PRELIMINARY CHROMATOGRAPHIC INVESTIGATION OF FOUR *STACHYS* SPP. (*LAMIACEAE*) FROM OLTENIA FLORA

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Abstract

Concerning four Stachys spp. (Lamiaceae) from the Oltenia flora, the paper highlights the polyphenolic content of aerial parts through high-performance thin-layer chromatography coupled with photodensitometry. Chlorogenic acid (CA) was identified and quantified in all 20% methanolic extracts of Stachydis herba. The highest CA amount was determined in S. recta (59.88 mg%), followed by S. sylvatica (32.90 mg%), S. officinalis (31.34 mg%), and S. germanica (25.16 mg%), respectively.

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

INTRODUCTION

Commonly known as hedge nettle, *Stachys* genus, *Lamiaceae* family, contains more than 300 different annual or perennial species (herbs or small shrubs) originating from Mediterranean region, Asia, Americas, and southern Africa. In the Romanian flora, 12 *Stachys* spp. are also found (Ciocârlan, 2009; Imbrea *et al.*, 2011).

The flowering aerial part of *Stachys* spp. contains important active principles, such as: essential oil (germacrene D, limonene, *β*-caryophyllene, *β*-pinene, nerolidol, linalyl acetate, linalool, caryophyllene oxide, spathulenol, *α*-cadinene, *α*-pinene, *β*-myrcene, *α*-terpineol, *β*-bourbonene, *β*-ylangene); iridoids (ajugoside, aucubin, harpagide, acetyl-harpagide, harpagoside); flavonoids (apigenin, luteolin, chrysoeriol, isoscutellarein, kaempferol, quercetin, isorhamnetin, naringenin and their glycosides); diterpenes with labdane, *neo*-clerodane, rosane and *ent*-kaurene type (deoxyandalusol, stachaegyptins, roseo-stachone, stachyrosanes); triterpenes (ursolic acid and oleanolic acid derivatives); phytosterols; phenylethanoid glycosides (acteoside, verbascoside, martynoside, leucosceptoside A, lavandulifoliosides); phenylpropanoid glycosides (coniferin, syringin); lignans (sesamin, paulownin, urolignoside); phenolic acids (chlorogenic acid, caffeic acid); fatty acids (linoleic, oleic and palmitic acids): oligosaccharides (Giuliani *et al.*, 2009; Goren *et al.*, 2011; Hajdari *et al.*, 2012; Háznagy-Radnai *et*

al., 2006 & 2012; Khanavi *et al*., 2009; Kiliç *et al.*, 2017; Lazarević *et al*., 2013; Sarikurkcu *et al.*, 2021; Stegăruş *et al.*, 2021; Tomou *et al*., 2020; Tundis *et al*., 2014; Venditti *et al.*, 2013).

In Turkey, *Stachys* spp. are used as wild (mountain) tea mainly against stomatitis, diabetes, rheumatism, cough and cold, or as functional foods for a healthy diet (Carović-Stanko *et al.*, 2016; Gören, 2014; Satıl & Açar, 2020; Tomou *et al*., 2020).

Stachys spp. exhibited significant pharmacological actions, such as: antimicrobial (against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Candida albicans*), antioxidant, anti-inflammatory (useful for polycystic ovary syndrome), hepatoprotective, anti-diabetes, wound healing, antiviral, cytotoxic and antiproliferative, enzyme inhibitory (Alizadeh *et al.*, 2020; Goren *et al.*, 2011; Hajdari *et al.*, 2012; Háznagy-Radnai *et al.*, 2012; Khanavi *et al*., 2009 & 2012; Lazarević *et al*., 2010 & 2013; Paun *et al.*, 2018; Sarikurkcu *et al.*, 2021; Tomou *et al*., 2020; Tundis *et al*., 2014).

The purpose of our research was the preliminary analysis of polyphenols in the aerial parts of four *Stachys* spp. from Oltenia flora, by high-performance thin-layer chromatography (HPTLC)–densitometry.

MATERIALS AND METHODS

Plant material

The plant material was harvested during the flowering period, in May–June 2022, from the Oltenia Region, South-West of Romania. It was represented by the aerial parts of four *Stachys* spp. (*S. germanica* L., *S. officinalis* (L.) Trevis. sin. *Betonica officinalis* L., *S. recta* L., and *S. sylvatica* L.) Our study did not involve endangered or protected herbal species. The voucher specimens (StG-20220602/1, StO-20220623/1, StR-20220524/1, and StS-20220607/1, respectively) were deposited in the Herbarium of the Department of Pharmaceutical Botany, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova.

