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DETERMINATION OF PHENOLIC COMPOUNDS AND WATER-SOLUBLE VITAMINS IN THE EXTRACT OF A POPLAR (POPULUS ALBA) AND WILLOW (SALIX) MIXTURE BY HPLC METHOD AND EVALUATION OF THEIR BIOLOGICAL SIGNIFICANCE

Turdiboyev Azamjon Xasanboy ugli [1],
[1]Basic Doctoral Student, Fergana State University

Imomova Mukammal Yormuhamatovna [2]
[2]Doctor of Philosophy (PhD) in Chemistry,
Associate Professor, Fergana State University

ABSTRACT

This study presents a comprehensive analysis of **phenolic compounds and water-soluble vitamins** in an ethanolic extract obtained from a 1:1 mixture of poplar (Populus alba) and willow (Salix) using high-performance liquid chromatography (HPLC). The qualitative and quantitative determination of gallic acid, salicylic acid, rutin, quercetin, apigenin, as well as vitamins C, B1, B2, B3, B6, B9, and PP was performed.

The results revealed that **salicylic acid (38.053 mg/100 g)** and **rutin (12.970 mg/100 g)** were the predominant phenolic compounds, while **folic acid (vitamin B9, 48.188 mg/100 g)** was the most abundant water-soluble vitamin.

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Vitamin B12 was not detected, which is consistent with the plant origin of the raw materials.

These findings confirm that the poplar–willow mixture is rich in biologically active substances and represents a promising natural source for the development of **anti-inflammatory, antioxidant, and functional food or medicinal products**.

Keywords: Poplar, willow, phenolic compounds, water-soluble vitamins, HPLC, polyphenols.

INTRODUCTION

In recent years, the study of natural extracts rich in biologically active compounds derived from medicinal plants has become one of the priority directions in the pharmaceutical, medical, and functional food industries. In particular, phenolic compounds and water-soluble vitamins play an important role in reducing oxidative stress, strengthening the immune system, and regulating metabolic processes in the human body.

Phenolic compounds—flavonoids and phenolic acids—possess strong antioxidant, anti-inflammatory, and antimicrobial properties and play a significant role in the prevention of cardiovascular, oncological, and inflammatory diseases. Water-soluble vitamins, especially B-group vitamins and vitamin C, participate as coenzymes in metabolism, nervous system function, and immune defense.

Poplar (Populus alba) and willow (Salix) plants have long been widely used in traditional medicine. Salicylic acid found in willow served as the basis for the development of aspirin in modern pharmaceuticals, while poplar is recognized as a rich source of flavonoids and polyphenols. However, a

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comprehensive analysis of biologically active compounds in mixtures of these plants has not been sufficiently studied.

Therefore, the aim of this study is to identify phenolic compounds and water-soluble vitamins in extracts of a poplar–willow mixture using the HPLC method and to scientifically evaluate their biological significance.

EXPERIMENTAL PART

Reagents and instruments

Gallic acid was obtained from "Macklin" (China), salicylic acid from "Rhydburg Pharmaceuticals" (Germany), quercetin, apigenin, and kaempferol from "Regal" (China), while rutin was isolated from natural sources using extraction and column chromatography methods. HPLC-grade water and acetonitrile, as well as chemically pure acetic acid and sodium hydroxide, were used as reagents. The determination of polyphenol content in plant samples was carried out using an LC-40 Nexera Lite high-performance liquid chromatograph manufactured by Shimadzu (Japan).

Preparation of standard solutions

Gallic acid (5.2 mg), salicylic acid (5.2 mg), rutin (5 mg), quercetin (5 mg), apigenin (5 mg), and kaempferol (5 mg) were dissolved in 96% ethanol using an ultrasonic bath for 20 minutes, transferred into a 50 mL volumetric flask, and diluted to the mark with ethanol. From each solution, 200 µL was taken and mixed, and a total of four different solutions were prepared by serial dilution. Each solution was transferred into vials and used for analysis.

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Preparation of plant extracts

For the extraction of phenolic compounds, 1 g of the test sample was weighed with an accuracy of 0.01 g using an NV222 balance (OHAUS, USA), placed into a 50 mL conical flask, and 25 mL of 96% ethanol was added. The mixture was extracted in a GT SONIC-D3 ultrasonic bath (China) at 60 °C for 20 minutes. After cooling, the mixture was filtered and diluted to 25 mL with ethanol in a volumetric flask. A 1.5 mL aliquot of the extract was centrifuged at 7000 rpm using a Mini-7 centrifuge (BIOBASE, China) and filtered through a 0.45 µm syringe filter before analysis.

