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DETERMINATION OF VITAMIN CONTENT IN OURET LANATA (AERVA LANATA JUSS.) PLANT USING HPLC METHOD

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Annotation
The article provides information about the chemical composition of the Ouret lanata (Aerva lanata Juss.) plant and its significance for human health. The content of water-soluble vitamins in the plant's aqueous extract was determined using high-performance liquid chromatography (HPLC), showing an increasing order of vitamin content as C < B9 < B1.

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INTRODUCTION

Many medicinal preparations used in traditional and modern medicine are derived from plants or prepared using plant-based materials. Medicinal plants typically contain various amounts of alkaloids, glycosides, flavonoids, essential oils, vitamins, and other compounds beneficial to human health. One such plant with these properties is Ouret lanata. Ouret lanata is a natural medicinal herb traditionally used to treat various diseases. It is known for its abundant bioactive compounds that have positive effects on the human body. Scientific literature highlights its composition and its potential applications in traditional medicine[1,2].

Due to its unique composition, Ouret lanata exhibits numerous therapeutic effects. The plant contains various chemical components, including β-sitosterol, campesterol, and chrysine. Studies from Egypt have identified vanillic acid, syringic acid, and daucosterol in the plant, while research in Russia has found flavonoids, lupeol derivatives, and feruloyltyramine. Other compounds such as alkaloids, terpenoids, sterols, flavonoids, glycosides, and polyphenols have been isolated by scientists from Russia and India.

Ouret lanata is rich in vitamins, minerals, and antioxidants, each providing significant health benefits. Vitamin C strengthens the immune system and protects against cardiovascular diseases.

Vitamin B_1 (Thiamine) plays a crucial role in the metabolism of carbohydrates, proteins, and fats, as well as in neural excitation processes at synapses. It protects cell membranes from the toxic effects of peroxidation products. Thiamine improves brain function, memory, attention, and thinking, stabilizes mood, enhances learning ability, and promotes bone and muscle growth.

The findings emphasize the importance of Ouret lanata as a valuable source of bioactive compounds that can contribute to human health. Normalization of appetite, slowling down aging, and reducing the negative impacts of alcohol and tobacco Ouret lanata (*Aerva lanata* Juss.) offers numerous health benefits, such as regulating appetite, slowing the aging process, mitigating the harmful effects of alcohol and tobacco, maintaining the rhythmic contractions of digestive muscles, alleviating locomotor system disorders, and ensuring the normal functioning of the heart muscles. Additionally, it helps reduce toothache[3].

Key Properties of Ouret lanata-Based Preparations:

Powerful Diuretic Effect: Ouret lanata preparations gently remove excess fluids from the body without causing dehydration. Stone Dissolution: An essential property often utilized for treating urolithiasis, where the plant aids in breaking down stones. Body Detoxification: These preparations can eliminate chlorides, uric acid, heavy metals, and toxins from the body. Cold and Infection Treatment: Ouret lanata is used to treat colds, boost the immune system, and enhance the body's resistance to infections. Calming Effect: The plant's formulations improve memory and soothe the nervous system. Thrombosis Treatment: It is employed in managing thrombosis[4].

Alkaloids in Ouret lanata: The alkaloids found in Ouret lanata can relieve spasms, reduce pain, stop bleeding, and promote normal blood circulation. However, their effect depends on the dosage[5].

Applications and Medicinal Uses: The dried herb collected during the flowering period has prominent diuretic properties and a spasmolytic effect on the smooth muscles of the urinary, bile, and gastrointestinal tracts. Ouret lanata reduces inflammation, alleviates swelling, and assists in the expulsion of stones in conditions like pyelonephritis and urolithiasis. In bile duct inflammation, it supports bile secretion and reduces inflammation, while in chronic gastritis exacerbations, it eases painful stomach muscle spasms.

Conditions Treated with Ouret lanata: Ouret lanata is used to treat: Chronic pyelonephritis, Urolithiasis, Cystitis, Chronic persistent and moderately active hepatitis, Chronic cholecystitis, Biliary dyskinesia, Exacerbations of chronic gastritis, Pylorospasm [6].

Additional Benefits: Ouret lanata is highly regarded for its detoxifying properties, including: Removing toxins and heavy metal salts from the body, Dissolving kidney stones, Acting as a unique diuretic without causing dehydration, Furthermore, it is utilized as an infusion for managing diabetic nephropathy, arterial hypertension, and edema of various origins. Unlike many pharmaceutical diuretics, Ouret lanata prevents dehydration, making it a valuable natural remedy [7].

