HISTO-ANATOMICAL AND PRELIMINARY TLC ANALYSIS ON *TEUCRIUM CHAMAEDRYS* L. (*LAMIACEAE*) SPECIES

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ABSTRACT

For Teucrium chamaedrys L. (Lamiceae) species, harvested in July 2016, from Dolj County (southwestern Romania), the paper presents the histo-anatomical investigation of roots, rhizomes, stolons, aboveground stems and leaves, and the preliminary TLC analysis of the poyphenols from the aerial parts (Teucrii herba). Chlorogenic acid was identified starting from the 11 fingerprint chromatographic bands.

INTRODUCTION

Teucrium chamaedrys L., Germander, Wall germander, *Lamiaceae* family, is an old medicinal plant spontaneous in meadows, sunny shrubs and arid zones, from Europe (Mediterranean region), Northern Africa and Asia. It is an undergrowth species, often used for ornamental purposes [4].

Numerous active principles were evidenced for the aerial parts of *T. chamaedrys*, as follows: essential oil (*β*-caryophyllene, nonacosane, germacrene D, caryophyllene oxide, *α*-pinene, *β*-pinene, *α*-farnesene) [7, 14], *neo*-clerodane diterpenoids (teucrin A, dihydro-teugin, teucroxide, syspirensin A) [14], iridoids (ajugol and harpagide derivatives) [14], phenylpropanoid glycosides (verbascoside, teucrioside) [14], flavonoids (luteolin, quercetin, rutin, isoquercitrin, quercitrin) [13], polyphenolcarboxylic acids (caftaric, gentisic, caffeic, *p*-coumaric, chlorogenic and ferulic acids) [13, 14].

For the above-mentioned active principles, different pharmacological properties were highlighted: *in vitro* cytotoxic, antiproliferative and proapoptotic effects on P388 lymphocytic leukemia in mice and HCT-116 cells (diterpenoids, polyphenols) [11], antioxidant (essential oil, flavonoids, polyphenolic acids) [11, 13, 14], antihyperglycemic [14], analgesic and anti-inflammatory (flavonoids, iridoids) [10], antimicrobial (essential oil, polyphenols) [13], anti-amoebian [14]. *T. chamaedrys* extracts containing high concentrations of teucrin A (a *neo*-clerodane diterpenoid) are potentially hepatotoxic [9].

In the specialty papers, there are short and fragmentary information about the histo-anatomy of *T. chamaedrys* species [6, 8].

In this paper, we reported the histo-anatomical investigation of the root, rhizome, stolon, aboveground stem and leaf of *T. chamaedrys* species, as well as the preliminary TLC analysis of the polyphenols content from the aerial parts (*Teucrii herba*).

MATERIAL AND METHOD

Histo-anatomical investigation

The vegetal material was harvested from *T. chamaedrys* blooming plants, in July 2016, from the Radovan village environs, Valea Rea zone, Dolj County (southwestern Romania).

Fixation and preservation of the biological material (roots, rhizomes, stolons, above-ground stems, leaves) were performed in 70% ethanol. Cross-sections were made with the aid of a botanical razor.

After washing with distilled water, the sections were clarified in 10% solution of sodium hypochlorite (Javel water). The clarifying agent was removed through washing with distilled water. For the staining of sections, Genevese reagent (Congo red–chrysoidine mixture) was applied, getting various colors, depending on the chemical composition of cell membranes: pink–red for cellulose and mucilages, pale red for cytoplasm, yellow for suberin, brown for lignin [2].

For the analysis of stained and mounted sections, Krüss binocular photon microscope was used (objectives ×4, ×10, ×20, ×40). The photos were obtained on a Nikon Eclipse 55i binocular microscope coupled with Nikon DS–Fi1 high definition video camera. For images acquisition, Image-Pro Plus ver. 6.0 software package was applied.

The description of microscopic sections was made taking into account one classical Romanian work [12].

