DETERMINATION OF VITAMIN LEVELS IN THE “AS RHEUM” FOOD SUPPLEMENT BASED ON RHUBARB AND WILD GARLIC (JUSAI) USING THE HPLC METHOD

Shakhobidin Kh. Abdullayev
Andijan State University, Professor of the Department of Chemistry, Doctor of Chemical Sciences, Member of the Tabobat Academy of Uzbekistan
https://orcid.org/0000-0002-6069-1785

Marxabo A. Xolmatova
Fergana State University, Teacher of the Chemistry Department
https://orcid.org/0000-0003-0169-1555
E-mail: m_kholmatova_8787@mail.ru

Tokhir Kh. Abdullaev
Institute of Chemistry of the National Academy of Sciences of Tajikistan, Leading Researcher at the Laboratory of Organic Synthesis, Doctor of Chemical Sciences, Associate Professor
https://orcid.org/0000-0003-2630-8540

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Annotation. This article presents the results of determining the amounts of water-soluble vitamins in rhubarb leaves and the aerial part of the Allium odorum plant (jusai) growing in the Fergana Valley, as well as in a new dietary supplement (DS) “As rheum,” which is a composite mixture of these plants. Analysis by high-performance liquid chromatography (HPLC) revealed the presence of all B-group vitamins (except B12) and vitamin C. It was found that in the dietary supplement “As rheum,” the vitamin content is stabilized compared to their amounts in the original plant raw materials (rhubarb and jusai). The levels of vitamins B2, B6, and B9 in the DS “As rheum” exceed the human daily requirement by 1.95–5.08 times, indicating the potential for its effective use in traditional medicine for the prevention and treatment of conditions associated with deficiencies of these vitamins.

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Introduction. Vitamins are substances that are not synthesized, or are synthesized in very small amounts, in the human body but are essential for vital physiological processes. They are not an energy source; however, they play active roles in metabolic processes, enzyme activity, immune function, and the growth and regeneration of tissues. Water-soluble vitamins include the B-group vitamins (B_1, B_2, B_3, B_5, B_6, B_7, B_9, B_{12}) and vitamin C [1]. These vitamins are not stored in large quantities in the body, which makes their regular intake from food necessary [2, 3]. Numerous clinical studies have reported that deficiencies in B-group vitamins can lead to anemia, neurological disorders, fatigue, and problems associated with metabolic syndrome [2, 4]. Vitamin C has been shown to enhance immune response, exhibit antioxidant effects, and improve iron absorption [5].

In our previous studies [6, 7, 8], a new functional food additive (FA) called “As rheum” was developed based on a compositional mixture of rhubarb leaves and the aerial part of the Allium odorum plant (jusai), with the aim of creating a product standardized by its biologically active compounds. This article presents the results and analysis of determining the amounts of water-soluble vitamins present in these plant parts and in the “As rheum” FA using the high-performance liquid chromatography (HPLC) method.

Materials and Methods

Reagents and Equipment. As standard substances, the following reagents were used: vitamins B_1, B_2, B_3, B_6, and PP from *BLDPharm* [9]; vitamin B_{12} from *Rhydburg Pharmaceuticals*; vitamin C from *Carl Roth GmbH* [10]; and vitamin B_9 from *DSM Nutritional Products GmbX* [11]. Ultrapure HPLC-grade water, acetonitrile, glacial acetic acid, hydrochloric acid, and sodium hydroxide were also used in chromatographic analyses.

Preparation of Standard Solutions. To prepare standard solutions with a concentration of 100 mg/L, 5 mg of each standard substance—vitamin C (CAS 50-81-7), vitamin B_1 (CAS 59-43-8), vitamin B_6 (CAS 58-56-0), vitamin B_3 (CAS 59-67-6), vitamin B_{12} (CAS 68-19-9), and vitamin PP (CAS 98-92-0)—was placed in separate volumetric flasks and dissolved in 0.1 M aqueous HCl to a final volume of 50 mL. Standard solutions of vitamin B_2 (CAS 83-88-5) and vitamin B_9 (CAS 59-30-3) were prepared similarly, dissolving each substance in 0.025% aqueous NaOH solution. Then, 200 µL of each B-group vitamin and PP vitamin standard solution was mixed, resulting in a stock standard solution with a concentration of 14.286 mg/L for each vitamin. By diluting this stock solution, standard solutions with concentrations of 7.143, 3.571, and 1.786 mg/L were prepared. Separately, vitamin C standard solutions with concentrations of 286.0, 143.0, 71.5, and 57.2 mg/L in 0.1 M HCl were prepared. Ultrapure water was used as the 0 mg/L blank standard for calibration curve construction.