EXPERIMENTAL PART

Determining the amount of water-soluble vitamins in the hairy pea plant by the HPLC method.

Experimental part. **Reagents and equipments.** Vitamin B_{12} was obtained from Rhydburg Pharmaceuticals (Germany), vitamin C from Carl Roth GmbH (Germany), B_9 from DSM Nutritional Products GmbH (Germany), vitamins B1, B_2, B_3, B_6, and PP from «BLDPharm» (China). We used HPLC grade water, acetonitrile, and reagent grade acetic acid and sodium hydroxide. Quantitative determination of water-soluble vitamins in the plant was carried out using a highperformance liquid chromatograph LC-40 Nexera Lite manufactured by Shimadzu, Japan.

Preparation of standard solutions. C (CAS 50-81-7), B1 (CAS 59-43-8), B_6 (CAS 58-56-0), B_3 (CAS 59-67-6), B_{12} (CAS 68–19–9) and Vitamin PP solutions (CAS 98-92-0) (100 mg/l) are prepared by dissolving 5 mg of each vitamin in 50 ml of 0.1 N HCl solution. 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 µl of the original vitamins B_1, B_6, B_3, B_{12}, PP were mixed and a solution was prepared with a concentration of each vitamin of 14.286 mg/l. In this way, standard solutions of 7.143, 3.571, 1.786 mg/l were prepared. Standard solutionsof vitamin C were also prepared with concentrations of 286, 143, 71.5, 57.2 mg/l. Pure water with a concentration of 0 mg/l was used to construct the calibration graph.

Preparation of sample extract. Extracting water-soluble vitamins, 1 g of the test sample was weighed out, 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. Then the mixture was cooled, filtered and the volume of water was brought to 25 ml in a volumetric flask. 1.5 ml of extract was filtered through a syringe filter with a pore size of 0.22 µm, placed in a vial and used for analysis.

Chromatographic conditions. Identification of vitamins. Standard Solutions and Sample Extracts 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. Software 6.92 has been analyzed. A GIST C18 reverse-phase column (150 × 4.6 mm; 5 µm, Shimadzu, Japan) with a gradient mobile phase consisting of acetonitrile (A) and 0.25% acetic acid in water (B) (Table 1), used. The injection volume was 10 µL, the flow rate was 0.6 mL/min, and the column thermostat temperature was set to 40°C. The analytical signal (peak area) of each vitamin was recorded at three wavelengths: 265, 291, 550 nm. To determine vitamin C, a 15-minute gradient was used (Table 2), the analytical signal was measured at a wavelength of 265 nm.

Table 1. Mobile phase gradient program for determining vitamins

Time, minute	Acetonitrile (A),%	0.5% acetic acid (B), %
0	0	100
3	0	100
14	20	80
17	50	50
18	0	100
25	Termination	

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

Time, minute	Acetonitrile (A),%	0.5% acetic acid (B), %
0	0	100
2	0	100
6	50	50
6.01	0	100
15	Termination	

