HISTO-ANATOMICAL AND PRELIMINARY TLC INVESTIGATIONS ON *SCUTELLARIA HASTIFOLIA* L. (*LAMIACEAE*) SPECIES

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ABSTRACT

The cross-sections through rootlet, rhizome, aboveground stem and leaf of Scutellaria hastifolia L. species (Lamiaceae family) were obtained for the first time and examined using microphotography technique. Moreover, thin layer chromatography–densitometric method was applied for the determination of the polyphenols content in the flowering aerial parts (Scutellariae hastifoliae herba). Starting from 12 distinctive chromatographic bands, one caffeic acid derivative was identified.

INTRODUCTION

Scutellaria hastifolia L., Spear-leaved skullcap, Norfolk Skullcap (*Lamiaceae* family) is a perennial herbaceous species, 10–40 cm high, with bluish violet flowers in the summer (June–August), opposite and ovate lanceolate leaves. It is spontaneous mainly in temperate areas of Europe and Asia [7].

Due to their content of active principles (flavonoids, diterpenoids, essential oil, phenyl-ethanoid glycosides, lignans, polysaccharides, carotenoids, alkaloids) [15, 17], *Scutellaria* species exhibit important pharmacological actions, such as: antioxidant [3, 16], cytostatic (antitumoral), anti-angiogenesis and immunomodulatory [5, 10, 11, 14], anticonvulsant, antimicrobial and antiviral, hepatoprotective [13, 17], anti-inflammatory, antipyretic, tonic [6, 8, 18], antifeedant [9, 15].

In the specialty papers, there are scarce and incomplete data concerning *S. hastifolia* histo-anatomy [20] and chemical composition [17].

The aim of our paper was the histo-anatomical investigation of the rootlet, rhizome, aboveground stem and leaf of *Scutellaria hastifolia* species and the preliminary analysis of the polyphenols content from the aerial parts (*Scutellariae hastifoliae herba*).

MATERIAL AND METHOD

Histo-anatomical investigation

The vegetal material was harvested from *S. hastifolia* plants in blossom, in May 2016, from Bucovăţ Forest, Dolj County (southwestern Romania).

The fixation and preservation of rootlets, rhizomes, aboveground stems and leaves were achieved in 70% ethanol. The cross-sections and longitudinal-radial sections were obtained using botanical razor.

After washing with distilled water, the sections were clarified using 10% sodium hypochlorite solution (Javel water). Then, the clarifying agent was removed by washing with distilled water. Congo red–chrysoidine mixture (Genevese reagent) was used for the staining of sections. Depending on the chemical composition of cell membranes, the reactive induced various stains: pink to red for cellulose and mucilage, pale red for cytoplasm, yellow for suberin and brown for lignin [2]. Stained and mounted sections were analyzed

on a Krüss binocular photon microscope (objectives ×4, ×10, ×40) and then photographed using a Sony DSLR-A380 digital system adapted to the microscope.

The description of microscopic sections was realized according to some classical authors [19].

Thin-layer chromatography (TLC) analysis

Preliminary analysis of polyphenols was performed on *Scutellariae hastifoliae herba* (the aerial parts of *S. hastifolia* species), using a CAMAG (Muttenz, Switzerland) system, in the following experimental conditions [1, 4, 12]: stationary phase TLC silica gel 60 F_{254} 20×10 precoated glass plates (Merck, Darmstadt, Germany) pre-washed with chloroform–methanol (1:1, *v/v*); mobile phase chloroform–ethyl acetate–toluene–formic acid–methanol (15:20:10:10:1, in volumes) in a vapor-equilibrated chromatographic tank (20×10 cm twin trough chamber, CAMAG); sample – 20% methanolic extract of *Scutellariae hastifoliae herba*; standards (Merck) – 0.05% methanolic solutions of caffeic acid, chlorogenic acid, quercetin and rutin; migration distance 80 mm; sample (1–10 µL) and standards (2 µL) application – CAMAG Linomat 5 semiautomatic system (spray gas nitrogen, dosage speed 150 nL/s and band length 8 mm); detection – CAMAG TLC Scanner 3 photodensitometer, UV 254 nm, without derivatization, deuterium–wolfram lamp, scanning speed 20 mm/s, resolution 100 µm/step; measurement mode – absorption; spectra acquisition, processing and quantification analysis – winCATS ver. 1.4.3 software package.

