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COMPREHENSIVE HPLC ANALYSIS OF WATER-SOLUBLE B-GROUP AND VITAMIN C CONTENTS IN LIMONIUM GMELINII (ARSLONQUYRUQ)

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Abstract

In this study, the content of water-soluble B-group vitamins (B_1, B_2, B_6, B_9, B_{12}) and ascorbic acid (vitamin C) in the aqueous extract of Arslonquyruq (Limonium gmelinii) was determined using high-performance liquid chromatography (HPLC), and their practical significance in the fields of medicine and pharmacology was comprehensively evaluated. It is well known that water-soluble vitamins play a crucial role in essential metabolic and physiological processes in the human body, including regulation of nervous system function, strengthening of the immune system, hematopoiesis, and enhancement of antioxidant defense mechanisms. Therefore, the investigation of the vitamin composition of medicinal plant extracts using modern analytical techniques is of particular relevance and importance in contemporary scientific research. During the study, the precise concentrations of vitamins in the extract were determined using a Shimadzu LC-40 Nexera Lite HPLC system in combination with internationally recognized standard vitamin solutions. The results revealed that the Arslonquyruq extract contained high levels of vitamin C and B-group vitamins, substantiating the potential of this plant for extensive application in the pharmaceutical and dietary product industries as a natural source of antioxidants and bioactive compounds. In particular, the vitamin C content was recognized for its essential role in collagen synthesis, suppression of inflammatory processes, and activation of the immune system, while B-group vitamins were found to play a critical role in nervous system function and hematopoiesis. Overall, the findings indicate that Limonium gmelinii is a promising bioactive medicinal plant with significant potential for use in healthcare, pharmaceutical sciences, nutrition, and phytotherapy. In addition, the feasibility of developing natural antioxidant preparations with high biological activity, as well as vitamin-enriched beverages and nutraceutical products based on Arslonquyruq extract, was scientifically substantiated. This study contributes to an in-depth investigation of medicinal plants native to the republic and expands the possibilities for their utilization in the domestic pharmaceutical and food industries.

Keywords

Arslonquyruq (Limonium gmelinii), water-soluble vitamins, vitamin C, B-group vitamins, high-performance liquid chromatography (HPLC), biological activity, antioxidant, pharmacology, plant extract, Shimadzu LC-40 Nexera Lite.

Relevance of the Topic

At present, the extraction of biologically active compounds from natural sources and the determination of their qualitative and quantitative composition are of great scientific and practical importance. In particular, the analysis of the vitamin composition of extracts obtained from medicinal plants plays a significant role in the fields of healthcare, pharmaceuticals, and nutraceuticals. Although Arslonquyruq (Leonurus spp.) has long been widely used in traditional medicine, its richness in water-soluble vitamins has not yet been fully investigated. At the same

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time, B-group vitamins and ascorbic acid are known to regulate essential metabolic and physiological processes in the human body, support immune system function, and provide antioxidant protection. The determination of the vitamin composition of medicinal plants using sensitive and precise modern techniques, such as high-performance liquid chromatography (HPLC), serves as a fundamental basis for pharmaceutical quality control and scientific research. Therefore, the identification of vitamins B_1, B_2, B_6, B_9, B_{12}, and vitamin C in Arslonquyruq extract enables a more in-depth evaluation of the pharmacological and nutritional significance of this plant.

