

FLAVONOID COMPOSITION OF PURPLE CONEFLOWER (ECHINACEA PURPUREA L.) SEEDS AND THEIR MEDICAL RELEVANCE

Mo'minjonova Mukarramxon Sherzodbek kizi,
Department of Biological Chemistry,
Andijan State Medical Institute, Uzbekistan, Andijan.

ANNOTATION: This study focuses on determining the chemical composition and quantitative profile of flavonoids present in the seeds 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 the analysis, while extraction was carried out in an ultrasonic bath using 96% ethanol. According to the results, salicylic acid (464.528 mg/100 g) and kaempferol (54.033 mg/100 g) were identified as the most abundant compounds in the seeds. These polyphenols represent key metabolites responsible for the plant's antioxidant and anti-inflammatory activities, confirming their significant pharmacological and technological relevance.

Keywords: *Echinacea purpurea*, 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, phytotherapeutic, and nutraceutical industries, distinguished by its immunotropic, antioxidant, anti-inflammatory, and adaptogenic properties. In recent years, not only the aerial parts of this species but also its seeds have become the focus of extensive scientific investigation. This is because seeds serve as the plant's most concentrated biological "reserve," where phenolic metabolites—particularly flavonoids—are found to be more stable and, in some cases, present in higher amounts compared to leaves and flowers [1].

Flavonoids, a major class of polyphenolic compounds, serve as essential components of the plant's antioxidant defense system and act as universal bioactive modulators in the human body, holding great scientific and practical importance. Kaempferol, quercetin, luteolin, apigenin derivatives, and their glycosides detected in *Echinacea* seeds are known for their ability to regulate immune responses, neutralize free radicals, modulate intracellular signaling pathways, and influence key mechanisms underlying neurodegenerative and metabolic disorders [2].

Nevertheless, the flavonoid profile of *Echinacea purpurea* seeds remains significantly less studied compared to other morphological parts of the plant. Existing literature provides limited integrated and systematic information regarding the structural diversity, extraction characteristics, and biological activities of these compounds. In particular, studies on the flavonoid content, biosynthesis, and potential pharmacological value of ecotypes cultivated under the conditions of Uzbekistan's flora are extremely scarce [3,4].

This situation makes the comprehensive investigation of the flavonoid composition of Purple Coneflower seeds an important scientific and practical priority. New phytopreparations, biologically active supplements, antioxidant formulations, and immunomodulatory agents developed based on the chemical profile of the seeds hold substantial potential for the pharmaceutical industry [5,6].

Therefore, the main objective of this research is to identify the flavonoids present in *Echinacea purpurea* seeds, analyze their structural-functional properties, determine their quantitative profile, and scientifically substantiate their role in biological activity.

Reagents and Equipment Used. Quercetin, apigenin, and salicylic acid were obtained from *Rhydburg Pharmaceuticals* (Germany); gallic acid from *Macklin* (China); kaempferols from *Regal* (China); and rutin was isolated from natural sources using extraction and column chromatography methods. HPLC-grade water, acetonitrile, CH₃COOH, and NaOH of analytical purity were used throughout the study. The quantification of polyphenols in Purple Coneflower was carried out using the LC-40 Nexera Lite High-Performance Liquid Chromatograph (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. The solutions were transferred into 50 ml volumetric flasks and brought to volume with CH₃CH₂OH. From each stock solution, 200 μL was taken, mixed, and four different diluted solutions were prepared. All prepared solutions were transferred into vials and used for analysis.

