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PHYTOCHEMICAL AND ELEMENTAL PROFILING OF *LIMONIUM OTOLEPIS* GROWING IN THE FERGANA REGION, UZBEKISTAN: HPLC AND ICP-OES ANALYSIS*

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This study aimed to investigate the phenolic and elemental composition of the aerial parts of *Limonium otolepis* growing in the Fergana region, Uzbekistan. High-performance liquid chromatography (HPLC) analysis was employed to identify and quantify the phenolic compounds present in the plant. The analysis revealed four major phenolic compounds, *viz.* rutin, apigenin, gallic acid, and hyperoside. The concentrations of these compounds were measured, with gallic acid showing the highest concentration at 77.521 mg/100 g, followed by rutin at 38.968 mg/100 g, apigenin at 26.351 mg/100 g, and hyperoside at 18.254 mg/100 g. Additionally, elemental analysis using inductively coupled plasma-optical emission spectrometry (ICP-OES) was conducted to determine the macro- and microelemental content of the plant. Significant levels of essential elements such as potassium, calcium, magnesium, and iron were found, highlighting the plant's nutritional and therapeutic potential. Potassium, in particular, was present at 586.241 mg/10 g, underscoring its importance for metabolic processes. The absence of harmful heavy metals such as lead and mercury further supports the safety of the plant for use in food and medicinal applications. These findings underscore the potential of *L. otolepis* as a valuable source of bioactive compounds and essential nutrients, making it a promising candidate for the development of phytopreparations and nutritional supplements.

Keywords: *Limonium otolepis*, flavonoids, macroelements, microelements, HPLC, ICP-OES.

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Introduction

Limonium otolepis (Schrenk) Kuntze, a member of the *Plumbaginaceae* family, is a perennial herbaceous plant known for its distinctive morphology and ecological adaptability. Native to Afghanistan, Central Asia, Xinjiang and western Gansu in China, this species thrives in saline and arid environments, contributing to its unique biochemical profile [1, 2]. The genus *Limonium*, commonly referred to as sea lavender, comprises over 600 species distributed globally, often in coastal and saline habitats. These plants are characterized by their rosette leaves and inflorescences of small, papery flowers, making them popular in ornamental horticulture [3]. *Limonium* species have demonstrated a range of biological activities in both *in vitro* and *in vivo* studies [4, 5]. For example, a number of compounds isolated from *L. myrianthum, L. leptophyllum,* and *L. gmelinii* through comparative studies have demonstrated antimalarial activity [6]. The ethanolic extract of *L. vulgare* has shown significant cytotoxic activity against *Artemia salina* and *Daphnia magna*. Additionally, it exhibited anti-neoplastic effects in the potato disk assay, indicating its potential for cancer-related applications [7]. The essential oil of *L. oleifolium* exhibited strong biological activities, including antiamoebic, leishmanicidal, and antimicrobial effects, while demonstrating minimal toxicity in macrophage cells [8]. Phytopreparations from *Limonium* species have also demonstrated notable biological activities, including antiviral, hepatoprotective, anti-inflammatory, and anti-burn properties [9]. Earlier phytochemical and biological research on the *Limonium* genus has revealed the presence of various classes of compounds, including lignanamides and anthocyanins (Table 1).

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Table 1. Overview of compound classes identified in some *Limonium* species collected from various geographic regions

Species	Collection location	Compound classes	Reference
L. brasiliense	San Martin, Argentina	Flavonoids, anthocyanins, phenolic acids	[10]
L. axillare	Suez Canal Road, Egypt	Phytosterols, terpenes	[11]
L. densiflorum	Sebkha Sidi El Hani, Tunisia	Phenolic acids, flavonoids, lignanamides	[12]
L. algarvense	Ria de Alvor, Portugal	Flavonoids, fatty acids, aminoacids, polysaccharides	[13]
L. gmelinii	Xinjiang, China	Lignanamides	[14]
	Kazakhstan	Pigments, lipids	[15]

Very little is known about the chemical composition of *L. otolepis*, although it also has potential pharmacological uses. To the best of our knowledge, only lipophilic pigments, total lipid content, fatty acids from the aerial parts [15] and phytoecdysteroid composition [16] have been studied. In this study, we aimed to investigate the phenolic, macro- and microelemental composition of the aerial parts of *L. otolepis* growing in the Fergana region, Uzbekistan (Fig. 1).