Introduction

Arslonquyruq (belonging to the genus Leonurus in Latin) is widely used in traditional medicine of Central Asia, where its infusions and decoctions are considered to possess sedative and hypotensive properties [1]. The plant belongs to the family Lamiaceae, which is characterized by the presence of flavonoids, iridoids, essential oils, and other bioactive compounds exhibiting antibacterial, antioxidant, and various other biological effects [1]. Extracts obtained from medicinal plants rich in such biologically active substances play an important role in the pharmaceutical and food industries. Water-soluble vitamins, including B-group vitamins and vitamin C, are essential nutrients for the human body. They act as cofactors in numerous enzymatic reactions, supporting normal growth and development, skin, nervous and cardiac functions, as well as the formation of red blood cells. For instance, thiamine (B_1) participates in metabolic processes, while riboflavin (B_2), pyridoxine (B_6), folate (B_9), and cyanocobalamin (B_{12}) are essential for nervous system function and hematopoiesis. Vitamin C plays a critical role in collagen synthesis, resolution of inflammatory processes in wounds, bone formation, and immune system enhancement. Due to the instability and limited storage of water-soluble vitamins in the human body, determining their content in natural sources is of great importance [3]. Such analyses allow for the evaluation of the nutritional and medicinal quality of biologically rich medicinal plants, such as Arslonquyruq.

Objective of the Study: To determine and quantify the content of water-soluble B-group vitamins (B_1, B_2, B_6, B_9, B_{12}) and vitamin C in Arslonquyruq (Leonurus spp.) extract using high-performance liquid chromatography (HPLC) with the Shimadzu LC-40 Nexera Lite system (Japan).

Materials and Methods: The content of water-soluble vitamins in the extract was precisely determined using the HPLC method. For this purpose, vitamin B_{12} was obtained from Rhydburg Pharmaceuticals (Germany), while vitamins B_1, B_2, B_6, B_9, and C were sourced from DSM Nutritional Products GmbH (Germany). HPLC-grade solvents, including water, acetonitrile, chemically pure acetic acid, and sodium hydroxide, were used as reagents. The quantification of water-soluble vitamins in the plant extract was carried out using a Shimadzu LC-40 Nexera Lite high-performance liquid chromatography system (Japan).

Preparation of Standard Solutions: Standard solutions of vitamin C (CAS 50–81–7), B_1 (CAS 70–16–6), B_6 (CAS 65–23–6), and B_{12} (CAS 68–19–9) were prepared at a concentration of 100 mg/L by dissolving 5 mg of each vitamin in 50 mL of HPLC-grade water. Standard solutions of vitamins B_2 (CAS 83–88–5) and B_9 (CAS 59–30–3) were prepared by dissolving 5 mg of each vitamin in 50 mL of 0.025% sodium hydroxide solution. Subsequently, all initial B-vitamin solutions were combined to prepare a mixed stock solution. The stock solution was stored in sealed amber bottles at –18 °C to prevent degradation. Working standard solutions at concentrations of 5, 10, 15, and 20 mg/L were prepared by diluting the mixed stock solution accordingly.

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Preparation of Plant Extract: To extract water-soluble vitamins, a 2 g sample of the plant material was accurately weighed to 0.01 g using an NV222 analytical balance (OHAUS, USA) and transferred to a 100 mL conical flask. Then, 50 mL of 0.1 N HCl solution was added. The mixture was subjected to extraction in a GT SONIC-D3 ultrasonic bath (China) at 60 °C for 20 minutes. After extraction, the mixture was cooled, filtered, and the volume was adjusted to 100 mL with water in a volumetric flask. A 1.5 mL portion of the extract was filtered through a 0.45 µm syringe filter into a vial and used for subsequent HPLC analysis.

Chromatographic Conditions for B-Group Vitamin Analysis: Standard solutions and plant extract samples were analyzed using a Shimadzu LC-40 Nexera Lite high-performance liquid chromatography (HPLC) system, consisting of an LC-40D pump, SIL-40 autosampler, and SPD-M40 photodiode array (PDA) detector, controlled via LabSolutions software ver. 6.92. A Shim-pack GIST C18 reversed-phase column (150 × 4.6 mm, 5 mkl; Shimadzu, Japan) was used, with a gradient mobile phase composed of acetonitrile (A) and 0.5% acetic acid in water (B) (Table 1). The injection volume was set to 10 mkl, the flow rate to 0.9 ml/min, and the column temperature to 35 °C. The analytical signals (peak areas) of each vitamin were recorded at four wavelengths: 361, 291, 265, and 247 nm (Figures 1–4).

