PRELIMINARY CHROMATOGRAPHICRESEARCH ON SOME *SALVIA* SPP. (*LAMIACEAE*)

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Abstract
Salvia genus, representative for the Lamiaceae family as it comprises approximately one thousand different species, is considered for a wide plethora of therapeutic actions, such as antioxidant, anti-inflammatory, hepatoprotective, antitumoral and antidiabetic activity. The paper presents the preliminary chromatographic investigations of the polyphenols in the aerial parts of some Salvia spp. Using high-performance thin-layer chromatography (HPTLC) coupled with photodensitometry, caffeic acid was identified and quantified in the 20% methanolic extracts of Salviaeherba, in descending order, as follows: S. nemorosa (3.096%) > S. verticillata (3.041%) > S. sclarea (2.663%) > S. glutinosa (1.962%) > S. aethiopis (0.926%).
Key words*:photodensitometry, polyphenols, Salvia spp., thin-layer chromatography*

INTRODUCTION

Salvia genus, commonly known as sage, *Lamiaceae* family, includes almost one thousand different annual, biennial, and perennial herbaceous species originating mainly from the Central America, South America, Asia, and Mediterranean region. Fifteen *Salvia* spp. are also found in the Romanian flora (Ciocârlan, 2009).

From the phytochemical point of view, the aerial parts and leaves of *Salvia* spp. contain various active principles, such as: essential oil (*β*-caryophyllene, germacrene-B, spathulenol, and *cis*-*β*-farnesene, *β*-ocimene, *α*-gurjunene, germacrene-D, hexyl acetate, aromadendrene); flavonoids (acacetin, kaempferol, pinocembrin, catechin, quercetin, glycosidesof apigenin, luteolin and scutellarein); diterpenoids of abietane, clerodane, pimarane and labdanetypes (carnosol, rosmanol, salvinolone, horminone); steroids (brassicasterone); triterpenoids of ursane,

oleanane, and lupane types; polyphenolcarboxylic acids (caffeic acid, rosmarinic acid, chlorogenic acid, salvianolic acid, ferulic acid); tannin; resins and oleoresins (Aćimović *et al.*, 2018; Afonso *et al.*, 2019; Bidabadi *et al.*, 2020; Boufadi*et al.*, 2021; Gîrd*et al.*, 2014; Hanganu *et al.*, 2019; Ivanov *et al.*, 2022).

In ethnopharmacology, *Salvia* spp. are used for rheumatism, diabetes, inflammation, ulcers, dizziness, seizures, and in various brain tonic recipes (Boufadi *et al.*, 2021; Lopresti, 2017). For *Salvia* spp., up to date research highlighted some important pharmacological properties: anti-inflammatory, antioxidant, hypoglycemic, antimicrobial, wound healing, enhancer of the cognitive activity, protective towards neurodegenerative diseases, immuno-modulatory, hepatoprotective, cytotoxic *in vitro* against HepG2 (hepatocellular), K562 (chronic myelogenous leukemia), HL60 (human acute promyelocytic leukemia cells), MCF-7 (human breast), HeLa (cervical) carcinoma cells, enzyme inhibitory, anti-leishmanial (Aćimović *et al.*, 2018; Afonso *et al.*, 2019; Bahadori *et al.*, 2018; Boufadi*et al.*, 2021; Firuzi*et al.*, 2013; Gülçin*et al.*, 2004; Hanganu *et al.*, 2019; Ivanov *et al.*, 2022; Lopresti, 2017; Tosun *et al.*, 2009; Vasilache *et al.*, 2021; Wu *et al.*, 2012).

The aim of our paper was the preliminary chromatographic analysis of polyphenols in the aerial parts of five *Salvia* spp. from Oltenia flora, using high-performance thin-layer chromatography (HPTLC) coupled with photodensitometry.

MATERIALS AND METHODS

Plant material

The plant material (aerial parts of five *Salvia* spp. – *S. aethiopis* L., *S. glutinosa* L., *S. nemorosa* L., *S. sclarea* L., and *S. verticillata* L.) was collected during the flowering period, in May–June 2022, from the Oltenia Region, South-West of Romania. The research did not involve endangered or protected herbal species. Voucher specimens (SaA-20220624/1, SaG-20220607/1,SaN-20220524/1, SaS-20220602/1, and SaV-20220607/1) were stored in the Herbarium of the Department of Pharmaceutical Botany, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova.

