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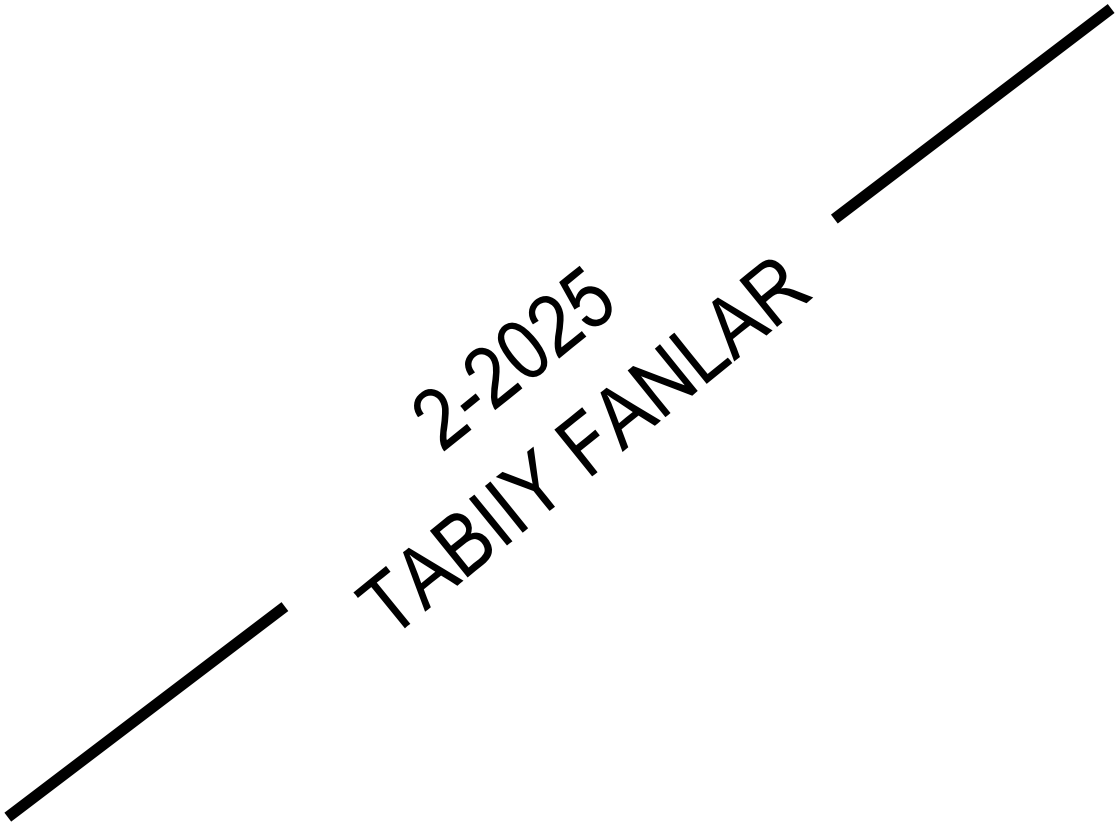

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AS-RAZZOQ OZIQ-OVQAT QO‘SHILMASIDAGI SUVDA ERUVCHAN VITAMINLAR MIQDORI TAHLILI

АНАЛИЗ СОДЕРЖАНИЯ ВОДОРАСТВОРИМЫХ ВИТАМИНОВ В ПИЩЕВОЙ ДОБАВКЕ AS-RAZZOQ

ANALYSIS OF THE CONTENT OF WATER-SOLUBLE VITAMINS IN THE FOOD SUPPLEMENT AS-RAZZOQ

Ibrohimjon Rahmonovich Asqarov[1]
[1]Andijon davlat universiteti, Kimyo kafedrasi professori, kimyo fanlari doktori, O‘zbekistonda xizmat ko‘rsatgan ixtirochi, O‘zbekiston TABOBAT Akademiyasi raisi

Obidjon Shaxabidinovich Abdulloyev[2]
[2]Andijon davlat universiteti, Kimyo kafedrasi professori v.b., kimyo fanlari doktori (DSc), O‘zbekiston Tabobat akademiyasi a’zosi

Qobiljon Qaxramonovich Otaxonov[3]
[3]Andijon davlat universiteti, Kimyo kafedrasi mudiri, kimyo fanlari bo‘yicha falsafa doktori (PhD), dotsent

