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DETERMINATION OF WATER-SOLUBLE VITAMINS IN EXTRACT OF POPLAR AND WILLOW MIXTURE USING AND EVALUATION OF THEIR HEALTH BENEFITS

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Annotation

In this study, the content of water-soluble vitamins in a 1:1 mixture of poplar (Populus spp.) and willow (*Salix spp.*) plants was determined using the HPLC (High-Performance Liquid Chromatography) method. The results revealed the presence of vitamins C, B_1, B_2, B_3, B_6, B_9, and PP, while vitamin B_{12} was not detected. The obtained data indicate that this mixture may serve as a valuable source of biologically active micronutrients with significant scientific and practical importance for supporting human health.

How to Cite

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Introduction. Vitamins are among the most essential micronutrients in the human body. They play a direct role in cellular metabolism, energy exchange, enzymatic reactions, and tissue regeneration processes. In particular, water-soluble vitamins- B-group vitamins and vitamin C- are not stored in the body, which makes their regular intake through food or biologically active supplements essential. A deficiency of these substances can lead to central nervous system disorders, circulatory problems, decreased immunity, and the development of diseases associated with oxidative stress.

In recent years, phytotherapeutic research aimed at strengthening human health through natural sources has been widely conducted. Special scientific attention has been given to plant extracts that are ecologically clean and rich in phenolic and flavonoid compounds, which can serve as natural sources of vitamins. Among these plants, poplar (Populus spp.) and willow (Salix spp.) have long been used in folk medicine as anti-inflammatory, antipyretic, and analgesic agents. Their composition includes tannins, organic acids, salicylates, flavonoids, and other biologically active compounds; however, their content of water-soluble vitamins has not been sufficiently studied.

The scientific novelty of this study lies in the fact that, for the first time, the water-soluble vitamins of a 1:1 mixture of poplar and willow were comprehensively analyzed using HPLC (High-Performance Liquid Chromatography). The aim of the research is to determine the vitamin composition of this mixture, evaluate the quantitative ratios of the vitamins, and substantiate their biological importance for human health.

This approach not only identifies the micronutrient potential of plant raw materials but also expands their possible applications in the pharmaceutical and nutraceutical industries as dietary supplements or components of functional foods.

Experimental Part. Reagents and Equipment. Vitamin B12 (Rhydburg Pharmaceuticals, Germany), vitamin C (Carl Roth GmbH, Germany), vitamin B9 (DSM Nutritional Products GmbX, Germany), and vitamins B1, B2, B3, B6, and PP (BLDPharm, China) were utilized in the study. HPLC-grade reagents-water, acetonitrile, glacial acetic acid, and sodium hydroxide of high chemical purity- were employed. The quantification of water-soluble vitamins in plant material was performed using an LC-40 Nexera Lite high-performance liquid chromatograph (Shimadzu, Japan) [1].

Preparation of Standard Solutions. Standard solutions 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) at 100 mg/L were prepared by dissolving 5 mg of each vitamin in 50 mL of 0.1 N HCl. For vitamins B2 (CAS 83-88-5) and B9 (CAS 59-30-3), 0.025 % sodium hydroxide solution was used to dissolve 5 mg in 50 mL. Subsequently, 200 µL of each initial B1, B6, B3, B12, and PP vitamin solution were mixed to obtain a combined standard with a concentration of 14.286 mg/L for each vitamin. Dilutions were then prepared to obtain 7.143, 3.571, and 1.786 mg/L solutions. Vitamin C standards were prepared at 286, 143, 71.5, and 57.2 mg/L. Distilled water was used as the 0 mg/L point in the calibration curve.

Preparation of the Sample Extract. For extraction of water-soluble vitamins, 1 g of the sample was weighed into a 50 mL conical flask and 25 mL of 0.1 N HCl was added. The mixture was subjected to ultrasonic extraction at 60 °C for 20 minutes using a GT SONIC-D3 ultrasound bath (China). After cooling, the mixture was filtered and brought to 25 mL with water in a volumetric flask. A 1.5 mL aliquot of the extract was filtered through a 0.22 µm syringe filter, transferred into a vial, and used for chromatographic analysis.

