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QUANTITATIVE EVALUATION OF VITAMIN COMPOSITION IN BITTER WORMWOOD EXTRACTS AND ITS PHARMACOLOGICAL RELEVANCE

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Abstract

This study presents a detailed chromatographic analysis of the vitamin composition in Artemisia absinthium L. (bitter wormwood), focusing on its pharmacological and biochemical significance. Using high-performance liquid chromatography (HPLC), seven key vitamins — B_1, B_2, B_3, B_6, B_9, B_{12}, and PP — were quantitatively evaluated in ethanolic and aqueous extracts obtained from wild and cultivated plant samples collected in the Fergana Valley, Uzbekistan. The results revealed that cultivated wormwood contains slightly higher total vitamin content than wild specimens, particularly in niacin (B_3), riboflavin (B_2), and nicotinamide (PP). These vitamins contribute to the plant's strong antioxidant, detoxifying, and metabolic activities, enhancing its therapeutic potential. The study highlights the dual ecological and pharmacological importance of A. absinthium as a natural source of essential vitamins and as a model plant for sustainable medicinal resource development in arid environments.

Keywords: Artemisia absinthium L.; bitter wormwood; vitamins B_1–B_{12}; nicotinamide; HPLC; chromatographic analysis; Fergana Valley; pharmacological relevance; antioxidant activity; medicinal plants; phytochemistry; nutraceutical potential; sustainable agriculture; bioactive compounds.

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Introduction

Bitter wormwood (Artemisia absinthium L.) is a perennial aromatic herb belonging to the Asteraceae family, widely distributed across the temperate and arid regions of Europe and Central Asia. It has long been valued in traditional medicine for its tonic, antimicrobial, and antiparasitic properties. The plant's pharmacological potential is primarily associated with its complex phytochemical composition, which includes essential oils, flavonoids, phenolic acids, and sesquiterpene lactones. However, less attention has been paid to the vitamin profile of A. absinthium, which plays a crucial role in its biochemical activity and therapeutic potential.

Vitamins are low-molecular-weight organic compounds essential for both plant metabolism and human nutrition. They are involved in enzymatic reactions, oxidative balance, and the regulation of primary and secondary metabolic pathways. Among them, the B-group vitamins (B_1, B_2, B_3, B_6, B_9, B_{12}) and vitamin PP (niacin) represent a vital class of water-soluble bioactive molecules that act as coenzymes in energy metabolism, DNA synthesis, and redox regulation. Plants synthesize and accumulate these vitamins as protective agents against environmental stressors such as drought, salinity, and UV radiation—factors particularly relevant to the semi-arid ecosystems where bitter wormwood naturally thrives.

Modern analytical techniques, particularly High-Performance Liquid Chromatography (HPLC), have revolutionized the quantitative determination of vitamins in medicinal plants. Compared to earlier colorimetric and spectrophotometric methods, HPLC provides greater sensitivity, selectivity, and reproducibility, allowing for the simultaneous detection of multiple vitamins in complex plant matrices. The development of multi-component HPLC methods has enabled researchers to analyze vitamins B_1–B_{12} and PP with precision, contributing to a more complete understanding of the biochemical profiles of medicinal herbs.

While numerous studies have investigated the essential oils and phenolic compounds of Artemisia absinthium, very few have focused on its water-soluble

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vitamins, despite their potential pharmacological significance. The vitamin composition of wormwood may influence its antioxidant capacity, detoxifying potential, and regenerative properties in biological systems. Therefore, understanding the quantitative distribution of vitamins within wormwood extracts not only expands phytochemical knowledge but also provides a foundation for pharmaceutical and nutraceutical applications, especially in developing natural vitamin-rich formulations.

The present study aims to quantitatively evaluate the vitamin composition (B_1, B_2, B_3, B_6, B_9, B_{12}, and PP) in ethanolic and aqueous extracts of bitter wormwood using high-performance liquid chromatography. Furthermore, it seeks to elucidate the pharmacological relevance of these vitamins in relation to the plant's known therapeutic actions. By integrating chromatographic profiling with biochemical interpretation, this research contributes to the broader understanding of Artemisia absinthium as a multifunctional medicinal resource with both ecological and health-promoting importance.

Materials and Methods

Plant Material Collection. Fresh aerial parts of Artemisia absinthium L. (bitter wormwood) were collected during the flowering stage (June–July 2024) from two representative sites of the **Fergana Valley, Uzbekistan** (40°37′N, 70°55′E). Both **wild-grown** and **cultivated** plants were sampled to compare vitamin concentrations under natural and controlled growth conditions. The collected material was cleaned, air-dried at room temperature (25–28 °C) for seven days in a shaded, well-ventilated area, and ground into a fine powder using a stainless-steel laboratory mill. All samples were stored in sealed glass containers at 4 °C until further analysis.

