PRELIMINARY HPTLC INVESTIGATION OF TWO *GALEOPSIS* SPP. (*LAMIACEAE*) FROM SOUTHWEST ROMANIA FLORA

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Abstract

The paper highlights the phenolic acids content of roots, aerial parts and leaves of two Galeopsis spp. (Lamiaceae) from the southwest Romania (Oltenia Region) flora, using high-performance thin-layer chromatography (HPTLC) coupled with photodensitometry. Chlorogenic acid (CGA) was identified and quantified in all 70% ethanolic extracts of Galeopsis spp. The highest CGA amount was determined in G. speciosa leaves (9.192 mg/g), followed by G. bifida leaves (9.017 mg/g), G. speciosa roots (8.283 mg/g), G. speciosa aerial parts (7.317 mg/g), G. bifida aerial parts (3.392 mg/g), and G. bifida roots (1.825 mg/g). Our research used HPTLC for the assessment of phenolic acid content, emphasizing the pharmacological potential of Galeopsis spp., mainly the antioxidant properties, which may contribute to their traditional applications in treating respiratory and inflammatory disorders.

Key words*: *Galeopsis spp.*, *Lamiaceae*, *high-performance thin-layer chromatography*, *photodensitometry*, *polyphenols

INTRODUCTION

Galeopsis genus (*Lamiaceae* family) includes annual herbaceous species, usually known as hemp-nettle, originating from Europe and Asia. *Galeopsis* spp. are common in the Romania flora, around oak–boreal (spruce) forests, in ponds, forest clearings, near springs and along streams, in cultivated or ruderal zones, gardens (Bendiksby *et al.*, 2011; Ciocârlan, 2009; Maslova, 2008; Pozhidaev & Petrova, 2023; Tutin *et al.*, 1972).

From the phytochemical point of view, important active principles have been detected in the flowering aerial parts of *Galeopsis* spp., such as essential oil rich in monoterpenes (linalool, linalyl acetate, limonene, *α*-pinene, *β*-pinene) and sesquiterpenes (*β*-caryophyllene, *β*-farnesene, germacrene D, bicyclogermacrene), flavonoids (hypoletin, scutellarein, isoscutellarein, luteolin, apigenin), iridoid glycosides (harpagoside, harpagoside-8-O-acetate, antirrhinoside, 5-O-glucosyl-antirrhinoside), labdane diterpenoids (hispanolone, galeopsin,

pregaleopsin), betaines (stachydrine or L-proline betaine), phenylethanoid glycosides (verbascoside, martynoside), saponins (daucosterol and hederagenin heterosides), polyphenolic acids (chlorogenic acid, caffeic acid, rosmarinic acid), fatty acids (epoxy- and hydroxy-derivatives), carbohydrates, amino acids, enzymes, mineral salts (soluble salts of silicic acid) (Asilbekova *et al.*, 1987; Gusakova & Asilbekova, 1991; Gusakova & Khomova, 1984; Gusakova *et al.*, 1981; Khomova *et al.*, 1983; Olennikov, 2020; Olennikov *et al.*, 2010; Rodriguez & Savona, 1980; Tomás-Barberán & Wollenweber, 1990; Tomás-Barberán *et al.*, 1991; Tyunnikova *et al.*, 2004; Venditti *et al.*, 2013; Zhang *et al.*, 2002).

In Romanian ethnopharmacology, *Galeopsidis herba* is used as an expectorant and antispasmodic, in respiratory diseases (bronchial catarrh, bronchitis, tracheitis, cough, bronchial asthma), but also as a diuretic, hematopoietic and healing agent (Grigorescu *et al.*, 1986; Istudor, 2005).

Recent research on various *Galeopsis* spp. extracts (dichloromethane, ethyl acetate, methanol, water) evidenced relevant pharmacological actions, as follows: neuroprotective effect *in vitro* (flavonoids and diterpenoids on rat pheochromocytoma PC12 cell cultures), antioxidant (flavonoids, polyphenolic acids; DPPH assay), anti-acetylcholinesterase (flavonoids), anti-inflammatory (iridoid glycosides), diuretic (saponins, flavonoids), expectorant (saponins) (Matkowski & Piotrowska, 2006; Matkowski & Wołniak, 2005; Matkowski *et al.*, 2008; Uriarte & Calvo, 2009).