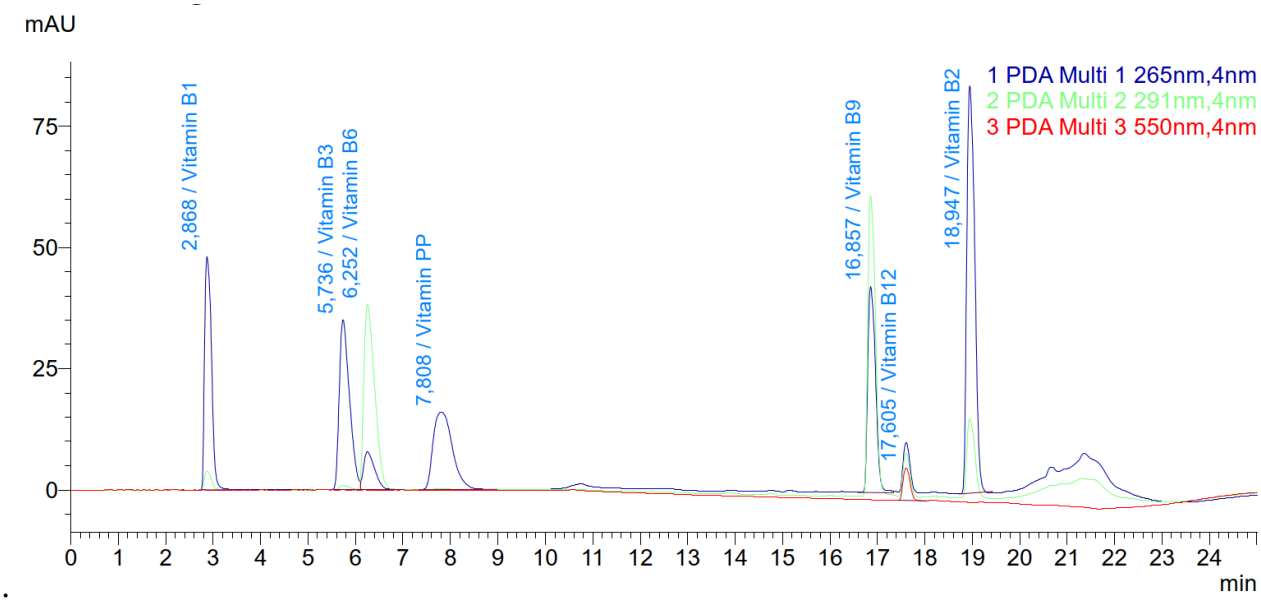


Figure 1. Chromatogram of Standard Vitamins Solution

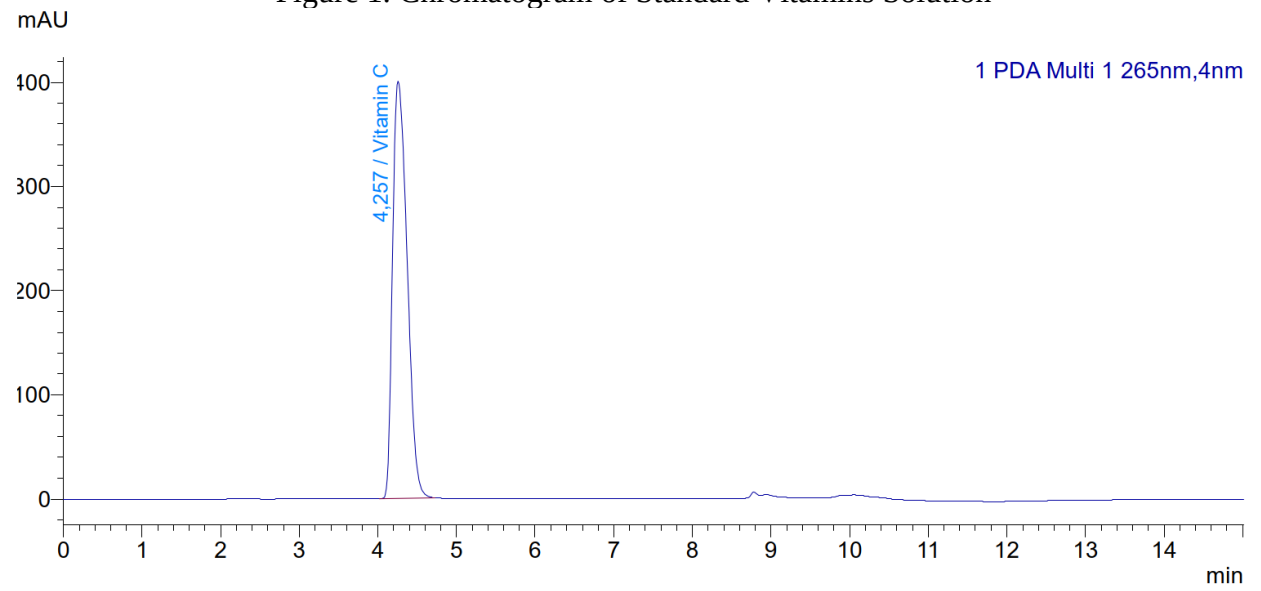


Figure 2. Chromatogram of Standard Vitamin C Solution

RESULTS AND DISCUSSION

L.A. Bagdanova and a group of Russian researchers developed a methodology for determining water-soluble vitamins in premixes (feed additives). Their method ensures the required accuracy (relative error within 10%) by optimizing the conditions for obtaining, chromatographically separating, and identifying vitamins [8,9,10,11].

Another team led by A.V. Pirogov at Moscow State University designed a simultaneous determination method for 14 types of water-soluble vitamins using high-performance liquid chromatography (HPLC) [12].