Ziyodulloh Nabijon o‘g'li Razzaqov[4]
[4]Andijon davlat universiteti, Kimyo mutaxassisligi tayanch doktoranti

Annotatsiya

Mahalliy dorivor o‘simliklar, xususan, na’matak (Rosa canina L.) va qizilmiya (Glycyrrhiza glabra L.) suvli ekstraktlaridagi vitamin tarkibi eksperimental tarzda o‘rganildi. Ekstraktlardagi vitamin miqdorini aniqlash uchun turli standartlardan foydalanilgan: Rhydburg Pharmaceuticals (Germaniya) dan B_{12} vitamini, Carl Roth GmbH (Germaniya) dan C vitamini, DSM Nutritional Products GmbH (Germaniya) dan B_9 vitamini va B_1, B_2 vitaminlari, BLDPharm (Xitoy) dan B_3, B_6 va PP. Suv, asetonitril, kimyoviy toza sirka kislotasi va natriy gidroksid kabi HPLC toifali reagentlar ham ishlatilgan. Vitaminlar LC-40 Nexera Lite (Shimadzu, Yaponiya) yuqori samarali suyuq xromatografi yordamida tahlil qilindi. Suvli ekstrakti 75:25 nisbatda na’matak mevalari (Rosa canina L.) va qizilmiya ildizlari (Glycyrrhiza glabra L.) aralashmasidan tayyorlangan. Ushbu ekstrakt vitaminlarga boy ekanligi aniqlandi. Uning foydali kimyoviy tarkibiga asoslanib, immunitetni mustahkamlash, yuqori nafas yo‘llari kasalliklarini davolash va balg'amni yumshatishda foydalanish bo‘yicha tavsiyalar ishlab chiqilgan.

Аннотация

Содержание витаминов в водных экстрактах местных лекарственных растений, в частности шиповника (Rosa canina L.) и солодки (Glycyrrhiza glabra L.), изучалось экспериментально. Для определения содержания витаминов в экстрактах использовались различные источники: витамин B_{12} от Rhydburg Pharmaceuticals (Германия), витамин С от Carl Roth GmbH (Германия), витамин B_9 от DSM Nutritional Products GmbH (Германия), а также витамины B_1, B_2, B_3, B_6 и PP от BLDPharm (Китай). Также использовались реагенты класса HPLC, такие как вода, ацетонитрил, химически чистая уксусная кислота и гидроксид натрия. Анализ витаминов проводился с использованием высокоэффективного жидкостного хроматографа LC-40 Nexera Lite от Shimadzu (Япония). Водный экстракт был приготовлен из смеси плодов шиповника (Rosa canina L.) и корней солодки (Glycyrrhiza glabra L.) в соотношении 75:25. Этот экстракт оказался богатым витаминами. На основе его полезного химического состава были разработаны рекомендации по его использованию для укрепления иммунной системы, лечения заболеваний верхних дыхательных путей и облегчения мокроты.

Abstract

The vitamin content in water extracts of local medicinal plants, specifically Rosehip (Rosa canina L.) and licorice (Glycyrrhiza glabra L.), was studied experimentally. To determine the vitamin content in the extracts, various sources were utilized: Vitamin B_{12} from Rhydburg Pharmaceuticals (Germany), vitamin C from Carl Roth GmbH (Germany), vitamin B_9 from DSM Nutritional Products GmbH (Germany), and vitamins B_1, B_2, B_3, B_6, and PP from BLDPharm (China). HPLC-grade reagents such as water, acetonitrile, chemically pure acetic acid, and sodium hydroxide were also used. The vitamins were analyzed using an LC-40 Nexera Lite high-performance liquid chromatograph by Shimadzu

(Japan). The water extract was prepared from a blend of rosehip fruits (Rosa canina L.) and licorice roots (Glycyrrhiza glabra L.) in a ratio of 75:25. This extract is found to be rich in vitamins. Based on its beneficial chemical composition, recommendations have been developed for its use in strengthening the immune system, treating upper respiratory tract diseases, and alleviating phlegm.

Kalit so'zlar: *Rosa canina L., Glycyrrhiza glabra L., vitamin, HPLC, vitamin C, immunostimulyator.*

Ключевые слова: *Rosa canina L., Glycyrrhiza glabra L., витамин, ВЭЖХ, витамин С, иммуностимулятор.*

Key words: *Rosa canina L., Glycyrrhiza glabra L., vitamin, HPLC, vitamin C, immunostimulant*.

