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HISTO-ANATOMICAL AND PRELIMINARY CHROMATOGRAPHIC ANALYSIS ON *POLYGALA VULGARIS* L. (*POLYGALACEAE*) SPECIES

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ABSTRACT

The paper highlights the histo-anatomical investigation of root, rhizome, aboveground stem and leaf of Polygala vulgaris L. (Polygalaceae) species, harvested in August 2016, from southern Romania, as well as the preliminary thin-layer chromatographic analysis of the polyphenols content of the aerial parts (Polygalae vulgaris herba). Rutin was identified and quantified starting from the 11 TLC fingerprint bands.

INTRODUCTION

Polygala vulgaris L., Common milkwort, Milkwort, *Polygalaceae* family, is a perennial herbaceous species, widespread in Europe, Asia, Northern America, in forest edges, wet meadows, grasslands, heaths and dunes, on neutral, sandy or calcareous soils [5, 12].

For the aerial parts of *P. vulgaris*, the modern researches highlighted useful active principles, such as: flavonoids [4, 11], mucilages [7], saponins [7], lignans (aucuparin) [6], xanthones, chloroxanthones, xanthone C-glycosides [4, 6, 13, 14], polyphenolcarboxylic acid esters [6], biphenyl derivatives [4], sucrose esters [13]. *Polygala* sp. exhibited some important pharmacological properties, as follows: *in vitro* cytotoxic activity against colon carcinoma LoVo cell line (lignans, xanthones, methylsinapate) [6], *in vitro* antioxidant effect [4, 16], antinociceptive properties in chemical and thermal behavioral models of pain in mice (flavonoids) [11], *in vitro* inhibitory properties of *P. myrtifolia* acetone extract on drug-resistant and drug-sensitive *Mycobacterium tuberculosis* H37Rv strain [10], antihyperglycemiant and antihyperlipidemic actions (*P. chinensis* and *P. javana*) [9], antitussive and expectorant [7].

Concerning *P. vulgaris* species histo-anatomy, the specialty papers contain very few and incomplete data [15, 17].

The histo-anatomical investigation of the root, rhizome, aboveground stem and leaf of *P. vulgaris* species and TLC analysis of the polyphenols from the aerial parts (*Polygalae vulgaris herba*) represent the aims of our paper.

MATERIAL AND METHOD

Histo-anatomical investigation

The biological material was collected from *P. vulgaris* plants in blossom, in August 2016, from the surroundings of Măldăreşti village, Vâlcea County (southern Romania).

Fixation and preservation of roots, rhizomes, aboveground stems and leaves were performed in 70% ethanol. Cross-sections and longitudinal-radial sections were obtained from the biological material, using botanical razor.

After the washing with distilled water, the sections were clarified using 10% sodium hypochlorite solution (Javel water). Then, the clarifying agent was eliminated by washing with distilled water. Genevese reagent (Congo red and chrysoidine mixture) was used for

the staining of sections, bringing different colors, depending on the chemical composition of cell membranes: pink to red for cellulose and mucilages, pale red for cytoplasm, yellow for suberin and brown for lignin [2].

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

The description of microscopic sections was accomplished starting from the classical works [18].

Thin-layer chromatography (TLC) analysis

For the aerial parts of *P. vulgaris* species (*Polygalae vulgaris herba*), the preliminary TLC analysis of the polyphenols content was made in the following experimental conditions, using a CAMAG (Muttenz, Switzerland) system [1, 3, 8]:

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

▪ mobile phase: 10 mL of chloroform–ethyl acetate–toluene–formic acid–methanol (15:20:10:10:1, in volumes) in the chromatographic tank (20×10 cm twin trough chamber, CAMAG), with no oversaturation;

▪ sample: 20% methanolic extract of *Polygalae vulgaris 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: 254 nm, CAMAG TLC Scanner 3 photodensitometer, without derivatization (deuterium–wolfram lamp, scanning speed 20 mm/s, resolution 100 µm/step);

▪ measurement mode: absorption;

▪ spectra acquisition, peak/area processing and quantification analysis: 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 secondary structure due to the presence of two meristematic secondary areas: subero-phellodermic cambium (phellogen) and libero-ligneous cambium. From the outside towards the inside of the root, the following histological sequence was evidenced in cross-section. The periderm is made up of suber, phellogen and phelloderm. The suber consists of 3–4 layers of large, flattened cells impregnated with suberin. From point to point, the suber is exfoliated. The subero-phellodermic cambium is made up of a single layer of antero-posterior flattened cells, with thin walls and slightly curled radial walls. The phelloderm is composed of 2–3 cell layers with cellulosic thin walls. Into the cortical parenchyma of the primary structure, ergastic substances are deposited. The conducting tissues are disposed on two concentric rings. The phloem tissue forms a thin, external ring made up of sieve tubes, phloem parenchyma and annex cells. The libero-ligneous cambium is located between xylem and phloem tissues. The xylem tissue forms the thick, inner ring consisting of numerous metaxylem vessels of different calibers, disorderly placed into the libriform tissue. The protoxylem vessels with small diameter, accompanied by xylem parenchyma are pushed to the center. The medullary

rays are multi-cellular, uniseriate, cellulosic at the level of the phloem tissue and lignified into the xylem tissue. The medullary parenchyma is missing (Figure 1).

