. The plant is widely used medicinally for the main complications of neurasthenia - skin ulcers, eczema, digestive disorders and psychological disorders.

Among the most effective and widespread pharmaceutical uses of H. perforatum in Europe after the 16th century was the use of distilled oil of the herb as a therapy for wounds and bruises. It was so effective that surgeons not only used it to clean wounds but also included it in the first official pharmacopeia of London under the name Oleum Hyperici.

Research Objective: The plant retains its place in the modern list of medicinal plants of pharmaceutical importance. Over the past 30 years, the plant has been extensively studied in clinical and laboratory studies. Many important physiological, chemical, pharmacological, and pharmacokinetic properties have been presented in the medical use of pharmaceutical preparations of the medicinal plant. The plant contains a wide range of vitamins, among which the B vitamins play a role in the main pharmacological effect. This plant is considered very effective as an effective alternative treatment for the recently emerging complications of depression.

Experimental section.

Reagents and equipment used. Vitamin B12 was obtained from “Rhydburg Pharmaceuticals” (Germany), vitamins B1, B2, B6, B9 and C from “DSM Nutritional Products GmbX” (Germany). Water, acetonitrile, acetic acid of chemically pure brand and sodium hydroxide reagents of HPLC purity were used.

The content of water-soluble vitamins in the plant was determined using an LC-40 Nexera Lite high-performance liquid chromatograph manufactured by Shimadzu, Japan.

Preparation of standard solutions. Solutions (100 mg/l) of vitamins C (CAS 50–81–7), B1 (CAS 70–16–6), B6 (CAS 65–23–6) and B12 (CAS 68–19–9) were prepared by dissolving 5 mg of each

vitamin in 50 ml of HPLC-grade water. 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, all the original B vitamins were mixed together to prepare a common solution. (The stock solution was stored in closed brown vials at -18 °C to prevent decomposition. Working standards of these vitamins at 5, 10, 15, 20 mg/l were prepared by diluting the stock solution. Preparation of plant extract. For the extraction of water-soluble vitamins, 2 g of the test sample was weighed to the nearest 0.01 g on an NV222 balance manufactured by OHAUS (USA), placed in a 100 ml conical flask, and 50 ml of 0.1 N HCl solution was added. The mixture was extracted in an ultrasonic bath GT SONIC-D3 (China) at 60 °C for 20 minutes. Then the mixture was cooled, filtered, and made up to 100 ml with water in a volumetric flask. 1.5 ml of the extract was transferred to a 0.45 µm syringe It was filtered on a filter, placed in a vial, and used for analysis.

Chromatographic conditions. Determination of vitamin B group. Standard solutions and sample extracts were analyzed using an LC-40 Nexera Lite high-performance liquid chromatograph equipped with an LC-40D pump, SIL-40 autosampler, SPD-M40 photodiode array detector (PDA) and LabSolutions ver. 6.92 software. 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 0.5% acetic acid in water (B) (Table 1) were used. The injection volume was 10 µl, the flow rate was 0.9 ml/min, and the column thermostat temperature was set at 35 °C. The analytical signal (peak area) of each vitamin was recorded at four wavelengths of 361, 291, 265, and 247 nm (Figures 1-4).

Determination of Vitamin C. Standard solution and sample extract A Shim pack GIST C18 reversed-phase column (150 × 4.6 mm; 5 µm, Shimadzu, Japan) and an isocritical mobile phase consisting of a 0.5% solution of acetic acid in water were used. The injection volume was 10 µl, the flow rate was 0.9 ml/min and the column thermostat was set to room temperature. The analytical signal (peak area) of Vitamin C was recorded at 244 nm (Figure 5).

Table 1. Mobile phase gradient program.