Chromatographic Conditions for Vitamin C Analysis: Standard solutions and plant extract samples were analyzed using a Shim-pack GIST C18 reversed-phase column (150 × 4.6 mm, 5 µm; Shimadzu, Japan) with an isocratic mobile phase consisting of 0.5% acetic acid in water. The injection volume was set at 10 mkl, the flow rate at 0.9 mL/min, and the column temperature was maintained at room temperature. The analytical signal (peak area) of vitamin C was recorded at 244 nm (Figure 5).

Results and Discussion:

Table 1. Gradient Program of the Mobile Phase.

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	End	

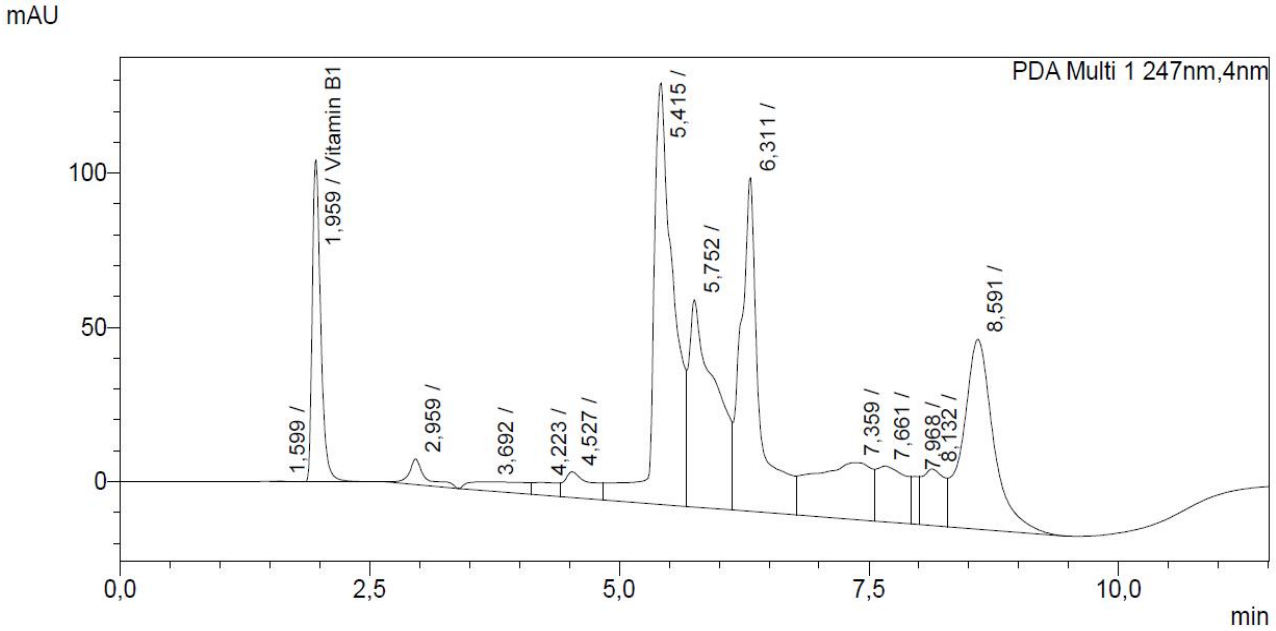

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Figure 1. Chromatogram of the B_1 vitamin standard solution recorded at 247 nm.

