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HPLC-BASED ANALYSIS OF PHENOLIC COMPOUNDS IN ZIZIPHORA EXTRACT

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Abstract
This study investigates the content of phenolic compounds in the extract of *Ziziphora* (locally known as kiyik o‘ti) using High-Performance Liquid Chromatography (HPLC) method. Standard solutions of phenolic compounds such as gallic acid, salicylic acid, rutin, quercetin, apigenin, and kaempferol were prepared and compared with the plant extract. The results confirmed the presence of apigenin, rutin, salicylic acid, and quercetin in the extract, while gallic acid and kaempferol were not detected. These findings are important for evaluating the biochemical composition of the plant and its potential medicinal properties.
Keywords: *Ziziphora*, wild thyme, phenolic compounds, flavonoids, high-performance liquid chromatography (HPLC), gallic acid, salicylic acid, rutin, quercetin, apigenin, kaempferol, extraction, chromatographic analysis, polyphenols, medicinal plants, antioxidants, analytical chemistry, bioactive compound, plant extract, pharmacognosy.

Introduction

Medicinal plants have long been used in traditional medicine, and in recent years, there has been growing scientific interest in studying their biochemical composition and pharmacological potential. The genus *Ziziphora*, particularly *Ziziphora clinopodioides* subsp. *rigida*, belonging to the family *Lamiaceae*, is widely distributed in Central Asia and traditionally used as an antiseptic, sedative, and anti-inflammatory agent [1]. In folk medicine, the aerial parts of this plant are employed in the treatment of digestive, respiratory, and cardiovascular disorders, which is attributed to its diverse phytochemical composition [2].

Among the bioactive compounds present in *Ziziphora* species, phenolic compounds—especially flavonoids—play a significant role. These compounds exhibit antioxidant, anti-inflammatory, antimicrobial, and antitumor properties [3,4]. Phenolics exert therapeutic effects by scavenging free radicals, modulating enzyme activity, and influencing cellular signaling pathways [5]. Therefore, the qualitative and quantitative analysis of these phenolic compounds is crucial for a deeper understanding of the plant extract’s medicinal potential.

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Among modern analytical techniques, High-Performance Liquid Chromatography (HPLC) is considered one of the most accurate and reliable methods for identifying and quantifying phenolic compounds [6]. Due to its high precision, sensitivity, and reproducibility, HPLC is widely regarded as the "gold standard" in the phytochemical analysis of medicinal plants [7].

In this study, phenolic compounds in the plant *Ziziphora clinopodioides* subsp. *rigida* were identified using the HPLC method. Standard solutions of gallic acid, salicylic acid, rutin, quercetin, apigenin, and kaempferol were prepared and compared with the plant extract. The results contribute to a deeper understanding of the phytochemical profile of this medicinal plant.

Materials and Methods

Reagents and Equipment. Gallic acid was obtained from Macklin (China), salicylic acid from Rhydburg Pharmaceuticals (Germany), while quercetin, apigenin, and kaempferol were sourced from Regal (China). Rutin was isolated from natural sources using extraction and column chromatography techniques. High-purity HPLC-grade solvents including water, acetonitrile, glacial acetic acid, and sodium hydroxide were used throughout the analysis. Quantification of polyphenols in the plant extract was performed using the LC-40 Nexera Lite High-Performance Liquid Chromatography (HPLC) system, manufactured by Shimadzu (Japan).

Preparation of Standard Solutions. The following standards were prepared: gallic acid (5.2 mg), salicylic acid (5.2 mg), rutin (5 mg), quercetin (5 mg), apigenin (5 mg), and kaempferol (5 mg). Each compound was dissolved in 96% ethanol using an ultrasonic bath for 20 minutes, and then transferred to 50 mL volumetric flasks and brought up to volume with ethanol. From each standard solution, 200 µL was mixed and serially diluted to prepare four different standard concentrations. Each solution was placed in vials and used for analysis.

Preparation of Plant Extract. To extract phenolic compounds, 1.00 g of *Ziziphora clinopodioides* subsp. *rigida* aerial parts was weighed using an NV222 analytical balance (OHAUS, USA) with 0.01 g precision. The sample was placed into a 50 mL conical flask, and 25 mL of 96% ethanol was added. Extraction was carried out using an ultrasonic water bath (GT SONIC-D3, China) at 60 °C for 20 minutes. After cooling, the mixture was filtered and diluted with ethanol to a final volume of 25 mL in a volumetric flask. A 1.5 mL portion of the filtrate was centrifuged using a Mini-7 centrifuge (BIOBASE, China) at 7000 rpm, filtered through a 0.45 µm syringe filter, and used for HPLC analysis.