Time	Acetonitrile (A), %	0.5% acetic acid (B), %
0	0	100
0,76	0	100
2,26	17	83
5,26	17	83
5,32	0	100
11	Termination	

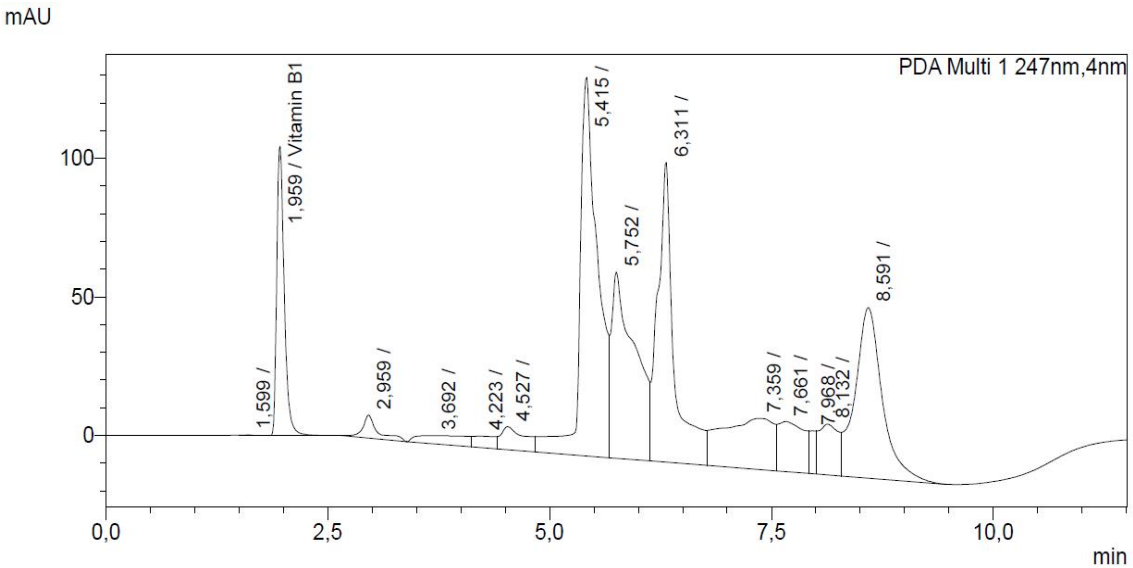


Figure 1. Chromatogram of vitamin B1, standard solution at 247

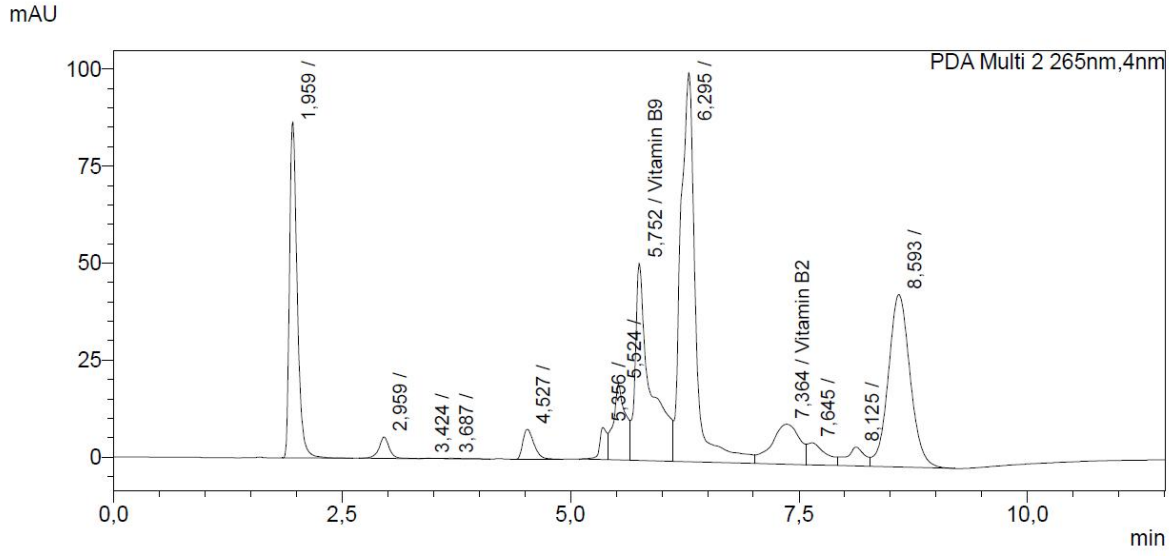


Figure 2. Chromatogram at 265 nm of standard solutions of vitamins B2 and B9.