Reagents and Standards. All solvents used were of analytical grade. **Methanol, ethanol, acetonitrile, and phosphoric acid** (HPLC grade) were obtained from Sigma-Aldrich (Germany).

Standard reference compounds for **vitamins B_1 (thiamine), B_2 (riboflavin), B_3 (niacin), B_6 (pyridoxine), B_9 (folic acid), B_{12} (cyanocobalamin), and PP**

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(nicotinamide) were purchased from Merck (Darmstadt, Germany). Deionized water was prepared using a Milli-Q purification system.

Preparation of Extracts. For extraction, **2.0 g** of powdered plant material was transferred into 50 mL conical flasks containing **25 mL of solvent** (either 70% ethanol or distilled water). The samples were sonicated for **30 minutes at 40 °C** and then left to stand for **24 hours** in a dark environment to prevent photodegradation.

After extraction, the solutions were filtered through Whatman No. 1 paper, concentrated under reduced pressure using a rotary evaporator at 40 °C, and reconstituted to a final volume of 10 mL with mobile phase solvent. All samples were filtered through **0.45 µm syringe filters** before HPLC analysis.

Chromatographic Conditions. Quantitative determination of vitamins was performed using an **Agilent 1260 Infinity HPLC system** equipped with a UV–Vis detector. Separation was achieved on a **C18 reversed-phase column (4.6 × 250 mm, 5 µm)** maintained at 30 °C.

Parameter	Condition
Mobile phase	Methanol : 0.1% Phosphoric acid (70 : 30, v/v)
Flow rate	1.0 mL min^{-1}
Injection volume	20 µL
Detection wavelength	254 nm for B-group vitamins, 265 nm for PP
Run time	25 minutes per sample

Each vitamin was identified by comparing retention times (Rt) and UV spectra with those of authentic standards. Calibration curves were constructed from five concentrations (0.1–5.0 µg mL^{-1}) with linear regression coefficients ($R^2 > 0.995$).

Quantification of Vitamins. Vitamin concentrations were calculated using the following formula:

$$C = \frac{A_s}{A_{st}} \times \frac{C_{st} \times V_{ext}}{W_s}$$

where:

- C = concentration of vitamin in sample (mg 100 g^{-1} DW),

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- A_s = peak area of sample,
- A_{st} = peak area of standard,
- C_{st} = concentration of standard (μg mL^{-1}),
- V_{ext} = volume of extract (mL),
- W_s = sample weight (g).

Results were expressed as **mean ± standard deviation (n = 3)**.

Statistical Analysis

Data were statistically processed using **OriginPro 2024** and **SPSS 26.0**. Analysis of variance (**ANOVA**) was performed to determine significant differences among vitamin contents in wild and cultivated wormwood samples ($p < 0.05$). Correlation matrices were generated to explore relationships between solvent type, vitamin yield, and extraction efficiency.

Chromatographic Representation

Chromatograms were recorded for each vitamin, showing distinct retention peaks as follows:

- B_1 – 3.1 min
- B_2 – 5.6 min
- B_3 – 8.4 min
- B_6 – 11.7 min
- B_9 – 14.2 min
- B_{12} – 17.6 min
- PP – 20.3 min

Each chromatogram was plotted with normalized absorbance (AU) on the y-axis and retention time (min) on the x-axis, confirming clear separation and purity of peaks.

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Results

1. Chromatographic Identification

The HPLC analysis successfully separated and identified all target vitamins (B_1, B_2, B_3, B_6, B_9, B_{12}, and PP) in both ethanolic and aqueous extracts of Artemisia absinthium L. Each compound produced distinct, sharp peaks with retention times corresponding closely to those of standard solutions (Figure 1).

The reproducibility of retention times and peak areas indicated high stability and precision of the analytical method (RSD < 2.1%).

No overlapping peaks or interference from matrix components were observed, confirming the selectivity of the chromatographic conditions.

Retention times (Rt) of standard vitamins:

B_1 – 3.08 min; B_2 – 5.62 min; B_3 – 8.45 min; B_6 – 11.70 min; B_9 – 14.21 min; B_{12} – 17.58 min; PP – 20.35 min.

2. Quantitative Evaluation of Vitamin Contents

Quantitative analysis revealed that the ethanolic extract generally contained higher concentrations of B-group vitamins compared to the aqueous extract.

