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Identification Of Selected Polyphenols In The Leaves Of Purple Coneflower (*Echinacea Purpurea*)

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This study presents the results of the identification of selected polyphenolic compounds in the leaves of Echinacea purpurea (purple coneflower) cultivated in Uzbekistan, using high-performance liquid chromatography (HPLC). The analysis focused on quantifying key bioactive compounds such as rutin, salicylic acid, quercetin, and apigenin, which are known for their therapeutic and antioxidant properties. The findings contribute to the phytochemical characterization of Echinacea purpurea and highlight its potential as a natural source of health-promoting substances in the development of functional foods and herbal medicines.
Keywords: Echinacea purpurea, leaf, polyphenol, rutin, salicylic acid, quercetin, apigenin, medicinal properties.

Introduction

Echinacea purpurea (commonly known as purple coneflower) is a flowering plant belonging to the Asteraceae family and is widely recognized for its medicinal properties. Native to North America, this plant holds considerable ecological and agronomic significance and has been extensively utilized in both natural ecosystems and cultivated settings for its therapeutic applications. Numerous scientific investigations have demonstrated its relevance in various environmental conditions and its positive effects on human health [1, pp. 191–202].

The stem of *E. purpurea* is green, dense, and approximately 5 mm thick, containing an inner white membranous layer, typically segmented into 10–12 sections. Its fruits are light yellow in color [2, pp. 11630–11639]. Several researchers have examined the chemical composition of *Echinacea purpurea*, reporting that the seed contains 7–30% protein, along with approximately 583 mg of essential and 169 mg of non-essential amino acids per 100 grams of dry matter. These results were demonstrated in the work of Merali S., Binns S., Paulin-Levasseur M., Ficker C., Smith M., Baum B.R., Brovelli E., and Arnason J.T. [3, pp. 171–179].

The fruit pericarp contains about 20% minerals, vitamins, and lipids, with water constituting the remaining 80%. The upper layer of the pericarp contains approximately 0.6% essential oil, composed primarily of terpenes (90%) and citral aldehyde (6%). This essential oil has a pale yellow color, a pleasant aroma, and exhibits strong bactericidal properties. The plant is also rich in macroelements such as potassium (K), calcium (Ca), magnesium (Mg),

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phosphorus (P), iron (Fe), copper (Cu), selenium (Se), sodium (Na), and zinc (Zn) [4, pp. 102–108].

The micronutrient profile of *E. purpurea* leaves and stems has also been analyzed by researchers including Bhatia H., Pal Sharma Y., Manhas R.K., and Kumar K. [5, pp. 1298–1308]. These chemical constituents are responsible for the plant's notable medicinal properties. *E. purpurea* has been reported to help reduce blood cholesterol levels, prevent hair loss, and support the functioning of the liver and gallbladder. It also aids in the formation of functional leukocytes, thus enhancing immune response against viral and infectious diseases [6, pp. 63–72; 7, pp. 233–241].

This study presents the results of a high-performance liquid chromatography (HPLC) analysis conducted to identify selected polyphenolic compounds in the leaves of *Echinacea purpurea* cultivated in Uzbekistan.

Materials and Methods

Reagents and Equipment

Gallic acid was obtained from Macklin (China), and salicylic acid from Rhydburg Pharmaceuticals (Germany). Quercetin, apigenin, and kaempferol were purchased from Regal (China), while rutin was isolated from natural plant sources through extraction and column chromatography. Analytical-grade reagents used for HPLC analysis included ultrapure water, acetonitrile, glacial acetic acid, and sodium hydroxide. Quantitative analysis of polyphenolic compounds in the leaf extract was performed using a high-performance liquid chromatography (HPLC) system LC-40 Nexera Lite (Shimadzu, Japan).

Preparation of Standard Solutions

Stock solutions of gallic acid (5.2 mg), salicylic acid (5.2 mg), rutin (5 mg), quercetin (5 mg), apigenin (5 mg), and kaempferol (5 mg) were prepared in 96% ethanol and sonicated in an ultrasonic bath for 20 minutes. Each solution was transferred to a 50 mL volumetric flask and brought to volume with ethanol. A 200 µL aliquot from each standard solution was mixed, and four different diluted working solutions were prepared by serial dilution. The resulting solutions were transferred into vials for HPLC injection.

