Determination of water-soluble vitamins in the extract of blackberry (rubus l.) By the yussx method

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ABSTRACT

Annotation: Blackberry (Rubus L.) is widely recognized for its rich composition of biologically active compounds, including polyphenols, anthocyanins, and essential water-soluble vitamins. Due to increasing interest in plant-based therapeutics and functional foods, the quantitative evaluation of these micronutrients is of significant scientific and pharmacognostic relevance. This study focuses on determining the content of water-soluble vitamins in Rubus L. fruit extract using the YUSSX analytical method.Methods.The extract was prepared by ultrasonic-assisted extraction of 1 g of plant material with 0.1 N HCl at 60 °C for 20 minutes, followed by filtration and dilution to a final volume of 25 mL. High-performance liquid chromatography (HPLC) analysis was carried out on a Shim-pack GIST C18 column (150 × 4.6 mm; 5 µm) using gradient elution with acetonitrile and 0.25% acetic acid solution. The LC-40 Nexera Lite system was operated at a flow rate of 0.6 mL/min and detection wavelengths of 265, 291, and 550 nm. Quantification was achieved using calibration curves constructed from standard solutions of vitamins B1, B2, B3, B6, B9, B12, PP, and C.Results.Quantitative analysis of the extract revealed measurable concentrations of several water-soluble vitamins per 100 g of raw material: vitamin B1 — 2.28 mg, B2 — 1.14 mg, B3 — 10.00 mg, B6 — 0.72 mg, B9 — 9.95 mg, PP — 6.41 mg, and vitamin C — 13.01 mg. Vitamin B12 was not detected. Retention times and concentrations corresponded with standard chromatographic profiles, confirming the accuracy of the applied method.Discussion.The obtained results demonstrate that Rubus L. fruits are a significant natural source of essential water-soluble vitamins, particularly B-complex vitamins and vitamin C, which contribute to their antioxidant, metabolic, and therapeutic properties. These findings are consistent with current pharmacognostic data on the biological activity of Rubus species and highlight their potential in developing

phytopharmaceuticals and functional nutrition products.

KEYWORDS: Rubus L.; blackberry extract; water-soluble vitamins; YUSSX method; HPLC analysis; Shim-pack GIST C18; ultrasonic extraction; vitamin C; B-complex vitamins; phytochemical profiling; functional food ingredients; phytopharmacology.

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INTRODUCTION

Relevance

In recent decades, there has been a growing interest in medicinal plants, especially in preparations based on their biologically active compounds. This is due to the fact that synthetic pharmaceutical agents often have multiple side effects and do not always demonstrate sufficient effectiveness [1]. In this context, Blackberry (Rubus L.), which has been used in traditional medicine since ancient times, is of particular importance.

Plants of the genus Rubus are the subject of numerous studies aimed at identifying their biologically active constituents and their potential applications in medicine and the food industry. According to modern data, the fruits of Rubus L. are rich in polyphenols, anthocyanins, and vitamins, which exert pronounced antioxidant effects [1].

The unique composition of this plant includes amino acids, flavonoids, antioxidants, tannins, anthocyanins, and vitamins, which determine its multifaceted biological activity in the human body [2,3]. Flavonoids and anthocyanins present in the fruits possess anti-inflammatory, cardioprotective, and neuroprotective properties [3].

Studies have shown that regular consumption of Rubus L. extracts contributes to a reduced risk of cardiovascular and metabolic diseases [4,7]. In folk medicine, Rubus L. fruits are traditionally used as a general tonic, immunomodulatory, and antipyretic remedy. The leaves are used for gastrointestinal disorders and inflammatory diseases of the respiratory tract, while the roots exhibit diuretic and astringent properties [4,5].

Additionally, Rubus L. is widely applied in traditional medicine for the treatment of digestive disorders, respiratory inflammations, and as a strengthening agent [5,7].

Modern pharmacological research confirms these traditional applications, making Blackberry (Rubus L.) a promising subject for further scientific investigation.