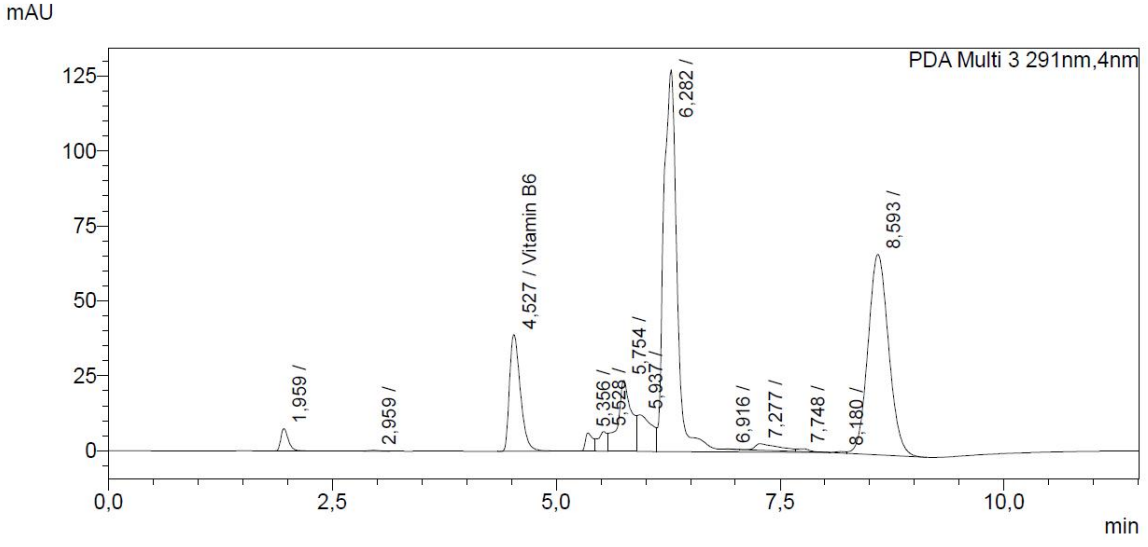


Figure 3. Chromatogram of vitamin B6 standard solution at 291 nm.