Materials and Methods

Plant Material. The plant samples were collected during the flowering season in June-July, 2024 from the Dangara district, Fergana region, Republic of Uzbekistan (40°37'52.3"N 70°51'28.6"E). The specimens were identified as *L. otolepis* by the Department of Botany at Fergana State University. A total of 1 kg of aerial parts were collected, dried at room temperature for 72 hours in a dark, ventilated space, and ground to a fine powder using a Wiley-Mill plant grinder. The ground material was sieved to obtain fractions of the same particle size.

Extraction Procedure for HPLC analysis. The extraction of 10 gr sample was performed twice using 70% ethanol (900 ml) at 70–75 °C for 3 hours with intensive stirring (250 rpm), maintaining a solvent-to-plant ratio of 90 : 20 (v/w). The solutions were filtered using Whatman No. 1 filter paper and combined. The final extract was concentrated under reduced pressure using a rotary evaporator (RE-501, China) and dried to obtain a crude extract (0.8 g).

Chemicals and Reagents. All chemicals used for analysis were of analytical grade. Acetonitrile and standard compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA).

HPLC Analysis. The analysis was performed using an Agilent Technologies 1260 Infinity II LC System equipped with an Eclipse XDB-C18 column (5.0 µm, 4.6 × 250 mm) and a diode-array detector (DAD) in isocratic elution mode. The mobile phase consisted of acetonitrile and acetate buffer (pH 2.92) in a 30 : 70 (v/v) ratio. The DAD detector was set to a scanning range of 200–400 nm. A sample injection volume of 5 µL was employed, and the analysis was carried out at a flow rate of 0.75 mL/min. The total runtime for the analysis was 15 minutes. Chromatograms were monitored at three wavelengths: 254, 265 and 281 nm. Stock solutions were prepared by dissolving each standard in methanol to obtain concentrations of 1 mg/mL. Calibration curves were generated using five concentrations (0.05, 0.1, 0.2, 0.5 and 1 mg/ml). The retention times and UV spectra of the phenolic compounds in the sample were compared with those of the standards for identification.

Fig. 1. Left: Plant collection site in Fergana region (black dot) with Uzbekistan's border marked in red. Right: Photograph of the *L. otolepis* on the site

Elemental Analysis. A precise 0.1000 g of the sample was transferred into the Teflon autoclave. To this, 3 ml of purified concentrated nitric acid (HNO_3) and 2 ml of purified hydrogen peroxide (H_2O_2) were added. The autoclave was sealed and placed in a Berghof microwave digester. The digestion was carried out for 40 minutes under conditions of a minimum temperature of 50 °C, a maximum temperature of 230 °C, and a maximum pressure of 40 bar. The autoclave was then cooled to room temperature, and the resulting liquid mixture was transferred into a 50 ml volumetric flask. The flask was then filled to the mark with ultrapure water (by the Millipore Milli-Q system). The mineralized solution was analyzed using a Perkin Elmer Avio-200 Inductively coupled plasma-optical emission spectrometer (ICP-OES). The content of macro and microelements in the sample was quantitatively analyzed relative to the standard sample introduced.

Results and Discussion

A total of eight compounds, *viz.* rutin, apigenin, quercetin, hyperoside, isorhamnetin, hypolaetin, gallic acid and hypolaetin-7-O-D-glycoside, were used as standards for the identification of phenolic compounds. The chromatograms are given in the supplementary material. Among these compounds, four phenolic compounds were identified from the aerial parts of the sample: rutin, apigenin, gallic acid, and hyperoside (Table 2).