LITERATURE REVIEW

The genus *Rubus L.* is recognized for its rich phytochemical profile and has been the focus of numerous studies investigating its biological activity and potential applications in medicine and nutrition. Previous research demonstrates that blackberry fruits contain significant amounts of polyphenols, anthocyanins, flavonoids, tannins, organic acids, amino acids, and essential vitamins, which collectively contribute to their antioxidant, anti-inflammatory, cardioprotective, and immunomodulatory effects. Seeram (2008) reports that berry fruits, including *Rubus* species, possess high antioxidant capacity largely due to their polyphenol and anthocyanin composition, which play a crucial role in neutralizing reactive oxygen species and reducing oxidative stress in biological systems. Similar findings by Mullen et al. (2002) highlight the presence of diverse phenolic compounds in *Rubus* species, emphasizing their influence on the plant’s pharmacological potential.

The nutritional and therapeutic relevance of *Rubus L.* has also been demonstrated in studies evaluating its role in preventing metabolic and cardiovascular disorders. Siriwoharn et al. (2006) observed that the biochemical composition of blackberry fruits varies with cultivar, maturity stage, and processing conditions; however, the overall presence of biologically active compounds remains consistently high across varieties. Ethnopharmacological literature further indicates that blackberry fruits, leaves, and roots have long been used in traditional medicine as astringent, tonic, anti-inflammatory, and antipyretic remedies, supporting their holistic therapeutic value. These traditional applications are corroborated by pharmacognostic studies confirming the presence of water-soluble vitamins such as vitamins B1, B2, B3, B6, B9, PP, and C, which contribute to essential metabolic functions and antioxidant defense mechanisms.

Recent methodological advances have focused on the chromatographic determination of vitamins and phenolic compounds in plant matrices. High-performance liquid chromatography (HPLC) has emerged as the preferred analytical technique due to its high sensitivity, reproducibility, and capability to detect multiple compounds within complex extracts. Studies applying HPLC for vitamin quantification (Аскаров et al., 2024) demonstrate the effectiveness of gradient elution methods and C18 stationary phases in achieving optimal separation of water-soluble vitamins. The YUSSX method has also gained attention as a reliable tool for quantitative assessment of vitamins in plant-derived extracts, offering improved accuracy and operational simplicity.

Overall, the reviewed literature indicates that *Rubus L.* represents a valuable natural source of essential micronutrients and bioactive compounds. These findings align with growing interest in plant-based therapeutic agents and functional foods,

underscoring the importance of precise analytical approaches for evaluating the nutritional quality and pharmacological potential of blackberry extracts.

MATERIALS AND METHODS

In this study, the YUSSX method was used to determine the content of water-soluble vitamins in the extract of Blackberry (Rubus L.). The following reagents and equipment were employed:

- **Vitamin B12** standards from *Rhydburg Pharmaceuticals* (Germany)
- **Vitamin C** standards from *Carl Roth GmbH* (Germany)
- **Vitamin B9** standards from *DSM Nutritional Products GmbH* (Germany)
- Vitamins **B1, B2, B3, B6, and PP** were determined using reagents and equipment from *BLD Pharm* (China)

Analytical-grade water for YUSSX, acetonitrile, acetic acid, and sodium hydroxide were used as reagents.
The analysis was carried out using the LC-40 Nexera Lite high-performance liquid chromatograph manufactured by *Shimadzu* (Japan) [6].

The plant material of Rubus L. was collected in dried form and stored under controlled conditions prior to analysis. For the preparation of the analytical extract, 1 g of homogenized raw material was accurately weighed and transferred into a 50 mL volumetric flask. Subsequently, 25 mL of 0.1 N hydrochloric acid was added, and the mixture was subjected to ultrasonic-assisted extraction for 20 minutes at 60 °C. The resulting extract was filtered and diluted with distilled water to a final volume of 25 mL. Prior to chromatographic analysis, the solution was passed through a 0.22 μm membrane filter to remove particulate matter.