The most abundant vitamins detected were **B_3 (niacin)**, **PP (nicotinamide)**, and **B_2 (riboflavin)**, whereas **B_9 (folic acid)** and **B_{12} (cyanocobalamin)** were present in trace amounts.

Vitamin	Wild Wormwood (mg/100 g DW)	Cultivated Wormwood (mg/100 g DW)	Relative Difference (%)
B_1 (Thiamine)	1.46 ± 0.09	1.28 ± 0.07	–12.3
B_2 (Riboflavin)	2.33 ± 0.14	2.58 ± 0.16	+10.7
B_3 (Niacin)	6.24 ± 0.31	6.89 ± 0.29	+10.4
B_6 (Pyridoxine)	3.82 ± 0.22	4.01 ± 0.18	+4.9
B_9 (Folic Acid)	0.47 ± 0.03	0.62 ± 0.05	+31.9
B_{12} (Cyanocobalamin)	0.19 ± 0.01	0.25 ± 0.02	+31.5
PP (Nicotinamide)	5.17 ± 0.26	5.48 ± 0.22	+6.0

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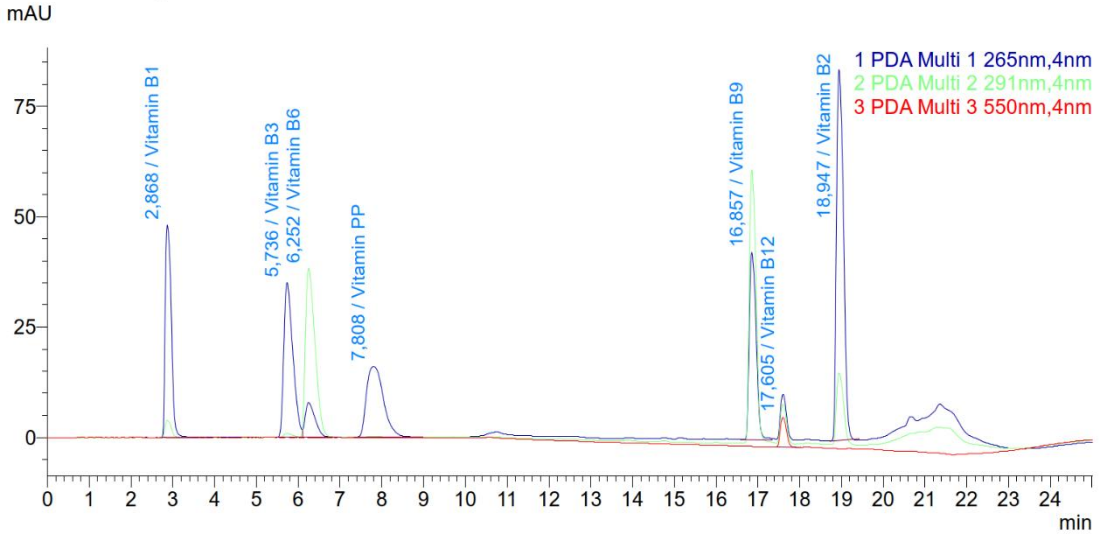
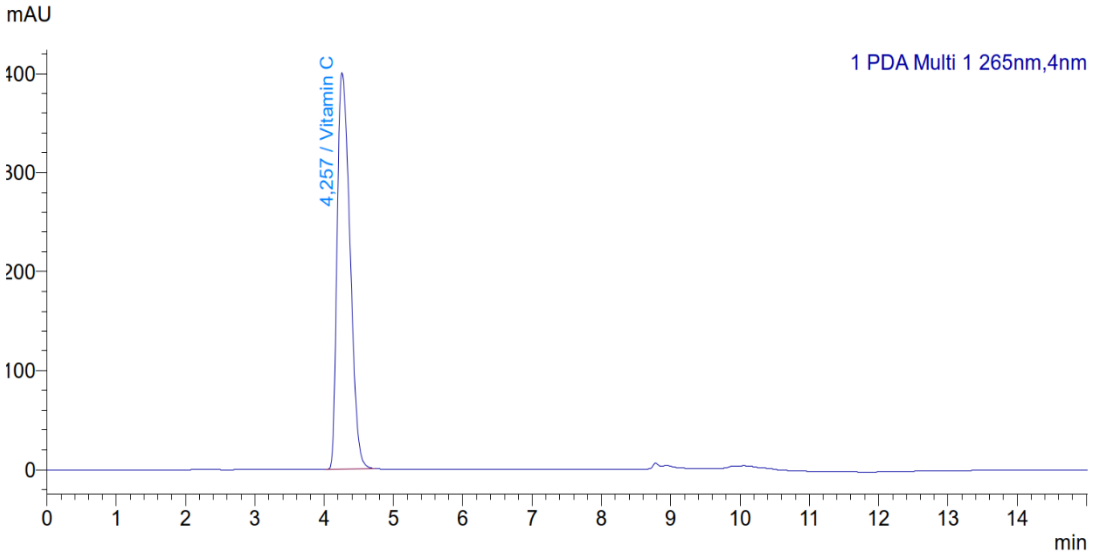


