# HISTO-ANATOMICAL AND PRELIMINARY TLC INVESTIGATIONS ON *HYPERICUM HIRSUTUM* L. (*HYPERICACEAE*) SPECIES

***GEORGE DAN MOGOŞANU[1], CORNELIA BEJENARU[2], ANDREI BIŢĂ[1]*, LUDOVIC EVERARD BEJENARU[1]***

*[1]Department of Pharmacognosy & Phytotherapy, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova, e-mail: andreibita@gmail.com*

*[2]Department of Vegetal & Animal Biology, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova*

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## ABSTRACT

*Starting from Hypericum hirsutum L. plants in blossom, harvested in May 2016, from Râşca Forest, Olt County (Romania), the cross-sections of root, aboveground stem and leaf were acquired and examined through microphotography technique. Also, the content of polyphenols was determined by thin layer chromatography as part of a comprehensive pharmacognostic analysis of aerial parts (Hyperici hirsuti herba). From the 12 separated chromatographic bands, one chlorogenic acid derivative was identified.*

## INTRODUCTION

*Hypericum hirsutum* L., Hairy St. John's wort, *Hypericaceae* family, is a perennial species spontaneous in Europe and Asia, growing mainly in forest edges and scrubs. It has downy stems and much longer leaves, compared to the most commonly known and used *H. perforatum* species [6].

*Hypericum* species contain some active principles (naphthodianthrones, flavonoids, essential oil, prenylated phloroglucinols, xanthones, proanthocyanidins, catechic tannin) [3, 7, 8, 13, 18, 21] with peculiar pharmacological activities: antidepressant [16], biostimulating, cicatrizing, antimicrobial [3, 5, 13, 14], antiviral [19], anti-inflammatory [17], antioxidant, anti-acetylcholinesterase [12], antieczematous, antihemorrhagic [3, 19], leishmanicidal [9].

In the specialty papers, there are scarce and incomplete data concerning *H. hirsutum* histo-anatomy [10, 15] and chemical composition [7, 8, 21].

The aim of our paper was the histo-anatomical investigation of the root, aboveground stem and leaf of the *H. hirsutum* species and the preliminary analysis of the polyphenols content from the aerial parts (*Hyperici hirsuti herba*).

## MATERIAL AND METHOD

### Histo-anatomical investigation

The vegetal material was collected from *H. hirsutum* plants in blossom, in May 2016, from Râşca Forest, Olt County, Romania.

The fixation and preservation of roots, 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 accomplished according to some classical authors [20].

### Thin-layer chromatography (TLC) analysis

Preliminary analysis of polyphenols was performed on the aerial parts of *H. hirsutum* species (*Hyperici hirsuti herba*), by using a CAMAG (Muttenz, Switzerland) system in the following experimental conditions [1, 4, 11]: stationary phase TLC silica gel 60 $F_{254}$ 20×10 cm 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 *Hyperici hirsuti 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, band length 8 mm); detection – CAMAG TLC Scanner 3 photodensitometer, UV 254 nm, without derivatization, deuterium–wolfram lamp, scanning speed 20 mm/s, data 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

#### *Root*

In cross-section, the root has circular shape and secondary structure due to the two secondary meristematic zones: phellogen and libero-ligneous cambium. From the outside towards the inside of the root, the following histological sequence was highlighted in cross-section. Peridermis is made of suber, phellogen and phelloderm. Suber is composed of 4–5 layers of suberin-impregnated flattened cells. From point to point, suber is exfoliated. One layer of subero-phellodermic cambium has anterior-posterior flattened cells, thin-walled, the radial walls being slightly undulated. Phelloderm is made of 2–3 cellular layers with slightly collenchymatized walls. Conducting tissues are arranged in two concentric rings. Phloem tissue forms a thin external ring, made of sieve tubes, phloem parenchyma and annex cells. Libero-ligneous cambium is disposed between xylem and phloem tissues. Xylem tissue forms the internal ring made of metaxylem vessels with different caliber, disorderly dispersed into the libriform tissue. Thus, the protoxylem vessels with small diameter, accompanied by few xylem parenchyma cells, are pushed toward the center of the root. Metaxylem vessels show reticulate thickenings, on longitudinal-radial sections. Medullary rays are multi-cellular, uniseriate, cellulosic, at the level of phloem tissue ring but lignified to the xylem tissue ring. Medullary parenchyma is missing (Figures 1 and 2).