The sample contained 26.351 mg/100 g of apigenin. This flavonoid is known for its anti-inflammatory, antioxidant, and anticancer properties, making it a valuable component for pharmaceutical and nutraceutical applications [17, 18]. With 38.968 mg/100 g, rutin was another major component. Rutin is renowned for its ability to strengthen blood vessels and its potent antioxidant effects, contributing to cardiovascular health [19, 20]. The highest concentration found was of gallic acid, at 77.521 mg/100 g. Gallic acid possesses strong antioxidant and antimicrobial properties, which can be beneficial in various therapeutic contexts [21, 22]. The sample also contained 18.254 mg/100 g of hyperoside, known for its antioxidant, anti-inflammatory, and anticancer activities [23, 24]. Hypolaetin, hypolaetin 7-O-D-Gly, isorhamnetin and quercetin were not detected. The absence of these compounds indicates that their presence is either negligible or undetectable within the sample.

In the course of the studies, it was observed that phenolic compounds in *Limonium* species exhibit considerable diversity in both quantity and composition. For instance, in the aerial parts of *L. duriusculum*, apigenin and apigenin-7-O-β-D-(6’’-methylglucuronide) were predominantly accumulated, with apigenin levels reaching 160 mg/100 g (compared to 26.351 mg/100 g in *L. otolepis*) [25]. *L. algervense* was notably rich in gallic acid, with a concentration of 585 mg/100 g (in contrast to 77.521 mg/100 g in *L. otolepis*) [26], while quercetin was not detected in either *L. algervense* or *L. otolepis*, despite its presence in other *Limonium* species, including *L. bicolor* [27]. HPLC analysis of *L. densiflorum* revealed that the major compounds were myricetin, trans-3-hydroxycinnamic acid, and isorhamnetin, with relative area percentages of 4.736%, 14.141%, and 12.604%, respectively [28].

The elemental analysis of the plant sample reveals a diverse and rich composition of both macro- and microelements, indicating its potential nutritional and therapeutic value (Table 3).

Potassium (K) has the highest concentration among the elements, measured at 586.241 mg/10 g. Calcium (Ca) and Magnesium (Mg) follow with concentrations of 251.130 mg/10 g and 75.070 mg/10 g, respectively. Sodium (Na) is also relatively high at 59.096 mg/10 g. Iron (Fe) is present at 11.289 mg/10 g. Phosphorus (P) and Silicon (Si) are present in significant amounts at 35.309 mg/10 g and 5.406 mg/10 g, respectively. The harmful heavy metals such as lead (Pb) and mercury (Hg) were not detected in the sample. This is a positive indication of the plant’s safety for consumption and its suitability for use in food and medicinal applications. The low levels of cadmium (Cd) at 0.014 mg/10 g and chromium (Cr) at 0.007 mg/10 g further support the plant’s safety profile. The absence of elements like Tellurium (Te), Selenium (Se), Antimony (Sb), Tin (Sn), Arsenic (As), and Silver (Ag) suggests that the plant does not accumulate these elements, which could pose health risks if present in significant amounts. This further emphasizes the plant’s potential as a safe nutritional supplement. The presence of elements like vanadium (V), manganese (Mn), boron (B), copper (Cu), and cobalt (Co) in trace amounts enhances the nutritional value of the plant. Among *Limonium* species, *L. bicolar* was studied for its macro- and micronutrients. A study by Wu *et al*. (2007) examined the flower, stem, leaf, and root of *L. bicolor* using Flame Atomic Absorption Spectroscopy (FAAS) [29] (Fig. 2).

Table 2. The HPLC analysis results for the aerial parts of *L. otolepis*

No.	Retention time (min)	Area (%)	Measured (mg/100 g)	Identificacion	λ (nm)
1	1.727	4.7464	26.351	Apigenin	265
2	2.262	18.8077	38.968	Rutin	281
3	1.857	27.1262	77.521	Gallic acid	254
4	2.815	22.0622	18.254	Hyperoside	265

Table 3. Inductively coupled plasma-optical emission spectrometer (ICP-OES) results for the *L. otolepis* aerial part. The values are expressed in milligrams per 10 g (mg/10 g)