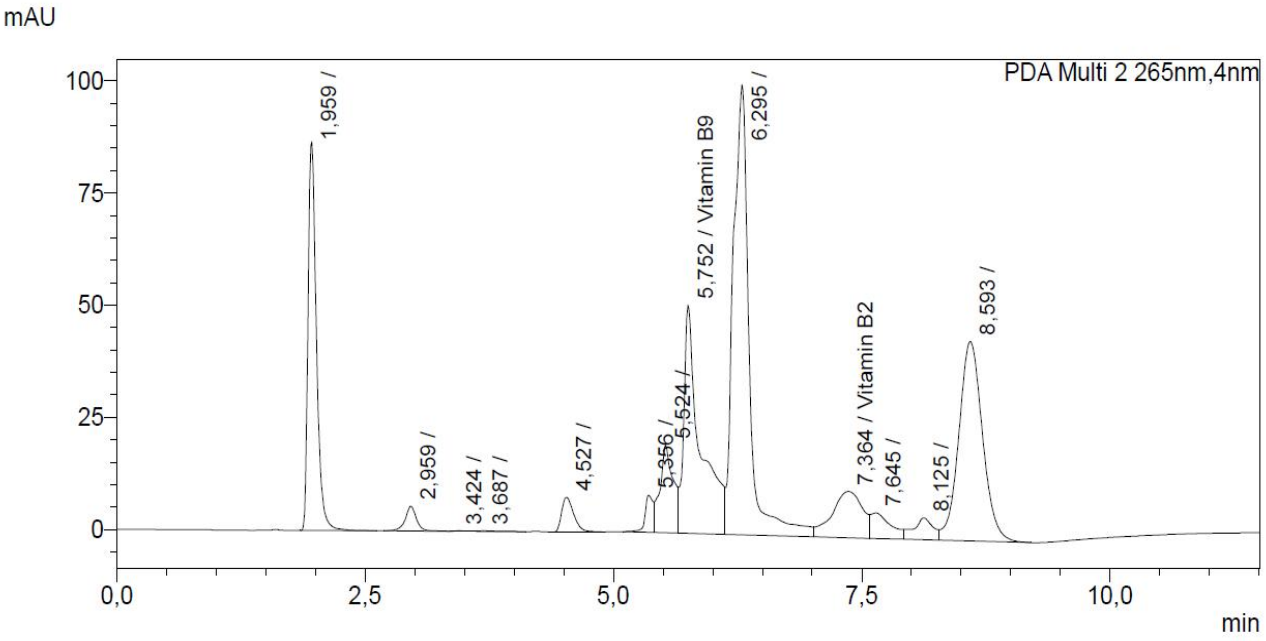

Figure 2. Chromatograms of the B_2 and B_9 vitamin standard solutions recorded at 265 nm.

Figure 3. Chromatogram of the B_6 vitamin standard solution recorded at 291 nm.