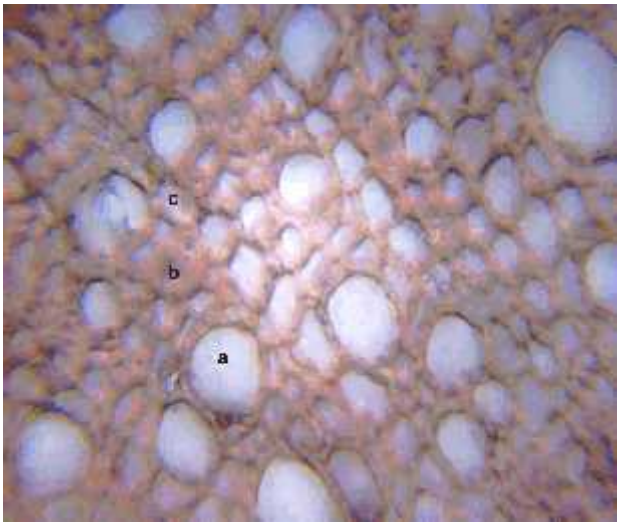


***Figure 1. Cross-section through H. hirsutum root: (a) metaxylem; (b) libriform tissue; (c) medullary ray (Congo red–chrysoidine staining, ×400).***

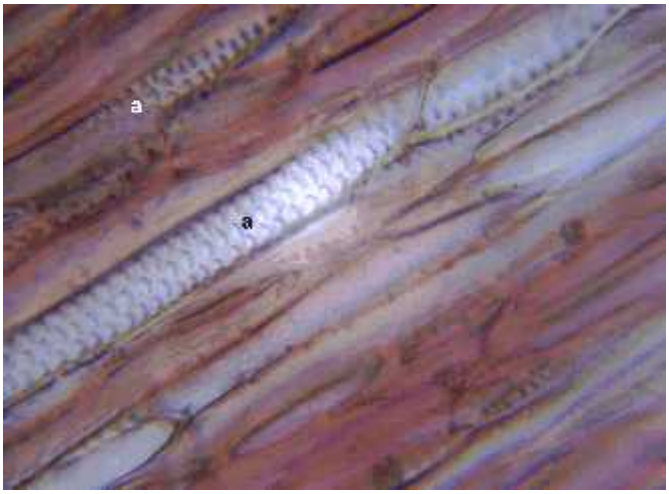


***Figure 2. Longitudinal-radial section through H. hirsutum root: (a) metaxylem with reticular thickenings (Congo red–chrysoidine staining, ×400).***

### *Aboveground stem*

In cross-section, the aboveground stem has circular shape and secondary structure generated by the libero-ligneous cambium. Epidermis is made of big heterodiametric cells with thickened external wall covered by a tick cuticle. The epidermal cells are tangentially elongated with thin radial walls and thickened tangential external and internal walls. From point to point, we found stomata and unicellular long tector hairs with sharp peak. The cortex is made up of two parts: external area of 2–3 layers of chlorenchyma and internal area, well represented, from parenchymatic type. The conducting tissues are arranged in two concentric rings. Phloem tissue forms a thin, external ring consisting of sieve tubes, phloem parenchyma and annex cells. Libero-ligneous cambium is situated between xylem and phloem tissues. Xylem tissue forms the internal ring composed of metaxylem vessels with various caliber, disorderly scattered into the libriform mass, pushing toward the center protoxylem vessels with small diameter, accompanied by few xylem parenchyma cells. In longitudinal-radial sections, xylem vessels exhibit reticulate and spiral thickenings. The medullary rays are multi-cellular, uniseriate, cellulosic at the level of phloem tissue ring but lignified into the xylem tissue ring. Medullary parenchyma is well developed, of meatus type (Figures 3–5).