Chromatographic Conditions. Phenolic compound analysis was performed using a Shim-pack GIST C18 reversed-phase column (150 × 4.6 mm; 5 µm, Shimadzu, Japan). The mobile phase consisted of a gradient elution using acetonitrile (Solvent A) and 0.5% aqueous acetic acid (Solvent B), as outlined in Table 1. The injection volume was 10 µL, flow rate was set at 0.5 mL/min, and the column oven temperature was maintained at 40 °C. Detection was carried out at a wavelength of 300 nm based on peak area measurements (Figure 1).

Table 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

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22	40	60
22,1	5	95
40	Finish	

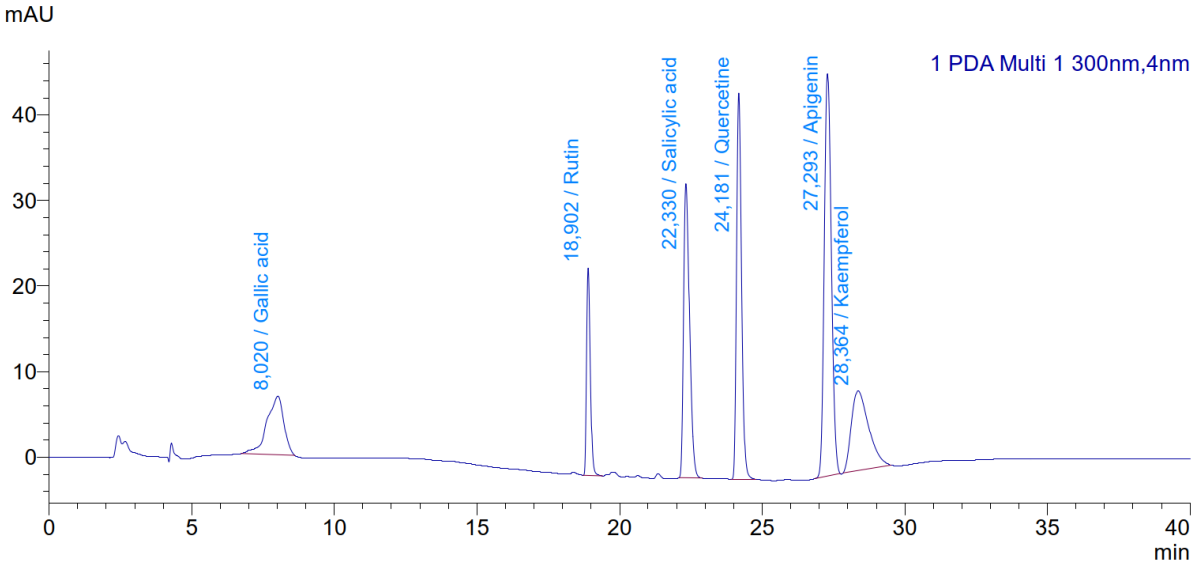

Figure 1. Chromatogram of standards at 300 nm.

Results and Discussion

Quantification of Phenolic Compounds in the Extract Sample. A chromatogram of the extract sample obtained from 1 g of *Ziziphora* plant material was recorded (Figure 2). Based on the chromatographic data and comparison with standard solutions, the concentrations of individual phenolic compounds present in 100 g of sample were calculated using the following formula and presented in Table 3.

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

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.

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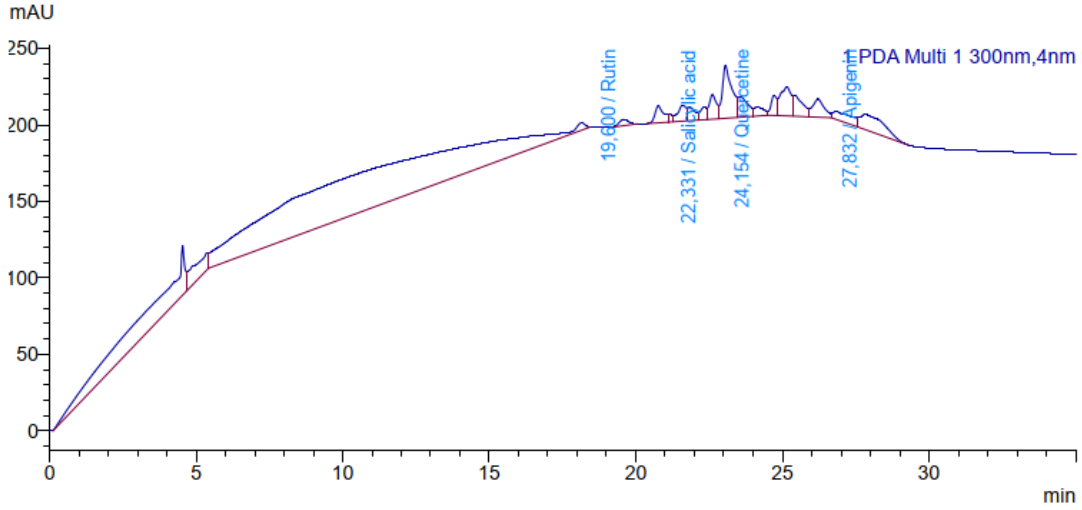