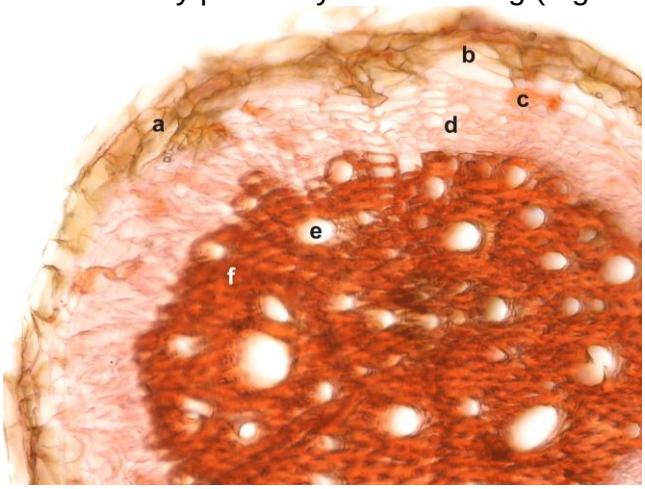

Figure 1. Cross-section through P. vulgaris root: (a) suber; (b) phellogen; (c) phelloderm; (d) phloem tissue; (e) metaxylem; (f) libriform tissue (Congo red–chrysoidine staining, ×200).

Rhizome

In cross-section, in the lower third, the rhizome has circular shape and secondary structure because of the two meristematic secondary zones: subero-phellodermic cambium and libero-ligneous cambium.

The following histological sequence was evidenced in cross-section, from the outside towards the inside of the rhizome. The periderm consists of suber, subero-phellodermic cambium and phelloderm.

The suber is made up of 3–4 layers of large, flattened cells impregnated with suberin. The suber is exfoliated in patches. A single layer of antero-posterior flattened cells, with thin walls and slightly curled radial walls represents the subero-phellodermic cambium. The phelloderm is formed of 2–3 cellular layers with cellulosic thin walls, which store ergastic substances.

The conducting tissues are arranged on two concentric rings.

The phloem tissue forms an externally well-represented ring, made up of sieve tubes, phloem parenchyma and annex cells. At this level, the medullary rays are multi-cellular, uniseriate, with cellulosic walls.

The libero-ligneous cambium was evidenced between the xylem and phloem tissues. The xylem tissue forms the inner ring made up of multiple metaxylem vessels of different sizes, widespread into the libriform tissue, pushing to the center the small-diameter protoxylem vessels. In the longitudinal-radial sections, the xylem vessels exhibit reticulate thickenings.

Accompanied by some xylem parenchyma, the protoxylem is poorly represented. The medullary rays are multi-cellular, uniseriate, lignified at the level of the xylem tissue. The medullary parenchyma is missing (Figures 2–4).

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

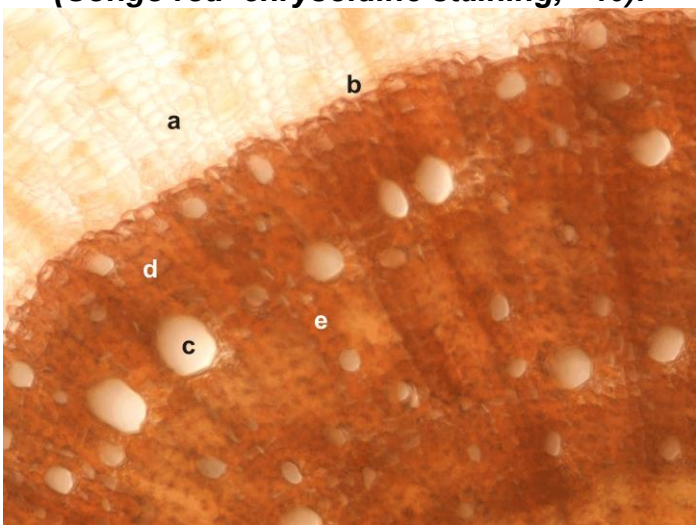

Figure 3. Cross-section through P. vulgaris rhizome: (a) phloem tissue; (b) libero-ligneous cambium; (c) metaxylem; (d) libriform tissue; (e) medullary ray (Congo red–chrysoidine staining, ×200).