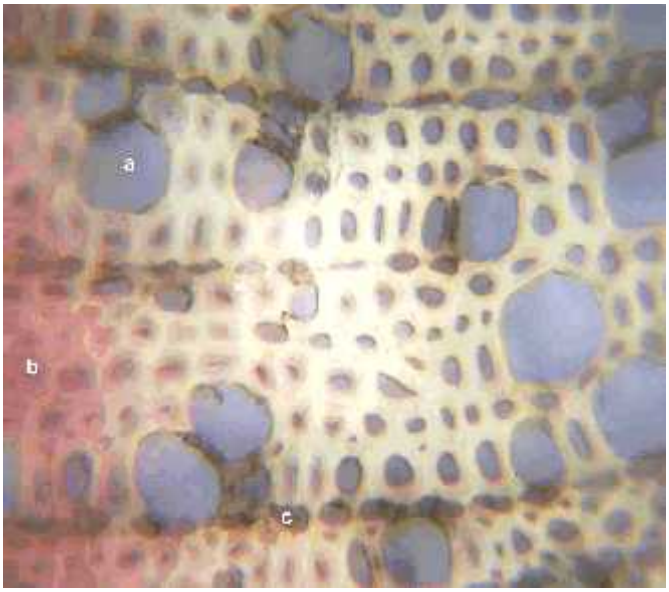


***Figure 3. Cross-section through H. hirsutum aboveground stem: (a) metaxylem; (b) libriform tissue; (c) medullary ray (Congo red–chrysoidine staining, ×400).***

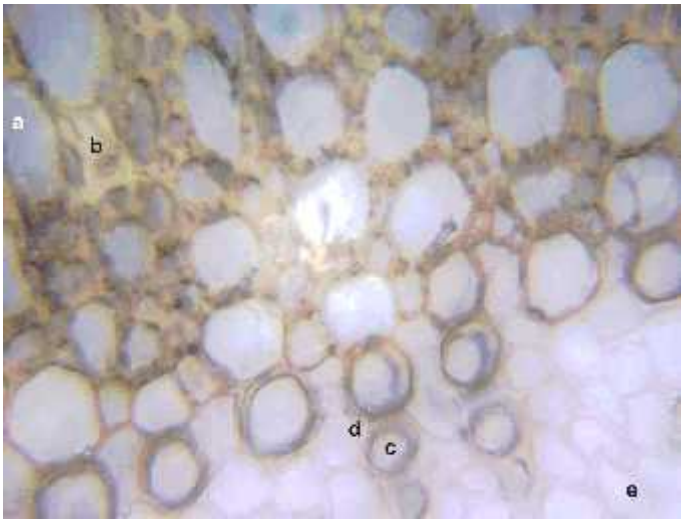


***Figure 4. Cross-section through H. hirsutum aboveground stem: (a) metaxylem; (b) libriform tissue; (c) protoxylem; (d) xylem parenchyma; (e) medullary parenchyma (Congo red–chrysoidine staining, ×400).***

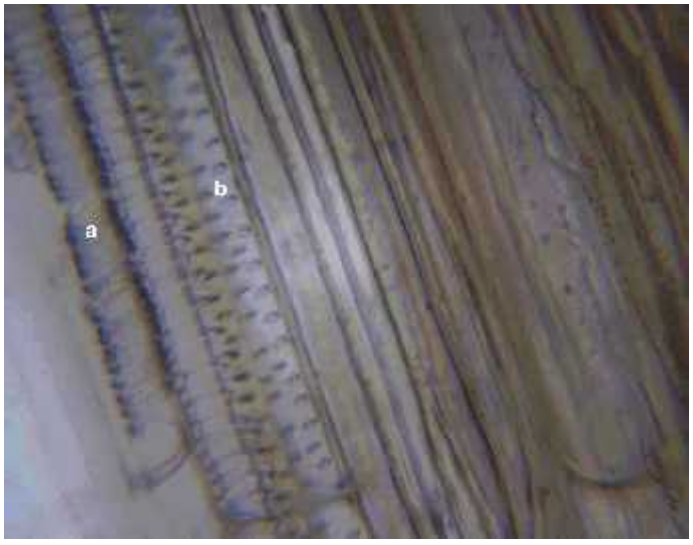


***Figure 5. Longitudinal-radial section through H. hirsutum aboveground stem: (a) metaxylem with spiral thickening; (b) metaxylem with reticular thickening (Congo red–chrysoidine staining, ×400).***