HPTLC analysis

Preliminary HPTLC analysis of polyphenols from the aerial parts of five *Salvia* spp.(*Salviae herba*) was performed on CAMAG (Muttenz, Switzerland) system, in thefollowing 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 *Salviae herba*;reference compounds (Merck): 0.1% methanolic solutions of caffeic acid, chlorogenic acid and rutin;migration distance: 62 mm (sample application line 8mm, solvent front 70 mm);application of sample (2µL) 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

HPTLC plates were sprayed with0.5 mM methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH), in the CAMAG TLC Spray Cabinet 2, and dried at room temperature, in the dark, for 90 seconds, then heated at 60°C, in an oven, for 30 seconds. Chromatograms were documented at white light illumination (Pozharitskaya*et al.*, 2008).

RESULTS AND DISCUSSIONS

Figures 1–10exhibited the experimental data on the preliminary HPTLC analysis of polyphenols from the aerial parts of five *Salvia* spp. (*Salviaeherba*).

Caffeic acid calibration curve (Figure 10) was achieved by linear regression mode, with 5% range deviation, 1.5% coefficient of variation and 0.9927 correlation coefficient (*R*): $y = 3.752\times10^{-9}x + 1.351\times10^{-2}$.

Caffeic acid (R_f 0.76) was quantified in the 20% methanolic extracts of *Salviaeherba*, as follows: *S. nemorosa* (3.096%) >*S. verticillata* (3.041%) >*S. sclarea* (2.663%) >*S. glutinosa* (1.962%) >*S. aethiopis* (0.926%).

The screening of the antioxidant activity for *Salviaeherba*methanolic extracts was determined *in situ*, after chromatographic separation (HPTLC–DPPH assay). The intensity of the yellow color for the HPTLC bands is directly correlated with the amount of polyphenols (*e.g.*, caffeic acid) identified and quantified in the analyzed samples (Figure 3).

Our results are comparable to those obtained in specialty papers regarding the polyphenolic content of *Salvia* spp. (Aćimović *et al.*, 2018; Hanganu *et al.*, 2019; Lu & Foo, 2002; Mocan *et al.*, 2020; Wu *et al.*, 2012).

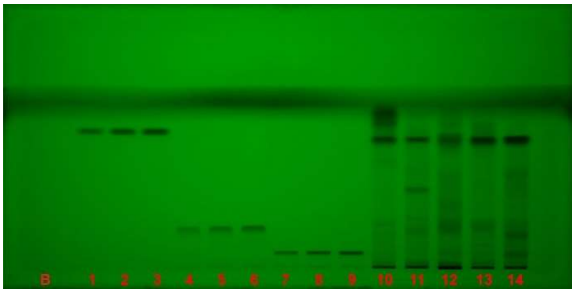

Figure 1. HPTLC chromatogram of polyphenols from *Salviaeherba* 20% methanolic extracts: UV 254 nm, without derivatization. B: Blank; Lanes 1–3: Caffeic acid, R_f 0.76; Lanes 4–6: Chlorogenic acid, R_f 0.22; Lanes 7–9: Rutin, R_f 0.08; Lanes 10–14: Samples (*S. aethiopis*, *S. glutinosa*, *S. nemorosa*, *S. sclarea* and *S. verticillata*, respectively).

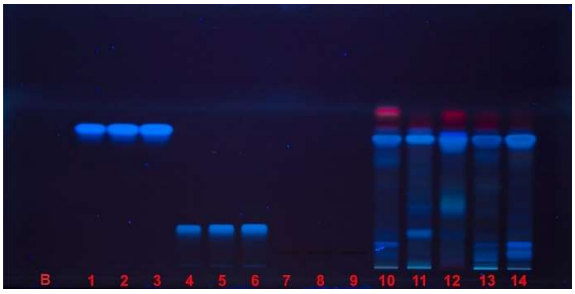

Figure 2. HPTLC chromatogram of polyphenols from *Salviaeherba* 20% methanolic extracts: UV 365 nm, without derivatization. B: Blank; Lanes 1–3: Caffeic acid, R_f 0.76; Lanes 4–6: Chlorogenic acid, R_f 0.22; Lanes 7–9: Rutin, not visualized; Lanes 10–14: Samples (*S. aethiopis*, *S. glutinosa*, *S. nemorosa*, *S. sclarea* and *S. verticillata*, respectively).