Preparation of *Echinacea purpurea* Leaf Extract

A 1 g portion of dried and powdered *Echinacea purpurea* leaf was accurately weighed using an NV222 analytical balance (OHAUS, USA) with a precision of 0.01 g. The sample was placed into a 50 mL conical flask and extracted with 25 mL of 96% ethanol in a GT SONIC-D3 ultrasonic bath (China) at 60 °C for 20 minutes. After cooling, the mixture was filtered and the volume was adjusted to 25 mL with ethanol in a volumetric flask. A 1.5 mL aliquot of the extract was centrifuged using a Mini-7 centrifuge (BIOBASE, China) at 7000 rpm. The supernatant was filtered through a 0.45 µm syringe filter and used for chromatographic analysis.

Chromatographic Conditions

The identification of phenolic compounds was carried out on a reversed-phase Shim-pack GIST C18 column (150 × 4.6 mm, 5 µm; Shimadzu, Japan) using a gradient mobile phase consisting of solvent A (acetonitrile) and solvent B (0.5% aqueous acetic acid), as detailed in **Table 1**. 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. Detection was performed at 300 nm, where the analytical signals (peak areas) of phenolic compounds were recorded.

Table 1. Mobile Phase Gradient Program

Time, min	Acetonitrile (A), %	0.5% acetic acid (B), %
0	5	95

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5	5	95
17	40	60
22	40	60
22,1	5	95
40	Finish	

Results obtained. Determination of the amount of phenolic compounds in the extract of red echinacea leaves. A chromatogram of a sample extract weighing 1 g was obtained (Figure 1), and based on the results, the amount of phenolic compounds in 100 g of the sample was calculated using the following formula and is presented in Table 2.

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

Here, X – The amount of phenolic compounds in 100 grams of fruit, mg;

C_{phen} – concentration of phenolic compounds in the extract determined by the HPLC method, mg/l;

$V_{extract}$ – volume of sample extract, l;

m_{sample} – mass of sample taken for extract preparation.

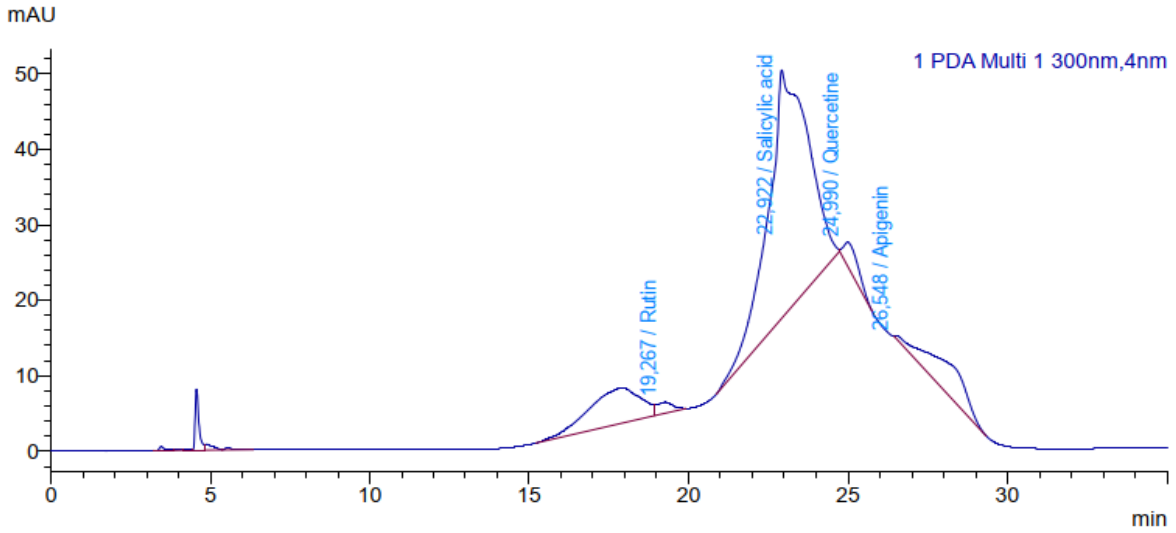

Figure 1. Chromatogram for the determination of polyphenols in red echinacea (leaf) extract.

Table 2. Amount of polyphenols in red echinacea (leaf) extract and retention times.

Phenol compound name	Holding time, sec	Concentration, mg/l	Amount in 100 ml of sample, mg
Gallic acid	Not specified	0	0,000
Routine	19,267	3,911	9,778
Salicylic acid	22,922	100,566	251,415
Quercetin	24,99	3,312	8,280
Apigenin	26,548	8,021	20,053

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Kaempferol	Not specified	0	0,000

Results and Discussion

As shown in Table 2, the high-performance liquid chromatography (HPLC) analysis of *Echinacea purpurea* leaves cultivated in Uzbekistan revealed that salicylic acid was present in the highest concentration among the identified polyphenolic compounds, amounting to 251.415 mg per 100 grams of dry extract. Other compounds such as apigenin, quercetin, and rutin were also detected in smaller quantities. However, gallic acid and kaempferol were not detected in the analyzed leaf samples.