Table 1. Mobile phase gradient program.

Time	Acetonitrile (A), %	0.5% Acetic acid (B), %
0	5	95
5	5	95
17	40	60
22	40	60
22,1	5	95
40	Tugatish	

Chromatographic conditions

Determination of phenolic compounds.

Standard solutions and sample extracts were analyzed using a Shim-pack GIST C18 reversed-phase column (150 × 4.6 mm, 5 µm; Shimadzu, Japan) with a gradient mobile phase consisting of acetonitrile (A) and a 0.5% aqueous solution of acetic acid (B) according to the gradient program shown in Table 1. The injection volume was 10 µL, the flow rate was set to 0.5 mL/min, and the column thermostat temperature was maintained at 40 °C. The analytical signal of phenolic compounds (peak area) was recorded at 300 nm (Figure 1).

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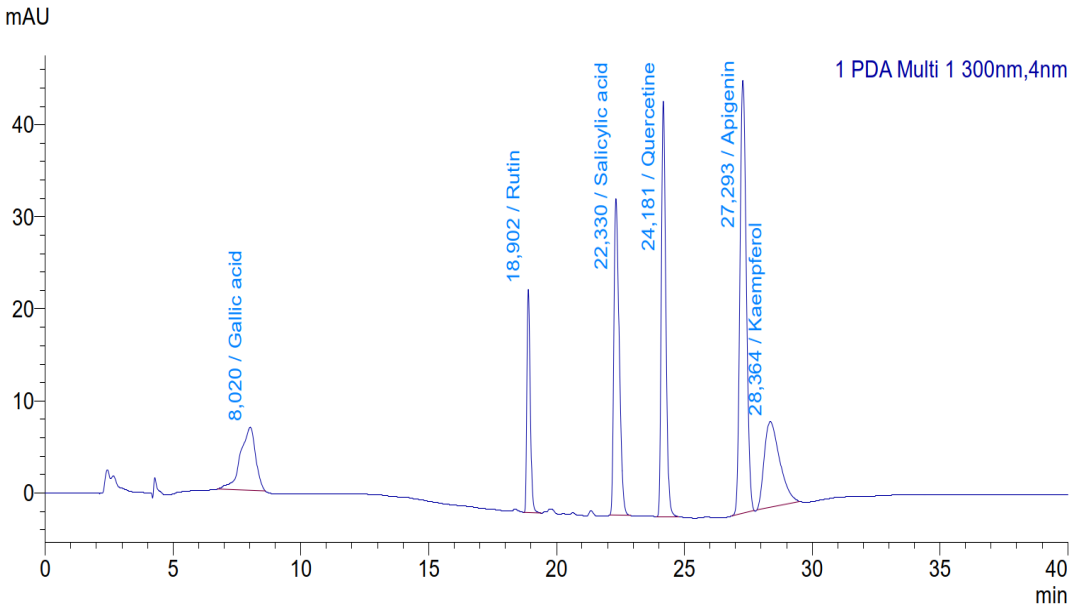

Figure 1. Chromatogram of standards recorded at 300 nm.

Results obtained

Determination of phenolic compound content in the sample extract. A chromatogram of the sample extract with a mass of 1 g was obtained (Figure 2), and based on the results, the amounts of phenolic compounds in 100 g of the sample were calculated using the following formula and are presented in Table 3.

$$X = \frac{C_{phen} \cdot V_{ectract}}{m_{sample}} \cdot 100\ g$$

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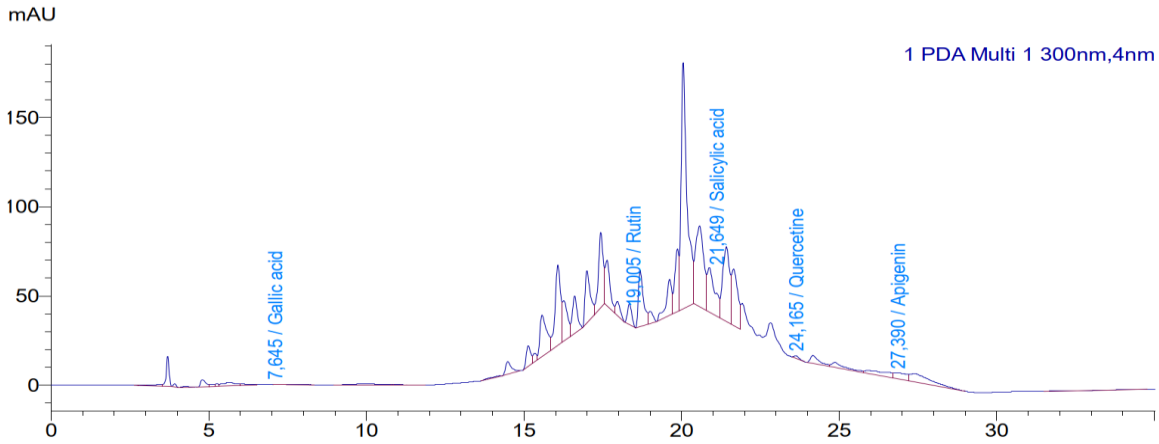

Figure 2. Chromatogram for the determination of polyphenols in the sample extract.