Thin-layer chromatography (TLC) analysis

The preliminary analysis of polyphenols from the aerial parts of *T. chamaedrys* species (*Teucrii herba*) was made using a CAMAG (Muttenz, Switzerland) system, in the following experimental conditions [1, 3, 5]:

▪ stationary phase: TLC silica gel G 60 F_{254}, 20×10 cm precoated glass plates (Merck, Darmstadt, Germany) pre-washed with chloroform–methanol (1:1, *v/v*) and activated by oven drying (110^0C, 30 min.);

▪ mobile phase: chloroform–ethyl acetate–toluene–formic acid–methanol (15:20:10: 10:1, in volumes), 10 mL in the chromatographic tank (20×10 cm twin trough chamber, CAMAG), without oversaturation;

▪ sample: 20% methanolic extract of *Teucrii herba*;

▪ standards: 0.05% methanolic solutions of caffeic acid, chlorogenic acid, quercetin and rutin (Merck);

▪ sample (1–10 µL) and standards (2 µL) applications: CAMAG Linomat 5 semiautomatic system (spray gas nitrogen, syringe volume 100 µL, predosage volume 0.2 µL, dosage speed 150 nL/s, band length 8 mm);

▪ migration distance: 80 mm (sample application line – 10 mm, solvent front – 90 mm);

▪ detection: CAMAG TLC Scanner 3 photodensitometer, without derivatization, UV 254 nm (deuterium–wolfram lamp, scanning speed 20 mm/s, resolution 100 µm/step);

▪ measurement mode and spectra acquisition, processing and quantification analysis: absorption, winCATS *ver*. 1.4.3 software package.

RESULTS AND DISCUSSIONS

Histo-anatomical investigation

Root

In cross-section, the root in the lower third has circular shape and primary structure. From the outside towards the inside of the root, the following histological sequence was highlighted in cross-section. The rhizodermis is made up of small cells with thin, cellulosic walls and long absorbent hairs. The exodermis consists of a single layer of large, hetero-diametric cells, with suberin-impregnated walls and passage cells in patches. The cortical parenchyma is made up of large oval cells with thin, cellulosic walls and intercellular spaces variable in size. The endodermis is formed by one layer of large, antero-posterior flattened cells provided with Casparian strips. A single cellular layer of cellulosic pericycle alternately disposed with endodermic cells delimits the central cylinder. The xylem vessels are alternately placed with the phloem ones and separated by multi-cellular, uniseriate, cellulosic medullary rays. Within the fascicles, the protoxylem and protophloem are arranged near the

pericycle, and the metaxylem and metaphloem in the central part. The metaxylem has reticulate thickenings and occupies the central part of the root, replacing the medullary parenchyma (Figure 1).

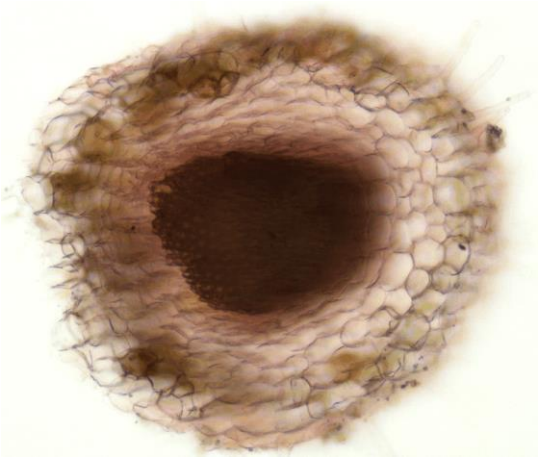

Figure 1. Cross-section through T. chamaedrys root: overview (Congo red–chrysoidine staining, ×200).

Rhizome

In cross-section, at the lower third level, the rhizome has circular-sinuous shape and secondary structure due to the presence of the libero-ligneous cambium. The following histological sequence was evidenced in cross-section, from the outside towards the inside of the rhizome. A single layer of epidermis consists of small cells with the outer wall bulged, thickened and covered with a thin cuticle. The epidermal cells are tangentially elongated, with thin radial walls and tangential outer and inner thickened walls. From place to place, the epidermis is exfoliated and replaced by the suber; it consists of large, flattened cells impregnated with suberin. The cortical parenchyma is made up of large, oval cells with cellulosic thin walls, disorderly arranged and generating intercellular spaces variable in size. In the inner part, there are sclerenchyma fiber bundles disposed in the periphloemic area. The conducting tissues are arranged on two concentric rings. The phloem tissue forms a thin, outer ring with sieve tubes, phloem parenchyma and annex cells. At the phloem tissue level, the medullary rays are multi-cellular, uniseriate, with antero-posterior flattened cells and cellulosic walls. The libero-ligneous cambium is placed between the xylem and phloem tissues. The xylem tissue forms the large inner ring, made up of numerous metaxylem vessels of different sizes, spread into the libriform tissue mass, pushing to the center the protoxylem vessels of small diameter. The xylem vessels exhibit reticulate thickenings. The protoxylem is poorly represented, accompanied by some xylem parenchyma. The medullary rays are multi-cellular, uniseriate, lignified at the level of the xylem tissue ring. The meatus-type medullary parenchyma is poorly developed, occupying the central area of the rhizome. A medullary gap is outlined (Figures 2–4).