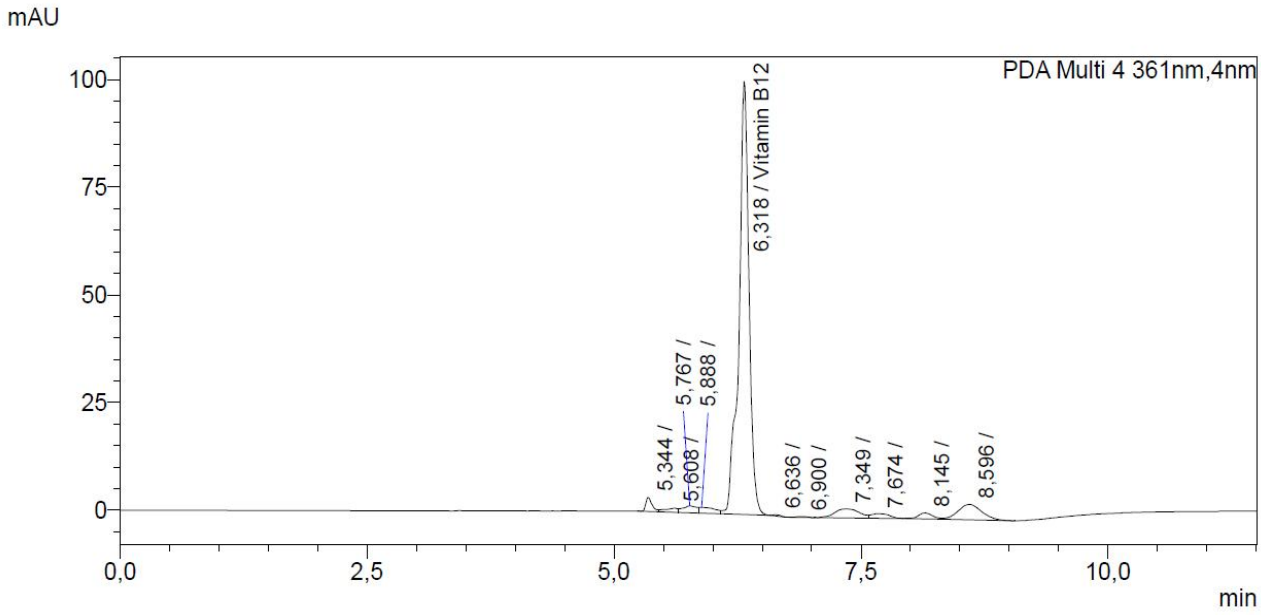

Figure 4. Chromatogram of the B_{12} vitamin standard solution recorded at 361 nm.