Figure 2. Chromatogram of polyphenols in the sample extract. Amount of polyphenols in the extract and retention times.

Table 2. Table 2. Retention times and concentrations of phenolic compounds identified in the Ziziphora plant extract.

Phenol compound name	Holding time, sec	Concentration, mg/l	Amount in 100 ml of sample, mg
Gallic acid	Not specified	0	0,000
Routine	19,6	6,856	17,140
Salicylic acid	22,331	4,508	11,270
Quercetin	24,154	4,063	10,158
Apigenin	27,832	12,781	31,953
Kaempferol	Not specified	0	0,000

The results of the chromatographic analysis of phenolic compounds present in the plant extract are presented in Table 2. Although six phenolic compounds were expected to be identified in the extract, only four were detected. The concentrations of *gallic acid* and *kaempferol* were below the detection limit and therefore recorded as 0.000 mg.

Among the compounds, *apigenin* was found to be the most abundant, with a concentration of 31.953 mg per 100 g of the sample. Apigenin is a flavonoid of notable biological significance, widely recognized for its anti-inflammatory, antioxidant, sedative, and anticancer properties. It contributes to nervous system relaxation, improves sleep quality, and helps inhibit the proliferation of cancer cells. The presence of apigenin at such a high concentration is a key indicator of the therapeutic potential of the extract.

Rutin was the second most abundant compound, with a concentration of 17.140 mg/100 g. Rutin is well known for strengthening capillary blood vessels, providing antioxidant effects, and reducing inflammation. It is extensively used in pharmaceutical preparations and functional foods.

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Salicylic acid and *quercetin* were also identified in appreciable quantities—11.270 mg/100 g and 10.158 mg/100 g, respectively. Salicylic acid is widely used in medicine as an analgesic and antiseptic agent, while quercetin is a powerful antioxidant that supports cardiovascular health, enhances immune function, and exhibits anti-allergic effects.

Conclusion

This study successfully identified the phenolic compounds present in the extract of *Ziziphora clinopodioides* subsp. *rigida* using High-Performance Liquid Chromatography (HPLC). Among the six expected phenolic compounds, four bioactive constituents—apigenin, rutin, salicylic acid, and quercetin—were detected in quantifiable amounts, while gallic acid and kaempferol were below the limit of detection.

The highest concentration was observed for apigenin (31.953 mg/100 g), a flavonoid known for its potent anti-inflammatory, antioxidant, anxiolytic, and anticancer properties. This high concentration suggests that apigenin may play a key role in the extract's therapeutic efficacy. Rutin (17.140 mg/100 g), another major flavonoid identified, is widely recognized for strengthening capillary vessels, modulating blood pressure, and providing anti-inflammatory and antioxidant effects. Salicylic acid (11.270 mg/100 g) and quercetin (10.158 mg/100 g), both well-documented for their analgesic, antipyretic, and cardiovascular protective properties, were also present in significant amounts.

The results indicate that *Ziziphora* extract is rich in bioactive polyphenols that contribute to a wide range of therapeutic actions, particularly in managing cardiovascular diseases, inflammation, oxidative stress, and immune-related disorders. These findings underscore the potential of *Ziziphora clinopodioides* as a natural source of medicinal compounds and support its traditional use in herbal medicine.

From a pharmacognostic and pharmaceutical development perspective, the presence of high-value phenolic constituents—especially apigenin and rutin—demonstrates the extract's potential in the formulation of phytopharmaceuticals or nutraceutical products. Moreover, further studies on the toxicological safety, bioavailability, and clinical efficacy of these compounds will be essential to validate their medicinal applications.

In conclusion, the phenolic profile of *Ziziphora* provides a strong scientific foundation for its use as a therapeutic agent and opens promising avenues for its integration into modern pharmaceutical and functional food industries.

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