HISTO-ANATOMICAL AND CHROMATOGRAPHIC RESEARCHES ON *ZIZIPHORA CAPITATA* L. (*LAMIACEAE*) SPECIES

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ABSTRACT

Concerning Ziziphora capitata L. (Lamiaceae) species, the paper presents the histo-anatomical analysis of root, aboveground stem and leaf, along with the chromatographic investigations of the polyphenols in the aerial parts. Caffeic acid (126.2 µg/mL) and rutin (171.3 µg/mL) were identified in the 20% methanolic extract of Ziziphorae capitatae herba, by thin-layer chromatography coupled with photodensitometry.

INTRODUCTION

Ziziphora capitata L., Ovate-leaved Ziziphora, *Lamiaceae* family, is an herbaceous, aromatic, annual species, of approximately 20 cm height, with pink, violet or purple flowers, spontaneous in the Mediterranean area, Balkans, Caucasus to Central Asia (northern Iran). It is found in the southwest but also in the southeast of Romania (Oltenia and Dobruja regions, respectively) [7, 21].

Several important active principles were isolated and characterized for *Ziziphora* sp., as follows: essential oil (pulegone, isomenthone, menthone, *α*-limonene, *α*- and *β*-pinene, thymol, *p*-cymene), flavonosides (baicalein, hyperoside, diosmin, linarin), anthocyanosides, triterpenoids (oleanolic acid, ursolic acid and related compounds), sterols (*β*-sitosterol), phenolic acids, monoterpene glucosides (ziziphorosides A–C), phenylpropane derivatives (rosmarinic acid), fatty acids (oleic acid) [1, 8, 9, 14, 19, 20, 23, 24].

Medicinal products (*herba*, *folium*, *flos*) and extractive preparations from *Ziziphora* sp. exhibited some useful pharmacological actions, such as: analgesic, anti-inflammatory, antireumatic, antioxidant, antimicrobial, antiparasitic, expectorant, vasodilator, carminative, astringent, cicatrizing, antitumoral, immunomodulatory [1, 3, 5, 8, 9, 13–18, 20, 21, 24, 26].

There are some specialty papers containing thorough information about the histo-anatomy of several *Ziziphora* species [12, 25]. In this work, we exhibit the histo-anatomical investigation of the root, aboveground stem and leaf of *Z. capitata* species but also the preliminary analyses of the polyphenols from *herba*, through thin-layer chromatography coupled with photodensitometry.

MATERIALS AND METHODS

Histo-anatomical investigation

The vegetal material was harvested in July 2016, from *Ziziphora capitata* species, in the blooming period, spontaneous in the environs of Radovan commune, Valea Rea zone, Dolj County (southwestern Romania).

Fixation and preservation of the

biological material (roots, aboveground stems and leaves) was accomplished in 70% ethanol solution. The cross-sections were made using a botanical razor.

The sections were clarified in 10% sodium hypochlorite solution (Javel water), after a pre-wash with distilled water. To remove the clarification agent, distilled water was also used for the sequential wash of the sections. For the sections' staining, Genevese reagent (Congo red–chrysoidine mixture) was employed; according to the chemical composition of cell membranes, certain colors were obtained: pink-red for cellulose and mucilages, pale red for cytoplasm, yellow for suberin, and brown for lignin [4].

For the examination of stained and mounted sections, at ×4, ×10, ×20, and ×40 objectives, Krüss binocular photon microscope was utilized. For the photographic capture, Nikon Eclipse 55i binocular microscope and Nikon DS–Fi1 high definition charge-coupled device (CCD) video camera were used. For acquisition and processing of the images, Image-Pro Plus *ver*. 6.0 software package (Media Cybernetics) was applied.

Concerning the histo-anatomical investigation, a Romanian reference piece of work was taken into account [22].

Thin-layer chromatography (TLC) analyses

Preliminary TLC analyses of polyphenols from the aerial parts of *Ziziphora capitata* species (*Ziziphorae capitatae herba*) were performed on CAMAG (Muttenz, Switzerland) system, in the subsequent experimental circumstances [2, 6, 10, 11]:

- stationary phase: TLC silica gel 60 F_{254} (Merck, Darmstadt, Germany) 10×10 cm pre-coated glass plates, pre-washed with chloroform–methanol (1:1, *v/v*) and activated by oven-drying (110^{0}C, 30 minutes);
- 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: 20% methanolic extract of *Ziziphorae capitatae herba*;
- standards (Merck): 0.05% methanolic solutions of caffeic acid, chlorogenic acid, quercetin and rutin;
- migration distance: 62 mm (sample application line 8 mm, solvent front 70 mm);
- application of sample (8 µL, 10 µL) and standards (2 µL): CAMAG Linomat 5 semi-automatic system – spray 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 25^{0}C (cold air dryer);
- chromatographic plate shooting: UV light (*λ* 254 nm);
- detection: CAMAG TLC Scanner 3 photodensitometer, for densitogram and *in situ* UV light (*λ* 280 nm) spectra, without derivatization, deuterium–tungsten lamp, scanning speed 40 mm/s, data resolution 200 µm/step, measurement mode absorption;
- winCATS *ver.* 1.4.3 software package.

