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ANALYSIS OF VITAMINS IN PINE POLLEN AND PLANTS OF EASTERN BIOTA

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Abstract

The article discusses the properties of medicinal plants containing vitamins. A small amount of vitamins also regulates biochemical processes, participates in cellular respiration, and plays an important role in metabolism in the body. The number of water-soluble vitamins in pine pollen and plants of eastern biota was determined using a high-performance liquid chromatograph LC-40 “Nexera Lite” manufactured by the Japanese company “Shimadzu”. Vitamins B1, B2, B6 B9, and C were found in all studied samples. Therefore, it was proposed to create new food supplements from the raw materials of this plant that help in the treatment of pulmonary tuberculosis, bronchitis, shortness of breath, prostatitis, prostate adenoma, and oncological diseases.

How to Cite

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Introduction. In folk medicine, in the treatment and prevention of diseases, raw materials and minerals obtained from plants and animals that have healing properties are used [1]. The fact that these natural remedies do not have a negative effect on the human body has been proven through ears of treatment. Integration of traditional medicine and modern medicine in the treatment of patients is important to prevent many problems.

In recent decades, despite the many synthetic drugs used in modern medicine and veterinary medicine, interest in traditional medicine has not disappeared, but, on the contrary, has been revived, which is explained to some extent by the increase in allergic reactions when taking synthetic drugs. Natural chemical compounds, as a rule, have a less harmful effect on the animal and human body than their synthetic analogues and substances with an artificially created structure. The chemical composition of plants began to be studied at the end of the 17th century, and at the end of the 19th century they began to study some alkaloids, glycosides, tannins, saponins, flavonoids, various organic acids, vitamins, fatty and essential oils and micro elements. [2]. Vitamins are organic substances of various chemical natures that are not produced in sufficient quantities by the cells of the human body, but are necessary for its normal functioning. The name of the vitamin comes from the Latin word vita - “life”. The reason for this name is their important vital role in the body. [3].

Vitamins were discovered by the Russian doctor N.I. Lunin (1880), and the term “Vitamin” was proposed by the Polish scientist Funk in 1912. Lack of vitamins in the body causes serious diseases, for example: scurvy, blurred vision, polyneuritis, etc. Lack of vitamin in the body - hypovitaminosis. A complete lack of vitamin is vitaminosis. Excess vitamins – hypervitaminosis.

All vitamins can be biosynthesized by plants. Also, the concentration of some vitamins (group B, folic acid, pantothenic acid, etc.) in most plants is small and approximately the same, while others (vitamins K, nicotinic acid, biotin, tocopherols, etc.) vary significantly. Only ascorbic acid (vitamin C), carotenoids (provitamin A) and some flavonoids (rutin, quercetin, etc.) are classified as vitamin P and can accumulate in high concentrations [4].

On the territory of Uzbekistan, about 4500 species of tall plants are distributed in nature, about 1200 species have medicinal properties. Currently, in our republic, 112 plant species are approved for use in medicine, 80% of them are plants that grow naturally. [5,6]. Medicinal plants that produce vitamins and accumulate them serve as raw materials for the production of pure preparations or vitamin concentrates in industry. Plants cannot serve as a direct source of vitamin A, but accumulate its provitamins and carotenes. Vitamin D is not produced in plants, but ergosterols are widely distributed in the world of the plants. They are the source of these vitamins in the body of animals and humans and are used for their artificial production [7]. Vitamins are mainly produced in plant cells, but in higher plants their main synthesis occurs in green leaves. Many vitamins are present in almost all types of medicinal plants, but in different organs and in different quantities; some are only available in certain plant species. Carotene (provitamin A) is synthesized by almost all higher medicinal plants. The main sources of carotene for humans are red and ellow plant fruits and colorful root vegetables. B complex vitamins are also widely distributed in the plant world. Vitamin C (ascorbic acid) is produced in almost all higher plants, mainly in leaves, and to a lesser extent in fruits, roots and stems [8]. Sterols (provitamin D) are present in all plants; their amount in vegetable oils is 0,5-0,6%. Vitamin E (tocopherol) is found in many medicinal plants, especially in the seeds of some cereals. Vitamin K (phylloquinone) is found in cabbage leaves, spinach, chestnuts, carrot roots, pumpkin, tomatoes, etc. Vitamin P (hesperidin and its related rutin) is found in roses and citrus fruits [9,10]. Vitamins are biologically active substances necessary for the normal functioning of the body. They participate in all metabolic processes and increase the body's resistance to disease. Of the 30 known vitamins, 20 are obtained from plant and animal products. Therefore, plant raw materials are a source of valuable vitamins for the human body, and its use helps prevent overdose and side effects that are inevitable with long-term and uncontrolled use of synthetic vitamins [10]. Below we have studied the analysis of vitamins from medicinal plants used in the treatment of a number of diseases.