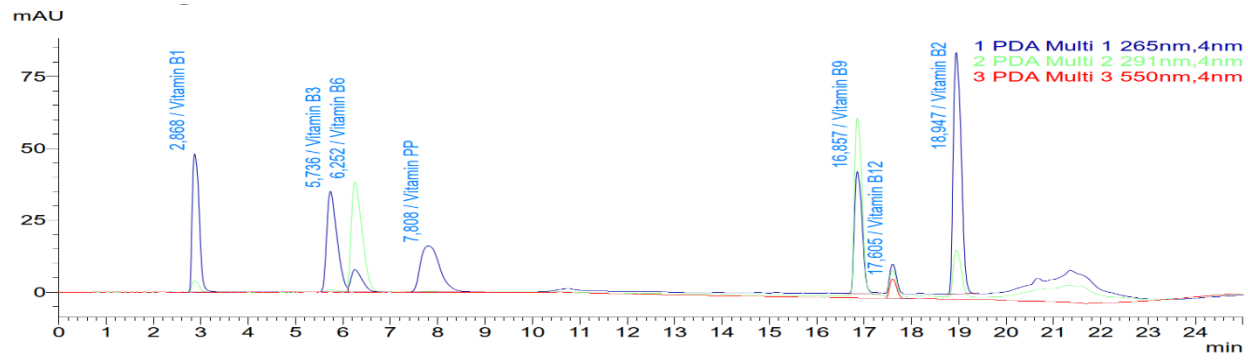
Chromatographic Conditions. Vitamin Determination. Standard solutions and the sample extract were analyzed on an LC-40 Nexera Lite HPLC system equipped with an LC-40D pump, SIL-40 autosampler, and SPD-M40 photodiode array detector (PDA), controlled by LabSolutions software version 6.92. A reversed-phase Shim-pack GIST C18 column (150 × 4.6 mm; 5 µm, Shimadzu, Japan) was used. The mobile phase consisted of acetonitrile (A) and 0.25 % aqueous acetic acid (B) in a gradient mode (Table 1). Injection volume was 10 µL, flow rate 0.6 mL/min, and column temperature 40 °C. Analytical signals were detected at 265, 291, and 550 nm (Figures 1-3). Vitamin C was determined using a 15-minute gradient (Table 2), with detection at 265 nm.

Gradient Program of the Mobile Phase for Vitamin Determination.

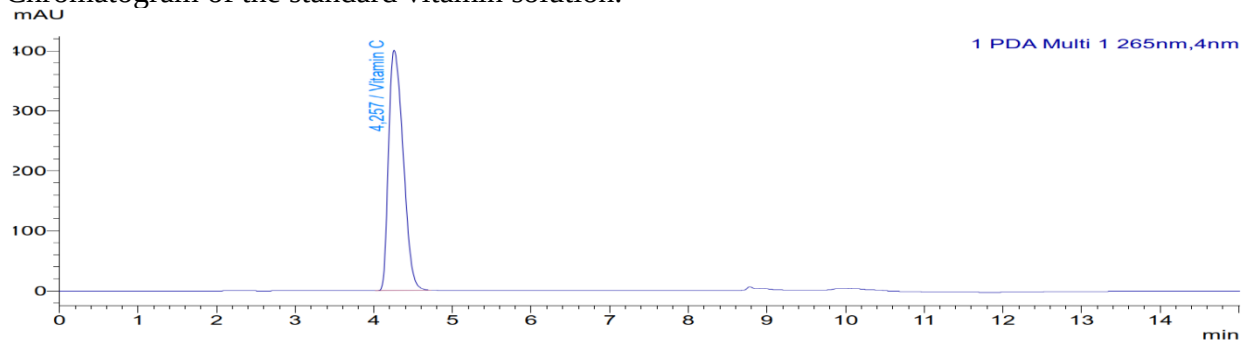
Время, мин	Acetonitrile (A), %	0.5% acetic acid (B), %
0	0	100
3	0	100
14	20	80
17	50	50
18	0	100
25	Завершение	

Vitamin C miqdorini aniqlashda harakatchan faza gradiyent dasturi.

Время, мин	Acetonitrile (A), %	0.5% acetic acid (B), %
0	0	100
2	0	100
6	50	50
6,01	0	100
15	Завершение	



Chromatogram of the standard vitamin solution.