INTRODUCTION

Scientific research is being conducted to develop natural food additives with immunostimulating properties and determine their chemical composition for the prevention and treatment of infectious diseases. In this regard, medicinal plants containing biologically active compounds that improve metabolism and stimulate the immune system are being used [1]. Examples of local medicinal natural sources rich in biologically active substances include Rosehip (Rosa) and Licorice (Glycyrrhiza).

LITERATURE REVIEW

Rosa canina L. belongs to the *Rosaceae* family, which contains over 100 species widely distributed in Europe, Asia, Africa, the Middle East, and North America [1]. This thorny shrub, also known as rosehip, dog rose, or briar rose, is mightily resistant to hard weather conditions [3]. Its pseudo-fruits [4], rose hips, ripen in August–September [5] and gain a brick-red to deep-red colour [6]. Rose hips are a valuable resource from both medical and economic perspectives [3]. It grows in gardens, fields, mountain slopes, and in the middle and upper parts of the mountains, in juniper groves, and in walnut groves in the Tashkent, Andijan, Syrdarya, Jizzakh, Samarkand, Fergana, Namangan, Kashkadarya, and Surkhandarya regions [7]. Therefore, there is a complete reserve of the plant, and in medicine, its fruits are mainly used as a natural source of medicinal herbs [8].

The licorice plant (Glycyrrhiza) belongs to the Fabaceae family, and 36 species are currently known to science. There are mainly 8 types of them in the territory of Uzbekistan. Among these species, the medicinal properties of smooth licorice (*Glycyrrhiza glabra* L.) are of particular interest. In Uzbekistan, licorice is found along the Sirdarya, Zarafshan, Chyrchik, Angren, and Amu Darya rivers. To create a reserve of this medicinal plant and its widespread use in the pharmaceutical industry and folk medicine, plantations of G. Glabra have been established in our country on an area of 2,858 hectares, and 23 thousand tons of products were produced in 2018 alone. Since the underground part of the plant is rich in biologically active substances, its root is widely used in medicine [9]

The antioxidant activity of different compositions of the samples was determined by spectrophotometric analysis to find the ratio of the mixture of rosehip and licorice plants with the highest biological activity. In the study, the antioxidant activity of extracts obtained using two different solvents (water and ethanol) of the plant mixtures was studied, which inhibited the autooxidation reaction of adrenaline. According to the results of the experiment, the ethanol extract prepared based on a ratio of 75:25 of rosehip (*Rosa canina* L.) fruits and licorice (*Glycyrrhiza glabra* L.) roots showed higher antioxidant activity than the other samples [10].

Considering that this sample has high antioxidant activity and that the composition of the fruit of the narcissus is rich in biologically active substances, has the property of stimulating the immune system, and that the licorice root is rich in glycyrrhizic acid, which has antioxidant, antimicrobial, anti-inflammatory properties, and has expectorant properties, the aim was to develop a new food supplement "AS-RAZZOQ" based on this composition, which has the properties of treating and preventing upper respiratory tract diseases and is rich in biologically active substances. Below, the amount of water-soluble vitamins in the composition of this food supplement was analyzed using the HPLC.

METHODS AND MATERIALS

Reagents and equipment used. Vitamin B12 was obtained from "Rhydburg Pharmaceuticals" (Germany), vitamin C from "Carl Roth GmbH" (Germany), B9 from "DSM Nutritional Products GmbX" (Germany), vitamins B1, B2, B3, B6, and PP from "BLDPharm"

(China). Water, acetonitrile, acetic acid of chemically pure brand and sodium hydroxide were used as reagents of HPLC purity. The content of water-soluble vitamins in plant extracts was determined using an LC-40 Nexera Lite high-performance liquid chromatograph manufactured by Shimadzu, Japan [11].

Preparation of standard solutions. Solutions (100 mg/L) of vitamins 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 PP (CAS 98-92-0) 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 the initial B1, B6, B3, B12, and PP vitamins 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 was used for the concentration of 0 mg/l to create a calibration graph.