Among the medicinal plants rich in such substances, we chose pine and a plant of eastern biota. Pollen from a pine plant and leaves from an eastern biota plant were used as raw materials. Having studied the chemical composition of both plants based on the literature, we settled on pine pollen and leaves of a plant of oriental biota.

Oriental biota (lat. Biota orientalis) belongs to the widespread thuja (lat. Thuja) of the cypress family (lat. Cupressaceae). The genus includes 6 species, united in two subfamilies (thuja and platycladus - lat. Platycladus), growing in North America and East Asia. The thuja subgenus has 5 species. Evergreen coniferous-woody plant. It is valued for its excellent decorative qualities, high resistance to the environment and the ability to purify the air from pathogens. Seeds (kernels) and leaves are used as medicinal raw materials. In addition to enriching the air with oxygen, the leaves of the biota (by constantly releasing essential oil) cleanse it of pathogenic bacteria. Essential oil helps regulate the menstrual cycle in women and sexual dysfunction in men, as it eliminates blockages in the pelvic organs (ovaries, uterus, prostate).

Its beneficial properties have been known for a long time. Representatives of American Indian tribes used the bark and leaves of the biota for the treatment and prevention of various inflammatory processes, infectious diseases, and rheumatism. That is why the biota has since received another wonderful name - “tree of life”.

Pine pollen is a unique natural antibiotic that has many beneficial properties and does not cause allergic reactions. [11] It is present in male pine buds that ripen at the end of May. They should be collected within a few days after the young buds appear. The wide distribution of various parts of pine trees, valuable medicinal properties and a wide range of medicinal effects determined their popularity in folk medicine [12]. Pine needles, buds, bark and cones have long been used in the treatment of various inflammatory diseases, and are also used as a general tonic and vitamin remedy.

A valuable source of biologically active substances is pine pollen, containing vitamins: A, D3, E, B1, B2, B6, PP, folic and ascorbic acids, as well as β-carotene, amino acids, macro- and microelements, enzymes and coenzymes, contains mono - and polysaccharides, fats [13]. Traditional Chinese medicine has used P massoniana pollen as medicine and food for thousands of ears. It was noted [14] that over the past 20 ears the number of publications in the field of pharmacological and clinical studies of pine pollen has increased significantly. Chinese researchers have found that extracts containing polysaccharide fractions of P massoni-ana pollen have immunomodulatory, anti-inflammatory and antiviral activity, as well as antioxidant and hepatoprotective effects [15-16]. An ethanolic extract of P. densiflora pollen grown in Korea has anti-inflammatory and antioxidant properties [17].

In Asian countries, pine pollen has long been used as a food supplement, as well as an effective means of healing and rejuvenating the whole body in traditional medicine and as a natural antibiotic:

Nervous system – due to the regular supply of nutrients, sleep is normalized, the psycho-emotional state is stabilized, the supply of oxygen to the brain improves, which reduces the risk of developing a pre-stroke condition;

Digestive system – improves metabolism, normalizes the functioning of all organs of the stomach and intestines, restores healthy intestinal microflora;

Cardiovascular system – the level of hemoglobin in the blood increases, blood circulation and vascular elasticity improve and blood pressure normalizes;

Reproductive system – during menopause, women’s hormonal balance stabilizes, the menstrual cycle becomes smooth; in men, potency improves and the risk of prostatitis decreases.