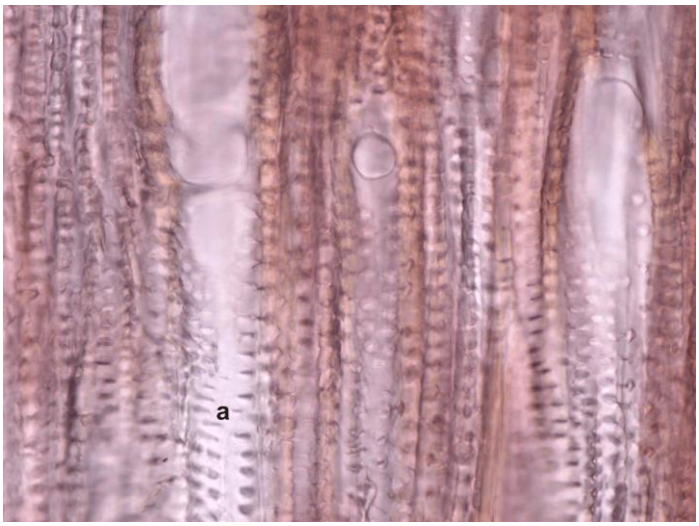

Figure 4. Longitudinal-radial section through P. vulgaris rhizome: (a) metaxylem with reticulate thickenings (Congo red–chrysoidine staining, ×400).

Aboveground stem

In cross-section, the aboveground stem into the upper third exhibits circular sinuous shape and secondary structure due to the presence of the libero-ligneous cambium. The epidermis is made up of approximately isodiametric cells, having thickened outer wall and covered by a thick cuticle with papilliform relief. The epidermal cells are slightly elongated tangentially, with thin radial walls and thickened tangential external and internal walls. From place to place, long unicellular tector hairs and stomata are observed. The cortex consists of two sectors: the external sector, made up of 2–3 chlorenchyma layers and the well-represented internal sector of parenchymatic type, with ergastic substances. At this level, sclerenchyma fibrous bundles are observed in the periphloemic area. Due to the activity of the libero-ligneous cambium, the conducting tissues are organized into concentric rings. The thin outer ring represents the phloem tissue, consisting of sieve tubes, phloem parenchyma and annex cells, the medullary rays being multi-cellular, uniseriate, cellulosic. The inner ring is made up of secondary xylem tissue composed of metaxylem with various sizes and well-represented libriform tissue. The xylem vessels have reticulate thickenings and 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 (Figures 5 and 6).

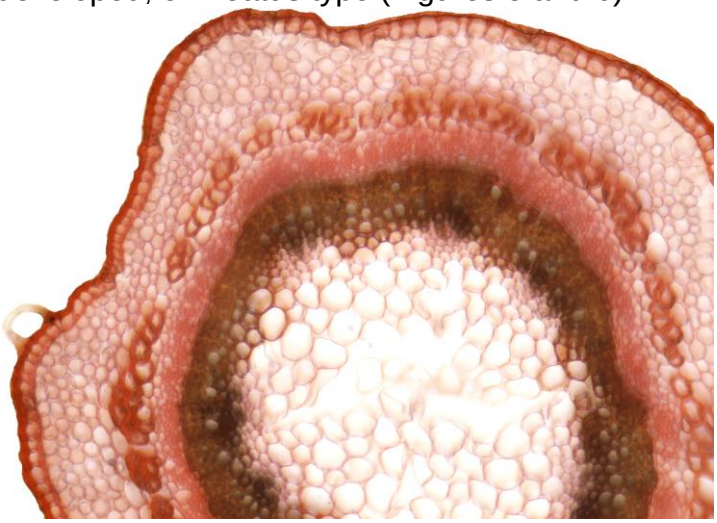

Figure 5. Cross-section through P. vulgaris aboveground stem: overview (Congo red–chrysoidine staining, ×100).