Preparation of sample extract. For the extraction of water-soluble vitamins, 1 g of the sample to be tested was weighed and placed in a 50 ml conical flask, and 25 ml of 0.1 N HCl solution was added. The mixture was extracted in an ultrasonic bath GT SONIC-D3 (China) for 20 minutes at 60 °C. Then the mixture was cooled, filtered, and made up to 25 ml with water in a volumetric flask. 1.5 ml of the extract was filtered through a 0.22 µm syringe filter, transferred to a vial, and used for analysis.

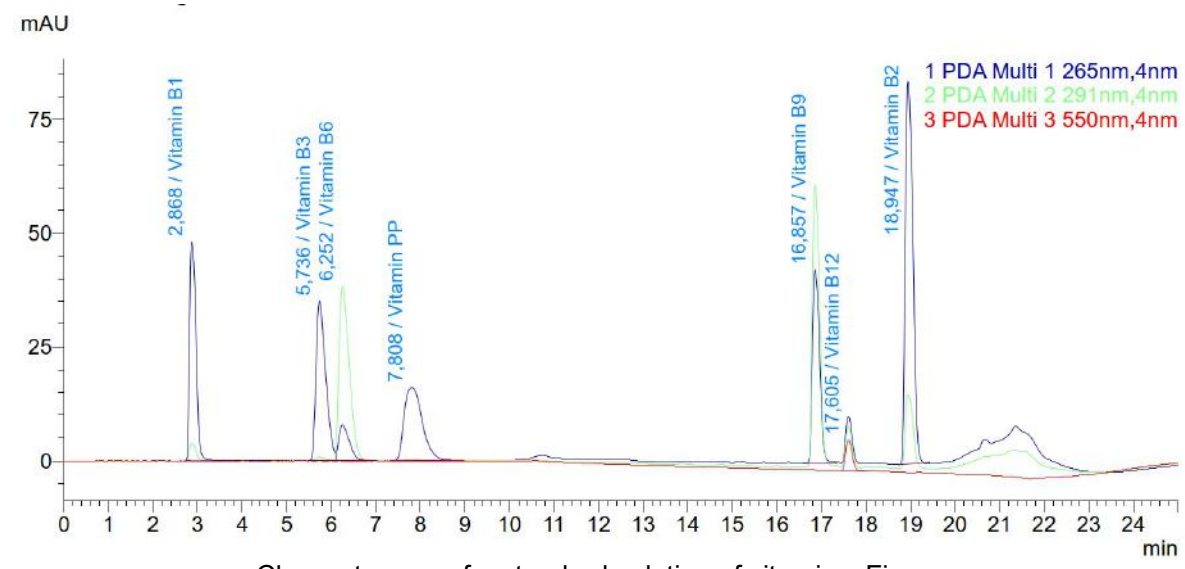
Chromatographic conditions. Determination of vitamins. Standard solutions and sample extract LC-40 Nexera Lite high-performance liquid chromatograph consisting of an LC-40D pump, SIL-40 autosampler, SPD-M40 photodiode array detector (PDA) and LabSolutions ver. 6.92 software. A Shim pack GIST C18 reversed-phase column (150 × 4.6 mm; 5 µm, Shimadzu, Japan) and a gradient mobile phase consisting of acetonitrile (A) and 0.25% acetic acid in water (B) (Table 1) were used. The injection volume was 10 µl, the flow rate was 0.6 ml/min, and the column thermostat temperature was set at 40 °C. The analytical signal (peak area) of each vitamin was recorded at three wavelengths: 265, 291, and 550 nm (Figures 1–3). A 15-minute gradient was used to determine vitamin C (Table 2), and the analytical signal was measured at a wavelength of 265 nm.

Mobile phase gradient program in the determination of vitamins. Table 1.