RESULTS AND DISCUSSIONS

Histo-anatomical investigation

Root

The root in the lower third has circular shape and secondary structure, due to the two meristematic secondary areas: subero-phellodermic cambium (phellogen) and libero-ligneous cambium. In cross-section, from the outside towards the inside of the root, the subsequent histological sequence was highlighted: Peridermis includes suber, phellogen and phelloderm. The suber is exfoliated in

patches; it contains 4–5 layers of large, flattened cells, impregnated with suberin. One layer of antero-posterior flattened cells, with thin walls and slightly curled radial walls forms the subero-phellodermic cambium. The phelloderm consists of 4–5 layers of cells with cellulosic thin walls. Disposed on two concentric rings, the conducting tissues are predominantly generated by the libero-ligneous cambium. A thin, external ring made up of sieve tubes, phloem parenchyma and annex cells represents the phloem tissue. The xylem tissue occupies the central area of the root, being composed of few metaxylem vessels of different calibers, disordered arranged in the libriform tissue mass, pushing to the center the small diameter protoxylem vessels accompanied by xylem parenchyma. The medullary rays are multicellular, uniseriate and cellulosic, at the level of phloem tissue, and multicellular, uniseriate and slightly lignified, into the xylem tissue. The medullary parenchyma is missing (Figures 1 and 2).

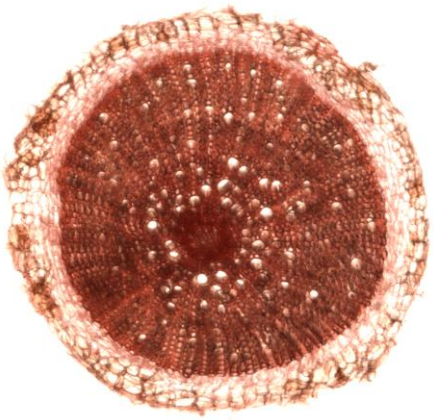

Figure 1. Cross-section through Z. capitata root: overview (Congo red–chrysoidine staining, ×40).

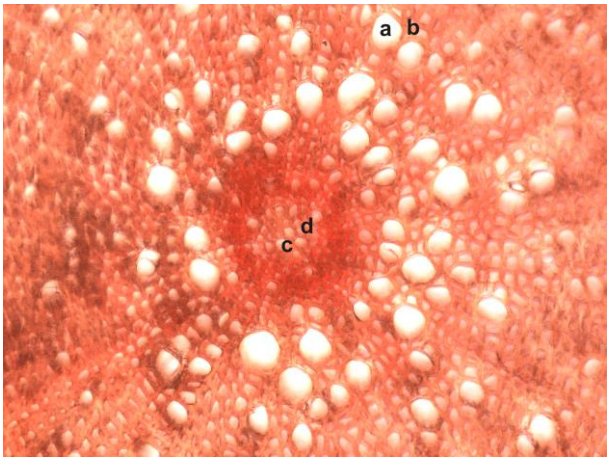

Figure 2. Cross-section through Z. capitata root: (a) metaxylem; (b) libriform tissue; (c) protoxylem; (d) xylem parenchyma (Congo red–chrysoidine staining, ×200).

Aboveground stem

The aboveground stem in the upper third has four-ribbed shape and secondary structure generated by the libero-ligneous cambium. The subsequent histological sequence was observed in cross-section, from the outside towards the inside of the aboveground stem: The epidermis has approximately isodiametric cells, with the thickened external wall covered by a thin cuticle with toothed relief. The epidermal cells are slightly tangential elongated, with thin radial walls and thickened tangential external and