### *Leaf's limb*

At the level of median rib, leaf's lamina is prominent on abaxial side. In cross-section, from the outside towards the inside of leaf's limb, the following histological sequence is observed. Upper epidermis is composed of a single layer of large cells, flattened, with thickened tangential external and internal walls and thin radial walls. External walls are bulged and covered by a thick cuticle. At this level, from point to point, are found long unicellular tector hairs with sharp peak. Mesophyll is made of a single layer of palisade parenchyma consisting of large, cylindrical cells, rich in chloroplasts, with small intercellular spaces, as well as of 3–4 layers of lacunose parenchyma composed of small cells, disorderly arranged, with large aeriferous spaces. Into the mesophyll are found many small libero-ligneous conducting fascicles, surrounded by assimilatory fascicular sheaths. At this level, cystoliths of calcium oxalate are also observed. Mesophyll has bifacial dorsiventral structure. Lower epidermis is made of a single layer of small cells, anterior-posterior slightly flattened, with thin radial walls and thickened tangential external and internal walls. At this level, there are many anisocytic stomata and unicellular long tector hairs, with sharp peak. In cross-section, the median rib has gutter aspect prominent on abaxial face. On the outside,

the epidermis consists of small cells, anterior-posterior slightly flattened, with the external wall covered with a thick cuticle. To the adaxial pole, exhibits rare unicellular sharp tector hairs. Under the epidermis, at both adaxial and abaxial poles, less angular collenchyma is found. At the central area, a single libero-ligneous conducting fascicle surrounded by a assimilatory fascicular sheath is located into a leaf's parenchyma mass. Xylem vessels are seriate disposed into the libero-ligneous fascicle. At this level, the medullary rays are uniseriate, cellulosic. The leaf's lamina has bifacial dorsiventral, hypostomatic structure (Figures 6 and 7).

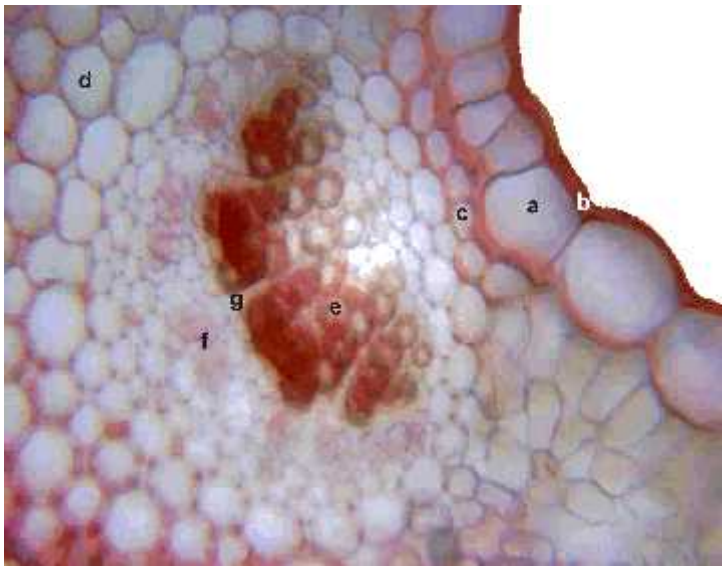


***Figure 6. Cross-section through H. hirsutum leaf's limb: (a) upper epidermis; (b) cuticle; (c) angular collenchyma; (d) leaf's parenchyma; (e) xylem tissue; (f) phloem tissue; (g) medullary ray (Congo red–chrysoidine staining, ×400).***