In addition, this remedy has a general strengthening and rejuvenating effect by normalizing metabolism and replenishing the body's internal reserves, which require large daily energy costs.

Determination of the amount of water-soluble vitamins in the extract using the YUSSX method

Experience part.

Used reagents and equipments. Vitamin B_{12} was obtained from “Rhydburg Pharmaceuticals” (Germany), vitamins B_1, B_2, B_6, B_9 and C from “DSM Nutritional Products GmbX” (Germany). HPLC grade water, acetonitrile, chemical grade acetic acid and sodium hydroxide were used.

The amount of water-soluble vitamins contained in the plant was measured using a high-performance liquid chromatography "LC-40 Nexera Lite" manufactured by the Japanese company "Shimadzu".

Preparation of standard solutions. Solutions of vitamins C (CAS 50–81–7), B_1 (CAS 70–16–6), B_6 (CAS 65–23–6) and B_{12} (CAS 68–19–9) (100 mg/l) 5 mg of each vitamin is prepared by dissolving 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 these vitamins in 50 ml of 0.025% sodium hydroxide solution. Then, 200 mkl of all the original vitamins were mixed and a stock solution was prepared with a concentration of each vitamin of 16.67 mg/l. By diluting it, solutions with

concentrations of 3.333 mg/l, 0.667 mg/l and 0.133 mg/l were prepared, poured into a vial and used for analysis.

Preparation of sample solution. To extract water-soluble vitamins, 1 ml of the test sample was measured, 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) at a temperature of 60 °C for 20 minutes. The mixture was then cooled, filtered and made up to 25 ml with water in a volumetric flask. 1,5 ml of extract was filtered through a syringe filter with a pore size of 0,45 μm, placed in a vial and used for analysis.

Chromatographic conditions.

Identification of vitamins. Standard solutions and sample extracts were analyzed using an LC-40 Nexera Lite high-performance liquid chromatograph equipped with an LC-40D pump, a SIL-40 autosampler, an SPD-M40 photodiode array detector (PDA) and LabSolutions ver. 6.92". Shimpack GIST C18 (150×4.6 mm; 5 μm, Shimadzu, Japan) reverse phase column with a gradient mobile phase consisting of acetonitrile (A) and a 0.25% solution of acetic acid in water (B) (table 1) was applied. The injection volume was set to 10 mkl, the flow rate to 0,9 ml/min, and the column oven temperature to 35°C. The analytical signal (peak area) of each vitamin was recorded at three wavelengths: 265, 291, 550 nm (Figures 1-3).

Table 1. Mobile phase gradient program.

Time	Acetonitrile (A),%	0,5% acetic acid (B), %
0	0	100
6,8	0	100
15,5	50	50
17,4	95	5
17,5	0	100
25	Termination	