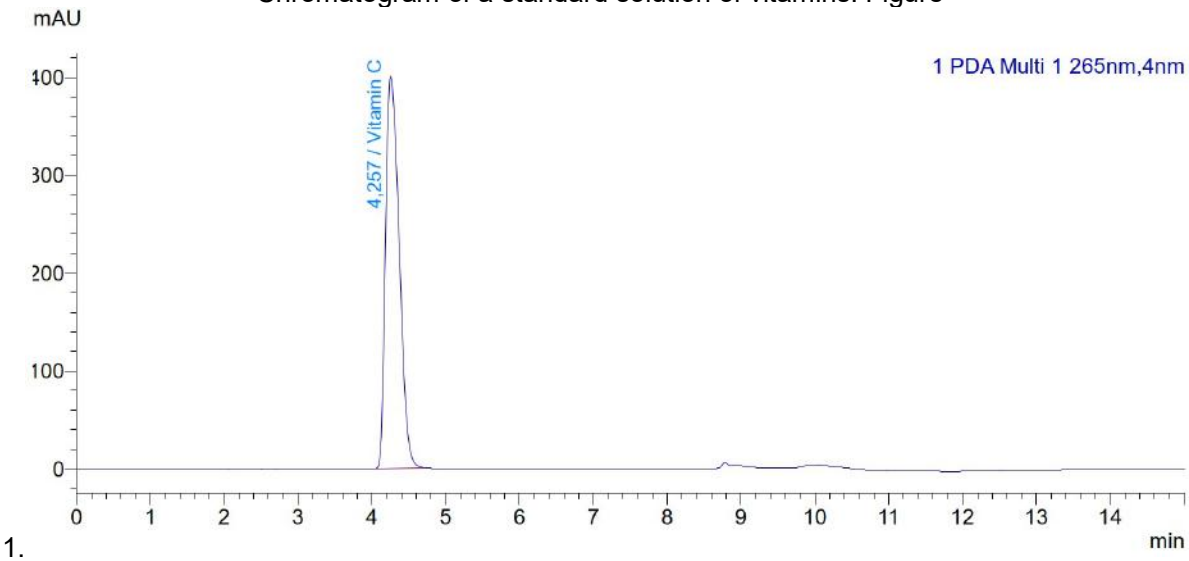
Time, min	Acetonitrile (A), %	0,5 % acetic acid (B), %
0	0	100
3	0	100
14	20	80
17	50	50
18	0	100
25	Finish	

Mobile phase gradient program for the determination of vitamin C. Table 2.

Time, min	Acetonitrile (A), %	0,5 % vinegar (B), %
0	0	100
2	0	100
6	50	50
6,01	0	100
15	Finish	



Chromatogram of a standard solution of vitamins. Figure



1.

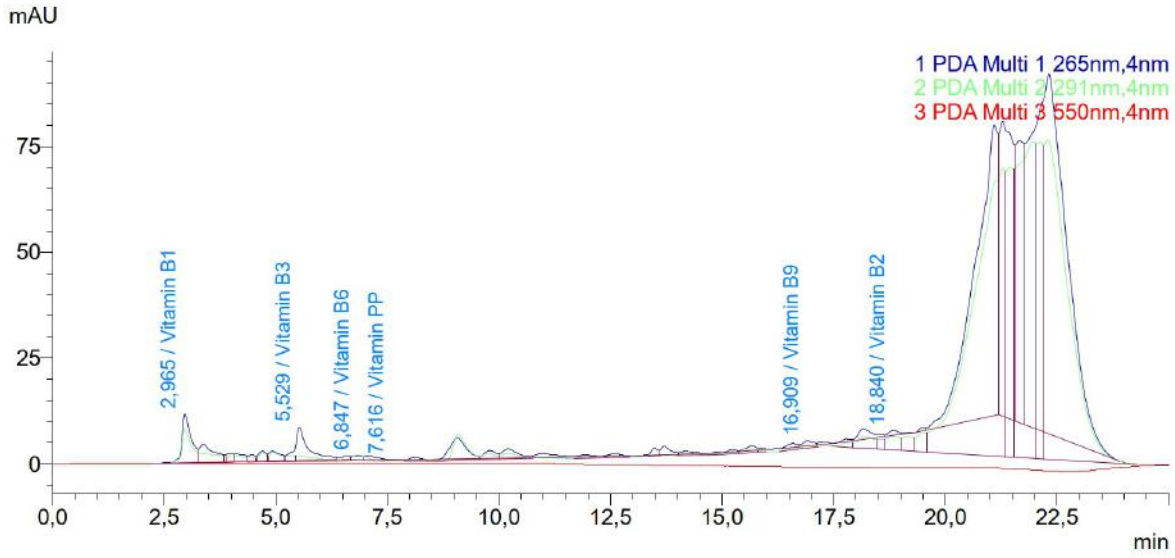
Chromatogram of a vitamin C standard solution. Figure 2.