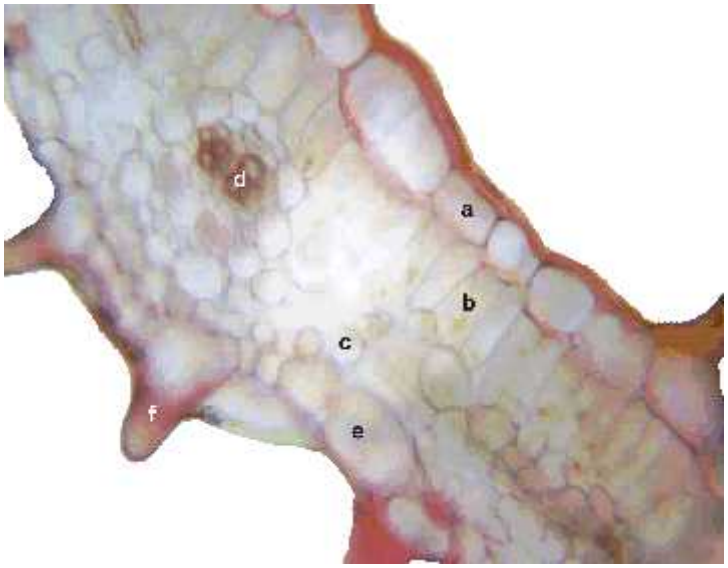


***Figure 7. Cross-section through H. hirsutum leaf's limb: (a) upper epidermis; (b) palisade parenchyma; (c) lacunose parenchyma; (d) libero-ligneous conducting fascicle; (e) lower epidermis; (f) tector hair (Congo red–chrysoidine staining, ×400).***

## TLC analysis

The experimental data on the preliminary TLC analysis of polyphenols from *Hyperici hirsuti herba* are highlighted in Figures 8–10. One chlorogenic acid derivative ($R_f$ 0.13) was identified from the 12 separated chromatographic bands, in an amount of 281.8 mg per 100 g of dried vegetal product.

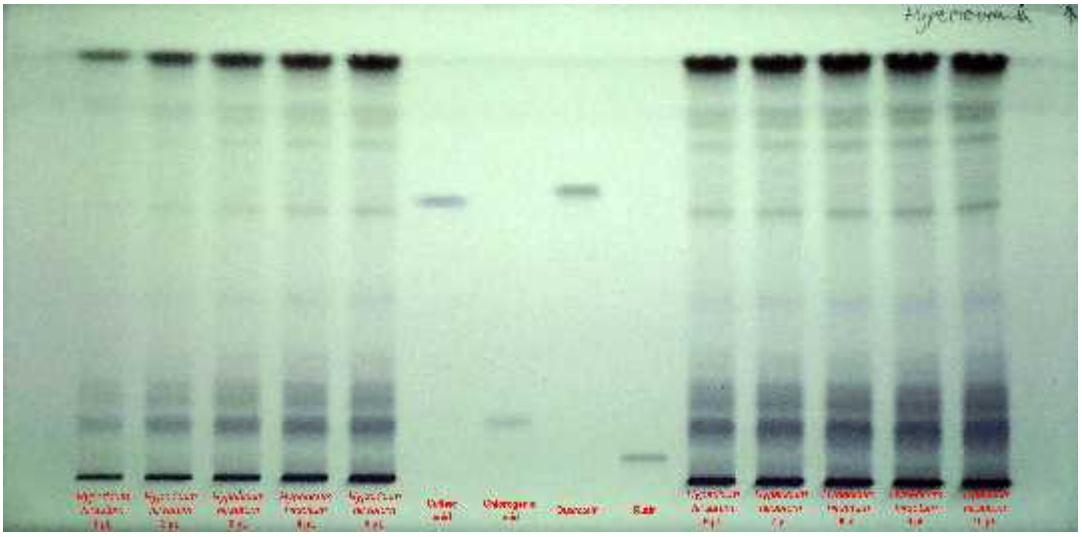

***Figure 8. TLC chromatogram of polyphenols from Hyperici hirsuti herba methanolic extract (UV 254 nm, without derivatization). From left to right: first five applications – sample (1–5 µL); subsequent four applications – standards (2 µL); last five applications – sample (6–10 µL).***