Galeopsidis Tinctura is recommended for bronchitis, bronchial asthma, dry cough, laryngeal irritation, anemia, for remineralization of joint tissue (silicic acid organic derivatives), and toning of the urinary tract (Grigorescu *et al.*, 1986; Istudor, 2005).

The aim of our paper was the preliminary analysis of phenolic acids content of roots, aerial parts and leaves of two *Galeopsis* spp. (*Lamiaceae*) from the southwest Romania flora, using high-performance thin-layer chromatography (HPTLC)–photodensitometry.

MATERIALS AND METHODS

Plant material

Harvested during the flowering period, in August 2024, from the Tismana surroundings, Gorj County (Oltenia Region, southwest Romania) flora, the plant material was represented by the roots, aerial parts and leaves of two *Galeopsis* spp.: *G. bifida* Boenn. (bifid hemp-nettle, split-lip hemp-nettle) and *G. speciosa* Mill. (large-flowered hemp-nettle, Edmonton hemp-nettle). The research did not involve endangered or protected plant species. The voucher specimens (GAL-BIF-2024-0819 and GAL-SPC-2024-0819, respectively) were deposited in the Herbarium of the Department of Pharmaceutical Botany, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova.

HPTLC analysis

A HPTLC analysis was employed to detect and quantify phenolic acids. Specifically, the target compound was chlorogenic acid (CGA), a prominent phenolic acid.

CAMAG (Muttenz, Switzerland) system and the following experimental conditions were used for preliminary HPTLC–densitometric analysis of *Galeopsis* spp.: stationary phase: HPTLC silica gel 60 F_{254} (Merck, Darmstadt, Germany) 20×10 cm precoated glass plates; mobile phase: ethyl acetate–formic acid–methanol–water (15:1:0.1:1, *v/v*); 10 mL of mobile phase were added in the developing twin-chamber (CAMAG) and then oversaturated for 20 minutes; sample:

six 70% ethanolic extracts of *Galeopsis* spp.; reference compound (Merck): 0.1% methanolic solution of CGA; migration distance: 62 mm (sample application line 8 mm, solvent front 70 mm); application of sample (2 µL) and reference solutions (2–10 µL): CAMAG Linomat 5 semi-automatic system – spraying gas nitrogen, syringe volume 100 µL, dosage speed 150 nL/s, pre-dosage volume 0.2 µL, bands length of 8 mm; plate drying: five minutes, at 25ºC (air dryer); plate shooting: ultraviolet (UV) light (254 nm, 365 nm); detection: CAMAG TLC Scanner 3 photodensitometer (for densitogram) and *in situ* UV (280 nm) spectra, without derivatization, deuterium–tungsten lamp, scanning speed 20 mm/s, data resolution 100 µm/step, absorbance measurement; visionCATS ver. 3.2 software package (CAMAG) (Altemimi *et al.*, 2015; Bojić *et al.*, 2013; Gîrd *et al.*, 2014; Jug *et al.*, 2018).

The calibration curve was an integral part of the study to quantify CGA in *Galeopsis* spp. samples using HPTLC analysis. CGA was used as the reference compound and was applied in varying volumes (2–10 µL) on the HPTLC plates to create a range of concentrations. A linear relationship was established between the concentrations of CGA and the intensity of the corresponding bands (densitometry at 280 nm).

DPPH *in situ* antioxidant assay

The HPTLC plates were sprayed, in the CAMAG TLC Spray Cabinet 2, with 0.5 mM of 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanolic solution; then, the plates were dried at room temperature, in the dark, for 90 seconds, heated at 60°C, in an oven, for 30 seconds, and analyzed in white light illumination. The antioxidant activity was assessed based on the intensity of yellow color bands (Balekundri *et al.*, 2024; Islam *et al.*, 2021; Pozharitskaya *et al.*, 2008).

RESULTS AND DISCUSSIONS

Figures 1–6 highlighted the results of the preliminary HPTLC analysis of phenolic acids in two *Galeopsis* spp.