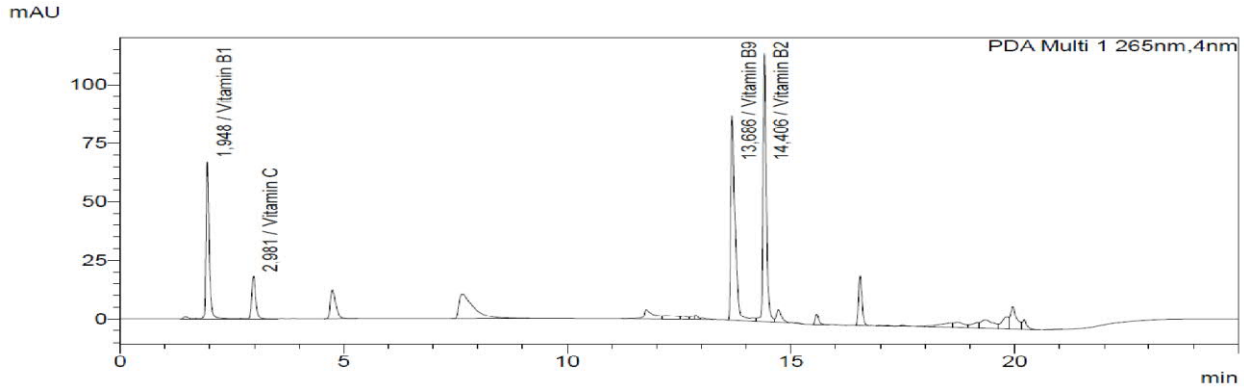


Figure 1. Chromatogram of a standard solution of vitamins B_1, B_2, B_9, C at 265 nm.

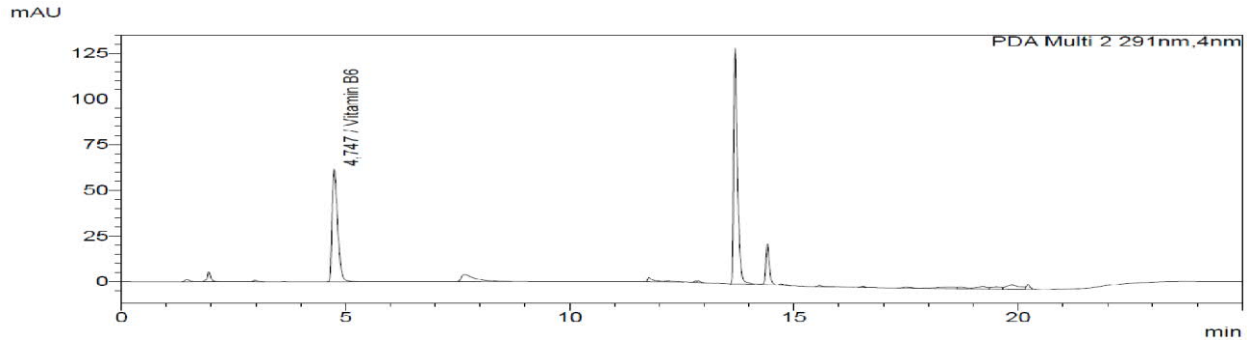


Figure 2. Chromatogram of a standard solution of vitamin B_6 at 291 nm

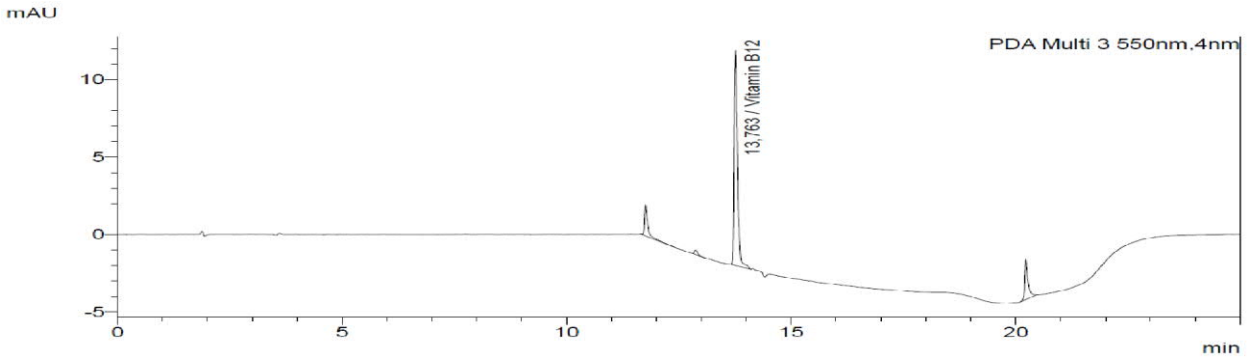


Figure 3. Chromatogram of a standard solution of vitamin B_{12} at 550 nm

Results.