internal walls. From point to point, there are stomata, multicellular, long and sharp tector trichomes, but also glandular trichomes with octocellular gland. The bark consists of 7–8 layers of angular collenchyma, at the ribs level, and of 2–3 layers of chlorenchyma, between the ribs. The inner area of the bark is parenchymatous; at this level, there is one layer of endodermis with large, suberin-impregnated cells. The conducting tissues are arranged into four large collateral open libero-ligneous fascicles, disposed at the four edges' level, and four small conducting fascicles made up of only secondary phloem and xylem, and resulting from the activity of libero-ligneous cambium. The phloem tissue is organized of sieve tubes, few phloem parenchyma and annex cells. At this level, the medullary rays are multicellular, multiseriate and cellulosic. The secondary xylem is made up of well-represented libriform tissue, situated in the vicinity of intrafascicular cambium, and of metaxylem vessels, with different sizes, arranged in radial strings, toward the inner side of the large conducting fascicles. In longitudinal-radial sections, the xylem vessels exihibited reticulate and helical thickenings. The primary xylem tissue is poorly represented by few primary xylem vessels and xylem parenchyma. Between the libero-ligneous conducting fascicles, in the xylem area, the medullary rays are wide and strongly lignified. The meatus-type medullary parenchyma is well developed (Figures 3 and 4).

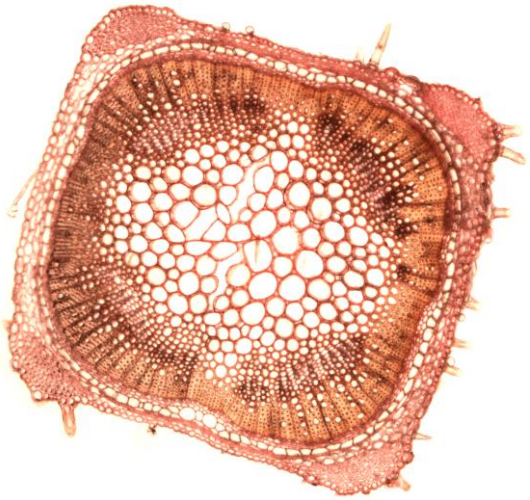

Figure 3. Cross-section through Z. capitata aboveground stem: overview (Congo red–chrysoidine staining, ×40).

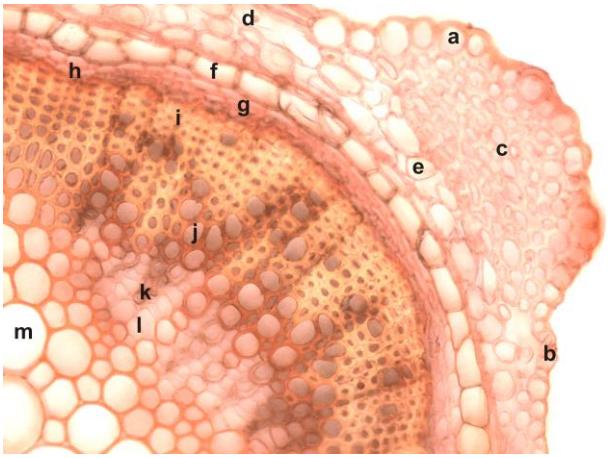

Figure 4. Cross-section through Z. capitata aboveground stem: (a) epidermis; (b) stomate; (c) angular collenchyma; (d) chlorenchyma; (e) cortical parenchyma; (f) endodermis;

(g) phloem tissue; (h) libero-ligneous cambium; (i) libriform tissue; (j) metaxylem; (k) protoxylem; (l) xylem parenchyma; (m) medullary parenchyma (Congo red–chrysoidine staining, ×200).

Leaf's limb

From the outside towards the inside of leaf's limb, in cross-section, the subsequent histological sequence was found: The upper epidermis consists of a single layer of large, flattened cells, with thickened tangential external and internal walls and thin radial walls. The external walls are bulged and covered by a cuticle with toothed relief. From place to place, unicellular, aculeiform tector trichomes and stomata are observed. The mesophyll is made up of two layers of palisade parenchyma, with large, elongated, chloroplast-rich cells, as well as of 4–5 layers of lacunose parenchyma, consisting of small cells, disordered disposed, leaving aeriferous spaces between them. Into the mesophyll, there are numerous small libero-ligneous conducting fascicles. The mesophyll has bifacial type, with dorsiventral structure. The lower epidermis is composed of a single layer of small, tangential elongated cells, with thin radial walls and thickened tangential external and internal walls. A toothed relief was highlighted for the cuticle. Numerous stomata and uni- or bi-cellular aculeiform tector trichomes are found at this level. In cross-section, the median rib is protruding and is rounded like a trough, on the abaxial side. In the central area, in the leaf's parenchyma mass, there is only one libero-ligneous fascicle, in which the xylem vessels have a serial disposition and the medullary rays are uniseriate, cellulosic. The leaf's limb has bifacial, dorsiventral, amphistomatic structure (Figure 5).