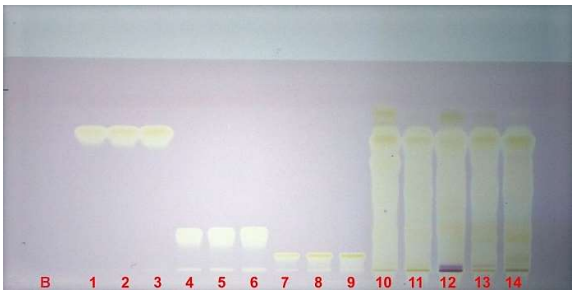

Figure 3. HPTLC chromatogram of polyphenols from *Salviaeherba* 20% methanolic extracts: white light illumination, derivatization with DPPH. B: Blank; Lanes 1–3: Caffeic acid, R_f 0.76; Lanes

4–6: Chlorogenic acid, R_f 0.22; Lanes 7–9: Rutin, R_f 0.08; Lanes 10–14: Samples (*S. aethiopis*, *S. glutinosa*, *S. nemorosa*, *S. sclarea* and *S. verticillata*, respectively).

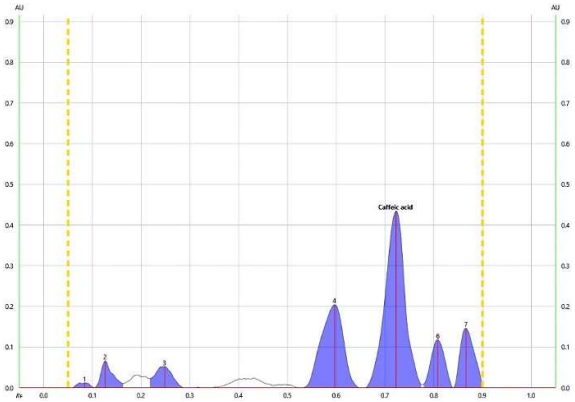

Figure 4. Densitogram of caffeic acid (UV 280 nm, without derivatization) separated from the *Salvia aethiopis* aerial parts 20% methanolic extract.

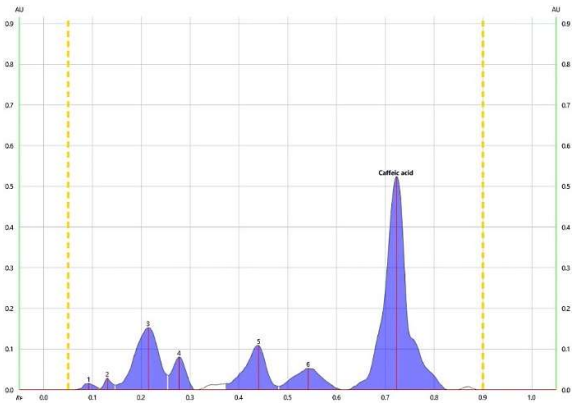

Figure 5. Densitogram of caffeic acid (UV 280 nm, without derivatization) separated from the *Salvia glutinosa* aerial parts 20% methanolic extract.

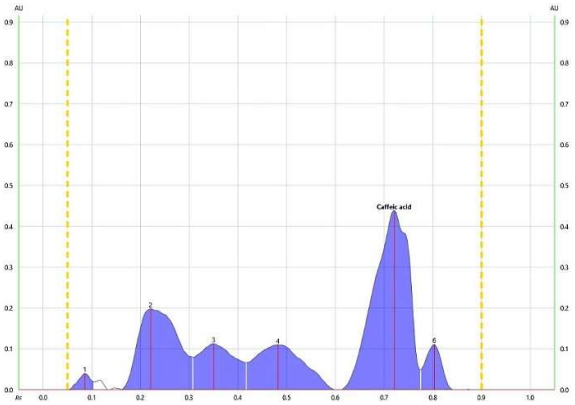

Figure 6. Densitogram of caffeic acid (UV 280 nm, without derivatization) separated from the *Salvia nemorosa* aerial parts 20% methanolic extract.

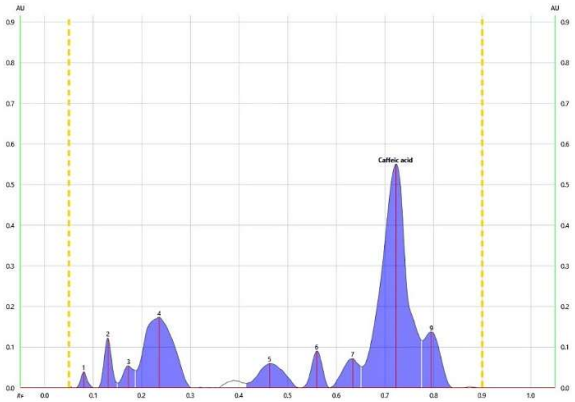

Figure 7. Densitogram of caffeic acid (UV 280 nm, without derivatization) separated from the *Salvia sclarea* aerial parts 20% methanolic extract.

