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DETERMINATION OF WATER-SOLUBLE FLAVONOIDS IN FIELD TEA HYPERICUM L. LEAF EXTRACT BY USSX METHOD

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Abstract: The article presents a brief botanical description of the Dalachoy (Hypericum L.) plant, information on its biologically active compounds and the importance of the plant in folk medicine. The article also presents the results of the determination of some flavonoids in the Dalachoy leaf extract using the HPLC (High Performance Liquid Chromatography) method.
Keywords: St. John's wort (Hypericum L.), folk medicine, biologically active compounds, flavonoids.

Introduction: For centuries, wild rose has been used in folk medicine to treat viral and bacterial diseases. Its aerial parts were mainly used [1]. Recently, modern research methods have shown that wild rose branches contain a compound called hypericin, which has strong antiviral and antibacterial properties and is considered one of the most active natural compounds in wild roses [2]. Also, in folk medicine, decoctions made from the stems and roots of the wild rose medicinal plant are used as astringents, antiseptics, and anti-inflammatory drugs for diarrhea, gastritis (to reduce stomach acidity), gastric and duodenal ulcers, and enterocolitis [3]. Regular use of these decoctions helps to activate the endocrine glands, regulate gastrointestinal function, and strengthen the body's protective functions [4.5].

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). HPLC grade water, acetonitrile, chemically pure acetic acid and sodium hydroxide were used as reagents.

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

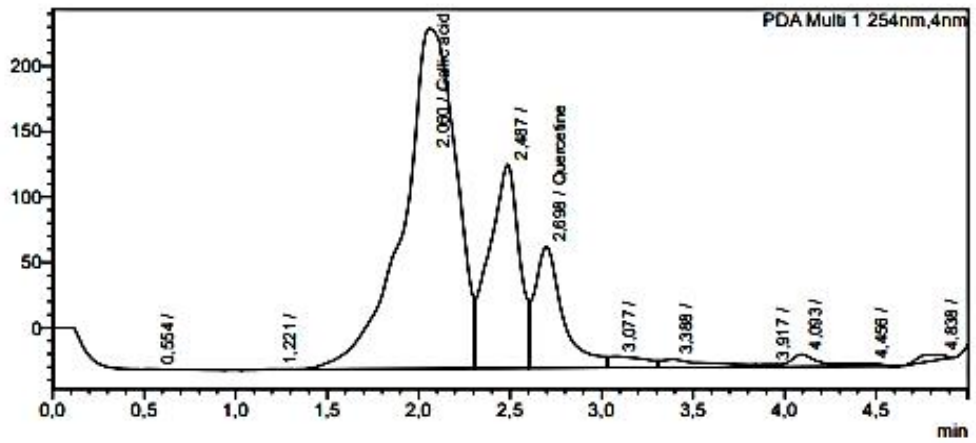
mm; 5 µm, Shimadzu, Japan) and a gradient mobile phase consisting of acetonitrile (A) and 0.5% acetic acid in water (B) (Table 1) were used. The injection volume was 10 µl, the flow rate was 0.9 ml/min, and the column thermostat temperature was set at 35 °C. The analytical signal (peak area) of each 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	

Results: The results obtained did not find rutin in the extract of field tea leaves. The extract of field tea leaves contained quercetin and gallic acid in the amounts of 112.075 mg/l and 889.678 mg/l, respectively, and 280.1875 mg and 2224.195 mg per 100 g of sample.



Of the flavonoids studied, gallic acid and quercetin flavonols were found to be the most effective superoxide reducers. These compounds have a free catechol group in the V-ring, and it is assumed that the hydroxyl groups that make up this structure react with the superoxide anion radical.

From the results obtained, it can be seen that the flavonoids in the extract of the field tea leaf participate in important biochemical processes in the human body as antioxidants, immunomodulators, and are of great importance in the prevention and treatment of antidepressants, atherosclerosis, diabetes, gout, and gallstone diseases.

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