Chromatogram of the standard vitamin C solution.

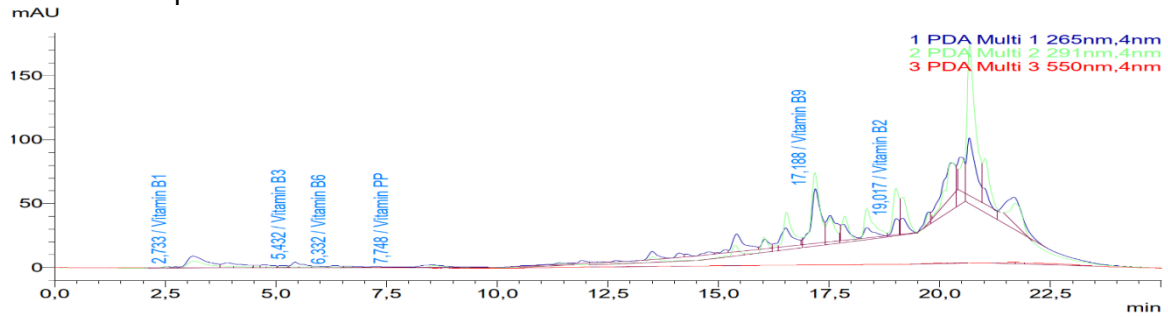
Results Obtained.

Determination of Vitamins in the Sample Extract. Chromatograms of the sample extract were obtained (Figures 3–4), and based on the results, the concentrations of vitamins in 100 g of the sample were calculated using the following formula. The calculated values are presented in Table 3.

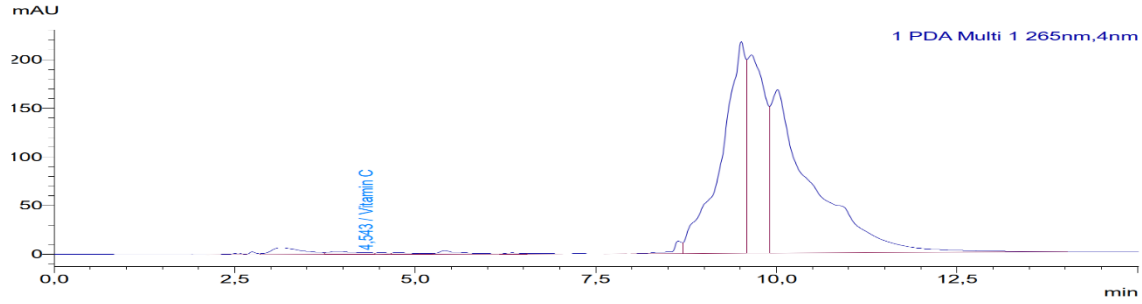
$$X = \frac{C_{vit} \cdot V_{ekstrakt}}{m_{namuna}} \cdot 100\ g$$

Results Obtained.

Determination of Vitamins in the Sample Extract.
Chromatograms of the sample extract were obtained (Figures 3–4), and based on the results, the concentrations of vitamins in 100 g of the sample were calculated using the following formula. The calculated values are presented in Table 3.



Chromatogram of vitamin determination in the sample extract.



Chromatogram of vitamin C determination in the sample extract.

Vitamin contents and their retention times in the extract.

Vitamin	Retention time, s	Concentration, mg/L	Content in 100 g of sample, mg
Vitamin B_1	2,733	0,275	0,688
Vitamin B_3	5,432	2,656	6,640
Vitamin PP	7,748	0,245	0,613
Vitamin B_9	17,188	19,275	48,188
Vitamin B_2	19,017	1,936	4,840
Vitamin B_6	6,332	0,129	0,323
Vitamin B_{12}	Not detected.	0	0,000
Vitamin C	4,543	0,731	1,828

Analysis of Results and Medical Significance. The analytical results demonstrated that the extract of the poplar–willow mixture contains seven water-soluble vitamins: B_1, B_2, B_3, B_6, B_9, PP, and C, while vitamin B_{12} was not detected. Among these, folate (B_9) showed the highest concentration (48.188 mg/100 g), indicating the hematopoietic and neuroprotective significance of the plant mixture. Folate plays a key role in DNA synthesis, red blood cell maturation, and neural tube formation during embryonic development.