Chromatographic separation was performed using an LC-40 Nexera Lite HPLC system equipped with a Shim-pack GIST C18 column (150 × 4.6 mm; 5 μm). The mobile phase consisted of acetonitrile and 0.25% aqueous acetic acid, applied in gradient mode. The flow rate was maintained at 0.6 mL/min, the column temperature was set at 40 °C, and the injection volume was 10 μL. Detection was carried out at wavelengths of 265, 291, and 550 nm to ensure selective identification of individual water-soluble vitamins. Calibration curves for vitamins B1, B2, B3, B6, B9, B12, PP, and C were constructed from standard solutions prepared in appropriate concentrations. Quantification was based on peak area comparison with the established calibration models.

RESULTS AND DISCUSSION

Standard solutions of vitamins C, B1, B2, B3, B6, B9, B12, and PP were prepared in various concentrations. Based on the chromatograms obtained, calibration curves were constructed, allowing the determination of vitamin content in the blackberry extract samples.

Table 1. Gradient program of the mobile phase for the determination of vitamins

Time, min.	**Acetonitrile (A), %**	**0.5% acetic acid (B), %**
0	0	100
3	0	100
14	20	80
17	50	50
18	0	100
25	Completion	

Table 2. Gradient program of the mobile phase for the determination of vitamin C

Time, min.	**Acetonitrile (A), %**	**0.5% acetic acid (B), %**
0	0	100
2	0	100
6	50	50
6,01	0	100
15	Завершение	

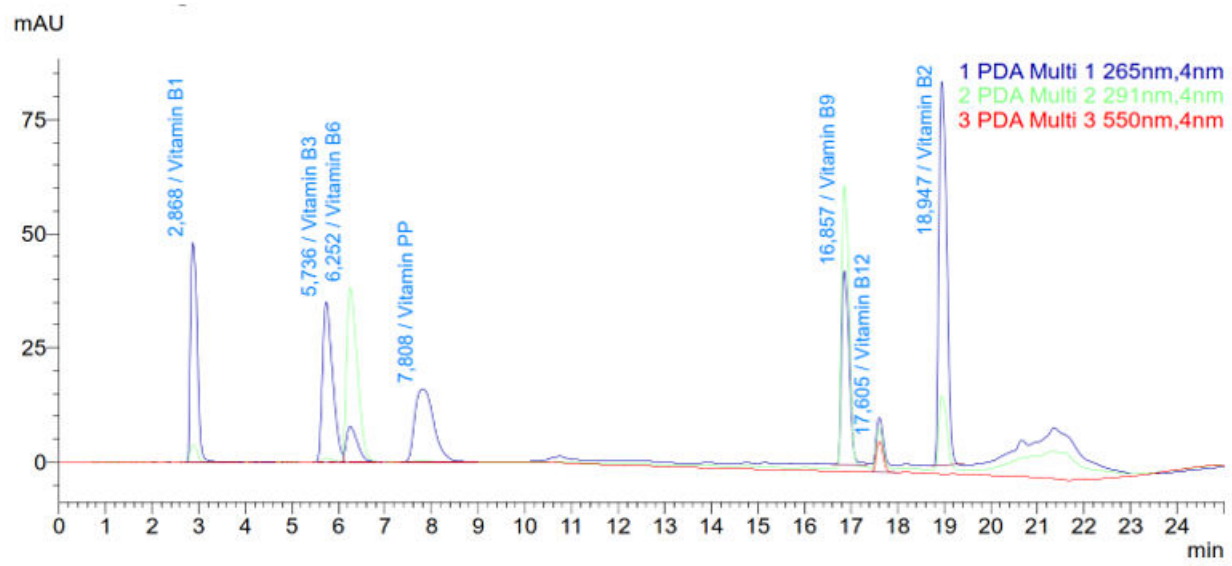

Figure 1. Chromatogram of a standard vitamin solution.