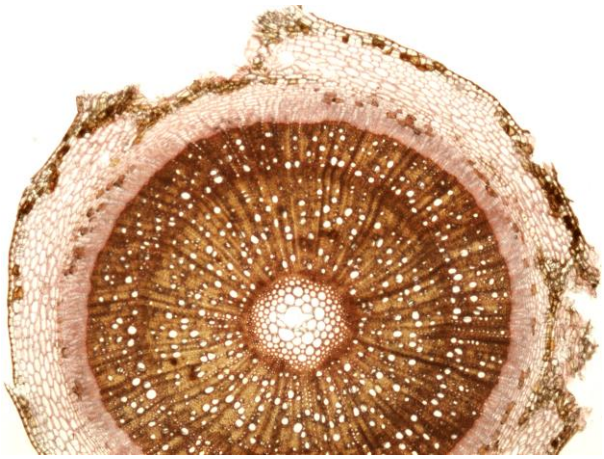

Figure 2. Cross-section through T. chamaedrys rhizome: overview (Congo red–chrysoidine staining, ×40).

Figure 3. Cross-section through T. chamaedrys rhizome: (a) metaxylem; (b) libriform tissue; (c) medullary ray; (d) protoxylem; (e) medullary parenchyma (Congo red–chrysoidine staining, ×100).

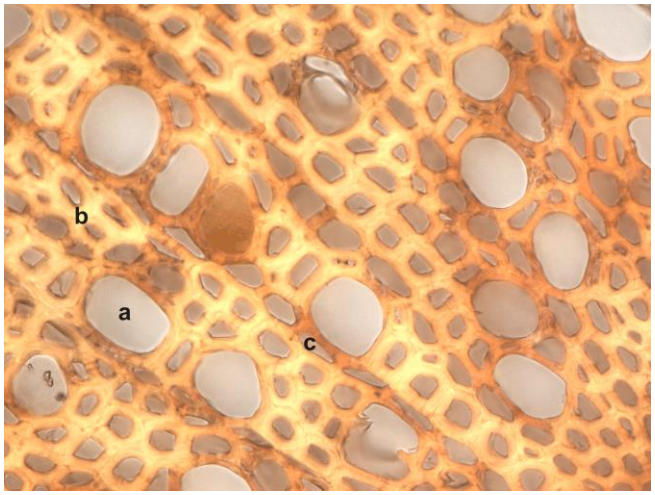

Figure 4. Cross-section through T. chamaedrys rhizome: (a) metaxylem; (b) libriform tissue; (c) medullary ray (Congo red–chrysoidine staining, ×400).

Stolon

In cross-section, in the upper third, the stolon has quadratic shape and secondary structure due to the libero-ligneous cambium. The epidermis has heterodiametric cells with

the outer wall bulged, thickened and covered by a thick cuticle. The epidermal cells are tangentially elongated, with thin radial walls and tangential outer and inner thickened walls. Long multi-cellular tector hairs are found in patches. The cortex consists of two areas: the outer area made up of 3–4 layers of angular collenchyma and the inner part of cortical parenchyma. In the inner part of the parenchymatic zone, there is one layer of endodermis exhibiting large, antero-posterior flattened cells, impregnated with suberin, as well as some sclerenchyma fiber bundles with periphloemic disposition. The conducting tissues are placed in two concentric rings, due to the activity of the libero-ligneous cambium. The phloem tissue is the thin, outer ring, consisting of sieve tubes, phloem parenchyma and annex cells. The medullary rays are multi-cellular, uniseriate, cellulosic. The xylem tissue forms the inner ring made up of metaxylem vessels of various calibers, scattered into the well-represented libriform tissue. The xylem vessels have spiral and reticulate thickenings. At this level, the medullary rays are multi-cellular, uniseriate, lignified. The primary xylem tissue is poorly represented, consisting of few primary xylem vessels and xylem parenchyma. The medullary parenchyma is well developed, of meatus type, and occupies the central area of the stolon (Figure 5).