Vitamins B_3 (niacin, 6.64 mg/100 g) and B_2 (riboflavin, 4.84 mg/100 g) are essential for maintaining optimal mitochondrial energy metabolism, redox balance, and lipid metabolism. They support the proper functioning of the musculoskeletal, cardiovascular, and nervous systems. Thiamine (B_1) and pyridoxine (B_6) are involved in neural impulse transmission, stress regulation, and normalization of muscle function.

Vitamin C (1.828 mg/100 g) activates the antioxidant defense system, neutralizes free radicals, and enhances immune protection. It is also a key biochemical factor in collagen synthesis, skin regeneration, and wound healing.

Considering the totality of results, the mixture of poplar and willow can be regarded as a promising phytocomposition that supports nervous and cardiovascular system function, strengthens immunity, and enhances antioxidant protection. It is recommended for inclusion in the diet of children, the elderly, and individuals with immune deficiencies or metabolic disorders.

Conclusion. Using High-Performance Liquid Chromatography (HPLC), the water-soluble vitamin composition of a mixture of poplar (Populus spp.) and willow (Salix spp.) was systematically and comprehensively analyzed for the first time. The extract was found to contain vitamins B_1, B_2, B_3, B_6, B_9, PP, and C, while vitamin B_{12} was not detected.

The identified vitamin complex performs vital biological functions across different physiological systems. Vitamin B_1 (thiamine) supports cardiac and neural function, acting as a coenzyme in energy metabolism. Vitamins B_2 (riboflavin) and B_3 (niacin) are integral to redox enzyme systems that generate metabolic energy. Vitamin B_6 (pyridoxine) regulates amino acid transamination and hormonal balance. Folate (B_9) contributes to DNA synthesis, erythropoiesis, and neural tube development, making it particularly important during pregnancy. Vitamin PP (nicotinamide) is a structural component of NAD/NADP coenzymes, which control cellular redox reactions and maintain metabolic stability. Vitamin C (ascorbic

acid) serves as a potent antioxidant, enhances immunity, stimulates collagen synthesis, and reinforces anti-inflammatory defense.

The findings suggest that this plant mixture, in addition to its vitamin content, possesses antioxidant and immunomodulatory properties. Therefore, the poplar willow extract can be considered not only a general health-promoting agent but also a potential functional food ingredient or pharmaceutical supplement. Future clinical and toxicological studies will allow scientific validation of its nutraceutical and phytotherapeutic effectiveness and expand its applications in the pharmaceutical field.

References

1. Asqarov I.R. va boshqalar. (2024). Suvda eruvchi vitaminlar miqdorini YuSSX usulida aniqlash metodikasi (Chilonjiyda misolida). Farg‘ona Davlat Universiteti, 30(5), 61.
2. Institute of Medicine (2000). Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. National Academies Press.
3. Ball, G. F. M. (2006). Vitamins in Foods: Analysis, Bioavailability, and Stability. CRC Press.
4. Zeisel, S. H. (2010). Choline: Critical Role During Fetal Development and Dietary Requirements in Adults. Annual Review of Nutrition, 29(1), 229-250.
5. Yadav, D. K., et al. (2019). Plant-based natural products as a tool for neuroprotection and cognitive enhancement. Phytomedicine, 55, 1-14.
6. Sivolapov A., Blagodarova T., Sivolapov V. Forty-five-year monitoring of selection stands of white (Populus alba L.), gray (Populus canescens Sm.), black poplar (Populus nigra L.), and willow (Salix alba L.) in Khoper State Natural Reserve //IOP Conference Series: Earth and Environmental Science. – IOP Publishing, 2019. – T. 226. – №. 1. – C. 012068.
7. Pajević S. et al. Phytoremediation capacity of poplar (Populus spp.) and willow (Salix spp.) clones in relation to photosynthesis //Archives of Biological Sciences. – 2009. – T. 61. №. 2. – C. 239-247.
8. Zalesny Jr R. S., Bauer E. O. Selecting and utilizing Populus and Salix for landfill covers: implications for leachate irrigation //International journal of phytoremediation. 2007. – T. 9. – №. 6. – C. 497-511.