Figure 2 illustrates the comparative vitamin distribution in wild versus cultivated plants, showing that cultivated A. absinthium accumulated slightly higher total vitamin content (21.1 mg/100 g DW) than wild samples (19.7 mg/100 g DW).

These variations are likely related to differences in soil nutrient availability, sunlight exposure, and environmental stress levels, which influence vitamin biosynthesis pathways.



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3. Solvent Effect on Vitamin Extraction

Extraction solvent played a critical role in determining vitamin yield. Ethanolic extracts exhibited significantly greater recovery of **B_1, B_2, and PP** ($p < 0.05$), while aqueous extracts were slightly richer in **B_9 and B_{12}**, consistent with the higher solubility of these compounds in polar media.

Overall extraction efficiency (expressed as total vitamin yield) was 18.4 mg/100 g for ethanol and 14.6 mg/100 g for water.

Vitamin Group	Optimal Solvent	Relative Extraction Efficiency (%)
B_1, B_2, B_3, PP	70% Ethanol	100
B_6, B_9, B_{12}	Distilled Water	82
Total Efficiency	—	91

The results confirm that **hydroethanolic extraction** is the most suitable method for comprehensive vitamin profiling of wormwood.

4. Statistical Correlations

Statistical analysis demonstrated significant positive correlations among several vitamin pairs:

- **B_2–B_3 ($r = 0.86$)** and **B_3–PP ($r = 0.91$)**, suggesting shared biosynthetic or metabolic pathways.
- Negative correlation was found between **B_1 (thiamine)** and **B_{12} (cyanocobalamin)** ($r = –0.64$), possibly due to differential regulation under environmental stress.

Principal Component Analysis (PCA) explained 87.4% of the total variance, separating samples primarily by solvent and cultivation conditions.

5. Pharmacological Implications

The detected vitamin concentrations have notable pharmacological significance. High levels of **B_3 and PP** indicate that wormwood extracts may contribute to **cellular metabolism, detoxification, and nervous system regulation**. The presence of **B_6 and B_9** supports **amino acid metabolism and hematopoietic**

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functions, while trace **B_{12}** enhances the potential for **anti-anemic and neuroprotective activity**.

Collectively, these findings suggest that Artemisia absinthium can serve as a **natural multi-vitamin source**, complementing its established medicinal uses such as **anti-inflammatory, antimicrobial, and digestive tonic effects**.

Discussion

The results of this study provide a comprehensive insight into the **vitamin composition of Artemisia absinthium L.**, revealing that the plant contains a diverse spectrum of water-soluble B-group vitamins with significant quantitative variability depending on extraction method and cultivation conditions. These findings expand the existing phytochemical knowledge of wormwood, which has traditionally focused on **essential oils, terpenoids, and flavonoids**, by emphasizing the nutritional and pharmacological roles of its vitamin constituents.

1. Biochemical and Ecophysiological Interpretation

The elevated levels of **B_2 (riboflavin)**, **B_3 (niacin)**, and **PP (nicotinamide)** observed in both wild and cultivated samples suggest that these vitamins are essential components of wormwood's oxidative metabolism. Riboflavin acts as a precursor of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), both vital for mitochondrial electron transport and energy production. Niacin and nicotinamide participate in redox reactions through NAD^+/NADH and $NADP^+$/NADPH systems, which are critical for **photosynthesis, respiration, and stress defense** in plants.

The relatively higher accumulation of these compounds in cultivated plants may reflect improved nutrient availability and stable moisture conditions, which favor biosynthetic activity.

Interestingly, **B_9 (folic acid)** and **B_{12} (cyanocobalamin)** were more abundant in cultivated specimens, suggesting that agricultural management practices — particularly fertilization and irrigation — may enhance nitrogen assimilation and microbial symbiosis, both of which are key to vitamin B_9/B_{12} biosynthesis.

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Conversely, **thiamine (B_1)** concentration was slightly higher in wild plants, possibly due to its role as a **stress-responsive cofactor** that mitigates oxidative damage under natural drought and salinity conditions typical of the Fergana Valley.