Determination of vitamins in samples. A chromatogram of the eastern leaf biota was obtained (Fig. 4), the results were processed and presented in Table 2.

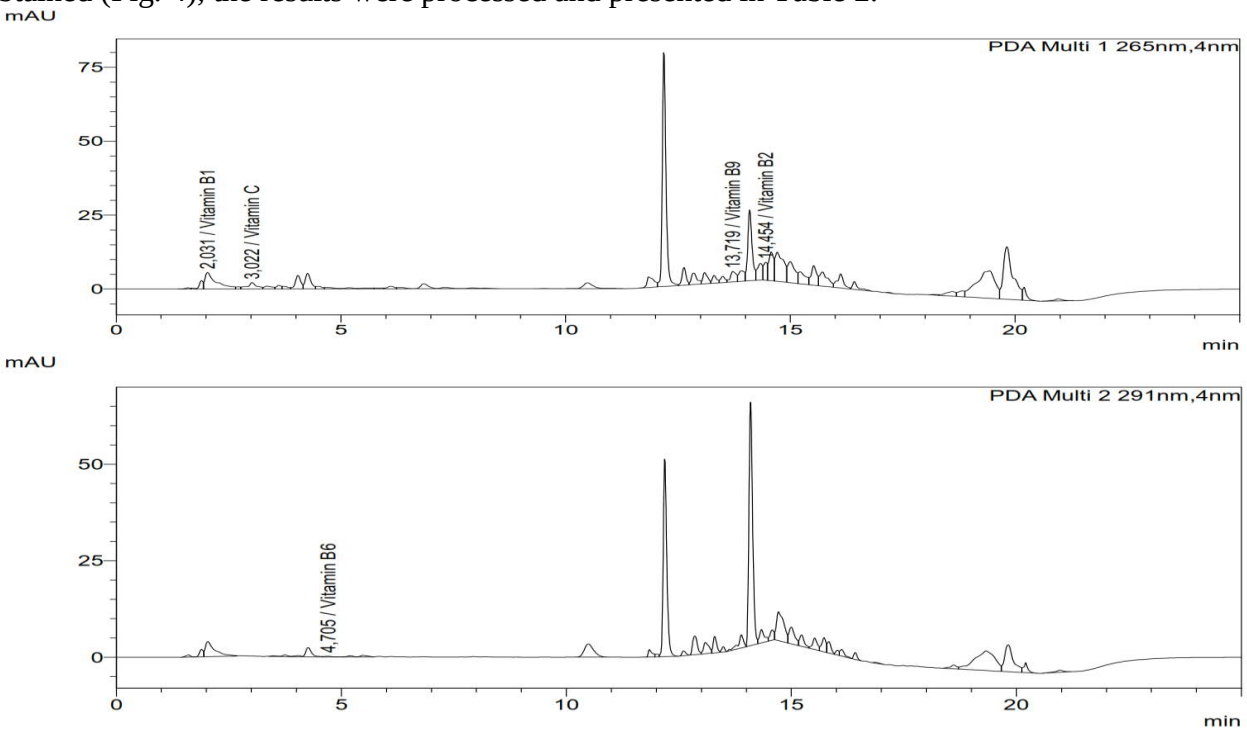


Figure 4. Chromatograms for determining vitamins in extracts of eastern biota.

Table 2. Amount and retention time of vitamins in the extract.

Vitamin	Retention time, sec	Concentration, mg/l	Quantity in 100 g sample, mg

Vitamin B_1	2,031	5,096	12,490
Vitamin C	3,022	4,958	12,152
Vitamin B_9	13,719	1,011	2,478
Vitamin B_2	14,454	0,96	2,353
Vitamin B_6	4,705	0,077	0,189
Vitamin B_{12}	Not identified	0	0,000

A chromatogram of pine pollen was obtained (Fig. 5), the results were processed and presented in Table 3.