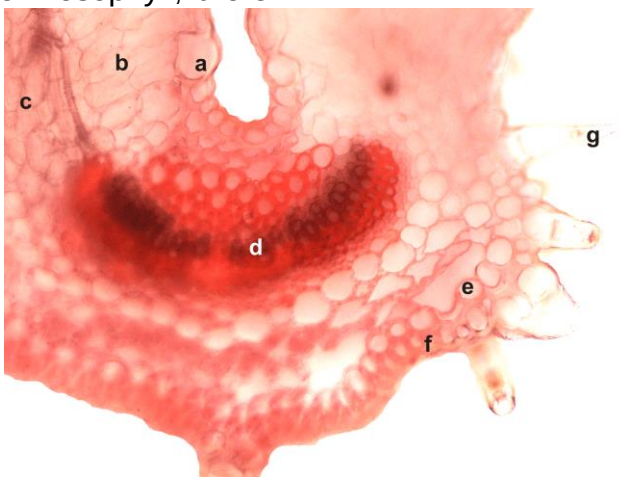

Figure 5. Cross-section through Z. capitata leaf's limb: (a) upper epidermis; (b) palisade parenchyma; (c) lacunose parenchyma; (d) libero-ligneous fascicle; (e) leaf's parenchyma; (f) lower epidermis; (g) tector trichome (Congo red–chrysoidine staining, ×200).

TLC analyses

Figures 6–9 highlighted the experimental data about the preliminary TLC analyses of polyphenols from *Ziziphorae capitatae herba*. Caffeic acid (R_f 0.89 – 126.2 µg/mL) and rutin (R_f 0.1 – 171.3 µg/mL) were quantified in the 20% methanolic extract, amounts which correspond to 63.1 mg and 85.65 mg/100 g of dried vegetal product, respectively.

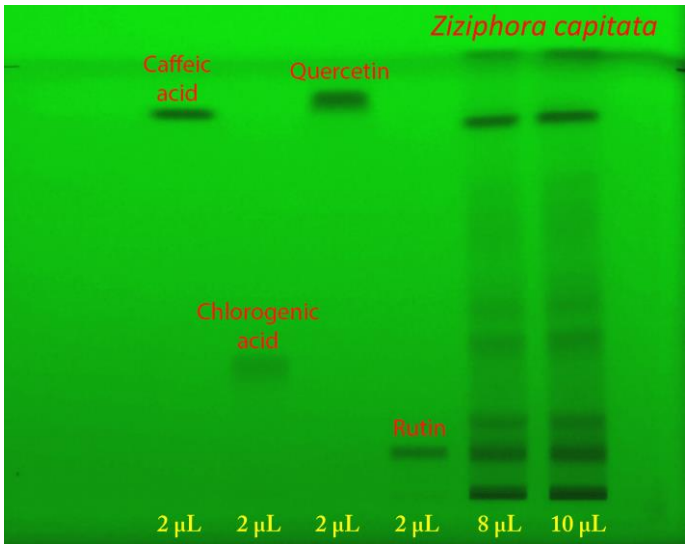

Figure 6. TLC chromatogram of polyphenols from Ziziphorae capitatae herba 20% methanolic extract (UV 254 nm, without derivatization). From left to right: first four bands – standards (2 µL); last two bands – sample (8 µL and 10 µL).