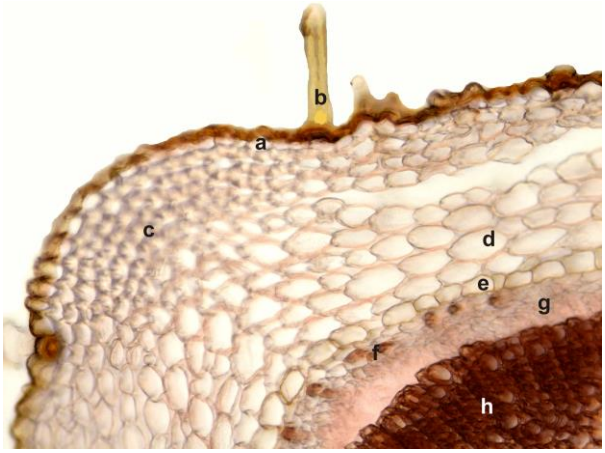

Figure 5. Cross-section through T. chamaedrys stolon: (a) epidermis; (b) tector hair; (c) angular collenchyma; (d) cortical parenchyma; (e) endodermis; (f) sclerenchyma fibers; (g) phloem tissue; (h) xylem tissue (Congo red–chrysoidine staining, ×200).

Aboveground stem

In cross-section, in the upper third, the aboveground stem has quadratic shape and secondary structure generated by the libero-ligneous cambium. Heterodiametric cells with the outer wall bulged, thickened and covered by a thick cuticle form the epidermis. The epidermal cells are tangentially elongated, with thin radial walls and tangential outer and inner thickened walls. From place to place, long multi-cellular tector hairs, glandular hairs and stomata are evidenced. The cortex is made up of two zones. The outer zone consists of 4–5 layers of angular collenchyma at the ribs' level and chlorenchyma between the ribs. The inner area comprises 4–5 layers of cortical parenchyma bounded internally by a single layer of primary-type endodermis, with large, heterodiametric, suberin-impregnated cells. Under the endodermis, sclerenchyma fiber bundles are evidenced in the periphloemic area. Placed near the four ribs, the conducting tissues are organized into four collateral-open libero-ligneous fascicles. The phloem tissue is made up of sieve tubes, phloem parenchyma and annex cells. Between the fascicles, the medullary rays are multi-cellular, pluriseriate, cellulosic. The xylem tissue is composed of metaxylem with different sizes spread into the well-represented libriform tissue. The xylem vessels have spiral and reticulate thickenings. The medullary rays between the conducting fascicles are wide, multi-cellular, pluriseriate, strongly lignified. The primary xylem tissue is poorly represented, with few primary xylem

vessels and xylem parenchyma. The meatus-type well-developed medullary parenchyma occupies the entire central area of the aboveground stem (Figure 6).

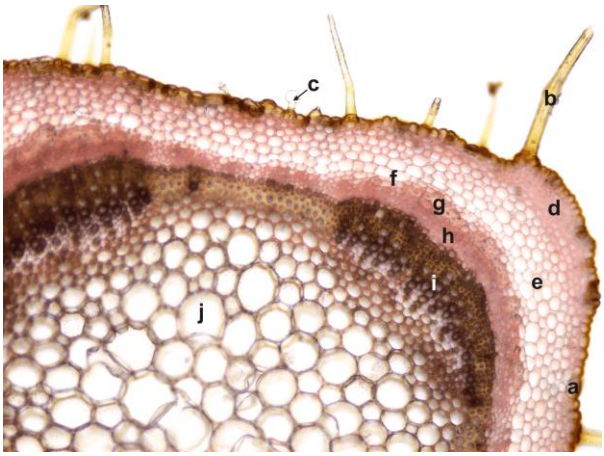

Figure 6. Cross-section through T. chamaedrys aboveground stem: (a) epidermis; (b) tector hair; (c) glandular hair; (d) angular collenchyma; (e) cortical parenchyma; (f) endodermis; (g) sclerenchyma fibers; (h) phloem tissue; (i) xylem tissue; (j) medullary parenchyma (Congo red–chrysoidine staining, ×100).

