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DETERMINATION OF WATER-SOLUBLE VITAMINS IN THE ROOTS AND LEAVES OF CHICORY (CICHORIUM INTYBUS L.) BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Umarova Odinaxon Maxmadamin qizi*, **Ibragimov Alijon**
FerSU
* ✉ odina@gmail.com

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ABSTRACT

In this study, the content of water-soluble vitamins in the roots and leaves of chicory (Cichorium intybus L.) was determined using High-Performance Liquid Chromatography (HPLC). Vitamins were extracted from the samples using a 40% ethanol solution. The obtained extracts were analyzed on an Agilent-1200 high-performance liquid chromatograph. The study focused on B-group vitamins (B2, B3, B6, and B9) as well as vitamin C. The results showed that the vitamin content in chicory leaves was higher than that in the roots. In particular, vitamins B2 and B9 were found in the highest concentrations. The findings confirm that chicory is a promising source of biologically active compounds for applications in the pharmaceutical and food industries.

Keywords: chicory, Cichorium intybus L., water-soluble vitamins, high-performance liquid chromatography (HPLC), chromatography, B-group vitamins, ascorbic acid, biologically active compounds.

INTRODUCTION

At present, the identification and comprehensive study of biologically active compounds in medicinal plants is one of the most important areas in the pharmaceutical, medical, and food industries. Vitamins contained in plants perform essential physiological functions in the metabolic processes of the human body. In particular, water-soluble vitamins participate in enzymatic reactions as coenzymes, enhance the activity of the immune system, and exhibit antioxidant properties [1,3].

Chicory (*Cichorium intybus* L.) is a perennial medicinal plant belonging to the Asteraceae family and has long been used in traditional medicine. The roots and aerial parts of this plant contain inulin, flavonoids, phenolic compounds, organic acids, and vitamins. Chicory is valued for its ability to improve liver function, normalize the digestive system, and help remove toxins from the body [1,4].

High-performance liquid chromatography (HPLC) is widely used for the quantitative analysis of vitamins. This method is characterized by high sensitivity, accuracy, and selectivity, making it possible to determine vitamins in complex biological samples [2,5,6]. Therefore, the aim of this study was to determine the content of water-soluble vitamins in the roots and leaves of chicory using the HPLC method.

MATERIALS AND METHODS

The roots and leaves of chicory were used as the research materials. During sample preparation, 5–10 g of crushed plant material was accurately weighed using an analytical balance and transferred into a 300 mL

flat-bottom flask. Then, 50 mL of 40% ethanol solution was added. The mixture was boiled under intensive stirring for 1 hour using a magnetic stirrer equipped with a reflux condenser. Subsequently, the mixture was stirred at room temperature for 2 hours. The obtained extract was allowed to settle and then filtered. An additional 25 mL of 40% ethanol was added to the residue, and the extraction procedure was repeated twice. All filtrates were combined in a 100 mL volumetric flask, and the volume was adjusted to the mark with 40% ethanol.

The resulting solutions were centrifuged at 7000 rpm for 10 minutes. The clear supernatant was used for analysis. Standard solutions of water-soluble vitamins were prepared at a concentration of 1 mg/mL. For this purpose, 50.0 mg of each vitamin standard was accurately weighed, dissolved in 40% ethanol in a 50 mL volumetric flask, and diluted to the mark.

Vitamin determination was carried out using an Agilent-1200 high-performance liquid chromatograph. The chromatographic conditions were as follows:

- **Column:** Eclipse XDB C18 (5 μm, 4.6 × 250 mm);
- **Detector:** Diode Array Detector (DAD), 250 nm;
- **Flow rate:** 0.8 mL/min;
- **Column temperature:** 25°C;
- **Injection volume:** 5 μL;
- **Mobile phase:** acetate buffer and acetonitrile.

The gradient elution program was as follows:

- 0–5 min — 96:4;
- 6–8 min — 90:10;
- 9–15 min — 80:20;
- 15–17 min — 96:4.

Initially, standard solutions were injected into the chromatograph to determine the retention times of the vitamins. Subsequently, plant extracts were analyzed, and the vitamin contents were calculated.

RESULTS AND DISCUSSION

The analysis results showed that water-soluble vitamins were present in different amounts in the roots and leaves of chicory.

Vitamin	Chicory Root (μg/g)	Chicory Leaf (μg/g)
B2	29.1	36.2
B6	9.2	11.6
B9	22.4	29.6
B3	8.5	15.2
C	9.3	16.8

According to the obtained results, the total vitamin content in chicory leaves was higher than in the roots. In particular, vitamin B2 was detected at the highest concentration, amounting to 29.1 μg/g in the roots and 36.2 μg/g in the leaves. Riboflavin (vitamin B2) plays an important role in oxidation-reduction reactions and actively participates in energy metabolism.

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Vitamin B9 (folic acid) was also detected in significant amounts, reaching 29.6 μg/g in the leaves. Folic acid plays a crucial biological role in cell division and blood formation processes. The amount of vitamin C in the leaves was almost twice as high as in the roots, indicating the strong antioxidant properties of the plant.

Ascorbic acid is important for strengthening the immune system and neutralizing free radicals. Vitamin B1 was not detected in either sample. This may be explained by its very low content in the plant material or its degradation during the extraction process. The results indicate that chicory leaves are an important source of biologically active compounds, particularly water-soluble vitamins.

CONCLUSION

The obtained data expand the possibilities for the use of chicory in the development of biologically active supplements, phytopharmaceuticals, and functional food products. High-performance liquid chromatography was found to be an effective method with high accuracy and sensitivity for the determination of water-soluble vitamins in chicory. The study demonstrated that B-group vitamins and vitamin C are accumulated in greater amounts in chicory leaves than in the roots. The identified vitamins enhance the biological and pharmacological value of the plant. Therefore, chicory can be considered a promising raw material for the production of pharmaceutical preparations, dietary supplements, and functional food products.

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