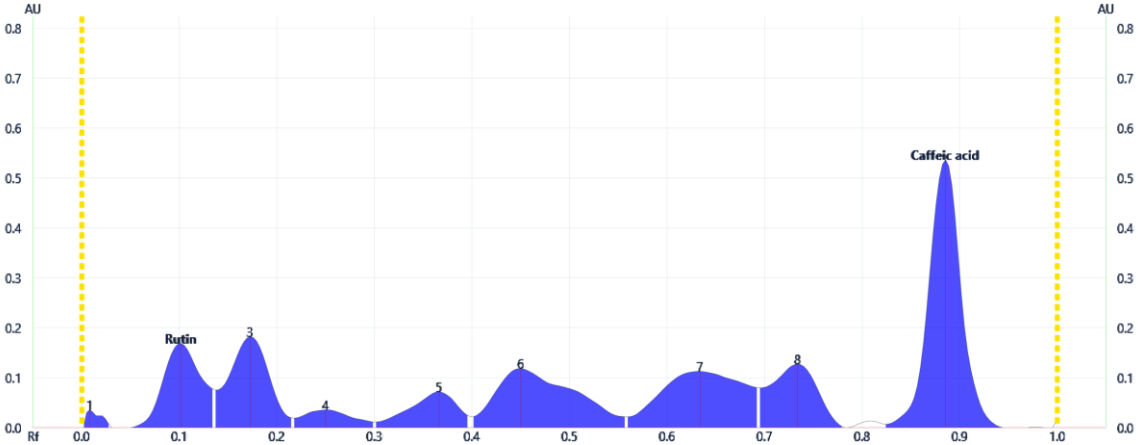

Figure 7. Densitogram of polyphenols (UV 280 nm, without derivatization) separated from Ziziphorae capitatae herba 20% methanolic extract. Caffeic acid and rutin were identified at R_f 0.89 and R_f 0.1, respectively.

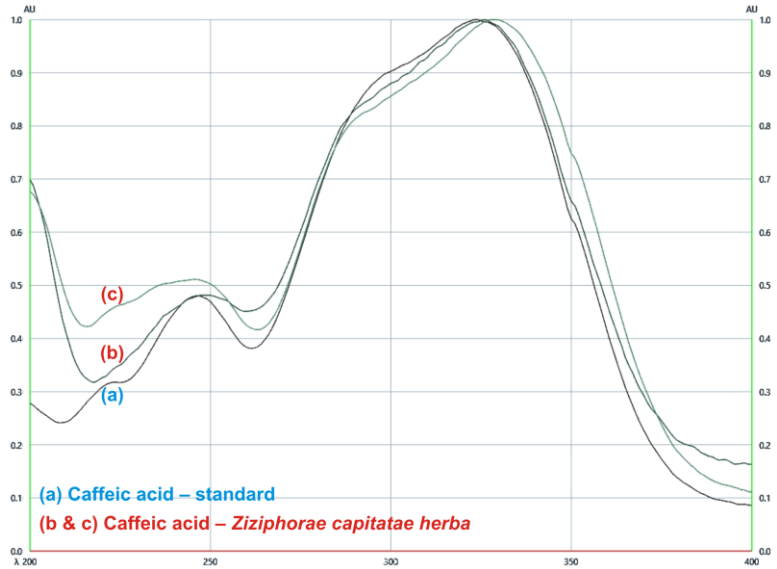

Figure 8. In situ UV spectra (280 nm) of caffeic acid standard and compound

separated from the analyzed sample.

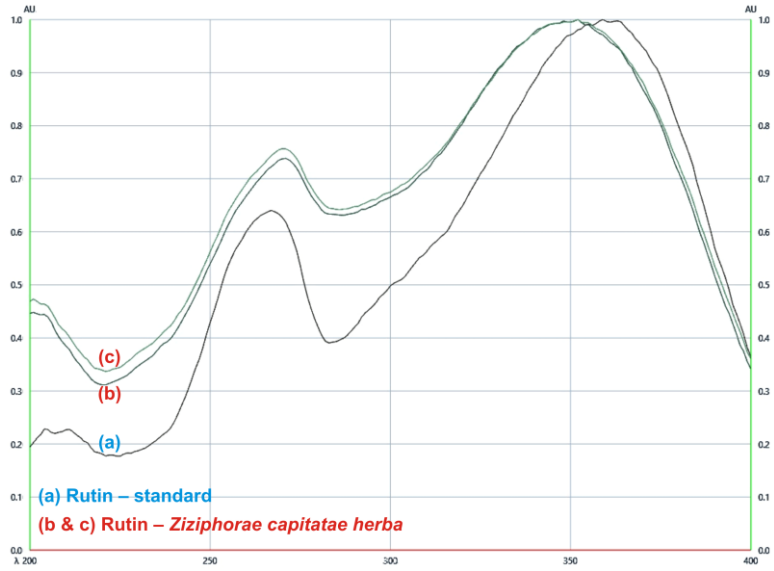

Figure 9. In situ UV spectra (280 nm) of rutin standard and compound separated from the analyzed sample.

CONCLUSIONS

The histo-anatomical analysis of roots, aboveground stems and leaves of *Ziziphora capitata* species, as well as the preliminary TLC analyses of polyphenols from the *herba* were achieved. The root in the lower third has circular shape and secondary structure. The aboveground stem in the upper third has four-ribbed shape and secondary structure. The leaf's limb has bifacial, dorsiventral, amphistomatic structure. Caffeic acid (126.2 µg/mL) and rutin (171.3 µg/mL) were quantified in the 20% methanolic extract by TLC coupled with photodensitometry.

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