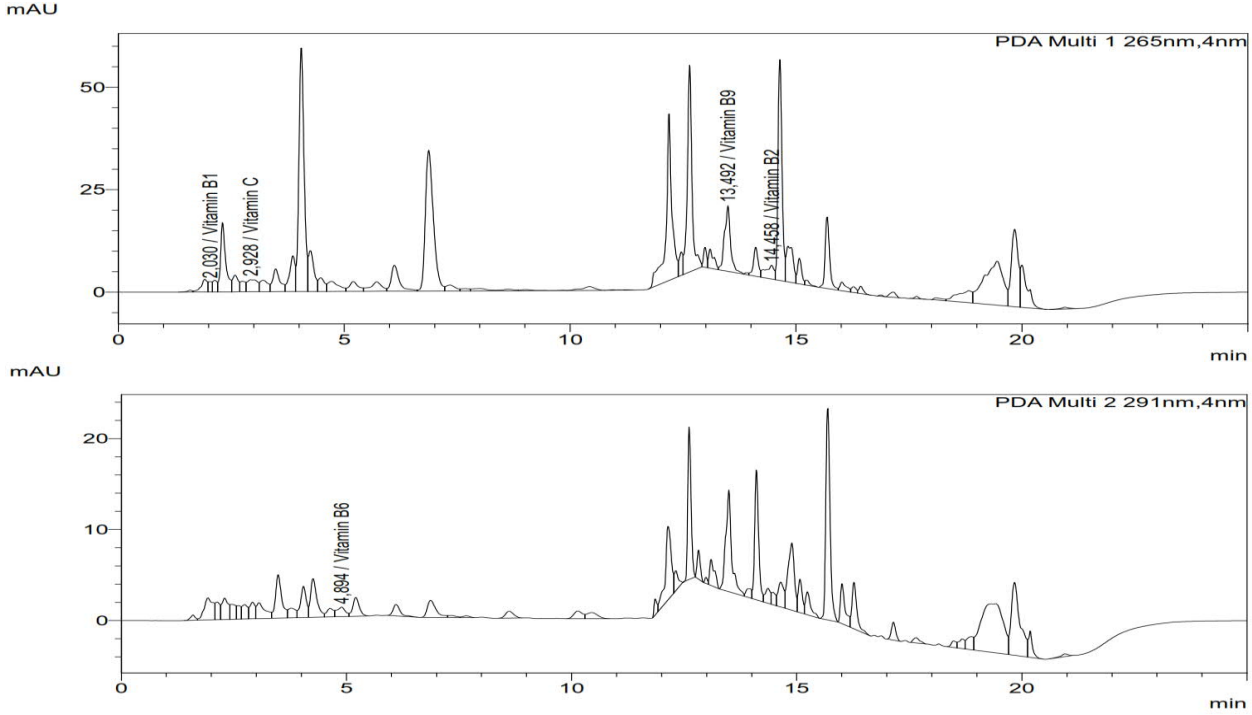


Figure 5. Chromatograms for determining vitamins in pine pollen extract.

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

Vitamin	Retention time, sec	Concentration, mg/l	Quantity in 100 g sample, mg
Vitamin B_1	2,03	0,988	2,422
Vitamin C	2,928	7,164	17,559
Vitamin B_9	13,492	4,323	10,596
Vitamin B_2	14,458	1,183	2,900
Vitamin B_6	4,894	0,455	1,115
Vitamin B_{12}	Not identified	0	0,000

A chromatogram of a mixture of pine pollen and leaves of eastern biota in a 1:1 ratio was obtained (Fig. 6), the results were processed and presented in table. 4.