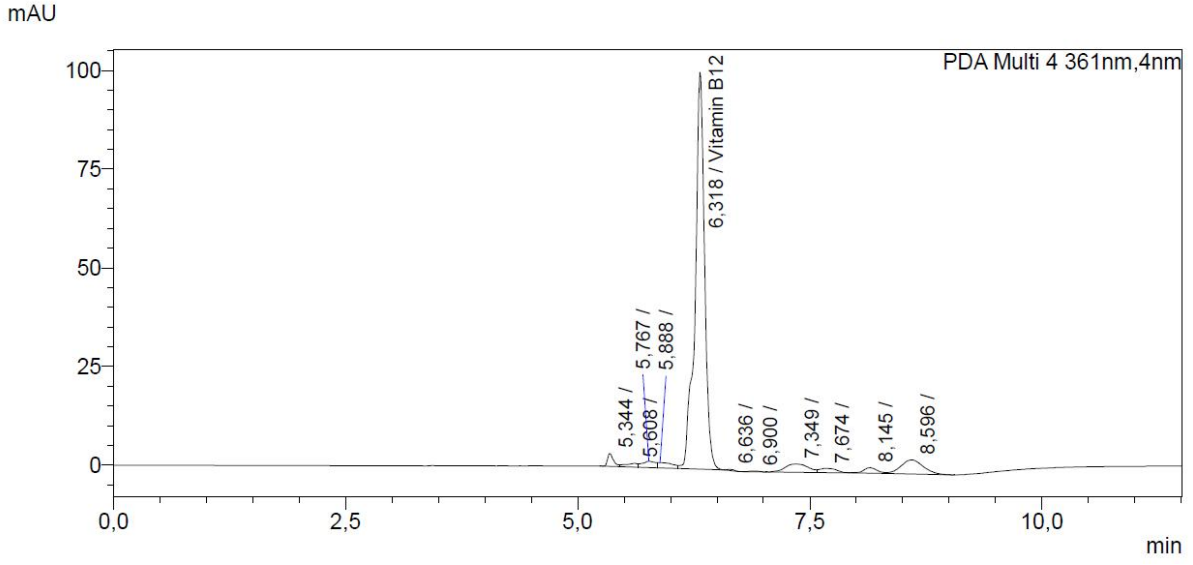
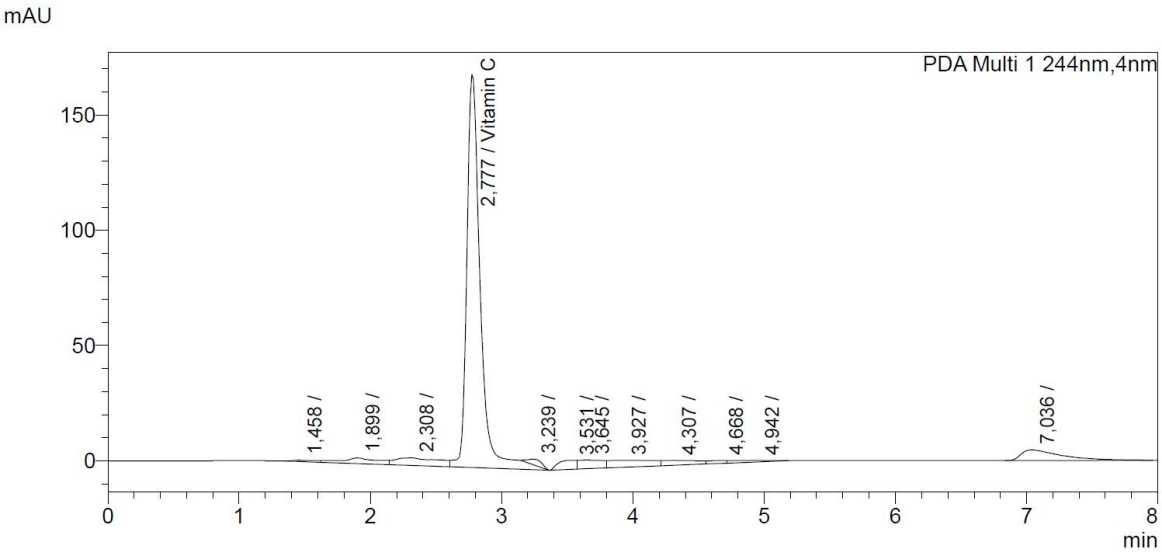


Figure 4. Chromatogram of vitamin B12 standard solution at 361 nm.



nm.

Figure 5. Chromatogram of a standard solution of vitamin C at 244 nm.

Results. A chromatogram of the sample extract in 0.1 N HCl was obtained (Figure 6) and the results were processed and presented in Table 2.

Water-soluble vitamins in the content of dalachai flowers and nettle leaves growing in the Andijan region were analyzed using the USSX method and compared with standard chromatograms.