Sample Extraction and Preparation for Analysis. To extract water-soluble vitamins, rhubarb leaves, the aerial part of jusai, and the “As rheum” FA were first naturally dried in the sampling area, then ground with a blender and sieved. The 0.1–0.2 mm fraction was used for analysis. A 1.00 g sample was placed in a 50 mL conical flask, and 25 mL of 0.1 M HCl was added. The mixture was extracted in a GT SONIC-D3 [12] ultrasonic bath at 60 °C for 20 minutes. After cooling, the extract was transferred to a volumetric flask and diluted with water to 25 mL. A 1.5 mL portion of the extract was centrifuged in a Bibse Mini-7 centrifuge at 7000 rpm. The supernatant was filtered through a 0.22 µm syringe filter, transferred to a chromatographic vial, and analyzed.

Chromatographic Analysis. Standard solutions and sample extracts were analyzed using an LC-40 Nexera Lite [13] high-performance liquid chromatograph. Data processing was carried out using LabSolutions ver. 6.92 software. Separation of components in the samples was performed on a Shim-pack GIST C18 column (150 × 4.6 mm; 5 µm, Shimadzu) using a gradient mobile phase consisting of acetonitrile (A) and 0.25% aqueous acetic acid (B) [14]. The injection volume was 10 µL, the flow rate was 0.6 mL/min, and the column temperature was maintained at 40 °C. Analytical signals (peak areas on chromatograms) of separated vitamins were recorded at wavelengths of 265, 291, and 550 nm. For vitamin C determination, a 15-minute gradient was used, and the analytical signal was measured at 265 nm [14].

Results and Discussion

Calibration curves showing the dependence of peak area on vitamin concentration were constructed for each vitamin, mathematically processed, and regression equations as well as R^2 values were obtained. The high accuracy of the calibration curves was confirmed by R^2 values differing from 1.000 by no more than 0.002 [14].

Table 1 presents the chromatographic results of determining the vitamin content in rhubarb leaves, the aerial part of jusai, and the “As rheum” FA.

Table 1.

Chromatographic determination of vitamin contents in rhubarb leaves, aerial part of jusai, and the "As rheum" functional food additive

Vitamin		Retention time, min			100 g sample content, mg		
		Rhubarb leaves	Jusai aerial part	"As rheum"	Rhubarb leaves	Jusai aerial part	"As rheum"
B_1	Thiamine	2,96	3,07	2,95	0,35	0,26	0,34
B_2	Riboflavin	18,90	18,55	18,90	0,99	20,52	3,31
B_3	Nicotinic acid	5,90	6,20	5,22	2,03	0,01	1,50
PP	Niacin	7,72	7,69	7,68	1,61	2,41	2,52
B_6	Pyridoxine	6,58	-	6,58	6,35	-	4,26
B_9	Folic acid	17,00	17,00	17,00	1,46	4,92	2,03
C	Ascorbic acid	4,45	4,67	4,46	0,95	0,45	1,29
B_{12}	Cyanocobalamin	*Not detected*					

From the table, it can be observed that under the chromatographic conditions selected, hydrophilic vitamins were well separated, which is confirmed by the differences in their retention times on the chromatographic column. In our study, vitamin B_{12} was not detected in any of the three sample extracts. Vitamin B_6 was also not detected in jusai.

Figure 1 compares the amounts of vitamins determined in 100 g of dry rhubarb, jusai, and the "As rheum" FA. The figure shows that the concentrations of vitamins B_2 and B_9 in jusai extract are high (20.52 and 4.92 mg, respectively), while in rhubarb their amounts are low (0.99 and 1.46 mg, respectively). Conversely, rhubarb accumulated comparatively larger amounts of vitamins B_6 and B_3 (6.35 and 2.03 mg). In the "As rheum" FA, which is a compositional mixture of rhubarb and jusai in a 3:1 mass ratio, the levels of vitamins that were initially low in the individual plants increased (e.g., B_6 and B_3 to 4.26 mg and 1.50 mg, respectively), while the amounts of vitamins that were initially high decreased proportionally (B_2 and B_9 to 3.31 mg and 2.52 mg, respectively).