HPTLC analysis

Starting from the aerial parts of four *Stachys* spp. (*Stachydis herba*), preliminary HPTLC–densitometric analysis of polyphenols was made on CAMAG (Muttenz, Switzerland) system, according with some specific experimental conditions (Altemimi *et al.*, 2015; Bojić *et al.*, 2013; Gîrd *et al.*, 2014; Jug *et al.*, 2018): 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, in volumes); 10 mL of mobile phase were added in the developing twin-chamber (CAMAG) and then oversaturated for 20 minutes; sample: five 20% methanolic extracts of *Stachydis herba*; reference compounds (Merck): 0.1% methanolic solutions of caffeic acid, chlorogenic acid and rutin; migration distance: 62 mm (sample application line 8 mm, solvent front 70 mm); application of sample (2 µL) and reference solutions (2 µL, 3 µl, 4 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: 5 minutes, at 25ºC (cold air dryer); plate shooting: ultraviolet (UV) light (254 nm and 365 nm); detection: CAMAG TLC Scanner 3 photodensitometer, for densitogram and *in situ* UV light (280 nm) spectra, without derivatization, deuterium–tungsten lamp, scanning speed 20 mm/s, data resolution 100 µm/step, measurement

mode absorbance; visionCATS ver. 3.1 software package (CAMAG).

DPPH *in situ* qualitative assay

In the CAMAG TLC Spray Cabinet 2, the HPTLC plates were sprayed with 0.5 mM methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH); 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 (Pozharitskaya *et al.*, 2008).

RESULTS AND DISCUSSIONS

The experimental results on the preliminary HPTLC analysis of polyphenols from the aerial parts of four *Stachys* spp. (*Stachydis herba*) were highlighted in Figures 1–9.

Through linear regression mode, with 5% range deviation, 1.68% coefficient of variation (CV), and 0.9961 correlation coefficient (*R*), the calibration curve for chlorogenic acid (Figure 9) was used for quantitative analysis: $y = 3.571\times10^{-9}x + 4.636\times10^{-3}$.

In the 20% methanolic extracts of *Stachydis herba*, the highest amount of chlorogenic acid (R_f 0.22) was quantified in *S. recta* (59.88 mg%), followed by *S. sylvatica* (32.90 mg%), *S. officinalis* (31.34 mg%), and *S. germanica* (25.16 mg%) aerial parts, respectively.

After the chromatographic separation, using HPTLC–DPPH assay, for *Stachydis herba* methanolic extracts, the screening of the antioxidant activity was performed *in situ*. For the HPTLC bands, the intensity of the yellow color is directly proportional with the concentration of polyphenols (chlorogenic acid) in the examined samples (Figure 3).

Starting from the polyphenolic content of *Stachys* spp., our results agree with the specialized research in the field of natural products chemistry (Khanavi *et al*., 2009; Lazarević *et al*., 2010; Stegăruş *et al.*, 2021; Tomou *et al*., 2020).

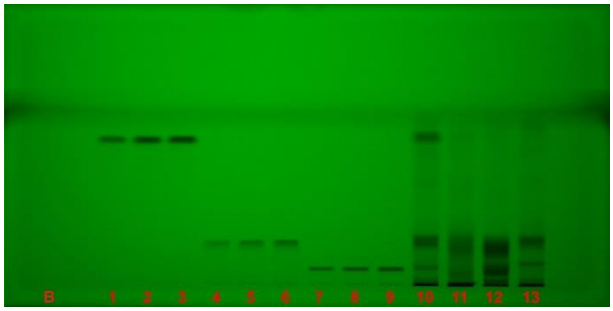

Figure 1. HPTLC chromatogram of polyphenols from *Stachydis herba* 20% methanolic extracts: UV 254 nm, without derivatization. B: Blank; Lanes 1–3: Caffeic acid, R_f 0.76; Lanes 4–6: Chlorogenic acid, R_f 0.22; Lanes 7–9: Rutin, R_f 0.08; Lanes 10–13: Samples (*S. germanica*, *S. officinalis*, *S. recta*, and *S. sylvatica*, respectively).