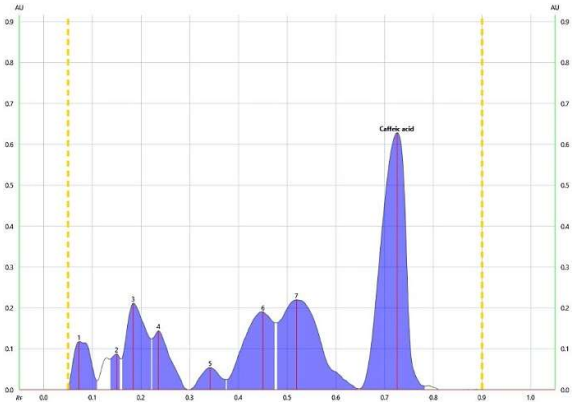

Figure 8. Densitogram of caffeic acid (UV 280 nm, without derivatization) separated from the *Salvia verticillata* aerial parts 20% methanolic extract.

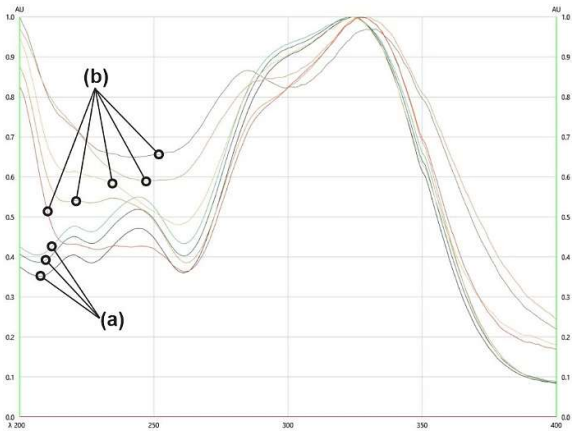

Figure 9. *In situ* UV spectra (280 nm) of caffeic acid reference (a) and compound separated from

the analyzed samples (b).

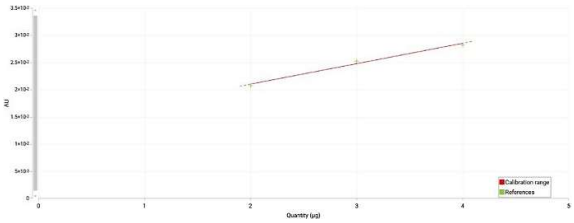

Figure 10. Caffeic acid reference calibration curve.

CONCLUSIONS

Salvia genusincludesabout 1000 different species, being considered for different therapeutic properties (anti-inflammatory, antioxidant, antitumoral, hepatoprotective, antidiabetic). Preliminary chromatographic analysis of the polyphenols in the aerial parts of five*Salvia* spp. was made using HPTLC–photodensitometry.In the 20% methanolic extracts, caffeic acid was identified and quantified: *S. nemorosa* (3.096%) >*S. verticillata* (3.041%) >*S. sclarea* (2.663%) >*S. glutinosa* (1.962%) >*S. aethiopis* (0.926%).

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REFERENCES

Aćimović, M., Kiprovski, B., Rat, M., Sikora, V., Popović, V., Koren, A., Brdar-Jokanović, M., (2018). *Salvia sclarea*: chemical composition and biological activity. *Journal of Agronomy, Technologyand Engineering Management*, 1(1), 18–28.

Afonso, A.F., Pereira, O.R., Fernandes, Â.,Calhelha, R.C., Silva, A.M.S., Ferreira, I.C.F.R., Cardoso, S.M., (2019). Phytochemical composition and bioactiveeffects of *Salvia africana*, *Salvia officinalis* '*Icterina'* and *Salvia mexicana* aqueous extracts. *Molecules*, 24(23), 4327.

Altemimi, A., Watson, D.G., Kinsel, M., Lightfoot, D.A., (2015). Simultaneous extraction, optimization, and analysis of flavonoids and polyphenols from peach and pumpkin extracts using a TLC–densitometric method.*Chemistry Central Journal*, 9, 39.

Bahadori, M.B., Eskandani, M., De Mieri, M., Hamburger, M., Nazemiyeh, H., (2018). Anti-proliferative activity-guided isolationof clerodermic acid from *Salvia nemorosa*L.: geno/cytotoxicity and hypoxia-mediatedmechanism of action. *Food and Chemical Toxicology*, 120, 155–163.

Bidabadi, S.S., VanderWeide, J., Sabbatini,P., (2020). Exogenous melatonin improvesglutathione content, redox state and increases essential oil production in two *Salvia* species under drought stress. *Scientific Reports*, 10(1), 6883.