Table 2. Content and retention times of polyphenols in the extract.

Phenolic compound	**Retention time, s**	**Concentration, mg/L**	**Content in 100 g of sample, mg**
Gallic acid	7.645	0.31	0.775
Rutin	19.005	5.188	12.970
Salicylic acid	21.649	15.221	38.053
Quercetin	24.165	2.478	6.195
Apigenin	27.390	4.537	11.343
Kaempferol	Not detected	0	0.000

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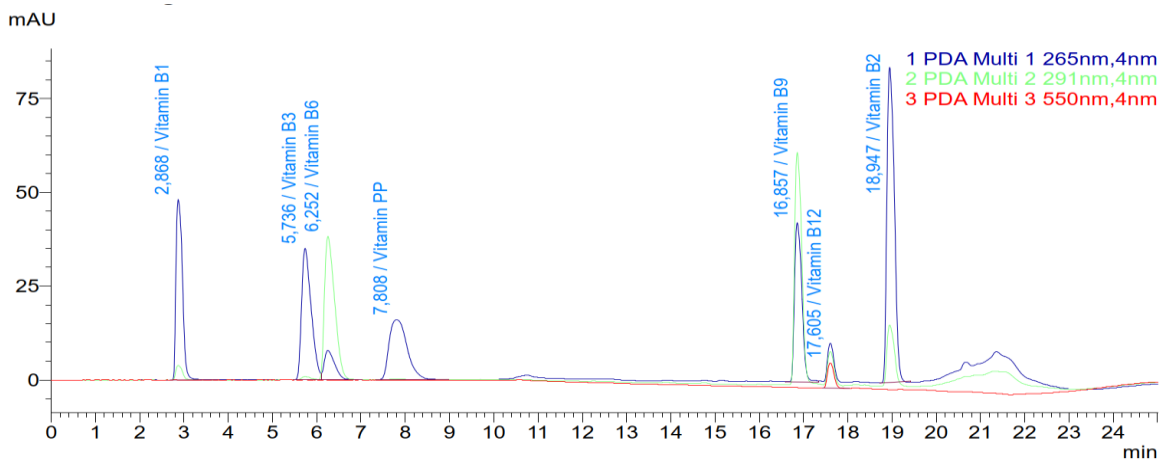

Figure 3. Chromatogram of the standard vitamin solution.

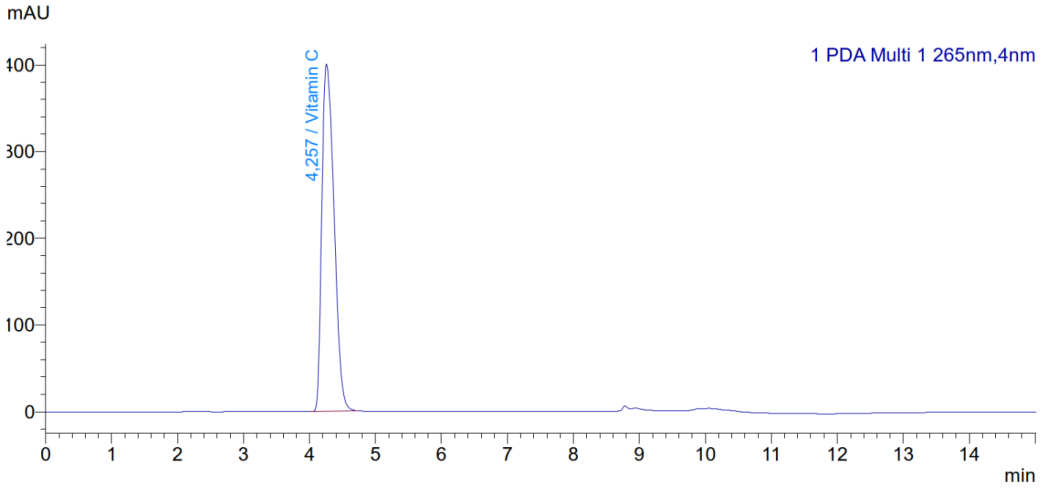

Figure 4. Chromatogram of the standard vitamin C solution.