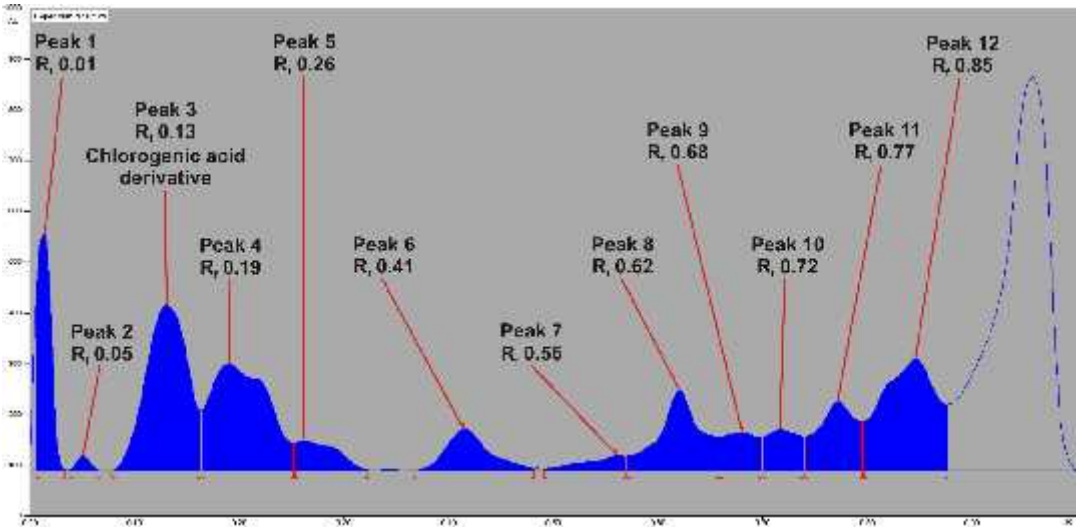


***Figure 9. Densitogram of polyphenols (UV 254 nm) separated from Hyperici hirsuti herba methanolic extract.***

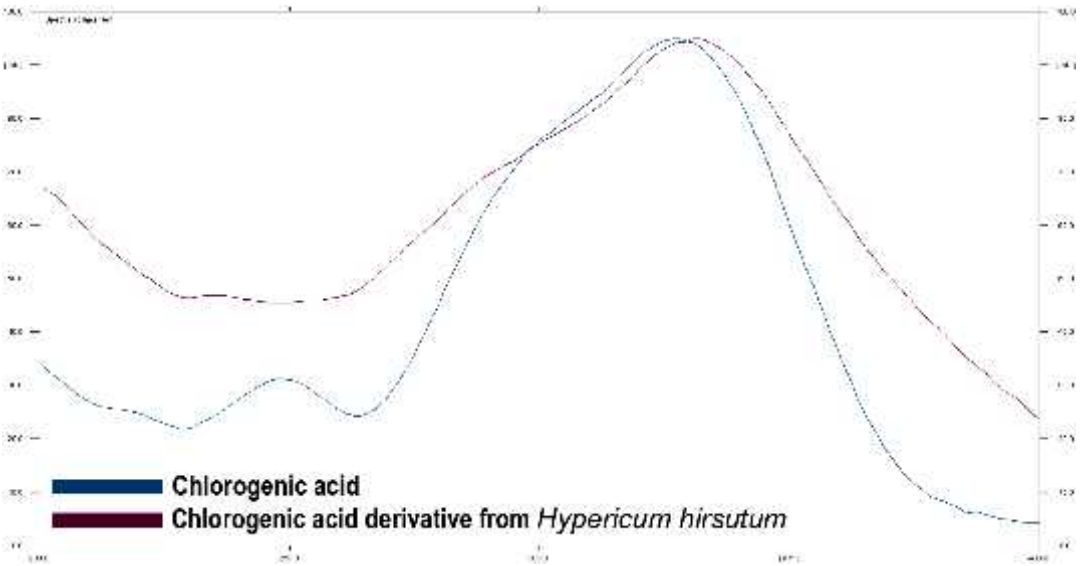


***Figure 10. Chlorogenic acid derivative in situ UV spectra of standard and compound separated from the analyzed sample.***

## CONCLUSIONS

Histo-anatomical investigation of the root, aboveground stem and leaf of *Hypericum hirsutum* species and preliminary TLC analysis of polyphenols from *Hyperici hirsuti herba* were realized. The root has circular shape and secondary structure due to the presence of two meristematic zones (phellogen and libero-ligneous cambium). The aboveground stem has circular shape and secondary structure generated by the libero-ligneous cambium. Leaf's limb has bifacial dorsiventral, hypostomatic structure. Using thin-layer chromatographic technique, a single chlorogenic acid derivative was isolated and quantified from the 12 separated chromatographic bands.

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