RESULTS AND DISCUSSIONS

Histo-anatomical investigation

Rootlet

In cross-section, rootlet has circular shape and primary structure. From the outside to the inside, the following histological sequence is evidenced (Figure1). The well-represented primary cortex consists of exodermis, cortical parenchyma and endodermis of primary-type. The rhizodermis is destroyed by soil particles. Exodermis is made up of single layer of large, polyhedral cells, with suberin-impregnated walls, and provided with passage cells. Cortical parenchyma is well represented, consisting of large, parenchymatic cells, delimiting small intercellular meatuses.

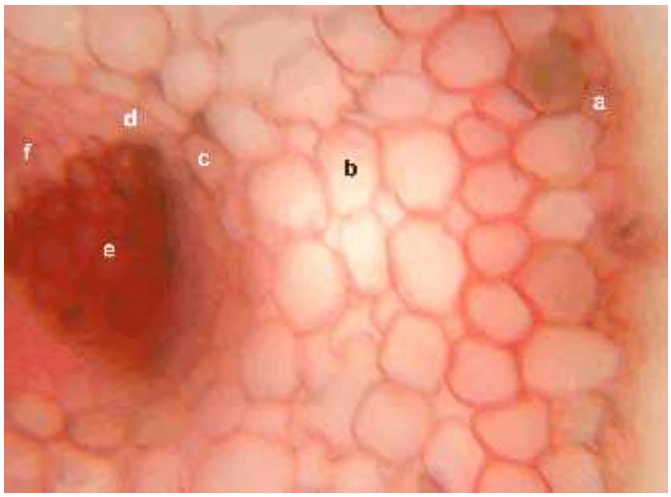

Figure 1. Cross-section through S. hastifolia rootlet: (a) exodermis; (b) cortical parenchyma; (c) endodermis; (d) pericycle; (e) xylem fascicle; (f) phloem fascicle (Congo red–chrysoidine staining, ×100).

Endodermis is composed of a single cellular layer, with suberin-impregnated walls and passage cells highlighted from place to place. Central cylinder is delimited by single-stratified, cellulosic pericycle. The conducting tissues are alternately disposed in simple

xylem and phloem fascicles, divided by cellulosic medullary rays. Phloem fascicles are made up of sieve tubes, phloem parenchyma and annex cells. Xylem bundles consist of protoxylem to the pericycle and metaxylem to the medullary parenchyma, but also of xylem parenchyma and annex cells. Medullary parenchyma is absent.

Rhizome

In cross-section, the rhizome has circular shape and secondary structure due to the libero-ligneous cambium. The epidermis consists of heterodiametric cells, with tangential external wall thickened, domed and covered by a thin cuticle. Epidermal cells are anterior-posterior flattened, with thin radial walls and thickened tangential external and internal walls. The cortex is made up of meatus-type cortical parenchyma. At this level, aeriferous spaces and isolated sclerenchyma fibers are observed. Into the periphloemic zone, packages of sclerenchyma fibers are stacked circular. Ursins are stored in some cortical parenchyma cells. As a result of the libero-ligneous cambium activity, it can be noted a thin external ring of secondary phloem consisting of sieve tubes, phloem parenchyma and annex cells, and also a thick internal ring of secondary xylem made up of large xylem vessels spread into the libriform tissue. Some cells of phloem parenchyma stored ursins. At the level of xylem tissue, medullary rays are multicellular, uniseriate, sclerified and lignified. Protoxylem is under-represented, being disposed near the medullary parenchyma and accompanied by xylem parenchyma. Meatus-type medullary parenchyma is underrepresented and occupies the center of the rhizome (Figure 2).