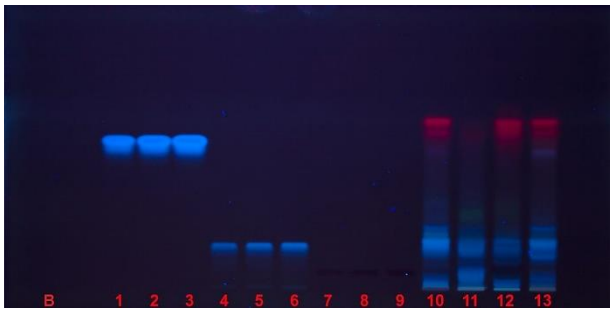

Figure 2. HPTLC chromatogram of polyphenols from *Stachydis herba* 20% methanolic extracts: UV 365 nm, without derivatization. B: Blank; Lanes 1–3: Caffeic acid, R_f 0.76; Lanes 4–6: Chlorogenic acid, R_f 0.22; Lanes 7–9: Rutin, not visualized; Lanes 10–13: Samples (*S. germanica*, *S. officinalis*, *S. recta*, and *S. sylvatica*, respectively).

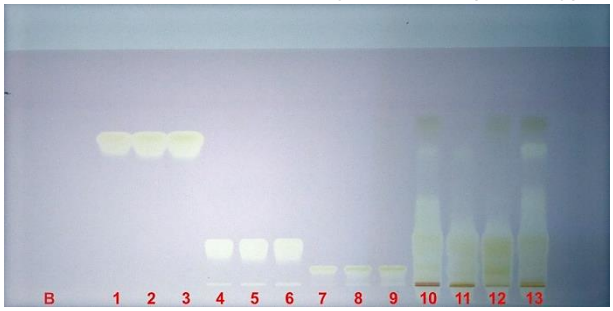

Figure 3. HPTLC chromatogram of polyphenols from *Stachydis herba* 20% methanolic extracts: white light illumination, derivatization with DPPH. B: Blank; Lanes 1–3: Caffeic acid, R_f 0.76; Lanes 4–6: Chlorogenic acid, R_f 0.22; Lanes 7–9: Rutin, R_f 0.08; Lanes 10–13: Samples (*S. germanica*, *S. officinalis*, *S. recta*, and *S. sylvatica*, respectively).

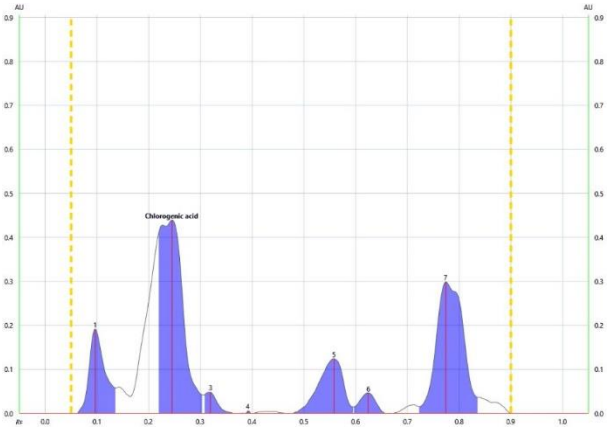

Figure 4. Densitogram of chlorogenic acid (UV 280 nm, without derivatization) separated from the *Stachys germanica* aerial parts 20% methanolic extract.

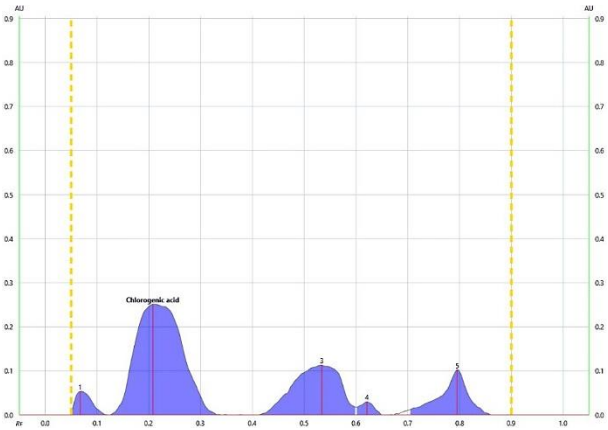

Figure 5. Densitogram of chlorogenic acid (UV 280 nm, without derivatization) separated from the *Stachys officinalis* aerial parts 20% methanolic extract.