These findings align partially with previous studies conducted by researchers such as Dr. David S. Sidwell and Dr. Larry C. Smee, who had earlier analyzed the polyphenol profile of *Echinacea purpurea* leaves grown in North America [8, pp. 478–483]. A comparative evaluation was conducted between our results (Table 2) and those reported by the aforementioned researchers, with the summary of this comparison presented in Table 3.

Table 3. Comparative analysis of phenolic compounds in *Echinacea purpurea* leaves grown in Uzbekistan and North America

Phenolic compound name	Amount in 100 g of red echinacea (leaf) growing in Uzbekistan, mg	Amount in 100 g of sample of red echinacea (leaf) growing in North America, mg	The difference
Rutin	9,778	7,785	*-1,993*
Salicylic acid	251,415	197,125	*-54,29*
Quercetin	8,280	6,781	*-1,499*
Apigenin	20,053	18,451	*-1,602*

As evident from Table 3, the content of polyphenolic compounds in *Echinacea purpurea* leaves cultivated in Uzbekistan is significantly higher compared to that in leaves of the same species grown in North America, based on equivalent dry mass (100 g). Specifically, the salicylic acid concentration in Uzbek-grown samples was found to be 54.29 mg higher than in the North American counterpart. In addition, the levels of other polyphenols—apigenin, quercetin, and rutin—also exceeded those reported for North American samples. This comparative abundance of polyphenols serves as a scientific basis for suggesting that *E. purpurea* grown in Uzbekistan may possess superior therapeutic properties relative to its North American variant.

Polyphenolic compounds are well known for their ability to reduce oxidative stress at the cellular level. They neutralize free radicals and protect cellular components such as DNA, membranes, and proteins from oxidative damage. Furthermore, polyphenols contribute to the maintenance of vascular elasticity, help lower cholesterol levels, and regulate blood pressure, all of which significantly reduce the risk of cardiovascular diseases.

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These compounds also exhibit anti-inflammatory activity, suppressing chronic inflammation and mitigating the progression of diseases such as arthritis, diabetes, and cancer. Certain polyphenols, including flavonoids and resveratrol, interact with enzymes that control cell proliferation, thereby inhibiting or slowing the growth of cancer cells. In addition, polyphenols enhance cognitive function, offering neuroprotection in the context of degenerative diseases such as Alzheimer's and Parkinson's. They also stimulate the immune system, strengthening the body's defense mechanisms against viruses, bacteria, and other pathogens [9, pp. 241–244].

Polyphenols are naturally found in a wide range of plant-based foods such as vegetables, fruits, tea, coffee, cocoa, and spices, and their regular intake through a balanced diet is crucial for long-term health.

Among the polyphenols found in *Echinacea purpurea* grown in Uzbekistan, salicylic acid ($C_7H_6O_3$) stands out due to its high concentration and broad pharmacological potential. It is a well-known natural and synthetic compound with strong anti-inflammatory and keratolytic (skin-exfoliating) effects. Originally isolated from the bark of willow (*Salix*) trees, salicylic acid has found extensive application in pharmaceuticals, dermatology, and cosmetic science. It is especially effective in the treatment of skin conditions, the reduction of inflammation and pain, and the prevention of cardiovascular complications [10, pp. 111–116].

These findings underscore the pharmacological promise of *E. purpurea* leaves cultivated in Uzbekistan and highlight their potential utility in the development of natural therapeutic agents and nutraceutical products.

Conclusion

In summary, the results of this study demonstrate that the leaves of *Echinacea purpurea* cultivated in Uzbekistan contain a significantly high concentration of salicylic acid (251.415 mg per 100 g of extract), which exceeds the levels of other identified polyphenolic compounds. Although smaller amounts of apigenin, quercetin, and rutin were also detected, gallic acid and kaempferol were not present in the analyzed samples. A comparative assessment with data reported for *Echinacea purpurea* grown in North America confirmed that the Uzbek-grown variant has a higher total polyphenol content.

These findings provide scientific evidence that *E. purpurea* cultivated in Uzbekistan possesses greater pharmacological potential than its North American counterpart, particularly due to its higher concentration of bioactive polyphenols. Given this, further research focused on developing natural, health-promoting food additives and herbal formulations from the leaves of Uzbek-grown *Echinacea purpurea* is of significant scientific and practical importance. Such studies could contribute to the production of effective, eco-friendly, and functional nutraceutical products aimed at enhancing human health.

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