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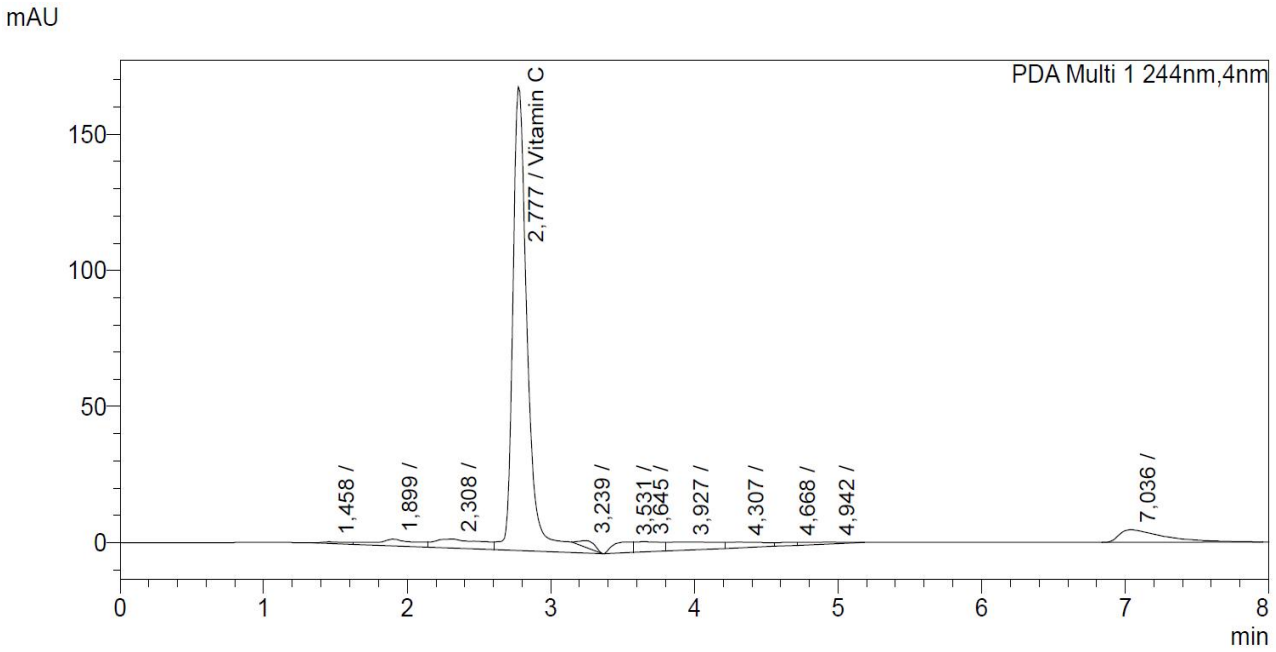
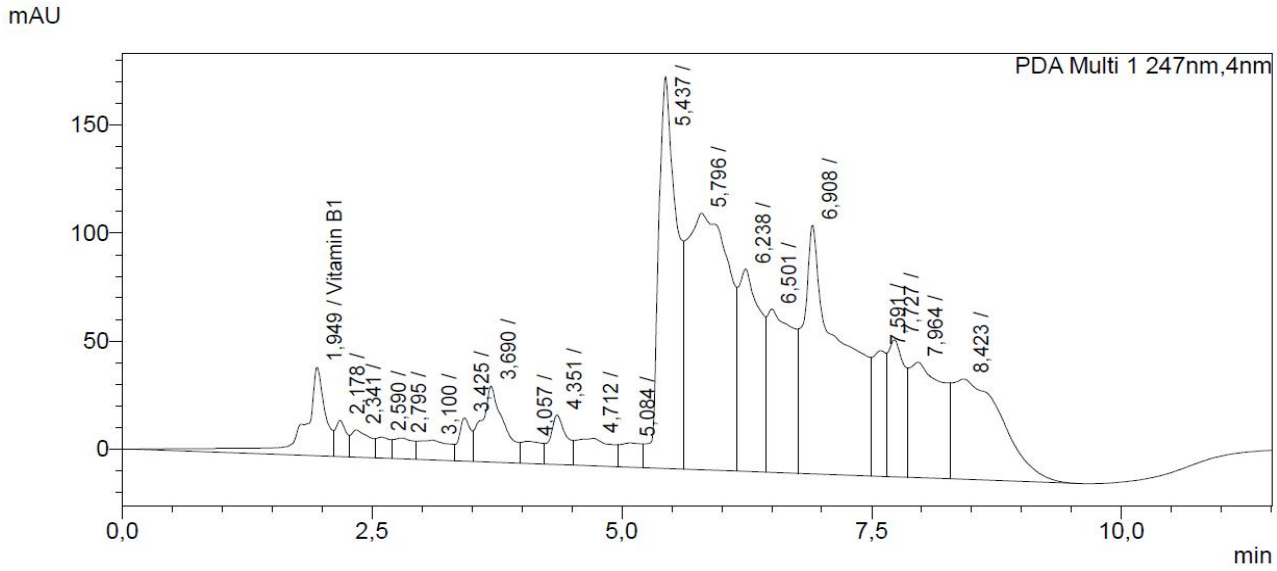