RESULTS AND DISCUSSION

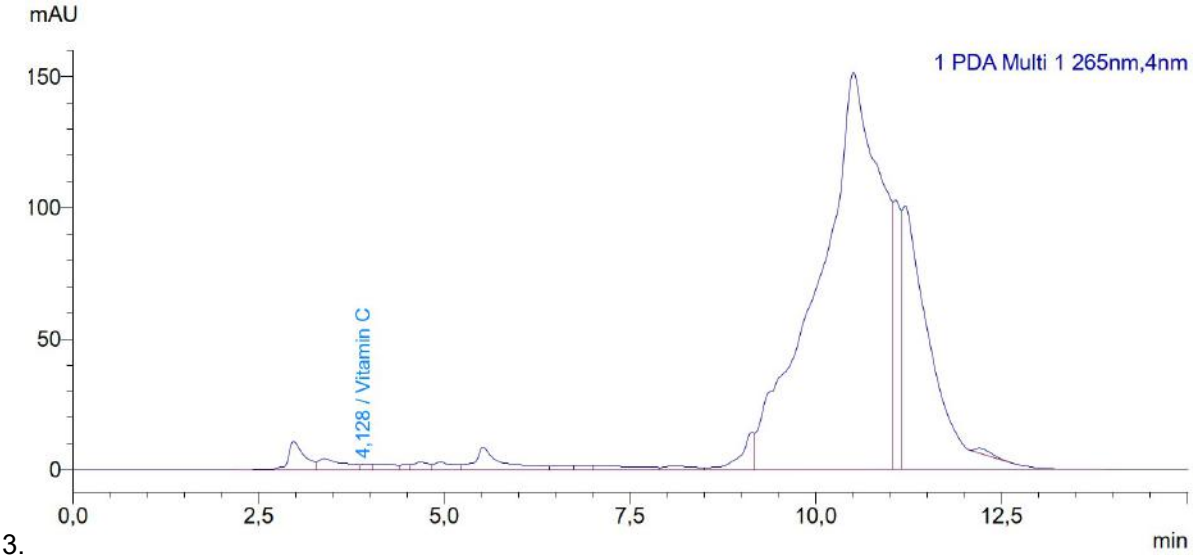
Determination of vitamins in the sample extract. A chromatogram of the sample extract (Figures 3-4) was obtained and based on the results, the amounts of vitamins in 100 g of fruit were calculated using the following formula and are presented in Table 3.

$$X = \frac{C_{vit} \cdot V_{extract}}{m_{sample}} \cdot 100\ g$$

Here, X is the amount of vitamins in 100 grams of fruit, mg;
C_{vit} – concentration of the vitamin in the extract determined by the HPLC method, mg/l;
$V_{extract}$ – volume of sample extract, l;
M_{sample} – the mass of sample taken for extract preparation.



Chromatogram of the determination of vitamins in the sample extract. Figure



3.

Chromatogram of the determination of vitamin C content in the sample extract. Figure 4.

Amount of vitamins in the extract and retention times. Table 3.

Vitamins	Retention time, sec	Concentration, mg/l	Amount in 100 g of sample, mg
Vitamin B_1	2,965	5,042	12,605
Vitamin B_3	5,529	4,737	11,843
Vitamin PP	7,616	0,098	0,245
Vitamin B_9	16,909	0,565	1,413
Vitamin B_2	18,84	0,524	1,310
Vitamin B_6	6,847	0,592	1,480
Vitamin B_{12}	No peak is detected	0	0,000
Vitamin C	4,128	1,932	4,830

CONCLUSION

The concentrate prepared from rosehip fruit contains a large number of vitamins, trace elements and minerals, amino acids, carbohydrates, proteins, fats, fatty acids and other substances that help strengthen human immunity [12, 13]. The results obtained showed that the amount of water-soluble vitamins B_1, B_3, PP, B_9, B_2, B_6, B_{12} and C, which are important for human

life, was studied in the sample under investigation. Vitamins PP, B_9, B_2, and B_6 were detected in moderate amounts in the extract, while vitamin B_{12} was not detected in the extract. In conclusion, it can be said that the extract is rich in vitamins, and of these, it is rich in vitamins B_1, B_3 and vitamin C.

Recommendation. Considering that the alcoholic extract of the plants of the genus Namatak and Shirinmia is rich in vitamins, micro- and macroelements, and is widely used in folk medicine, it is advisable to develop a new dietary supplement based on them that prevents and helps treat upper respiratory tract diseases.

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