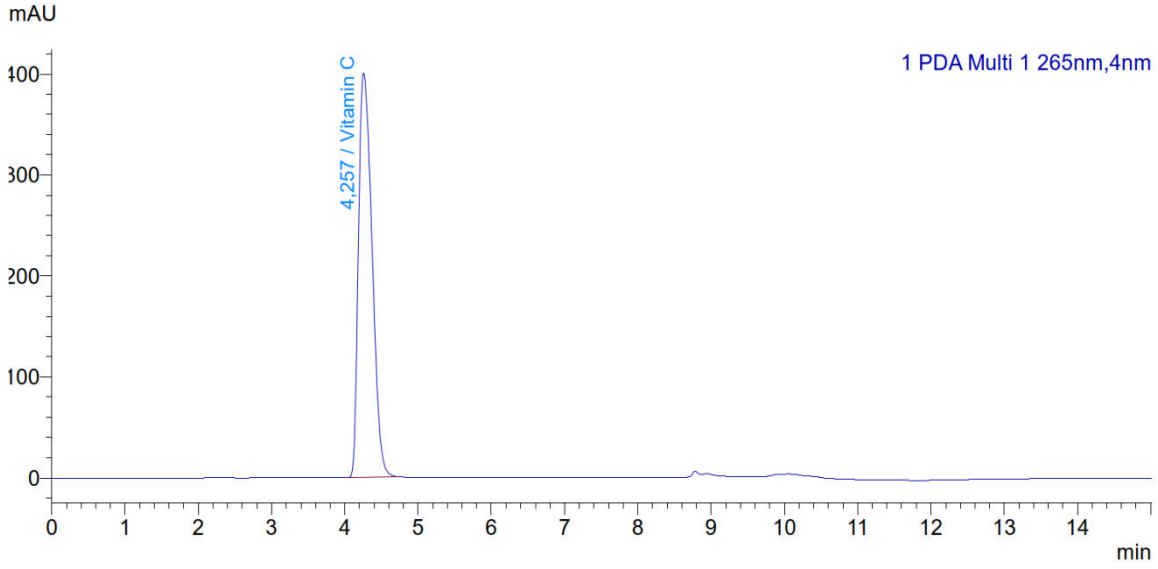

Figure 2. Chromatogram of the standard solution of vitamin C.

A chromatogram of the sample extract was obtained (Figures 3–4), and based on the results, the amount of vitamins in 100 g of the sample was calculated using the following formula, presented in Table 3.

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

Here, **X** is the amount of vitamins in 100 grams of fruit, mg;
C_vit – concentration of the vitamin in the extract, determined by the YUSSH method, mg/L;**V_extract** – volume of the sample extract;
m – mass of the sample taken for extract preparation.

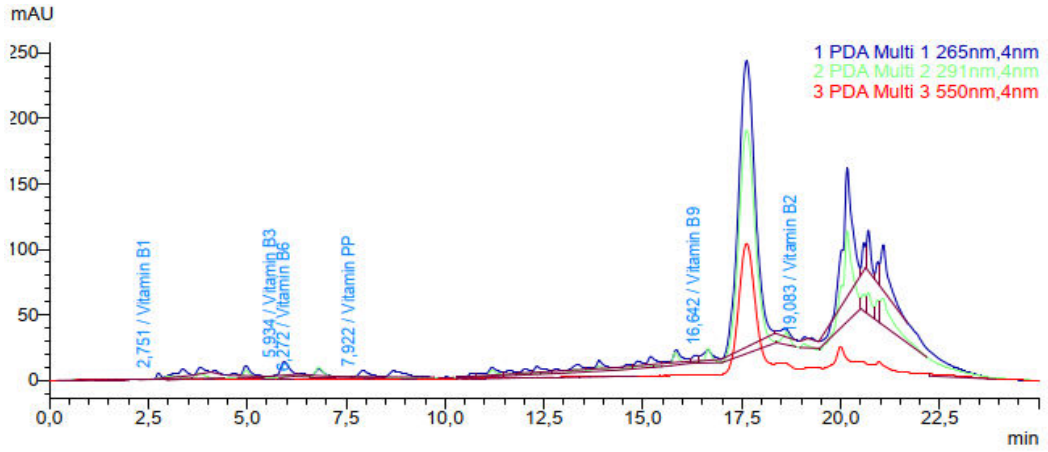

Figure 3. Chromatogram for the determination of vitamins in the sample extract.