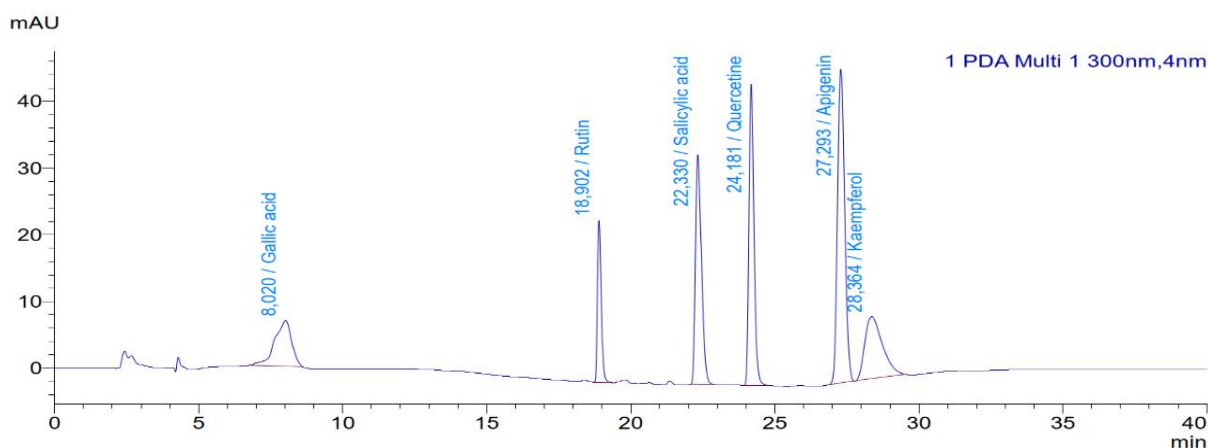
Preparation of Purple Coneflower Extract. To extract phenolic compounds from Purple Coneflower seeds, 1 g of sample was weighed with a precision of 0.01 g using an NV222 balance (OHAUS, USA). The weighed sample was placed in a 50 ml conical flask and mixed with 25 ml of 96% CH₃CH₂OH. The mixture was subjected to ultrasonic extraction at 60°C for 20 minutes in a GT SONIC-D3 ultrasonic bath (China). Afterwards, the extract was cooled, filtered, and brought to 25 ml with CH₃CH₂OH in a volumetric flask. A 1.5 ml aliquot of the extract was centrifuged at 7000 rpm using the Mini-7 centrifuge (BIOBASE, China), filtered through a 0.45 μm syringe filter, and used for analysis.

Chromatographic Conditions. Determination of phenolic compounds was performed using the 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 solution (B) (Table 2.3). The injection volume was 10 μL, the flow rate was set at 0.5 ml/min, and the column temperature was maintained at 40°C. Analytical signals (peak areas) of phenolic compounds were recorded at 300 nm (Figure 1.1).

Table 1.1.
Gradient Program of the Mobile Phase.

Time (min)	Acetonitrile (A), %	0.5% Acetic Acid (B), %
0	5	95
5	5	95
17	40	60
22	40	60
22,1	5	95
40	end	

Figure 1.1. Chromatogram of Standards at 300 nm.



Determination of Phenolic Compounds in the Extract of Purple Coneflower.

A chromatogram of the Purple Coneflower extract (1 g sample) was obtained (Figures 2–3). Based on the chromatographic results, the amounts of phenolic compounds present in 100 g of the extract were calculated using the following formula, and the results are presented in the tables above.

$$X = \frac{C_{phen} \cdot V_{ekstrakt}}{m_{namuna}} \cdot 100 \text{ g}$$

Here:

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

C_{phen} – the concentration of the phenolic compound in the extract determined by HPLC, mg/L;

$V_{extra}c_t$ – the volume of the Purple Coneflower extract, L;

m_{sample} – the mass of the sample taken for extraction.

The results of the study showed that the flavonoid content in the seeds of Purple Coneflower was quantitatively determined and analyzed using the HPLC method. The obtained data are presented in Figures 3.3 and Table 3.5.

Figure 1.2. Chromatogram for the Identification of Polyphenols in the Seed Extract of Purple Coneflower.