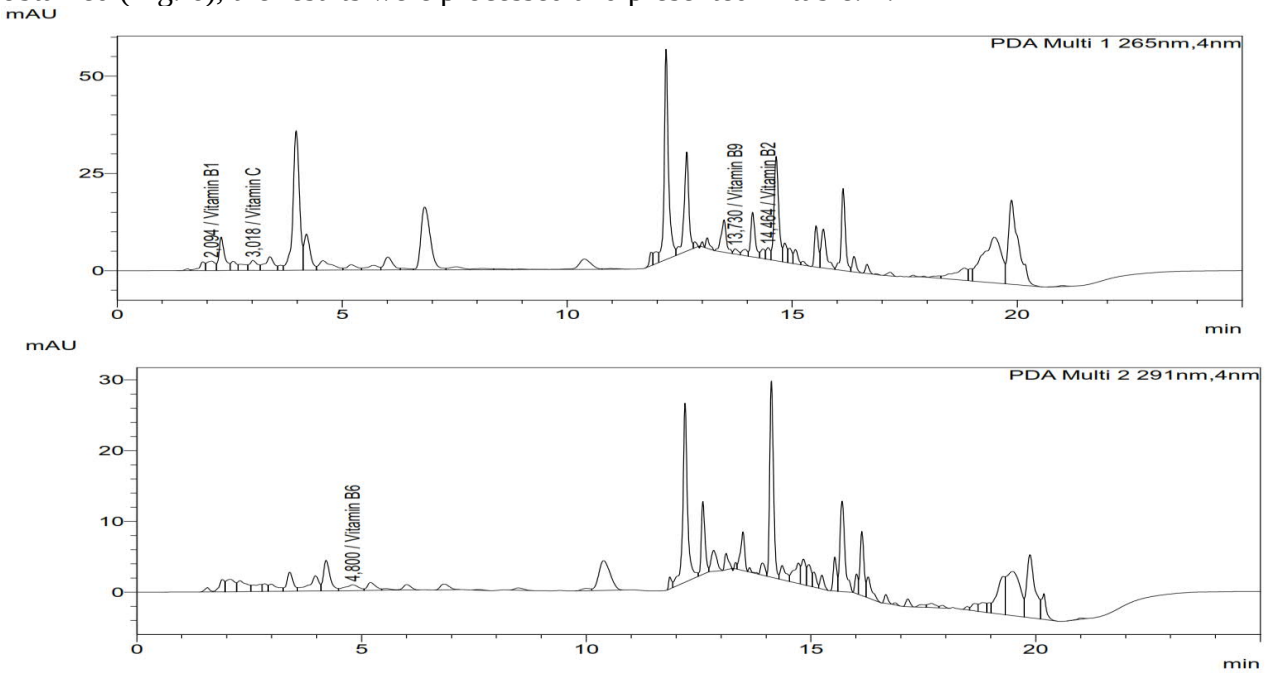


Figure 5. Chromatograms for determining vitamins in an extract of a mixture of pine pollen and leaves of eastern biota, 1:1.

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

Vitamin	Retention time, sec	Concentration, mg/l	Quantity in 100 g sample, mg
Vitamin B_1	2,094	1,833	4,493
Vitamin C	3,018	5,046	12,368
Vitamin B_9	13,73	1,967	4,821
Vitamin B_2	14,464	1,174	2,877
Vitamin B_6	4,8	0,892	2,186
Vitamin B_{12}	Not identified	0	0,000

Discussion of the results obtained. The above laboratory tests show that the medicinal plants studied contain sufficient amounts of B vitamins and especially ascorbic acid. This is appropriate if we take into account that these natural vitamins are involved in important processes in the human body and their use in the pharmaceutical industry in the production of medicines, in the treatment and prevention of diseases

Conclusion. In conclusion, extracts obtained from medicinal plants, the names and properties of which are listed above, if used in various forms of medicines: teas, decoctions, tinctures, tablets, capsules, ointments and preparations, and cheap raw materials with very low side effects can be prepared. The preparation technology is simple, we can use it for a long time, and also use it in patients with various diseases. During the research, water-soluble vitamins in the above-mentioned plants were studied and analyzed. Through a comprehensive analysis of plants, it is desirable to create new food additives used in folk medicine for the prevention and treatment of pulmonary tuberculosis, bronchitis, shortness of breath, prostatitis, prostate adenoma and oncological diseases.

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