A chromatogram of the sample extract was obtained, and based on the results, the amounts of vitamins in 100 g of the sample were calculated and presented in Table 1.

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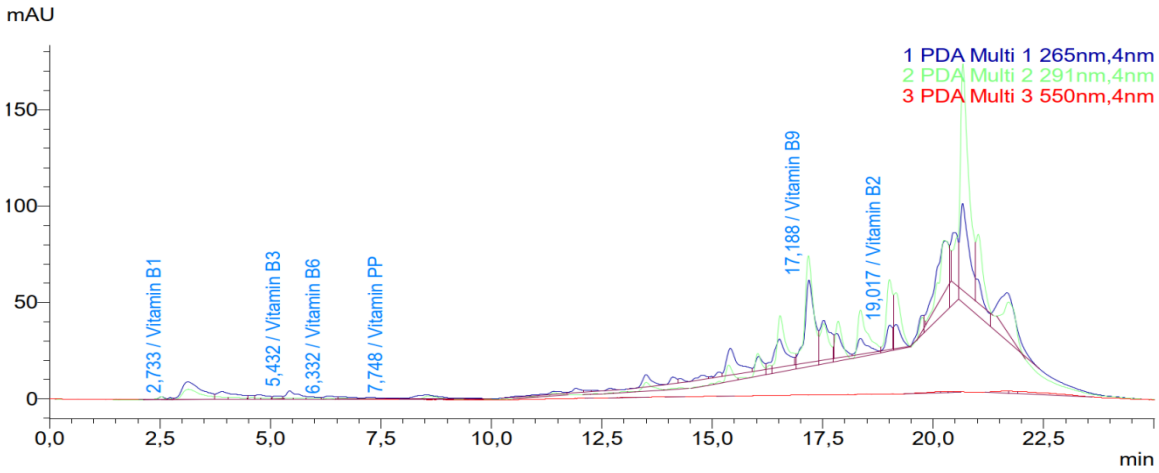

Figure 5. Chromatogram for the determination of vitamins in the sample extract.

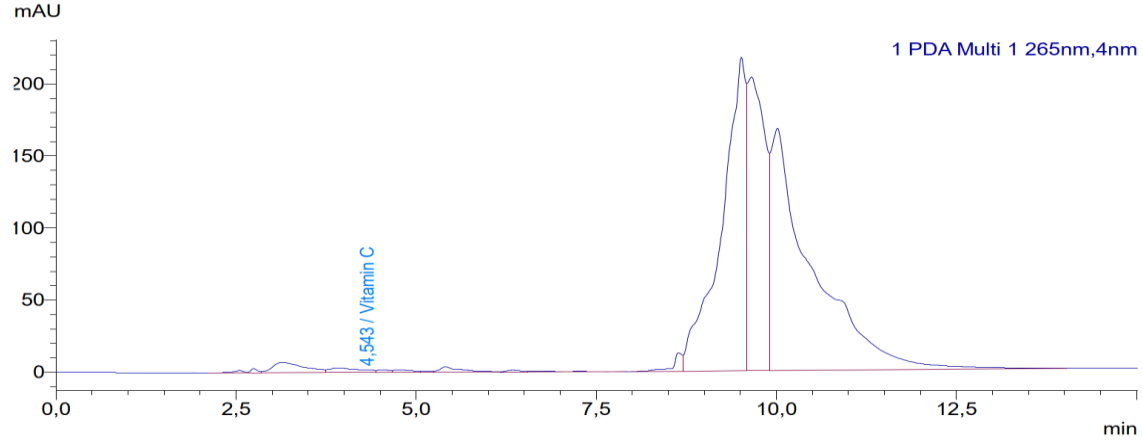

Figure 6. Chromatogram for the determination of vitamin C in the sample extract.

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

Vitamin	Retention time, s	Concentration, mg/L	Content in 100 g of sample, mg	Vitamin
Vitamin B_1	2.733	0.275	0.688	Vitamin B_1
Vitamin B_3	5.432	2.656	6.640	Vitamin B_3
Vitamin PP	7.748	0.245	0.613	Vitamin PP
Vitamin B_9	17.188	19.275	48.188	Vitamin B_9
Vitamin B_2	19.017	1.936	4.840	Vitamin B_2
Vitamin B_6	6.332	0.129	0.323	Vitamin B_6
Vitamin B_{12}	Not detected	0	0.000	Vitamin B_{12}
Vitamin C	4.543	0.731	1.828	Vitamin C

RESULTS AND DISCUSSION

In this study, the ethanol extract obtained from a mixture of poplar (Populus alba) and willow (Salix) plants was comprehensively analyzed for phenolic compounds and water-soluble vitamins using high-performance liquid chromatography (HPLC). HPLC provides high accuracy, sensitivity, and reproducibility in determining both qualitative and quantitative compositions of bioactive compounds in complex plant matrices. The selected chromatographic conditions allowed for efficient separation and reliable identification of phenolic compounds and vitamins.