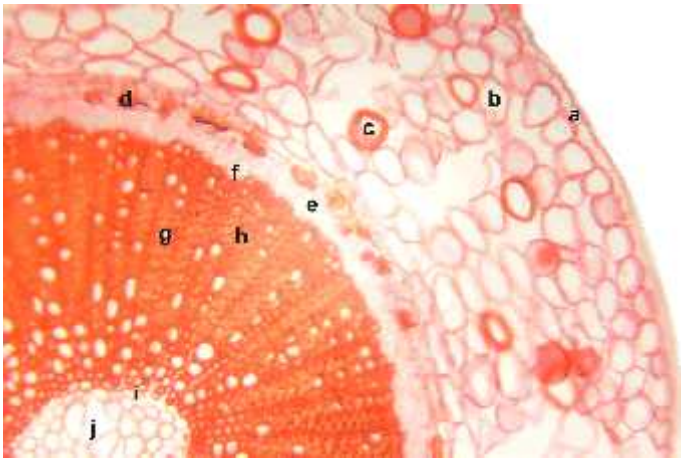

Figure 2. Cross-section through S. hastifolia rhizome: (a) epidermis; (b) cortical parenchyma; (c) sclerenchyma fiber; (d) bundle of sclerenchyma fibers; (e) phloem tissue; (f) libero-ligneous cambium; (g) metaxylem; (h) libriform tissue; (i) protoxylem; (j) medullary parenchyma (Congo red–chrysoidine staining, ×100).

Aboveground stem

In cross-section, into the upper third, aboveground stem has quadratic shape, with four prominent ribs, and secondary structure generated by the libero-ligneous cambium. Epidermis is made up of heterodiametric cells with tangential external wall thickened, domed and covered by a thin cuticle with toothed relief. The epidermal cells are anterior-posterior flattened, with thin radial walls and thickened tangential external and internal walls. From point to point are evidenced stomata and multicellular, uniseriate tector hairs, with sharp peak. The cortex is differentiated into angular collenchyma cords, arranged at the level of protruding ribs and intercostal chlorenchyma tissue, but also from cortical parenchyma. Conducting tissues are organized in four large collateral-open libero-ligneous fascicles disposed opposite the ribs and small libero-ligneous fascicles generated by the interfascicular libero-ligneous cambium and placed into the intercostal area. At the phloem pole, conducting fascicles are flanked by sclerenchyma tissue bundles placed semicircular.

Libero-ligneous cambium is intra- and interfascicular. Phloem tissue is represented by sieve tubes, phloem parenchyma and annex cells. Secondary xylem tissue is made up of xylem vessels with different sizes and well represented libriform tissue. The xylem vessels exhibit reticulate thickenings, in longitudinal-radial sections. The poorly represented primary xylem tissue consists of some protoxylem vessels accompanied by xylem parenchyma. Xylem vessels are arranged serially. The medullary rays are large, multi-cellular, pluriseriate and lignified. Medullary parenchyma is well developed, of meatus type (Figures 3 and 4).

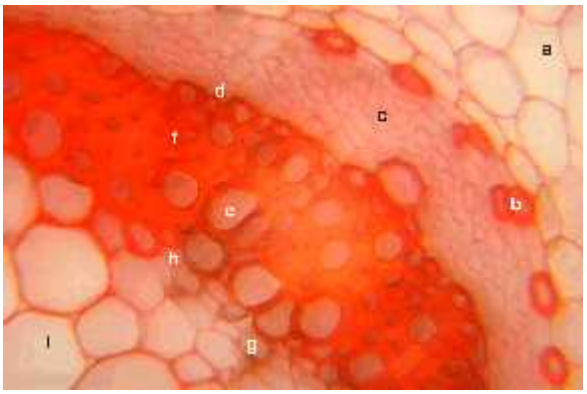

Figure 3. Cross-section through S. hastifolia aboveground stem: (a) cortical parenchyma; (b) bundle of sclerenchyma fibers; (c) phloem tissue; (d) libero-ligneous cambium; (e) metaxylem; (f) libriform tissue; (g) protoxylem; (h) xylem tissue; (i) medullary parenchyma (Congo red–chrysoidine staining, ×400).