Element	Wavelength (nm)	Measured (mg/10 g)	Element	Wavelength (nm)	Measured (mg/10 g)
Li	670.784	0.562	Fe	238.204	11.289
Al	396.153	1.121	Na	589.592	59.096
Mo	202.031	0.013	Pb	220.353	–
Te	214.281	–	Cd	228.802	0.014
Se	196.026	–	V	292.464	0.041
Sb	206.836	–	Zn	206.200	0.096
Sn	283.998	–	Cu	327.393	0.031
Sr	407.771	0.141	Ag	328.068	–
K	766.490	586.241	Hg	253.652	–
Ba	233.527	0.009	Co	228.616	0.004
Cr	267.716	0.007	Ni	231.604	0.051
Mn	257.610	0.121	P	213.617	35.309
B	249.677	0.281	Si	251.611	5.406
Ca	317.933	251.130	S	181.975	2.083
As	193.696	–	Mg	285.213	75.070

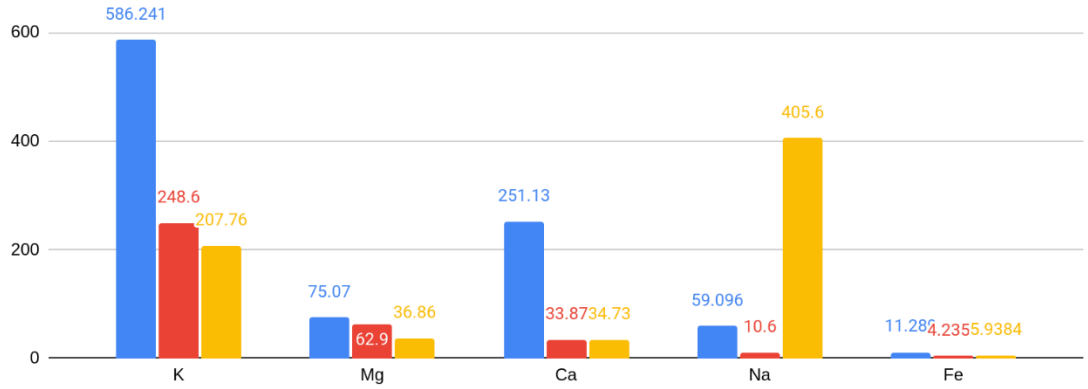

Fig. 2. The elemental composition comparison of the most abundant elements (mg/10 g) for *L. otolepis* and *L. bicolar*. Legend colors: blue – *L. otolepis* (aerial part), red – *L. bicolor* (stem), yellow – *L. bicolor* (flower)

As can be seen from the chart, *L. otolepis* exhibits a notably higher potassium concentration (586.241 mg/10 g) compared to the stem (248.6 mg/10 g) and flower (207.76 mg/10 g) of *L. bicolor*. Magnesium content is highest in *L. otolepis* (75.07 mg/10 g), with *L. bicolor*'s stem following at 62.9 mg/10 g. Calcium is most concentrated in *L. otolepis* (251.13 mg/10 g), surpassing *L. bicolor's stem* (74.73 mg/10 g). *L. bicolor*'s flower has a markedly higher sodium content (405.6 mg/10 g) than the aerial part of *L. otolepis* (10.6 mg/10 g). Additionally, *L. otolepis* contains more iron (11.284 mg/10 g) compared to the stem (2.355 mg/10 g) and flower (5.9384 mg/10 g) of *L. bicolor*.

Conclusion

The phenolic and elemental compositions of *L. otolepis* growing in Fergana region, Uzbekistan have been studied for the first time using HPLC and ICP-OES methods, respectively. The analysis has provided valuable insights into its chemical composition and potential health benefits. The identification of rutin, apigenin, gallic acid, and hyperoside, along with the significant presence of essential macro- and microelements, highlights the plant's potential for nutritional and therapeutic applications. The absence of toxic heavy metals further supports its safety for consumption. These results suggest that *L. otolepis* could be a promising candidate for the development of phytopreparations and nutritional supplements. Further research is warranted to explore its full pharmacological potential and to establish standardized extraction and processing methods.

Supplementary Information
The electronic supplement to the article (DOI: http://www.doi.org/10.14258/jcprm.20250215697s) provides additionalexperimental material that reveals the main points set out in the article

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Conflict of Interest
The authors of this work declare that they have no conflicts of interest.

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