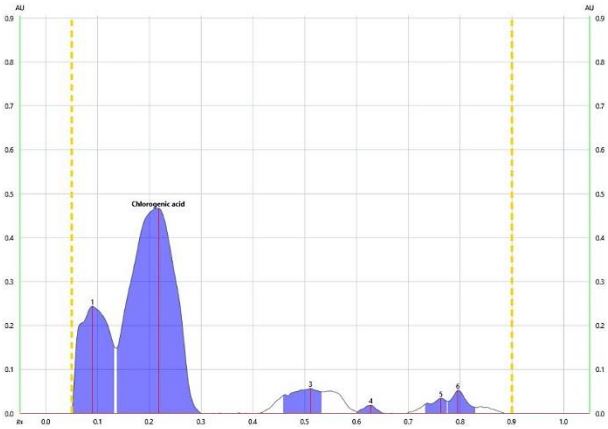

Figure 6. Densitogram of chlorogenic acid (UV 280 nm, without derivatization) separated from the *Stachys recta* aerial parts 20% methanolic extract.

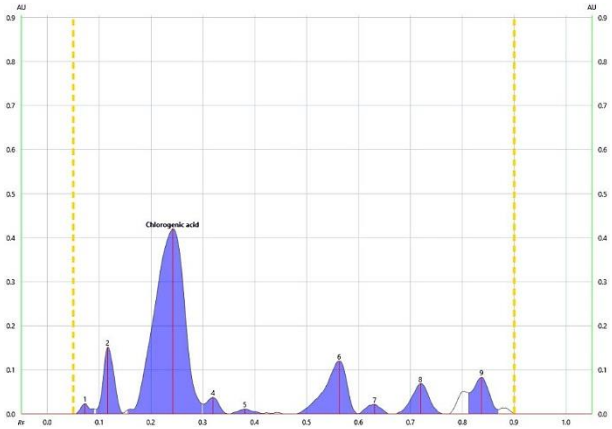

Figure 7. Densitogram of chlorogenic acid (UV 280 nm, without derivatization) separated from the *Stachys sylvatica* aerial parts 20% methanolic extract.

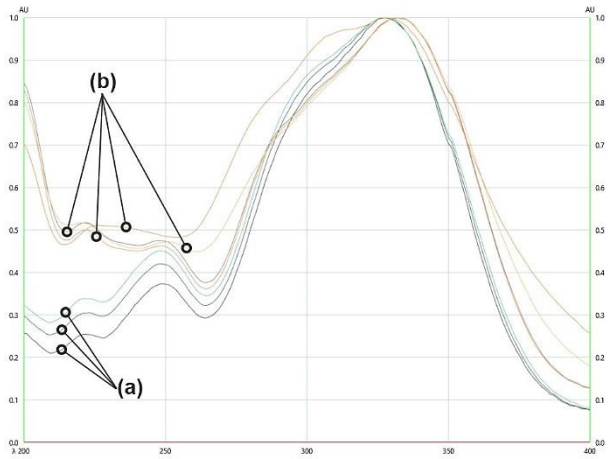

Figure 8. *In situ* UV spectra (280 nm) of chlorogenic acid reference (a) and compound separated from the analyzed samples (b).

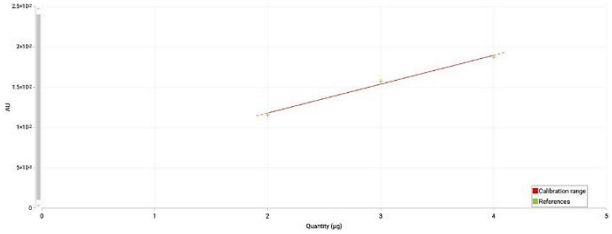

Figure 9. Chlorogenic acid reference calibration curve.

CONCLUSIONS

Preliminary chromatographic analysis of the polyphenols in the aerial parts of four *Stachys* spp. from the Oltenia flora was achieved by HPTLC coupled with photo-densitometry. Identified and quantified in

the 20% methanolic extracts of *Stachydis herba*, the amount of chlorogenic acid was fluctuating (mg%): *S. recta* (59.88) > *S. sylvatica* (32.90) > *S. officinalis* (31.34) > *S. germanica* (25.16).

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