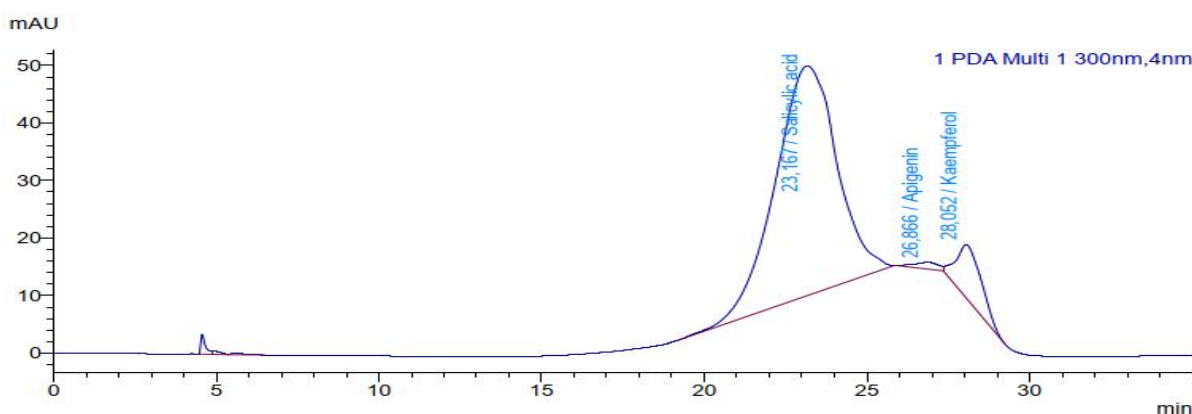


Table 1.2. Content and Retention Times of Polyphenols in the Seed Extract of Purple Coneflower.

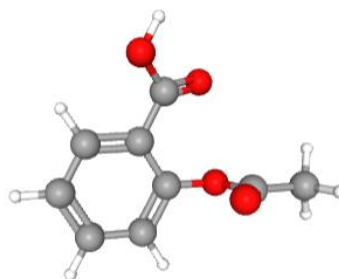
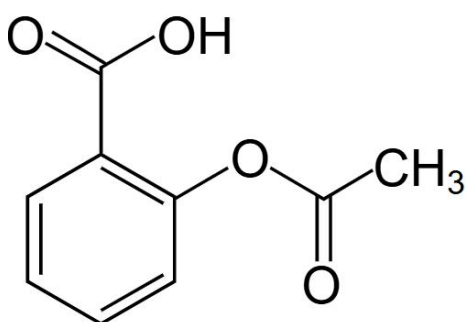
Phenolic Compound	Retention Time, s	Concentration, mg/L	Amount in 100 g Sample, mg
Gallic acid	Not detected	0	0.000
Rutin	Not detected	0	0.000
Salicylic acid	23.167	185.811	464.528
Quercetin	Not detected	0	0.000
Apigenin	26.866	1.27	3.175
Kaempferol	28.052	21.613	54.033

The quantitative determination of phenolic compounds in the seeds of Purple Coneflower was evaluated by comparing the results with literature data, and relevant conclusions were drawn. In this process, the specific phenolic compounds that were most abundant were identified, and their potential positive and negative effects on human health were analyzed using scientific sources, academic references, and published studies.

The amount of polyphenols in 100 g of Purple Coneflower seed extract was determined using the HPLC method. The results showed that salicylic acid (464.528 mg) and kaempferol (54.033 mg) were present in the highest quantities.

Salicylic acid is a naturally occurring organic compound belonging to the phenolic group and is found in many medicinal plants. Salicylic acid ($C_7H_6O_3$) has been used as a therapeutic agent for centuries. Its most widely known derivative, acetylsalicylic acid (aspirin), is one of the most extensively used analgesic and anti-inflammatory drugs worldwide today.

The pharmacological significance of salicylic acid is based on its multifunctional mechanisms of action. It reduces inflammatory processes by inhibiting prostaglandin synthesis. Therefore, it is considered beneficial in conditions such as arthritis, rheumatism, and other chronic inflammatory diseases.