The calibration curve of CGA (Figure 6), generated by linear regression mode, was used for quantitative analysis: $y = 1.048\times10^{-8}x$. Validation of the curve included the following parameters: range deviation 5%, ensuring a high level of precision in measurements; coefficient of variation (CV) 1.95%, indicating excellent repeatability and low variability in the data; correlation coefficient (R) 0.998, which reflects a near-perfect linear relationship between concentration and absorbance intensity. The high correlation coefficient (R=0.998) demonstrates the robustness of the calibration curve. The linearity and minimal variation validate the reliability of the method in quantifying CGA in complex plant extracts.

The investigation revealed variable amounts of CGA in herbal parts: *G. speciosa* leaves (9.192 mg/g) > *G. bifida* leaves (9.017 mg/g) > *G. speciosa* roots (8.283 mg/g) > *G. speciosa* aerial parts (7.317 mg/g) > *G. bifida* aerial parts (3.392 mg/g) > *G. bifida* roots (1.825 mg/g).

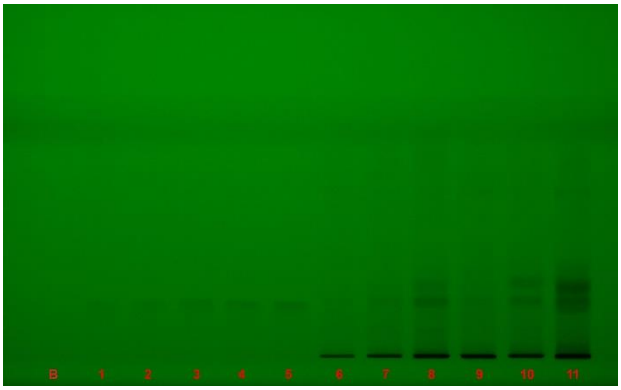

Figure 1. HPTLC chromatogram of phenolic acids from *Galeopsis* spp. 70% ethanolic extracts: UV 254 nm, without derivatization. B: Blank; Lanes 1–5: Chlorogenic acid (R_f 0.22); Lanes 6–8: *G. bifida* samples (*radix*, *herba* and *folium*, respectively); Lanes 9–11: *G. speciosa* samples (*radix*, *herba* and *folium*, respectively).

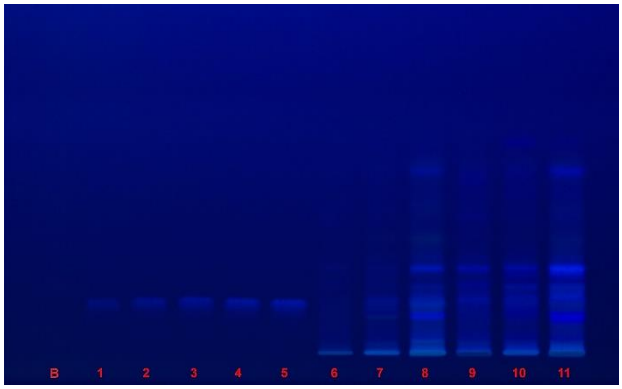

Figure 2. HPTLC chromatogram of phenolic acids from *Galeopsis* spp. 70% ethanolic extracts: UV 365 nm, without derivatization. B: Blank; Lanes 1–5: Chlorogenic acid (R_f 0.22); Lanes 6–8: *G. bifida* samples (*radix*, *herba* and *folium*); Lanes 9–11: *G. speciosa* samples (*radix*, *herba* and *folium*).

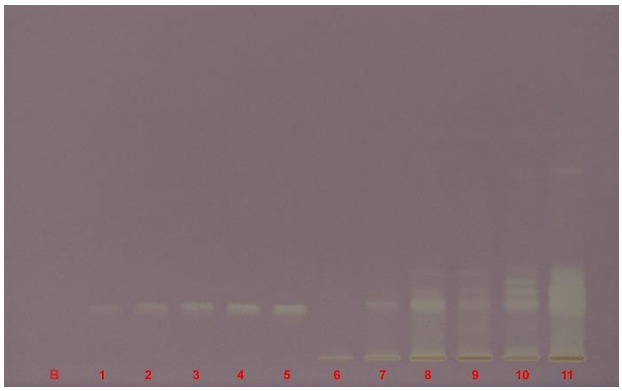

Figure 3. HPTLC chromatogram of phenolic acids from *Galeopsis* spp. 70% ethanolic extracts: white light, derivatization with DPPH. B: Blank; Lanes 1–5: Chlorogenic acid (R_f 0.22); Lanes 6–8: *G. bifida* samples (*radix*, *herba* and *folium*); Lanes 9–11: *G. speciosa* samples (*radix*, *herba* and *folium*).