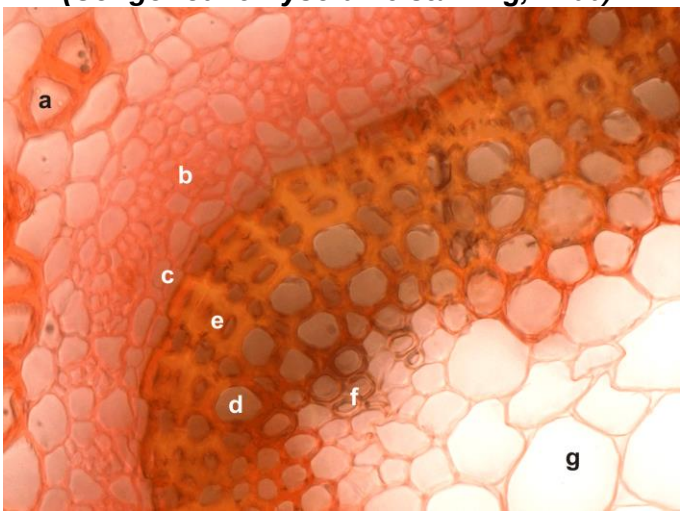

Figure 6. Cross-section through P. vulgaris aboveground stem: (a) sclerenchyma fibers bundle; (b) phloem tissue; (c) libero-ligneous cambium; (d) metaxylem; (e) libriform tissue; (f) protoxylem;(g) medullary parenchyma (Congo red–chrysoidine staining, ×400).

Leaf's limb

From the outside towards the inside of leaf's limb, the following histological sequence was observed in cross-section. The upper epidermis is made up of a single layer of large, flattened cells, with thickened outer and inner tangential walls and thin radial walls. The outer walls are bulged and covered with a thick cuticle. From point to point, stomata and tector hairs are evidenced. The mesophyll is organized from two cellular layers of palisade parenchyma, with large, elongated and chloroplast-rich cells, but also from 4–5 layers of lacunose parenchyma, made up of disorderly disposed small cells, with aeriferous spaces. Into the mesophyll, there are numerous small libero-ligneous conducting fascicles. The mesophyll has bifacial type and dorsiventral structure. The lower epidermis consists of one layer of tangentially elongated cells, with thin radial walls and thickened outer and inner tangential walls. At this level, stomata and tector hairs were found. In cross-section, the median rib is slightly protruding on the abaxial side. Only one libero-ligneous conducting fascicle is located into the central area, with some sclerenchyma fibers in the periphloemic area. Into the libero-ligneous fascicle, the xylem vessels have a serial disposition and the medullary rays are uniseriate, cellulosic. A bifacial, dorsiventral, amphistomatic structure was evidenced for the leaf's limb (Figure 7).

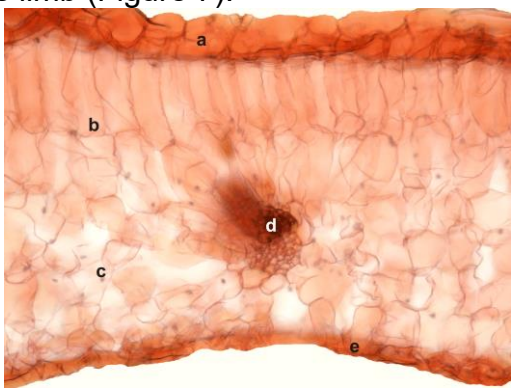

Figure 7. Cross-section through P. vulgaris leaf's limb: (a) upper epidermis; (b) palisade parenchyma; (c) lacunose parenchyma; (d) libero-ligneous conducting fascicle; (e) lower epidermis (Congo red–chrysoidine staining, ×200).

TLC analysis

The results of preliminary TLC analysis of polyphenols from *Polygalae vulgaris herba* are exhibited in Figures 8–10. Rutin (R_f 0.04, 96.41 mg/100 g of dried vegetal product) was