The analysis of phenolic compounds revealed the presence of gallic acid, rutin, salicylic acid, quercetin, and apigenin in the extract. Among these, salicylic acid was found in the highest concentration (38.053 mg/100 g), confirming the scientifically recognized significance of willow as a natural source of salicylic acid. Salicylic acid is known for its anti-inflammatory, analgesic, and antipyretic properties, which substantially enhance the potential pharmacological value of the extract.

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Rutin, the second most abundant compound (12.970 mg/100 g), plays a key role as a strong antioxidant, strengthening capillary walls, improving blood circulation, and reducing the effects of free radicals. The relatively high content of rutin indicates a strong antioxidant activity of the poplar–willow mixture.

Significant amounts of apigenin (11.343 mg/100 g) and quercetin (6.195 mg/100 g) were also detected, contributing notably to the extract's biological activity. These flavonoids are recognized for their anti-inflammatory, antimicrobial, and cell-protective properties. Although gallic acid was present in smaller amounts, its strong antioxidant activity contributes to the overall bioactivity of the extract. Kaempferol was not detected, which may be due to its concentration being below the detection limit or its absence in the sample.

Analysis of water-soluble vitamins revealed the presence of vitamins C, B_1, B_2, B_3, B_6, B_9, and PP in the extract. Among these, folate (vitamin B_9) was present in the highest amount (48.188 mg/100 g), highlighting the extract's potential role in supporting hematopoiesis, DNA synthesis, and nervous system function. The absence of vitamin B_{12} is consistent with the natural characteristics of plant raw materials.

Overall, the co-existence of phenolic compounds and water-soluble vitamins indicates a synergistic biological effect of the poplar–willow extract, suggesting its potential as a natural anti-inflammatory, antioxidant, and metabolically active agent.

The results demonstrated that phenolic compounds and vitamins are unevenly distributed in the extract. Salicylic acid and rutin were dominant among phenolic compounds, further confirming the pharmacological importance of willow and poplar. The high content of folate (B_9) among vitamins suggests that the extract may serve as a promising supplement during pregnancy, for

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anemia prevention, and in support of nervous system function. The combined presence of phenolic compounds and vitamins enhances the extract's synergistic biological activity.

CONCLUSION

In this study, the ethanol extract obtained from a 1:1 mixture of poplar (Populus alba) and willow (Salix) was thoroughly analyzed for phenolic compounds and water-soluble vitamins using high-performance liquid chromatography (HPLC). The results confirmed that HPLC allows accurate, sensitive, and reproducible analysis of bioactive compounds in complex plant extracts.

The analysis identified gallic acid, rutin, salicylic acid, quercetin, and apigenin, with salicylic acid being the most abundant (38.053 mg/100 g), confirming the scientifically based pharmacological significance of willow's anti-inflammatory and analgesic properties. This indicates the potential of the poplar–willow extract as a raw material for natural anti-inflammatory agents. The significant content of rutin (12.970 mg/100 g) demonstrates the extract's high antioxidant potential. Rutin supports capillary health, reduces oxidative stress, and promotes cardiovascular function. Apigenin and quercetin also contribute to the extract's overall biological activity through anti-inflammatory, antimicrobial, and cell-protective effects.

Although gallic acid was present in lower amounts, its strong antioxidant properties enhance the overall activity of phenolic compounds. The absence of kaempferol may indicate its nonexistence or presence below the detection limit.

The analysis of water-soluble vitamins showed the presence of C, B_1, B_2, B_3, B_6, B_9, and PP, with folate (B_9) being particularly abundant (48.188 mg/100

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g), making the extract a valuable natural source for supporting pregnancy, anemia prevention, and nervous system function. The absence of vitamin B_{12} is consistent with the natural composition of plant materials.

The co-presence of phenolic compounds and water-soluble vitamins confirms the synergistic biological effects of the extract. This synergy enhances its antioxidant, anti-inflammatory, and metabolic-supporting properties, making the extract a promising component for pharmaceutical and functional food applications.

In summary, the results scientifically confirm that the poplar–willow mixture is rich in bioactive compounds and possesses high practical significance. Future studies should explore the in vivo and in vitro pharmacological activity of this extract and its synergistic interactions with other bioactive components.

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