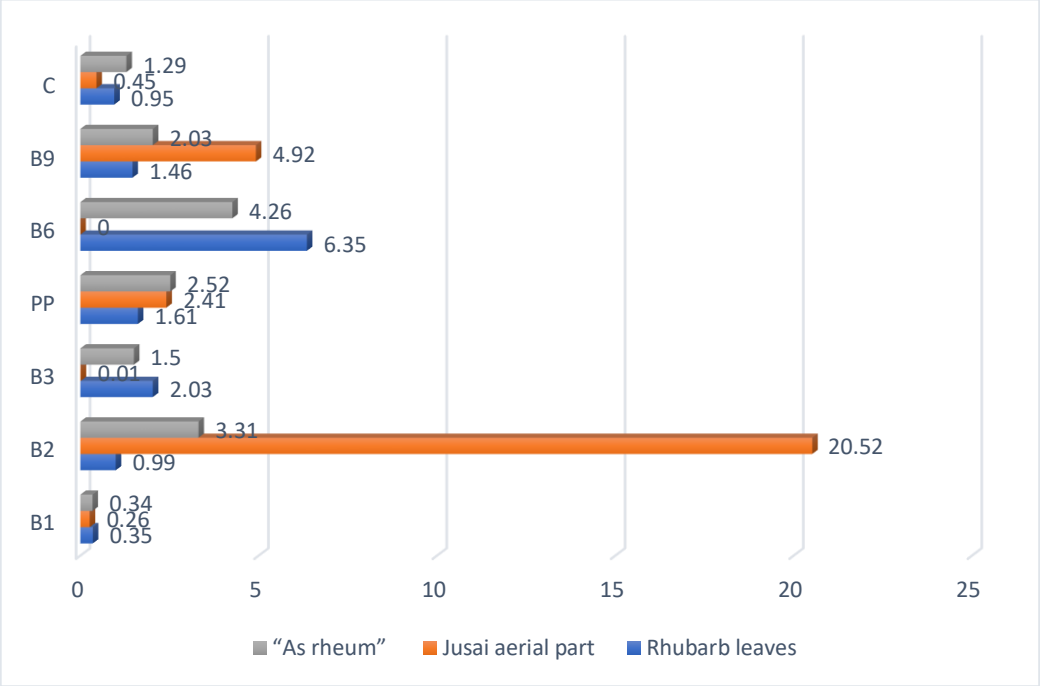


Figure 1. Vitamin content (mg per 100 g dry weight) in rhubarb, jusai, and the "As rheum" functional food additive.

100 g of the "As rheum" FA and the recommended daily intakes (RDI) for water-soluble vitamins in adult men (18+ years) along with the coverage ratios are presented in Table 2.

Table 2.

Daily requirement coverage of water-soluble vitamins for adult men (18+) provided by 100 g of "As rheum" FA

Vitamin	Content in "As rheum", mg/100 g	Daily requirement, mg	Relative to daily requirement
B_1	0,34	1,2	0,28
B_2	3,31	1,7	1,95
B_3	1,50	20	0,08
PP	2,52	20	0,13
B_6	4,26	2,0	2,13
B_9	2,03	0,4	5,08
C	1,29	90	0,014

From Table 2, it is evident that although the intake of vitamins B_1, B_3, PP, and C provided by consuming 100 g of "As rheum" FA is relatively low for adult individuals, the amounts of vitamins B2, B6, and B9 exceed the daily requirement by factors ranging from 1.95 to 5.08. Therefore, it can be stated that the newly developed "As rheum" FA may be effectively used in traditional medicine as a remedy for conditions associated with deficiencies of vitamins B_2, B_6, and B_9.

Conclusion. The results of determining the amounts of water-soluble vitamins in the extracts of the "As rheum" FA and its components—rhubarb leaves and the aerial part of jusai—using the high-performance liquid chromatography method showed that all B-group vitamins except B_{12}, as well as vitamin C, are present (vitamin B_6 was not detected in jusai). It was established that the vitamin contents in the "As rheum" FA were balanced compared with those in the original plants. The levels of vitamins B_2, B_6, and B_9 in the new "As rheum" FA exceed the daily requirement by factors of 1.95 to 5.08, which indicates its potential for effective use in traditional medicine for treating conditions associated with deficiencies of these vitamins.

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