Determination of vitamins in Ouret lanataextract. A chromatogram of the sample extract was obtained (Fig. 2, 3), and based on the results, the amount of vitamins in 100 g of fruit was calculated using the following formula, 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 g of extract, mg;

C_{vit}- concentration of vitamin in the extract, determined by the HPLC method, mg/l;

$V_{extract}$– volume of sample extract, l;

m_{sample}– weight of the sample that is taken to prepare the extract:

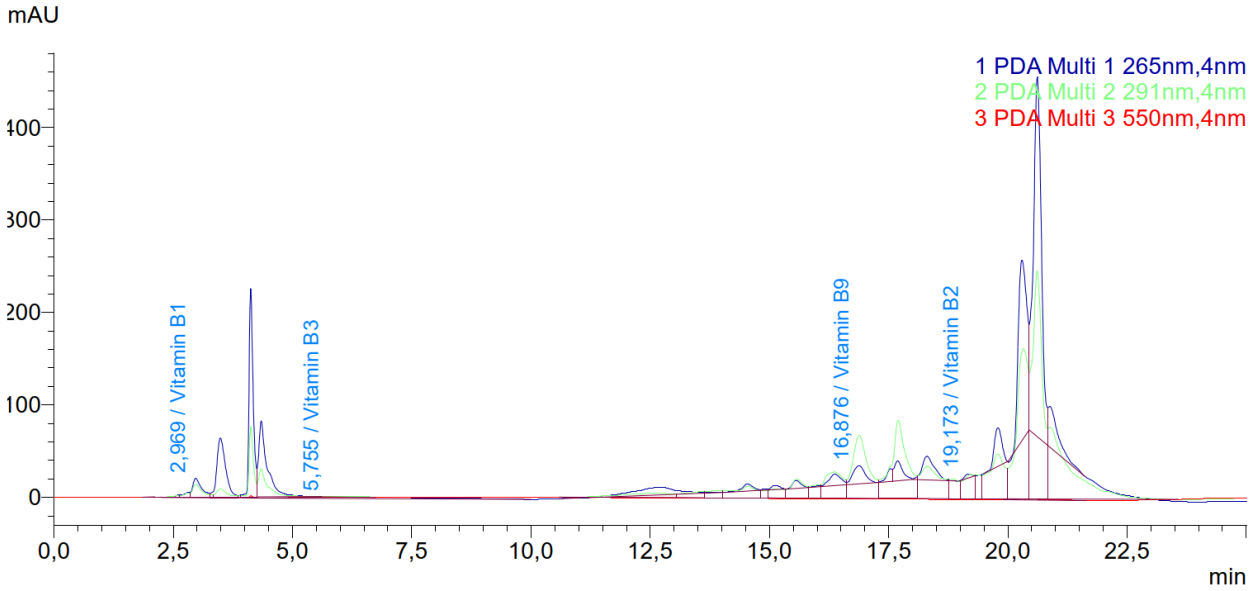


Figure 3. Chromatogram for determining the amount of vitamins in a sample extract