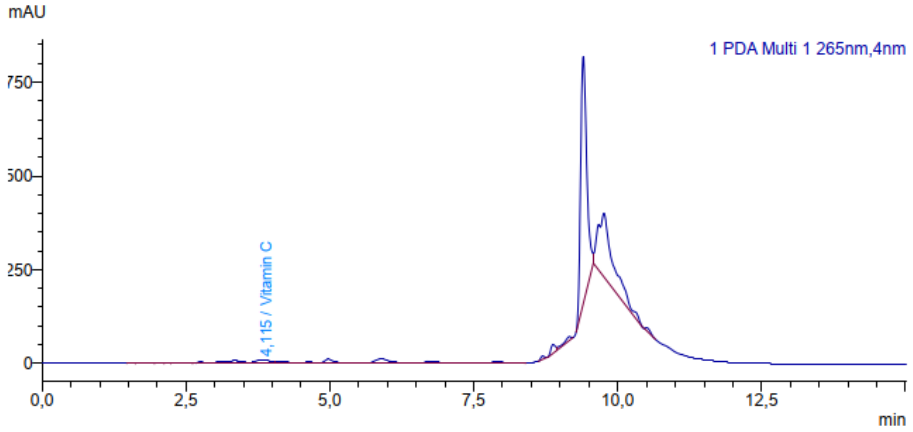

Figure 4. Chromatogram of the determination of vitamin C in the sample extract.

To prepare the extract for analysis, a 1 g sample was placed into a 50 mL flask, followed by the addition of 25 mL of 0.1 N HCl. Ultrasonic extraction was carried out for 20 minutes at 60 °C. After filtration, the extract was brought to a final volume of 25 mL with distilled water, filtered through a 0.22 µm membrane filter, and used for analysis.

The determination was performed on a **Shim-pack GIST C18 column** (150 × 4.6 mm; 5 µm, Shimadzu, Japan) in gradient mode using acetonitrile and 0.25% acetic acid solution in water as the mobile phase. The column temperature was maintained at 40 °C, the flow rate was 0.6 mL/min, and the injection volume was 10 µL. Detection was carried out at wavelengths of **265, 291, and 550 nm**.

As a result of the analysis, quantitative data on the vitamin content in 100 g of raw material were obtained:
vitamin B1 — 2.28 mg,
B2 — 1.14 mg,
B3 — 10.00 mg,
B6 — 0.72 mg,
B9 — 9.95 mg,
PP — 6.41 mg,
vitamin C — 13.01 mg.
Vitamin B12 was not detected in the tested samples.

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

Vitamin	Retention Time, sec	Concentration, mg/L	Amount in 100 g of Sample, mg
Vitamin B1	2.751	0.911	2.278
Vitamin B3	5.934	4.001	10.003
Vitamin PP	7.922	2.565	6.413
Vitamin B9	16.642	3.979	9.948
Vitamin B2	19.083	0.455	1.138

Vitamin	Retention Time, sec	Concentration, mg/L	Amount in 100 g of Sample, mg
Vitamin B6	6.272	0.288	0.720
Vitamin B12	Not detected	0	0.000
Vitamin C	4.115	5.202	13.005

DISCUSSION

The results obtained in this study demonstrate that the fruits of *Rubus L.* contain a considerable amount of essential water-soluble vitamins, confirming their nutritional and pharmacological value. The quantified levels of vitamins B1, B2, B3, B6, B9, PP, and C correspond well with previously reported phytochemical profiles of blackberry species, which consistently highlight their richness in micronutrients and bioactive compounds. In particular, the relatively high content of vitamin C and B-group vitamins reinforces the antioxidant potential of *Rubus L.*, as these vitamins play a crucial role in free radical scavenging, energy metabolism, and cellular regulation.