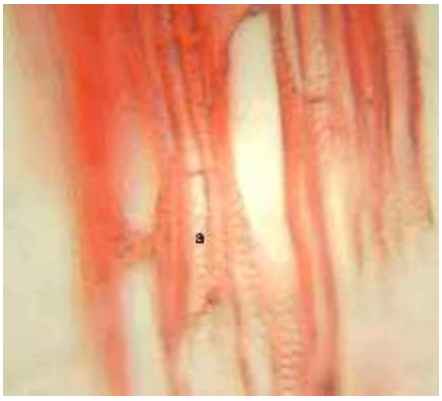

Figure 4. Longitudinal-radial section through S. hastifolia aboveground stem: (a) metaxylem with reticulate thickenings (Congo red–chrysoidine staining, ×400).

Leaf's limb

In cross-section, from the outside towards the inside of leaf's lamina, the following histological sequence is observed. The upper epidermis is composed of a single layer of flattened large cells with thickened tangential external and internal walls and thin radial walls. The external walls are bulged and covered by a thick cuticle. The mesophyll is made up of palisade and lacunose parenchymas. Palisade parenchyma has two layers of large, elongated cells rich in chloroplasts, arranged perpendicular to the upper epidermis, leading to small intercellular spaces. Lacunose parenchyma is composed of 3–4 layers of small cells, disorderly arranged, leaving large auriferous spaces. Into the mesophyll are many small libero-ligneous fascicles, each of them surrounded by an assimilatory fascicular sheath. Mesophyll has bifacial dorsiventral structure. Lower epidermis is made of a single layer of tangential elongated cells, with thin radial walls and thickened tangential external and internal walls. At this level, there are anomocytic stomata and unicellular, long tector hairs, with sharp peak, composed of three cells serially arranged. The median rib has gutter shape prominent on abaxial face. On the outside, both on the adaxial and abaxial faces, epidermis consists of small cells, anterior-posterior slightly flattened, with the external wall

covered by cuticle. Into the central zone is located a single libero-ligneous fascicle arranged in a leaf's parenchyma mass. Into the libero-ligneous fascicle, xylem vessels are seriate disposed and the medullary rays are uniseriate, cellulosic. Into the periphloemic area, bundles of sclerenchyma fibers are found. Leaf's limb has bifacial dorsiventral, hypostomatic structure (Figures 5–7).

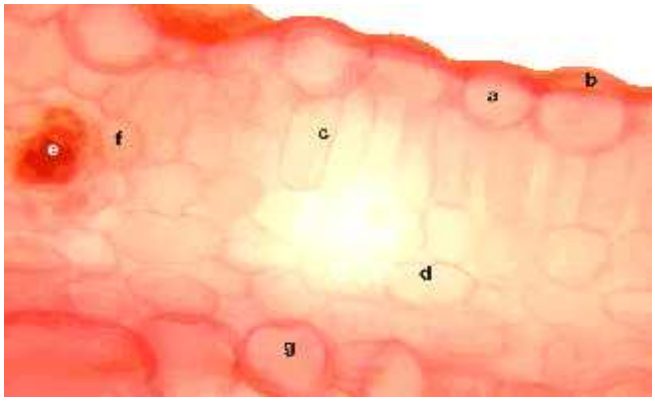

Figure 5. Cross-section through S. hastifolia leaf's limb: (a) upper epidermis; (b) cuticle; (c) palisade parenchyma; (d) lacunose parenchyma; (e) libero-ligneous conducting fascicle; (f) fascicular assimilatory sheath; (g) lower epidermis (Congo red–chrysoidine staining, ×400).