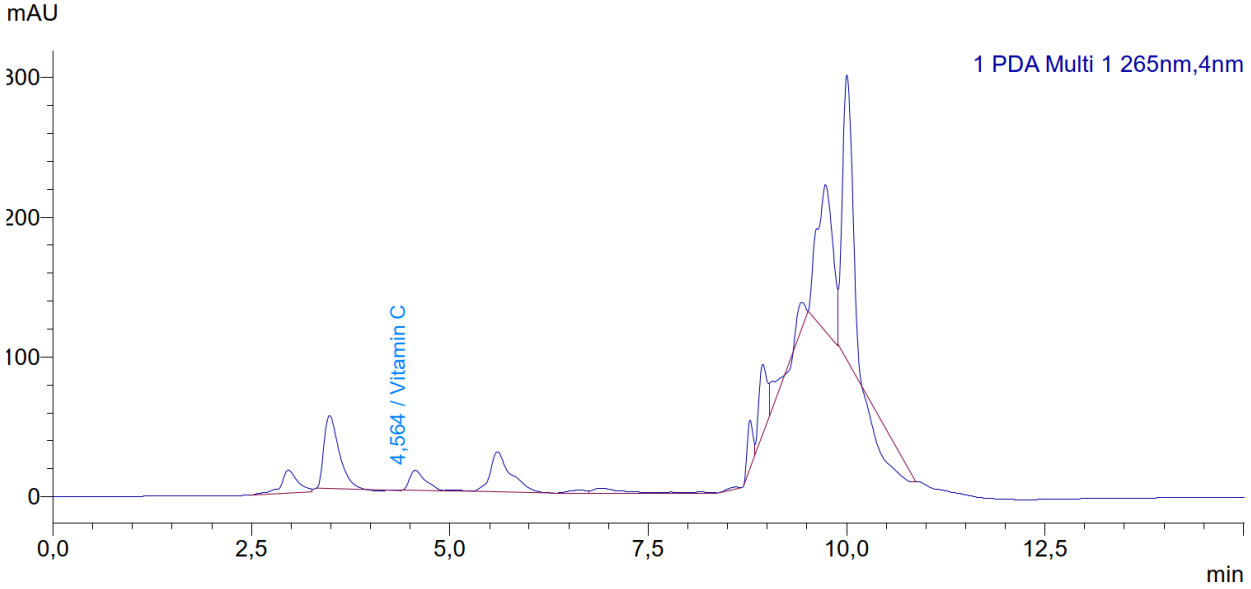


Figure 4. Chromatogram for determining the amount of vitamin C in a sample extract

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

Vitamins	Holding time, sec	Concentration, mg/l	Amount per 100 g sample, mg
Vitamin B_1	2,969	10,642	26,605
Vitamin B_3	5,755	0,142	0,355
Vitamin PP	Not detected	0	0,000
Vitamin B_9	16,876	10,25	25,625

Vitamin B_2	19,173	0,934	2,335
Vitamin B_6	Not identified	0	0,000
Vitamin B_{12}	Not identified	0	0,000
Vitamin C	4,564	10,232	25,580

If we analyze the chromatogram of vitamins contained in hairy erva extract (Figure 3), we can see that the peaks of vitamins B_1, B_9 and C are clearly visible among the peaks of 5 different vitamins identified in the chromatogram of vitamins. The absence of vitamins PP, B_6, B_{12} in the chromatogram determined from the extract indicates that vitamin B_{12} was not detected in the sample

Experimental analyzes of the chemical composition of the hairy pea plant (Table 3) among the 8 different vitamins analyzed in aqueous extracts prepared from 100 g of samples, the amount of B_1 was 26,605 mg, the amount of vitamin B_9 was 25,625 mg, and the amount of vitamin C the amount of 25,580 mg indicates that the amount is high.

These values demonstrate that the plant is particularly rich in B_1, B_9, and C vitamins, making it a valuable natural source for health benefits. Below is an overview of their roles and significance: Vitamin B_1 (Thiamine) Role in the body: Essential for carbohydrate, protein, and fat metabolism. Plays a crucial role in the nervous system by regulating nerve impulse transmission. Protects cell membranes from the toxic effects of oxidative stress. Health Benefits: Enhances brain function, memory, and focus. Supports mood regulation and cognitive abilities. Promotes the growth of bones and muscles.

Vitamin B_9 (Folic Acid) Role in the body: Water-soluble and critical for cell formation and DNA synthesis. Essential for the development of nervous tissue, particularly during pregnancy for fetal growth. Supports the production of red and white blood cells and energy metabolism. Health Benefits: Prevents neural tube defects during pregnancy. Assists in maintaining cardiovascular health by reducing homocysteine levels. Supports genetic material synthesis (DNA and RNA).

Vitamin C (Ascorbic Acid) Role in the body: One of the most potent antioxidants, counteracting oxidative stress caused by environmental factors like UV rays and pollution. Strengthens the immune system and provides anti-inflammatory and anti-allergic effects. Enhances the absorption of calcium and iron by converting ferric iron (Fe^{3+}) into ferrous iron (Fe^{2+}). Health Benefits: Protects against infections and boosts immunity. Reduces the risk of cardiovascular diseases by enhancing capillary permeability and elasticity. Supports collagen synthesis, which is crucial for maintaining skin elasticity, bone density, and joint flexibility. Aids adrenal gland function during stress. The abundance of these vitamins in *Aerva lanata* highlights its potential use in traditional and modern medicine for improving overall health and preventing various deficiencies. Additionally, the plant's richness in Vitamin C makes it an excellent source for enhancing skin health and immunity. If you have further questions or need assistance with the table or chromatograms, feel free to ask!

CONCLUSION

When reviewing the medicinal properties of the Hairy Plant (Ouret lanata), it has been found to exhibit strong diuretic effects, as well as anti-inflammatory, immune-boosting, and calming properties.

Through the use of the HPLC method to determine the water-soluble vitamin content in the extract of the Hairy Plant, it was revealed that the plant contains high levels of vitamins B1, B9, and C. These compounds provide the plant with strong diuretic, anti-inflammatory, and immune-strengthening properties, while also contributing to its calming effects. Thus, the Hairy Plant can be used as a natural remedy for various health benefits.

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