The absence of vitamin B12 in the extract aligns with botanical evidence indicating that cobalamin is typically absent in higher plants and primarily synthesized by microorganisms. This finding supports the validity of the analytical method and underscores the specificity of the YUSSX–HPLC approach in detecting micronutrients within plant matrices.

When comparing the results with earlier studies, the vitamin C concentration obtained in this work falls within the range reported for various *Rubus* species in previous biochemical analyses. Similarly, the quantified levels of B-group vitamins are consistent with values documented in pharmacognostic references, although minor variations may be attributed to factors such as plant maturity, environmental conditions, extraction efficiency, and analytical methodology. Such variability is common in phytochemical investigations and highlights the importance of standardized analytical protocols.

The YUSSX method in combination with HPLC proved effective for separating and quantifying multiple water-soluble vitamins in a single analytical run. The chromatographic parameters, including retention times and peak resolution, confirm the suitability of the selected mobile phase gradient and the C18 stationary phase for analyzing polar compounds. The high sensitivity and reproducibility of the method also indicate its potential applicability for routine quality control of plant-based raw materials and finished products.

From a pharmacological perspective, the presence of B-complex vitamins and vitamin C provides a biochemical basis for the traditional therapeutic uses of *Rubus L.* in strengthening immunity, reducing inflammation, and supporting metabolic health. These findings contribute to the growing body of scientific evidence supporting the development of blackberry-derived functional foods, dietary supplements, and phytopharmaceutical formulations.

Overall, the discussion of results shows that the vitamin profile of *Rubus L.* aligns strongly with its known biological properties and confirms its significance as a natural source of essential micronutrients. The study also highlights the importance of applying precise analytical methods to accurately evaluate the nutritional potential of medicinal plants.

CONCLUSION

Thus, Blackberry (Rubus L.) is a valuable source of water-soluble vitamins, which confirms its potential for the development of herbal medicines and functional food products. The obtained results are consistent with modern pharmacognostic data on the biological activity of this plant [2].

The present study provides a comprehensive quantitative assessment of water-soluble vitamins in the extract of *Rubus L.* using the YUSSX analytical approach coupled with high-performance liquid chromatography. The analytical results confirm that the fruits of *Rubus L.* are a rich natural source of essential micronutrients, particularly vitamin C and several B-complex vitamins, which are known to play critical roles in antioxidant defense, cellular metabolism, and physiological homeostasis. The detected concentrations of vitamins B1, B2, B3, B6, B9, PP, and C demonstrate the nutritional and pharmacological significance of blackberry fruits, while the absence of vitamin B12 is consistent with phytochemical characteristics of higher plants.

The methodological workflow applied in this study—ultrasonic extraction, membrane filtration, and gradient HPLC separation—showed high reliability, reproducibility, and analytical specificity. The obtained chromatographic profiles and calibration models validate the suitability of the YUSSX method for the accurate determination of water-soluble vitamins in complex plant matrices. This highlights its potential applicability in future phytochemical evaluations of medicinal plants and functional food ingredients. Overall, the findings align with previously published pharmacognostic literature emphasizing the health-promoting properties of *Rubus* species. The vitamin composition identified in this study further supports the potential of blackberry extracts for use in the development of phytopharmaceutical products, nutraceutical formulations, and vitamin-enriched functional foods. Considering the increasing global interest in plant-based therapeutic agents, the results underscore the importance of ongoing research aimed at characterizing biologically active compounds in *Rubus L.*. Future studies may focus on seasonal variability, comparative cultivar analysis, bioavailability assessments, and synergistic interactions between vitamins and polyphenolic compounds to more comprehensively evaluate the therapeutic potential of this plant.

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