Figure 5. Chromatogram of the vitamin C standard solution recorded at 244 nm.

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



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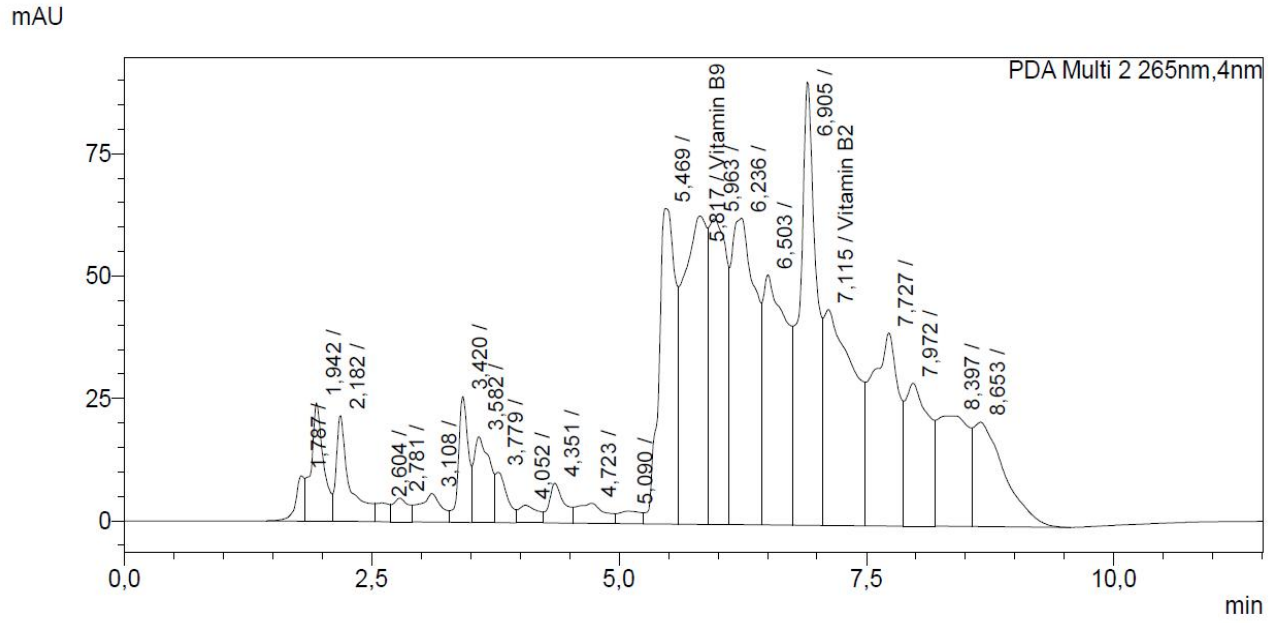