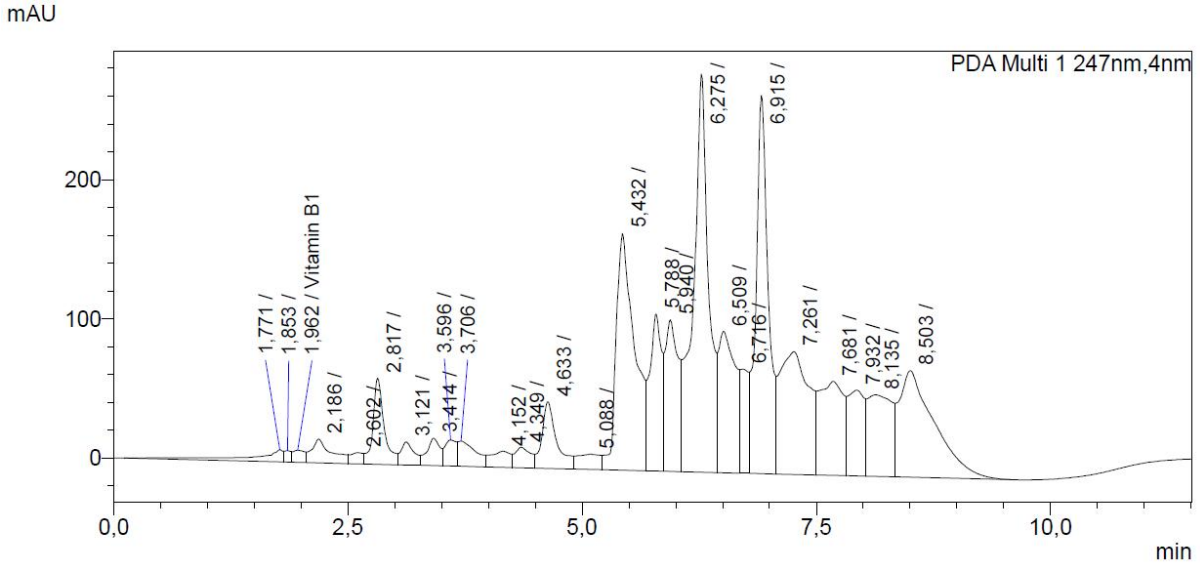


Figure 3.6. Chromatogram of a vitamin B1 sample solution at 247 nm.

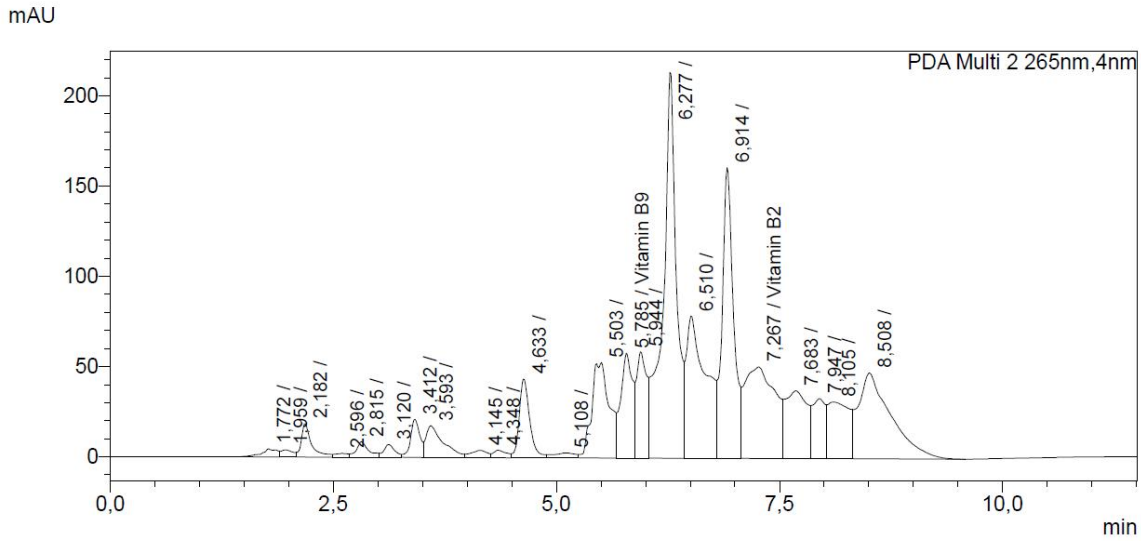


Figure 3.7. Chromatogram at 265 nm of vitamin B2 and B9 sample solutions.