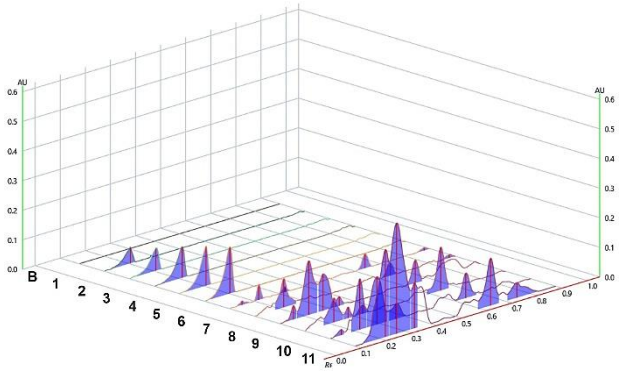

Figure 4. Densitogram of phenolic acids from *Galeopsis* spp. 70% ethanolic extracts: UV 280 nm, without derivatization. B: Blank; Lanes 1–5: Chlorogenic acid (R_f 0.22); Lanes 6–8: *G. bifida* samples (*radix*, *herba* and *folium*); Lanes 9–11: *G. speciosa* samples (*radix*, *herba* and *folium*).

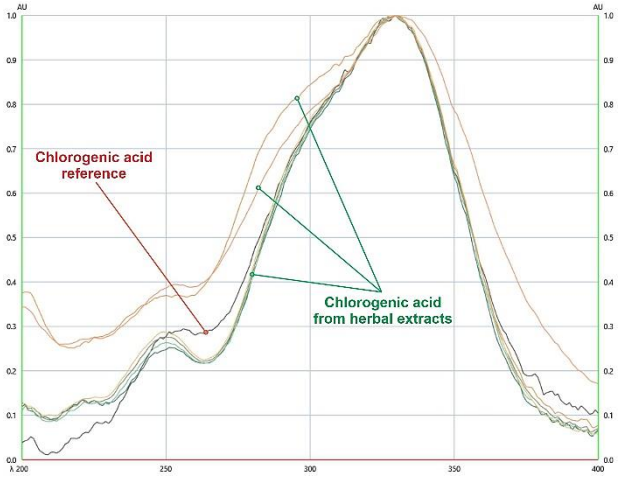

Figure 5. *In situ* UV spectra (280 nm) of chlorogenic acid reference and compound separated from the analyzed herbal samples.

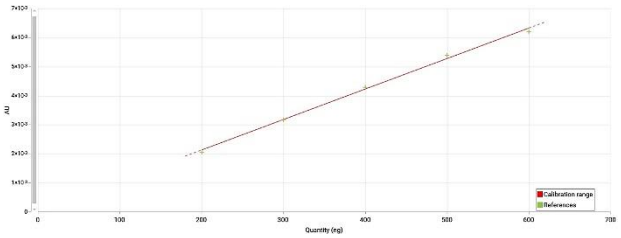

Figure 6. Chlorogenic acid reference calibration curve (UV 280 nm).

The analysis demonstrated a correlation between the concentration of CGA and antioxidant activity. High amounts of CGA were observed in leaves, followed by roots and aerial parts. The HPTLC–DPPH assay confirmed antioxidant activity, with yellow band intensity corresponding to polyphenol content (Matkowski & Piotrowska, 2006; Matkowski *et al.*, 2008; Olennikov, 2020; Tyunnikova, 2004).

CONCLUSIONS

The preliminary investigation of phenolic acids content of roots, aerial parts and leaves of two *Galeopsis* spp. (*Lamiaceae*) from the southwest Romania flora was made through HPTLC–densitometry. The amount (mg/g) of CGA in herbal samples was variable, as follows: *G. speciosa* leaves (9.192) > *G. bifida* leaves (9.017)

> *G. speciosa* roots (8.283) > *G. speciosa* aerial parts (7.317) > *G. bifida* aerial parts (3.392) > *G. bifida* roots (1.825). The study effectively used HPTLC to evaluate phenolic acid content, emphasizing the pharmacological potential of *Galeopsis* spp. It highlights their antioxidant properties, which may contribute to their traditional use for respiratory and inflammatory conditions.

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