Leaf's limb

The leaf's limb has a flat adaxial side and slightly rounded abaxial side. The following histological sequence is observed in cross-section, from the outside towards the inside of leaf's limb. The upper epidermis consists of one layer of large, flattened cells with thickened tangential outer and inner walls and thin radial walls. The outer walls are bulged and covered by a thin cuticle with dentate relief. From point to point, long multi-cellular tector hairs are observed. The mesophyll is made up of two layers of palisade parenchyma, with large, elongated and chloroplast-rich cells, as well as of three layers of lacunose parenchyma consisting of small cells, disorderly arranged, with aeriferous spaces. In the mesophyll, there are numerous small libero-ligneous conducting fascicles bordered by assimilation sheath. The mesophyll is of bifacial type with dorsiventral structure. The lower epidermis is made up of a single layer of small, tangential elongated cells, with thin radial walls and thickened tangential outer and inner walls. At this level, we found stomata, glandular hairs and multi-cellular, long tector hairs with pointed tip. The median rib is flat on the adaxial side and slightly rounded on the abaxial side. On the outside, the epidermis consists of small cells, slightly flattened antero-posterior; covered by a thin cuticle, the outer wall has dentate relief. Under the epidermis, both at the adaxial and abaxial sides are two layers of angular collenchyma. In the central area, a single libero-ligneous conducting fascicle, periphloemic flanked by a sclerenchyma cap, is placed in a leaf's parenchyma mass. The xylem vessels have a seriate layout and the medullary rays are multi-cellular, uniseriate, cellulosic. The leaf's limb has bifacial, dorsiventral, hypostomatic structure (Figures 7 and 8).

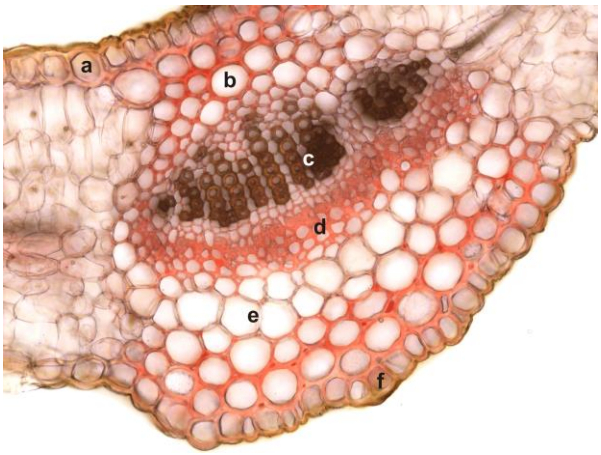

Figure 7. Cross-section through T. chamaedrys leaf's limb: (a) upper epidermis; (b) angular collenchyma; (c) libero-ligneous conducting fascicle; (d) sclerenchyma cap; (e) leaf's parenchyma; (f) lower epidermis (Congo red–chrysoidine staining, ×200).

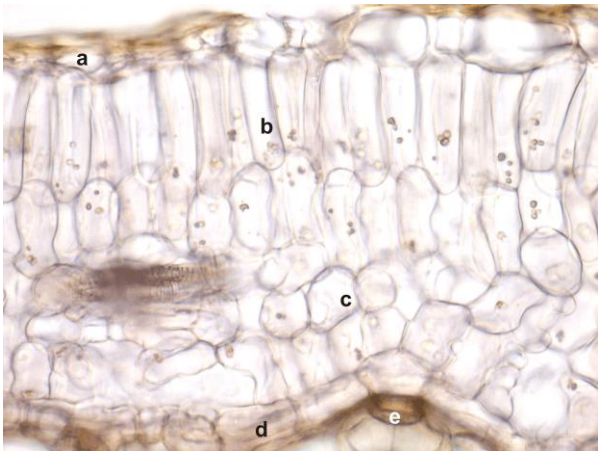

Figure 8. Cross-section through T. chamaedrys leaf's limb: (a) upper epidermis; (b) palisade parenchyma; (c) lacunose parenchyma; (d) lower epidermis; (e) glandular hair (Congo red–chrysoidine staining, ×400).

TLC analysis

Figures 9–11 highlighted the experimental data on the preliminary TLC analysis of polyphenols from *Teucrii herba*. Chlorogenic acid (R_f 0.13, 22.37 mg/100 g of dried vegetal product) was identified starting from the 11 fingerprint chromatographic bands.

