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Flavonoids In The Stem Of Purple Coneflower (Echinacea Purpurea) And Their Significance

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Abstract: This study was aimed at determining the chemical and quantitative composition of flavonoids in the stem of purple coneflower (Echinacea purpurea L.) using High-Performance Liquid Chromatography (HPLC; LC-40 Nexera Lite, Shimadzu). Quercetin, rutin, gallic acid, apigenin, kaempferol, and salicylic acid standards were used for analysis, while extraction was carried out in a 96% ethanol medium using an ultrasonic bath. The results showed that salicylic acid (464.528 mg/100 g) and kaempferol (54.033 mg/100 g) were present in the highest amounts in the plant material. These polyphenols are the main metabolites responsible for the antioxidant and anti-inflammatory activity of the plant, confirming their high pharmacological and technological significance.

Keywords: Purple coneflower, flavonoids, polyphenols, kaempferol, salicylic acid, HPLC, C18 column, extraction, antioxidant activity.

Introduction: Purple coneflower (Echinacea purpurea L.) is one of the most highly valued medicinal plants in the pharmaceutical, phytotherapy, and biologically active supplement industries, distinguished by its strong immunotropic, antioxidant, anti-inflammatory, and adaptogenic properties. In recent years, not only the aerial parts of this plant species but also its seeds have become the focus of in-depth scientific research. This is because seeds represent the most biologically concentrated “reservoir” of the plant, where phenolic metabolites, particularly flavonoids, are often more stable and sometimes present in higher amounts than in leaves and flowers [1].

Flavonoids are a large class of polyphenolic compounds that play a central role in the antioxidant defense system of plants and have significant scientific and practical importance as universal bioactive modulators in the human body. The kaempferol, quercetin, luteolin, and apigenin derivatives and their glycosides found in Echinacea seeds are known for their ability to regulate immune processes, neutralize free radicals, modulate intracellular signaling pathways, and influence key mechanisms of neurodegenerative and metabolic disorders [2]. However, compared to other morphological parts of the plant, the flavonoid profile of Echinacea purpurea stems has been insufficiently studied, and existing literature lacks comprehensive

and systematic data on their structural diversity, extraction properties, and biological activity. In particular, scientific studies on flavonoid content, biosynthesis, and potential pharmacological value in ecotypes cultivated under the conditions of Uzbekistan are extremely limited [3].

This situation makes an in-depth investigation of the flavonoid composition of purple coneflower stems not only scientifically relevant but also practically necessary. New phytopreparations, dietary supplements, antioxidant compositions, and immunomodulatory agents developed based on the chemical profile of the plant stems have great potential for the pharmaceutical industry [4]. Therefore, the main objective of this study was to identify flavonoids present in the stems of Echinacea purpurea, analyze their structural and functional properties, determine their quantitative profile, and scientifically substantiate their role in biological activity.

Reagents and Equipment

Quercetin, apigenin, and salicylic acid were obtained from Rhydburg Pharmaceuticals (Germany); gallic acid from Macklin (China); kaempferol from Regal (China); and rutin was isolated from natural sources using extraction and column chromatography methods. HPLC-grade water, acetonitrile, chemically pure CH_3COOH, and NaOH reagents were used. The determination of polyphenols in Echinacea plant material was performed using an LC-40 Nexera Lite high-performance liquid chromatograph manufactured by Shimadzu (Japan).

Preparation of Standard Solutions

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

Preparation of Echinacea Stem Extract

To extract phenolic compounds, 1 g of the test sample was weighed with an accuracy of 0.01 g using an OHAUS NV222 balance (USA) and placed into a 50 mL conical flask. Then, 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 portion of the extract was centrifuged at 7000 rpm using a Mini-7 centrifuge (BIOBASE, China) and filtered through a 0.45 µm syringe filter prior to analysis.

Chromatographic Conditions

Phenolic compounds were determined 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 0.5% aqueous acetic acid (B) (Table 2.3). The injection volume was 10 µL, flow rate 0.5 mL/min, and column temperature 40°C. Analytical signals (peak areas) of phenolic compounds were recorded at 300 nm (Figure 1).