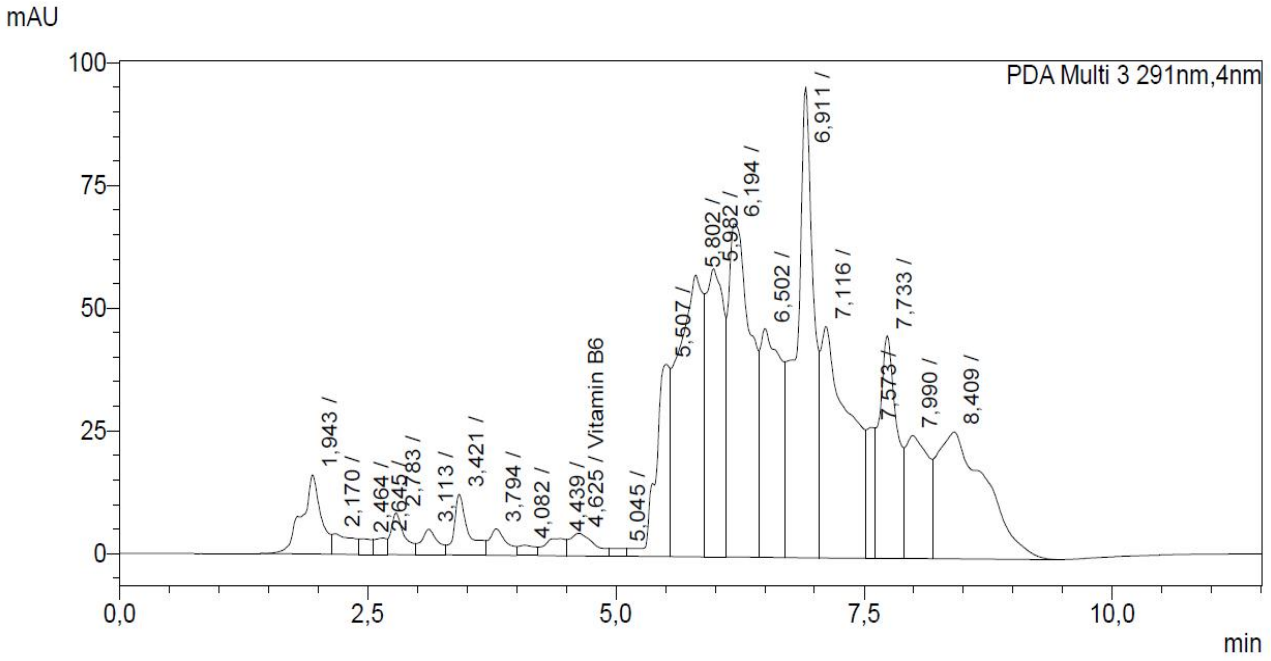

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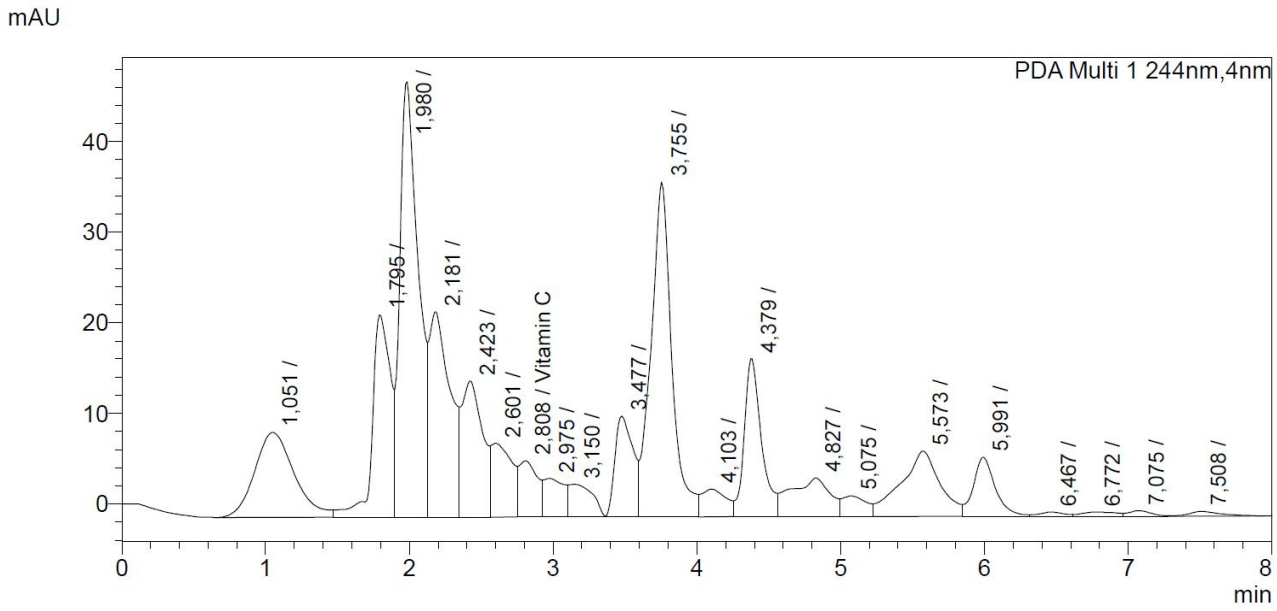

Figure 6. Chromatogram of the sample extract in 0.1 N HCl.

Table 2. Content and Retention Times of Vitamins in the Extracts

Vitamin	0.1 N HCl		
	Retention Time, s	Concentration, mg/L	Amount in 100 g of Sample, mg
Vitamin B_1	1,949	71,806	359,03
Vitamin B_9	5,817	0,64	3,2
Vitamin B_2	7,115	7,935	39,675
Vitamin B_6	4,625	9,627	48,135
Vitamin B_{12}	5,985	0,692	3,46
Vitamin C	2,808	5,031	25,155

Conclusion: In this study, the content of water-soluble B-group vitamins (B_1, B_2, B_6, B_9, B_{12}) and ascorbic acid (vitamin C) in the aqueous extract of Arslonquyruq (Limonium gmelinii) was successfully determined using high-performance liquid chromatography (HPLC). The results demonstrated that the extract contains relatively high amounts of vitamin C and B-group vitamins. This indicates the potential of this plant as a natural source of vitamins for use in the production of pharmaceutical, dietary, and nutraceutical products. In particular, the application of Arslonquyruq extract can be scientifically recommended to enhance the body's antioxidant defense system, improve immune function, and regulate metabolic processes.

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