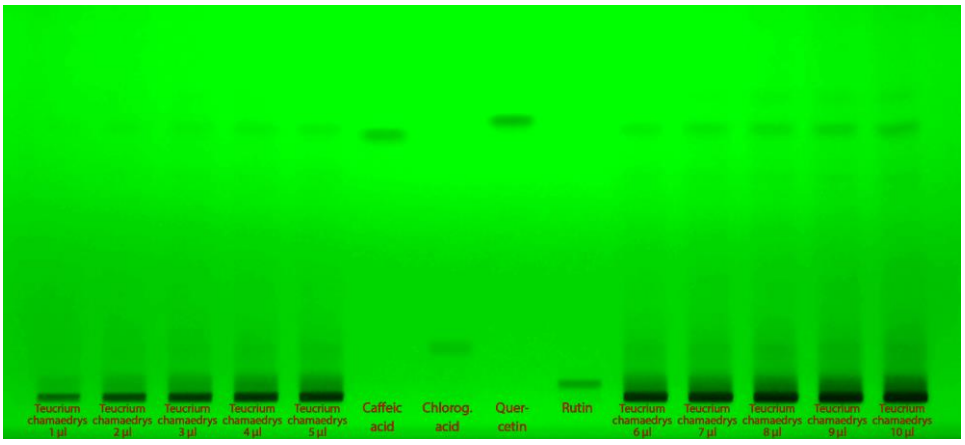

Figure 9. TLC chromatogram of polyphenols from Teucrii herba methanolic extract (UV 254 nm, without derivatization). From left to right: first five applications – sample (1–5 µL); subsequent four applications – standards (2 µL); last five applications – sample (6–10 µL).

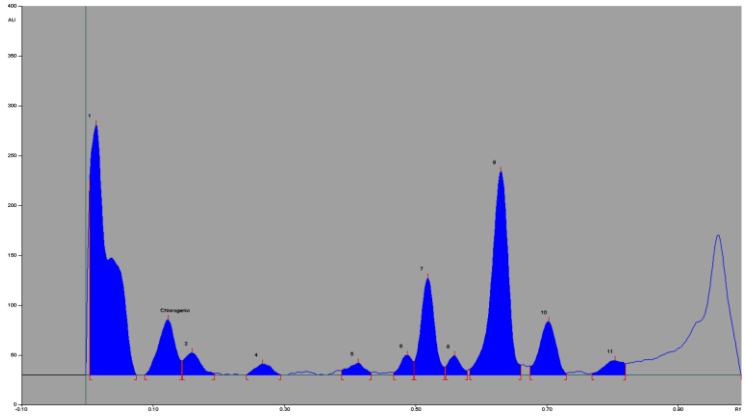

Figure 10. Densitogram of polyphenols (UV 254 nm) separated from Teucrii herba methanolic extract. From left to right, No. of peak/R_f: 1/0.02, 2/0.13 – chlorogenic acid, 3/0.16, 4/0.27, 5/0.42, 6/0.49, 7/0.52, 8/0.56, 9/0.63, 10/0.71, 11/0.81.

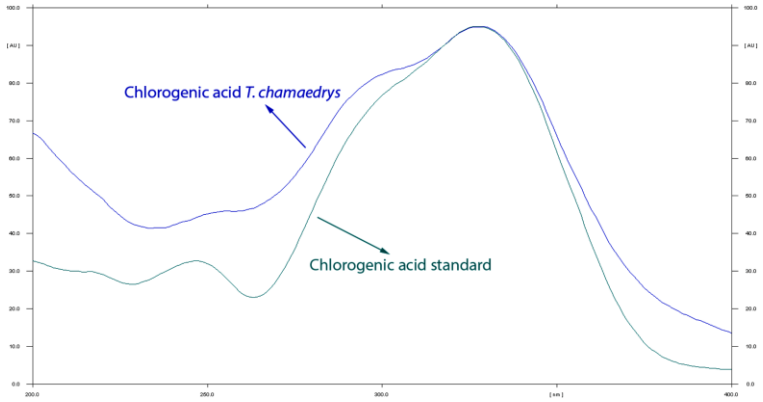

Figure 11. In situ UV spectra of chlorogenic acid standard and compound separated from the analyzed sample.

CONCLUSIONS

The histo-anatomical investigations of roots, rhizomes, stolons, aboveground stems and leaves of *T. chamaedrys* species and the preliminary TLC analysis of the polyphenols from the aerial parts (*Teucrii herba*) were achieved. The root has circular shape and primary structure. The rhizome has circular-sinuous shape and secondary structure. The stolon and the aboveground stem have quadratic shape and secondary structure (libero-ligneous cambium). The leaf's limb has bifacial, dorsiventral, hypostomatic structure. Chlorogenic acid was identified from the 11 TLC fingerprint bands.

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