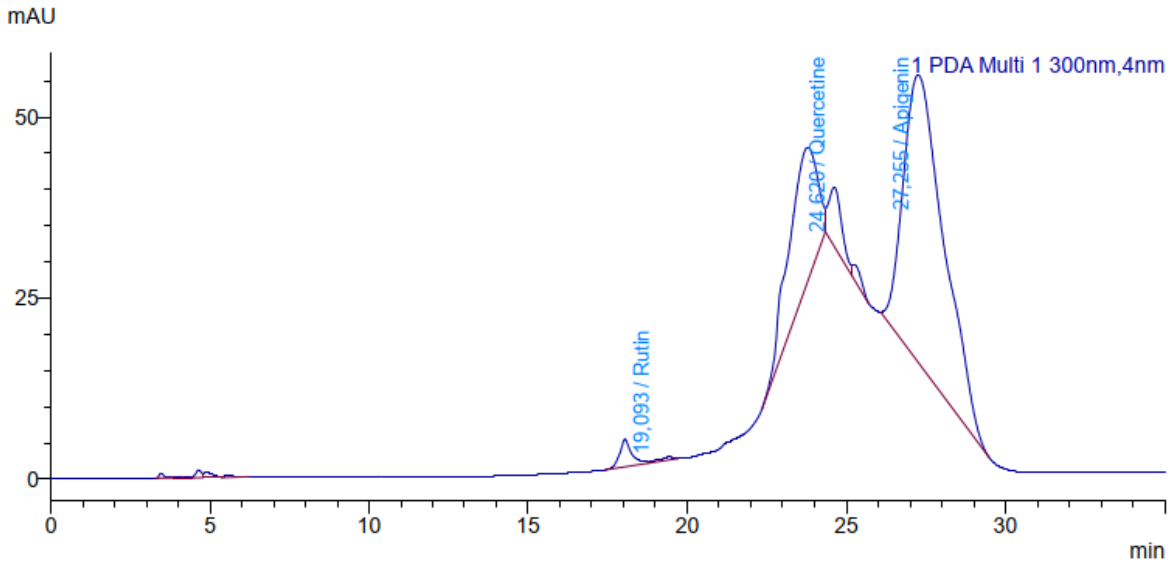

Figure 1. Chromatogram of polyphenols identified in the stem extract of Echinacea purpurea.

Determination of Phenolic Compound Content

A chromatogram of a 1 g Echinacea stem extract was obtained (Figures 1–2), and based on the results, the amounts of phenolic compounds in 100 g of extract were calculated using the following formula:

$$X = \frac{C_{\text{phen}} \cdot V_{\text{extract}}}{m_{\text{sample}}} \times 100$$

where

X – amount of phenolic compounds in 100 g of sample, mg;

$C_{(phen)}$ – concentration of phenolic compounds determined by HPLC, mg/L;

$V_{(extract)}$ – volume of the extract, L;

$m_{(sample)}$ – mass of the sample used for extraction.

The flavonoid content of Echinacea purpurea stems was determined and analyzed by HPLC. The results are presented in Figure 2 and Table 1.

Table 1

Retention times and concentrations of polyphenols in Echinacea purpurea stem extract

Phenolic compound	Retention time, s	Concentration, mg/L	Amount in 100 g sample, mg
Gallic acid	Not detected	0	0.000
Rutin	19.093	0.417	1.043
Salicylic acid	Not detected	0	0.000
Quercetin	24.62	7.00	17.500
Apigenin	27.255	73.69	184.225

The amount of polyphenols in 100 g of Echinacea purpurea stem extract was determined by HPLC, with apigenin being present in the highest concentration (184.225 mg).

Nature provides numerous bioactive compounds that support human health, among which phenolic compounds (polyphenols) occupy a special place. These organic compounds, mainly derived from plants, possess a wide range of beneficial properties and play an important role in maintaining health, preventing diseases, and improving overall quality of life [5]. Phenolic compounds are characterized by the presence of one or more phenolic groups and are commonly found as secondary metabolites in plants. They are present in fruits, vegetables, tea, coffee, cocoa, spices, and some legumes. Polyphenols are divided into four main groups: flavonoids, phenolic acids, stilbenes, and lignans. As strong antioxidants, phenolic compounds combat free radicals, reduce cellular damage, slow the aging process, and help prevent chronic diseases [6].

The content of health-beneficial polyphenolic compounds in the stems of purple coneflower grown in Austria was compared with results reported by Rudolf Bauer from the University of Graz, and the differences are presented in Table 1.

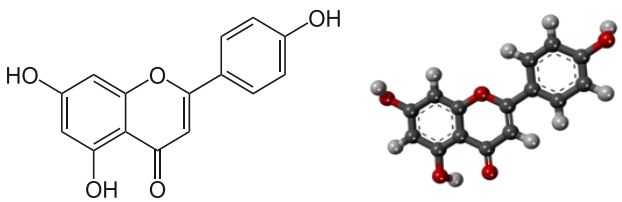

Figure 2. Chemical structure and spatial configuration of the flavonoid apigenin.

Apigenin is beneficial in lowering blood pressure, dilating blood vessels, and normalizing heart rhythm. It may also reduce “bad” cholesterol levels, playing an important role in preventing heart attacks, maintaining vascular elasticity, and improving blood circulation [7]. In addition, apigenin increases insulin sensitivity and helps regulate glucose metabolism [8], which is particularly important for individuals with diabetes or those at risk of developing it.

During the study, the amount of polyphenols in 100 g of Echinacea purpurea stem extract grown in Uzbekistan was determined by HPLC, revealing a high apigenin content (184.225 mg). Compared with Echinacea stems grown in Austria, the Uzbek-grown plants contained higher levels of phenolic compounds. Given the numerous positive effects of these polyphenols on human health, the development of food supplements based on Echinacea stems is considered a relevant and promising direction. Future studies will be devoted to further research in this area.

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