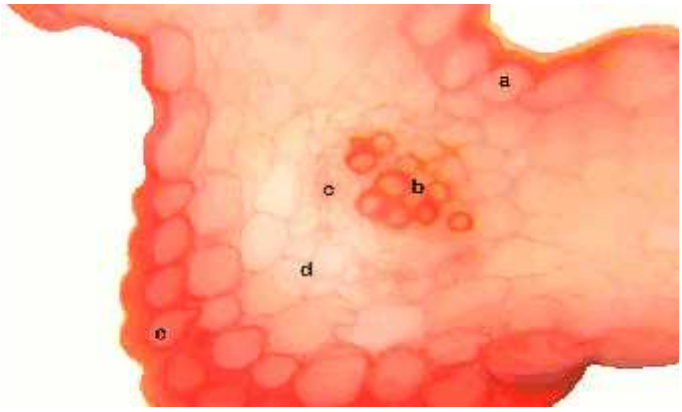

Figure 6. Cross-section through S. hastifolia leaf's limb: (a) upper epidermis; (b) libero-ligneous conducting fascicle; (c) bundle of sclerenchyma fibers; (d) leaf's parenchyma; (e) lower epidermis (Congo red–chrysoidine staining, ×400).

Figure 7. Cross-section through S. hastifolia leaf's limb: (a) anomocytic stomate (Congo red–chrysoidine staining, ×400).

TLC analysis

The experimental results from the preliminary TLC analysis of *Scutellariae hastifoliae herba* polyphenols are highlighted in Figures 8–10. One caffeic acid derivative was identified (R_f 0.64, 3.58 mg/100 g dried vegetal product) starting from 12 distinctive chromatographic bands.

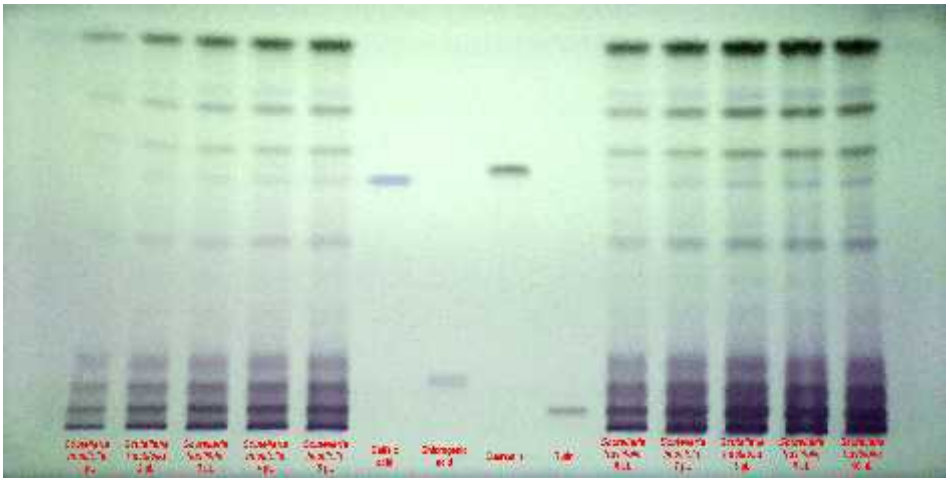

Figure 8. TLC chromatogram of polyphenols from Scutellariae hastifoliae herba methanolic extract (UV 254 nm, without derivatization). From left to right: first five applications – sample (1–5 µL); subsequent four applications – standards (2 µL); last five applications – sample (6–10 µL).