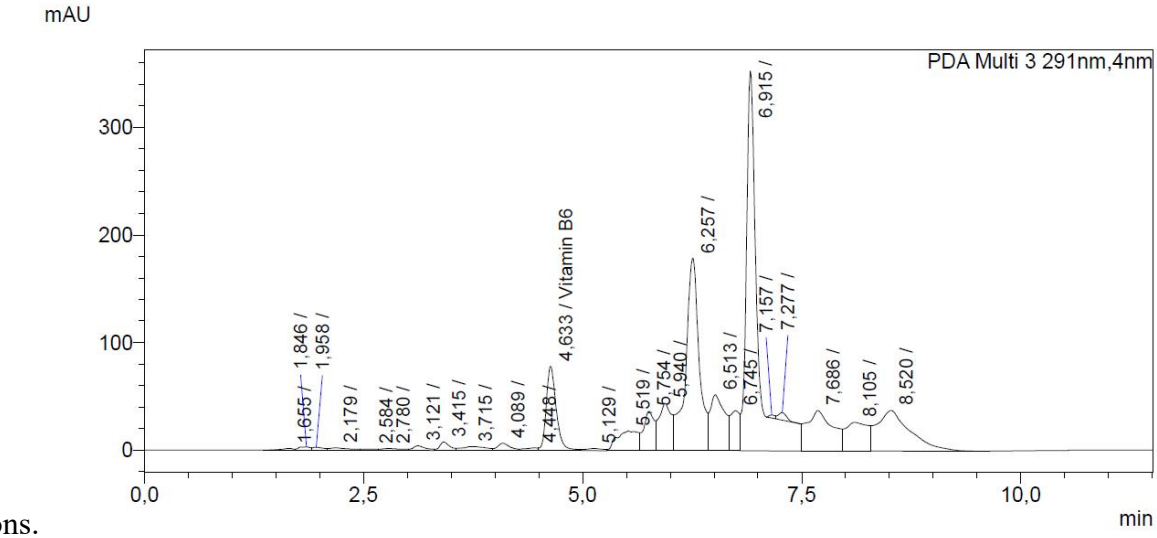


Figure 3.8. Chromatogram of a vitamin B6 sample solution at 291 nm.