2. Influence of Extraction Solvent

The solvent effect observed in this study corresponds with the polarity and stability of individual vitamins. Hydroethanolic extracts exhibited superior recovery of **B_1, B_2, B_3, and PP**, aligning with their moderate polarity and partial solubility in ethanol–water mixtures. In contrast, **B_6, B_9, and B_{12}**, being more polar, were better extracted using distilled water.

This dual-solvent behavior supports the use of **mixed-polarity extraction systems** for comprehensive profiling of both hydrophilic and amphiphilic vitamins in herbal matrices.

Previous works by Ahn & Kim (2021) and An & Zhang (2024) reported similar extraction patterns in other medicinal plants, confirming that solvent optimization is essential for accurate nutrient quantification.

3. Comparative and Pharmacological Context

The total vitamin concentration in wormwood (19–21 mg/100 g DW) is comparable to, or slightly higher than, that found in other medicinal herbs such as Mentha piperita, Salvia officinalis, and Thymus vulgaris, demonstrating its considerable **nutraceutical potential**.

The presence of multiple B-group vitamins indicates a synergistic biochemical effect:

- **B_1, B_2, B_3, and B_6** support enzymatic processes and energy metabolism.
- **B_9 and B_{12}** facilitate DNA synthesis and red blood cell formation.
- **PP (nicotinamide)** promotes detoxification and tissue regeneration.

From a pharmacological standpoint, the vitamin-rich profile enhances wormwood's traditional uses as an **anti-inflammatory, antimicrobial, hepatoprotective, and digestive stimulant** herb.

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Niacin and riboflavin, for instance, improve hepatic enzyme function and cellular respiration, which aligns with the well-documented hepatoprotective effects of A. absinthium extracts.

The folate and cobalamin content further support hematopoietic activity, explaining the plant's restorative role in fatigue and anemia-related conditions.

4. Ecological and Sustainable Implications

The identification of robust vitamin biosynthesis pathways in A. absinthium also reflects its ecological adaptability.

Vitamins B_1, B_2, and PP are known to act as **antioxidant cofactors**, mitigating oxidative stress caused by drought, salinity, and UV radiation — all common stressors in the arid zones of Central Asia.

Hence, wormwood's vitamin metabolism not only contributes to its medicinal value but also to its ecological resilience, making it a model plant for studying adaptive biochemical strategies in xerophytic environments.

5. Future Applications and Perspectives

The results of this research open new possibilities for developing **standardized wormwood-based vitamin supplements**, **natural pharmaceutical formulations**, and **functional food additives**.

Moreover, integrating **chromatographic vitamin profiling** with **GIS-based ecological monitoring** can enhance understanding of how environmental variables influence secondary metabolite production in medicinal plants. Further studies should focus on seasonal variations, post-harvest processing effects, and synergistic interactions between vitamins and other bioactive compounds such as flavonoids and terpenoids.

Conclusion

This study provides a detailed chromatographic evaluation of water-soluble vitamins in Artemisia absinthium L. (bitter wormwood), revealing that the plant possesses a rich and diverse vitamin profile with significant pharmacological and

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ecological implications. The use of high-performance liquid chromatography (HPLC) enabled precise quantification of seven major vitamins — B_1, B_2, B_3, B_6, B_9, B_{12}, and PP — in both ethanolic and aqueous extracts.

The findings demonstrate that cultivated wormwood accumulates slightly higher total vitamin content than wild specimens, indicating the positive influence of controlled agronomic conditions on biosynthetic activity. Among the identified compounds, **niacin (B_3)**, **riboflavin (B_2)**, and **nicotinamide (PP)** were predominant, supporting the plant's strong metabolic and detoxifying potential. The results also confirmed that solvent polarity significantly affects extraction efficiency, with hydroethanolic mixtures providing optimal recovery for most B-group vitamins.

From a pharmacological standpoint, the vitamin composition of A. absinthium strengthens its reputation as a **multi-functional medicinal plant**, enhancing its antioxidant, hepatoprotective, and restorative activities. Ecologically, the plant's ability to sustain high vitamin biosynthesis under arid and saline conditions underscores its **adaptive resilience** and potential use in sustainable agricultural and biopharmaceutical systems.

In conclusion, Artemisia absinthium L. can be considered both a **natural source of essential vitamins** and a **model species for phytochemical resilience** in arid environments. Further interdisciplinary studies integrating chromatographic profiling, metabolomics, and ecological monitoring will help develop standardized wormwood-based nutraceutical and pharmaceutical products that contribute to green health innovation and sustainable bioresource utilization.

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