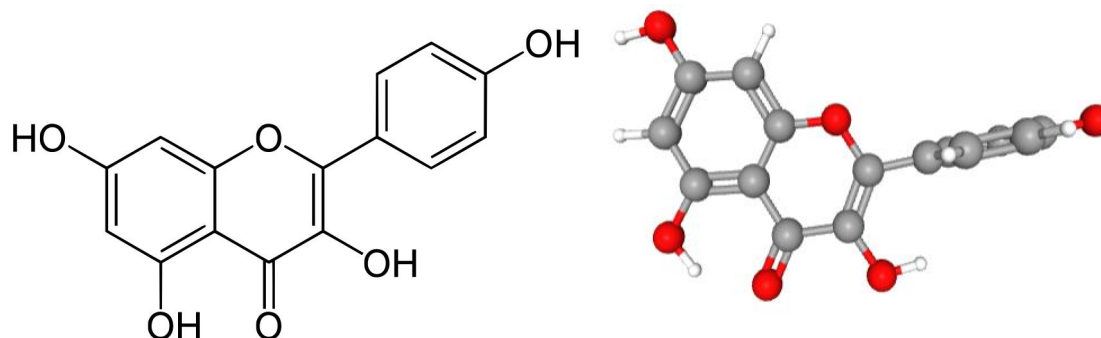


Salicylic acid

Salicylic acid suppresses mediators involved in the transmission of pain signals. It is particularly effective in reducing headaches, muscle pain, and rheumatic pain (Duke, 1998). By acting on the thermoregulatory center of the hypothalamus, salicylic acid lowers fever. This feature makes it widely applicable in conditions such as influenza and acute respiratory viral infections (ARVI). When used topically, salicylic acid is applied in the treatment of various skin conditions such as acne, warts (verrucae), eczema, and psoriasis. It possesses keratolytic activity, helping remove dead skin cells and accelerating skin regeneration.

Acetylsalicylic acid reduces platelet aggregation, which is essential in the prevention of ischemic heart disease, stroke, and myocardial infarction (Patrono et al., 2004). Salicylic acid is a multifunctional bioactive compound whose health benefits manifest in several directions: pain reduction, anti-inflammatory effects, antiseptic properties, and cardiovascular protection.

Moreover, its chemical derivative, acetylsalicylic acid, remains one of the most widely used pharmaceutical agents in modern medicine.



Kaempferol

Kaempferol is a biologically active compound belonging to the class of natural polyphenolic flavonoids, commonly found in many plants, especially leafy greens, onions, broccoli, green tea, pears, strawberries, and grape skins. It is recognized for its strong antioxidant, anti-inflammatory, anticancer, and cardioprotective properties.

Kaempferol neutralizes the effects of free radicals in the body. Free radicals damage cellular DNA, contributing to aging, chronic diseases, and increased cancer risk. Kaempferol protects cells from oxidative stress, slows down skin aging, and strengthens the immune system. It is also considered beneficial for cardiovascular health: it helps lower blood pressure, maintains vascular elasticity, reduces levels of “bad” cholesterol (LDL), prevents thrombus formation, decreases the risk of heart attacks and strokes, and improves blood circulation [7].

Kaempferol, as one of the major flavonoids found in many fruits and vegetables, demonstrates potent antioxidant and anti-inflammatory activities. Studies indicate that kaempferol may reduce the risk of cancer, cardiovascular diseases, diabetes, and other chronic disorders.

Main benefits of kaempferol: Antioxidant properties: Neutralizes free radicals, slows cellular aging, and reduces oxidative DNA damage.

Anti-inflammatory action: Decreases the production of pro-inflammatory cytokines (e.g., TNF- α , IL-6), thereby reducing the risk of chronic inflammatory disorders such as arthritis, asthma, and cardiovascular diseases.

Cardiovascular protection: Helps maintain normal blood pressure, preserves vascular elasticity, reduces LDL oxidation, and prevents atherosclerosis.

Neuroprotection (brain health): Protects neuronal cells from oxidative stress and may lower the risk of neurodegenerative diseases such as Alzheimer’s and Parkinson’s (based on preliminary studies).

Potential anticancer effects: Laboratory and animal studies suggest that kaempferol may slow the growth of breast, lung, prostate, liver, and colorectal cancer cells. However, these findings are not yet fully confirmed in humans.

Diabetes and glucose regulation: May increase insulin sensitivity and influence enzymes involved in glucose metabolism.

Kaempferol reduces inflammatory processes by lowering the activity of cytokines and enzymes such as COX-2, making it beneficial in chronic inflammatory diseases. It also exerts

neuroprotective effects by safeguarding nerve cells and reducing oxidative stress in the brain, potentially preventing neurodegenerative disorders (e.g., Alzheimer's, Parkinson's). Furthermore, kaempferol improves insulin sensitivity and helps regulate blood glucose levels, thereby reducing the risk of diabetic complications such as vascular damage, eye disorders, and kidney problems. It promotes skin cell regeneration, reduces skin inflammation, and protects against ultraviolet (UV) radiation, which is why it is used in cosmetic formulations.

During this research, the amount of polyphenols in 100 g of Purple Coneflower (seed) extract was determined using the HPLC method. The results showed that salicylic acid (464.528 mg) and kaempferol (54.033 mg) were present in high quantities. These polyphenols possess notable positive effects on human health, which significantly increases their scientific and practical importance. Taking this into account, the development of a food supplement derived from Purple Coneflower seeds is considered a relevant and promising direction. In future work, we aim to conduct further studies in this area.

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