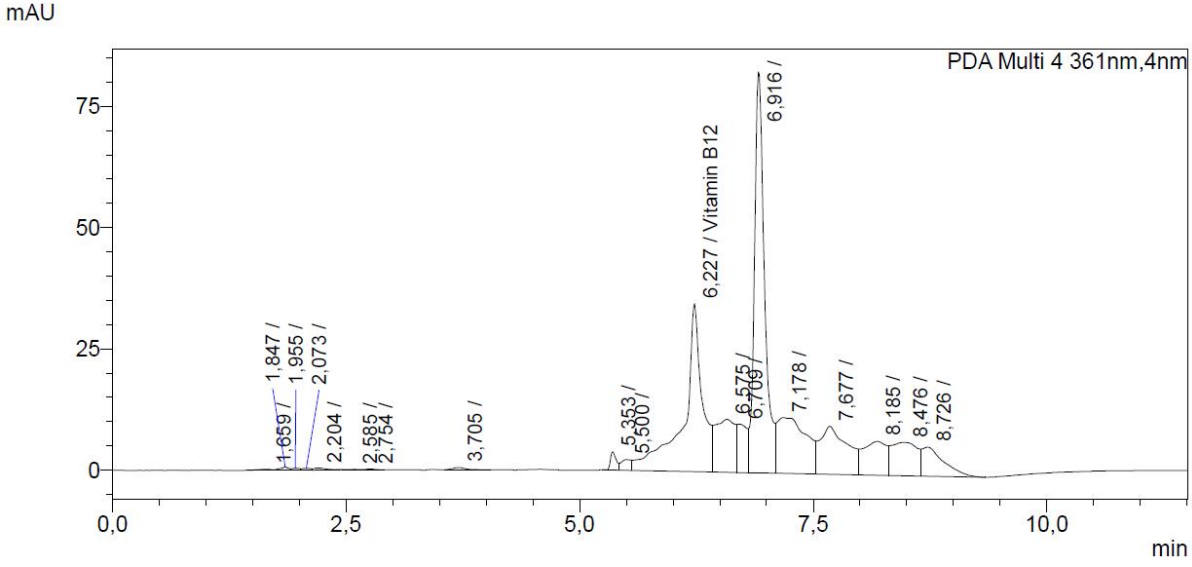
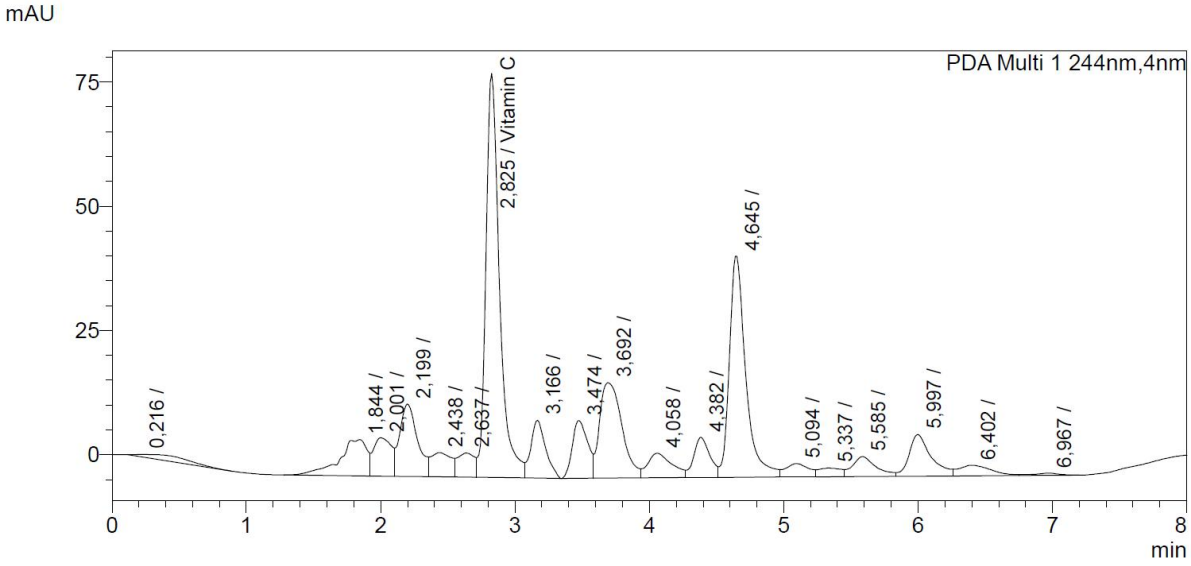


Figure 1.9. Chromatogram of a vitamin B12 sample solution at 361 nm.



nm.

Figure 1.10. Chromatogram of a vitamin C sample solution at 244 nm.

Table 3.2.

Vitamin content and retention times in the extract of Dalachoy flower and the amount per 100g, mg

Vitamin	0,1 N li HCl		
	Retention time, sec	Concentration, mg/l	Amount in 100 g of sample, mg
Vitamin B_1	1,962	8,725	43,625
Vitamin B_9	5,785	0,332	1,66
Vitamin B_2	7,267	10,308	51,54
Vitamin B_6	4,633	76,152	380,76
Vitamin B_{12}	6,227	1,327	6,635
Vitamin C	2,825	52,924	264,62

Conclusion. It was found that the composition of the Hypericum perforatum L. Dalachoy plant contains Vitamins that treat neurasthenia, one of the nervous diseases, Vitamin B6-380.76, Vitamin C-264.62, Vitamin B2-51.54, Vitamin B1-43.625. These results show that the use of Hypericum perforatum L. Dalachoy plant in the production of a food supplement used in the fight against and prevention of neurasthenia can achieve effective results.

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