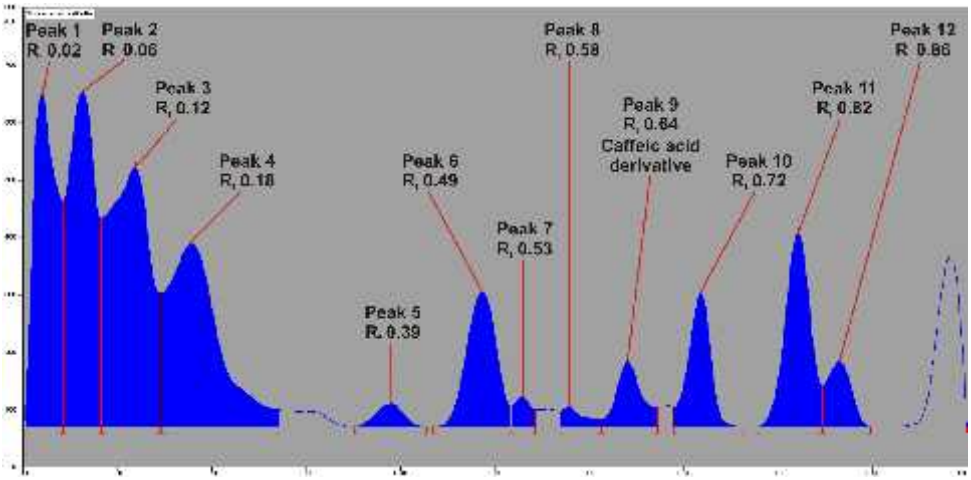

Figure 9. Densitogram of polyphenols (UV 254 nm) separated from Scutellariae hastifoliae herba methanolic extract.

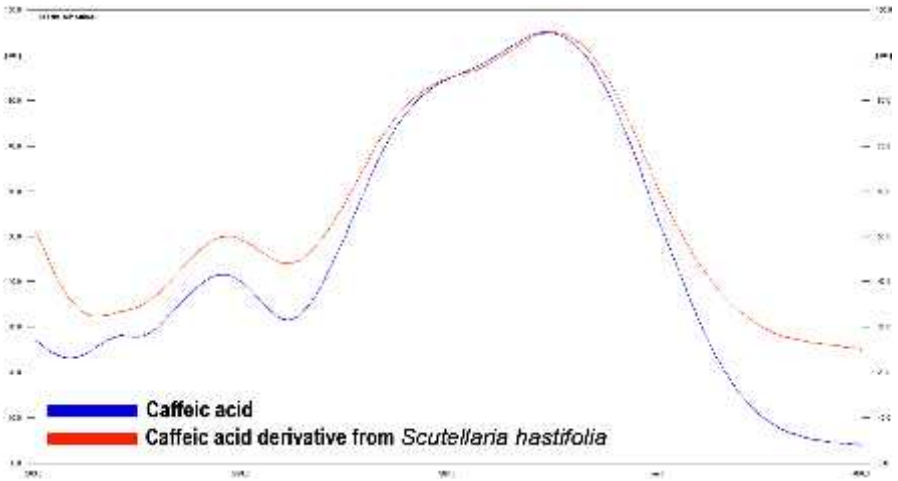

Figure 10. Caffeic acid derivative in situ UV spectra of standard and compound separated from the analyzed sample.

CONCLUSIONS

The histo-anatomical investigation of the rootlet, rhizome, aboveground stem and leaf of *Scutellaria hastifolia* species and the preliminary TLC analysis of *Scutellariae hastifoliae herba* polyphenols were effectuated. The rootlet has circular shape and primary structure. The rhizome has circular shape and secondary structure (libero-ligneous cambium). Into the upper third, the aboveground stem has quadratic shape, with four prominent ribs, and secondary structure. Leaf's limb has bifacial dorsiventral, hypostomatic structure. Starting from 12 distinctive chromatographic bands, one caffeic acid derivative was identified.

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