Bojić, M., Simon Haas, V., Sarić, D., Maleš, Z., (2013). Determination of flavonoids, phenolic acids, and xanthines in mate tea (*Ilex paraguariensis* St.-Hil.).*Journal of Analytical Methods in Chemistry*, 2013, 658596.

Boufadi, M.Y., Keddari, S., Moulaihacene, F., Chaa, S., (2021). Chemical composition, antioxidant and anti-inflammatory properties of *Salvia officinalis* extract from Algeria. *Pharmacognosy Journal*, 13(2), 506–515.

Ciocârlan V., (2009).*Flora ilustrată a României. Pteridophyta et Spermatophyta*. 3rd edition, Bucharest, RO: Ceres Publishing House, 656–659.

Firuzi, O., Miri, R., Asadollahi, M., Eslami, S., Jassbi, A.R., (2013). Cytotoxic, antioxidant and antimicrobial activities

and phenolic contents of eleven *Salvia* species from Iran. *Iranian Journal of Pharmaceutical Research*, 12(4), 801–810.

Gîrd, C.E., Nencu, I., Costea, T., Duţu, L.E.,Popescu, M.L., Ciupitu, N., (2014).Quantitative analysis of phenolic compounds from *Salvia officinalis* L. leaves.*Farmacia*, 62(4), 649–657.

Gülçin, İ., Oğuz, M.T., Oktay, M., Beydemir, Ş., Küfrevioğlu, Ö.İ., (2004). Evaluation of the antioxidant and antimicrobial activities of clary sage (*Salvia sclarea* L.). *Turkish Journal of Agriculture and Forestry*, 28(1), 25–33.

Hanganu, D., Olah, N.K., Pop, C.E., Vlase, L., Oniga, I., Ciocarlan, N., Matei, A., Puşcaş, C., Silaghi-Dumitrescu, R., Benedec, D., (2019). Evaluation of polyphenolic profile and antioxidant activity for some *Salvia* species. *Farmacia*, 67(5), 801–805.

Ivanov, M., Božunović, J., Gašić, U., Drakulić, D., Stevanović, M., Rajčević, N., Stojković, D., (2022). Bioactivities of *Salvia nemorosa* L. inflorescences are influenced by the extraction solvents. *Industrial Crops and Products*, 175, 114260.

Jug, U., Glavnik, V., Kranjc, E., Vovk, I., (2018). High-performance thin-layer chromatography and high-performance thin-layer chromatography–mass spectrometry methods for the analysis of phenolic acids.*Journal of Planar Chromatography*, 31(1), 13–22.

Lopresti, A.L., (2017) *Salvia* (sage): a review of its potential cognitive-enhancing and protective effects. *Drugs in R&D*, 17(1), 53–64.

Lu, Y., Foo, L.Y., (2002). Polyphenolics of *Salvia* – a review. *Phytochemistry*, 59(2),117–140.

Mocan, A., Babota, M., Pop, A., Fizeşan, I.,Diuzheva, A., Locatelli, M., Carradori, S.,Campestre, C., Menghini, L., Sisea, C.R.,Soković, M., Zengin, G., Păltinean, R., Badarau, S., Vodnar, D.C., Crişan, G., (2020). Chemical constituents and biologic activities of sage species: a comparison between *Salvia officinalis* L.,*S. glutinosa* L. and *S. transsylvanica* (Schur ex Griseb. & Schenk) Schur. *Antioxidants (Basel)*, 9(6), 480.

Pozharitskaya, O.N., Ivanova, S.A., Shikov,A.N., Makarov, V.G., (2008). Separation and free radical-scavenging activity of major curcuminoids of *Curcuma longa* using HPTLC–DPPH method. *Phytochemical Analysis*, 19(3), 236–243.

Tosun, M., Ercisli, S., Sengul, M., Ozer, H., Polat, T., Ozturk, E., (2009). Antioxidant properties and total phenolic content of eight *Salvia* species from Turkey. *Biological Research*, 42(2), 175–181.

Vasilache, A., Popa, M., Albu, C.C., Dragomirescu, A.O., Vasilache, A., Bencze, M.A., Suciu, I., Ionescu, E., (2021). Evaluation of the biocompatibilityof laser irradiated plant extracts used asadjuvants in irrigation and sanitization of root canals. *Farmacia*, 69(5), 934–940.

Wu, Y.B., Ni, Z.Y., Shi, Q.W., Dong, M., Kiyota, H., Gu, Y.C., Cong, B., (2012). Constituents from *Salvia* species and their biological activities. *Chemical Reviews*, 112(11), 5967–6026.