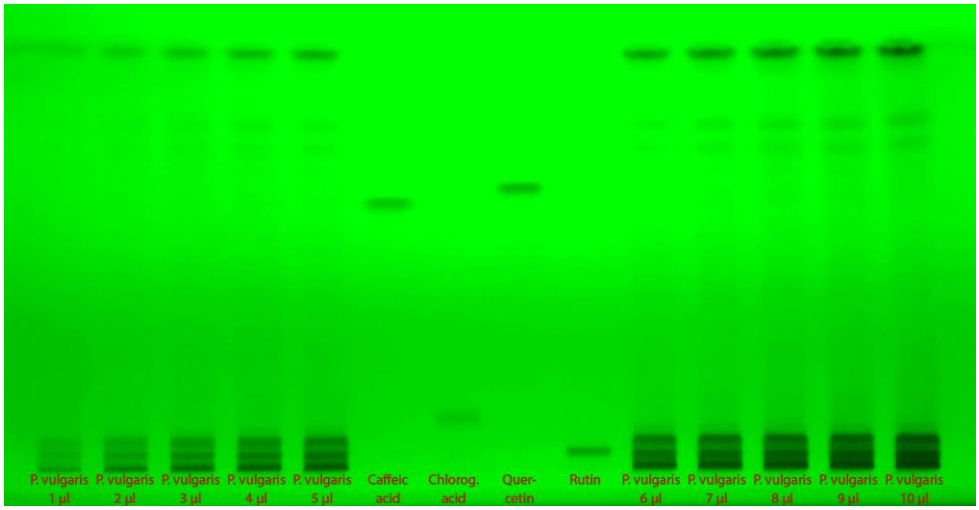

Figure 8. TLC chromatogram of polyphenols from Polygalae vulgaris herba methanolic extract (UV 254 nm, without derivatization). From left to right: first five applications – sample (1–5 µL); subsequent four applications – standards (2 µL); last five applications – sample (6–10 µL).

identified from the 11 fingerprint chromatographic bands.

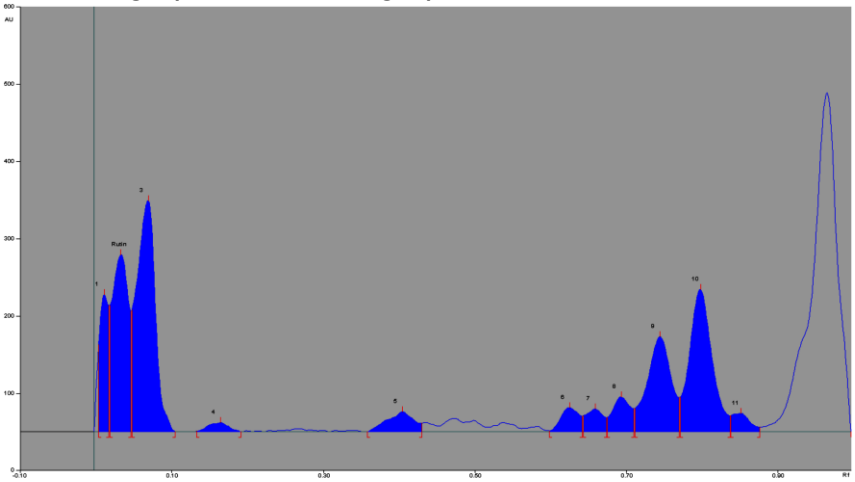

Figure 9. Densitogram of polyphenols (UV 254 nm) separated from Polygalae vulgaris herba methanolic extract. From left to right, No. of peak/R_f: 1/0.01, 2/0.04 – rutin, 3/0.07, 4/0.17, 5/0.41, 6/0.63, 7/0.66, 8/0.70, 9/0.75, 10/0.80, 11/0.85.

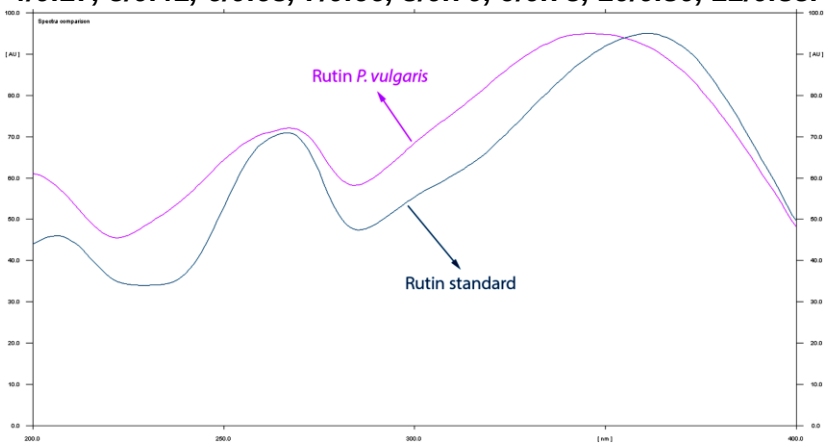

Figure 10. In situ UV spectra of rutin standard and compound separated from the analyzed sample.

CONCLUSIONS

The histo-anatomical investigation of roots, rhizomes, aboveground stems and leaves of *Polygala vulgaris* L. species, as well as the preliminary TLC analysis of polyphenols from *Polygalae vulgaris herba* were realized. The root and the rhizome have circular shape and secondary structure because of two meristematic secondary areas: subero-phellodermic cambium (phellogen) and libero-ligneous cambium. Into the upper third, the aboveground stem exhibits circular sinuous shape and secondary structure (libero-ligneous cambium). The leaf's limb has bifacial, dorsiventral, amphistomatic structure. Starting from the 11 TLC fingerprint bands, rutin was identified and quantified in the methanolic extract.

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