Histo-anatomical data concerning *Robinia Pseudoacacia* L. species (Fabaceae)

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Introduction: *Robinia pseudoacacia* L. (Fabaceae), black locust is a honey-bearing tree widespread in Romania from which only flowers and leaves are used in etno-medicine for their soothing, antiseptic and antispasmodic actions; the rest of the plant is toxic.
Material and methods: In this article we present for the first time the histo-anatomical structure of roots, stems and leaves of *Robinia pseudoacacia* L. species, by using photomicrography technique and staining with genovez reagent.
Results: The research results showed that the root and stem have secondary structure. The leaf has bifacial dorsiventral structure.
Conclusions: Microscopic analysis proved to be a valuable tool, very useful in specifying the characteristic anatomical features of the root, stem and leaf of *Robinia pseudoacacia* L.

Keywords: *Robinia pseudoacacia* L., locust tree, histo-anatomical structures, photomicrography technique

Introduction

Robinia pseudoacacia, commonly known as Black Locust (Fabaceae) is a thorny tree, native to North America and widespread in lowland areas. The seeds, bark and wood contain substances which are toxic to the human body. Only flowers and leaves are used for their antispasmodic, slightly sedative, anti-migraine, anti-cough effects, for digestive disorders and in fragrance industry [1].

The objective of this study was to analyse the cross-section of root, stem and leaf of *Robinia pseudoacacia* L using photomicrography technique. These sections were interpreted in order to specify the anatomical features of these plant organs. The microscopic analysis is part of the pharmacognostical and toxicological expertise required for herbal medicinal products [2–5].

Material and methods

Plant organs were harvested in full bloom, in July from the Mlecănești village, Dolj region, Romania. The preservation of the biological material (roots, stems, leaves) was made using a mixture of ethyl alcohol, glycerol and distilled water in equal proportions.

Root, stem and leaf cross-sections have been made using the anatomic razor. The cross-sections were washed with distilled water, and then clarified using a solution of sodium hypochlorite 10% (Javel water). Successive washings of the sections were made for removing the clarification agent.

The staining was made using genovez solution, resulted from the combination of two solutions: Congo red and chrysoidin. These were obtained separately as follows:

- Congo red – 5 g;
- Chrysoidin – 0.5 g;
- Distilled water – 100 ml;
- Ethyl alcohol 96° – 100 ml;
- Ammonia – 5 ml.

Coloration resulting from the use of these solutions varies depending on the chemical composition of cell membranes: red for cellulosic tissue, yellow or orange for lignified tissue; golden yellow for cutinizated tissue. Fixed and stained sections were studied using a binocular KRÜSS microscope (objectives ×10, ×20, ×40) and then photographed using a Nikon system adapted to the microscope with an automatic exposure, an FX-35DX camera and a Sony CCD-IRIS video camera.

Results

The anatomical structure of *Robinia pseudoacacia* L. root is presented on Figures 1–4, of the stem on Figures 5–8, and the anatomical structure of the leaf on Figures 9–12.

Discussions

1. Anatomical structure of *Robinia pseudoacacia* L. root

In cross-section, the root presents circular-ribbed contour and secondary structure builds on account of phloem and cambium [6]. The cork is well developed, made by 7–10 layers of flattened cells with suberized walls. The phellogen consists of only one layer of elongated cells. The phelloderm has 2–3 layers of cells, uniform distributed without spaces between them. The cortical parenchyma is well developed, made by cells containing starch and calcium oxalate crystals. In the parenchyma sclerenchymatous areas uniform distributed on three concentric circles can be distinguished. The secondary origin conducting tissues form two concentric rings, one thinner, the phloem to the outside and one thicker, the xylem to the inside. The phloem is made up of sieve tubes, annex cells and phloem parenchyma. The phloem cells contain simple calcium oxalate crystals. The xylem is made up of wide vessels dispersed in fundamental mass represented by libriform fiber; only in the primary xylem there can

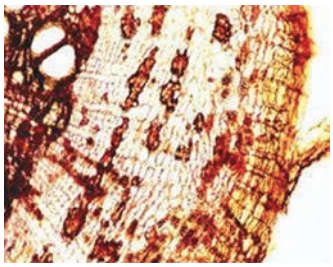

Fig. 1. Robinia pseudoacacia L., cross section through the root (Congo red and chrysoidin stain, x100) – original

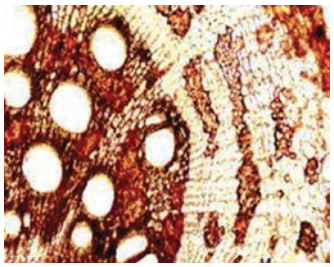

Fig. 2. Robinia pseudoacacia L., cross section through the root (Congo red and chrysoidin stain, x100) – original

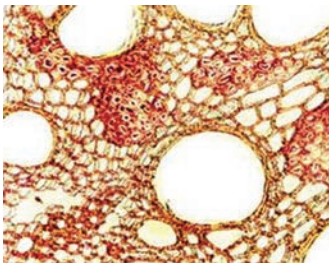

Fig. 3. Robinia pseudoacacia L., cross section through the root (Congo red and chrysoidin stain, x100) – original

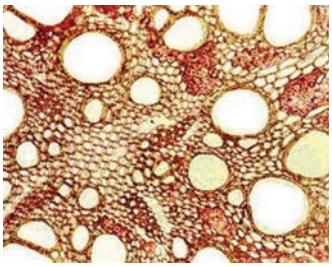

Fig. 4. Robinia pseudoacacia L., cross section through the root (Congo red and chrysoidin stain, x100) – original

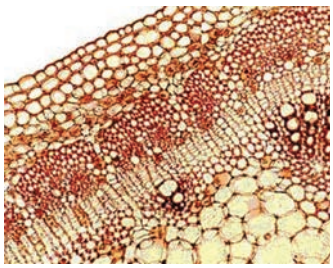

Fig. 5. Robinia pseudoacacia L., cross section through the stem (Congo red and chrysoidin stain, x100) – original

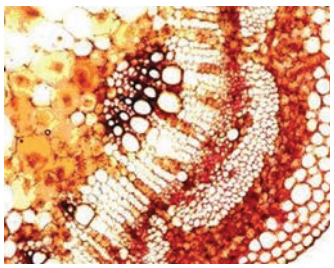

Fig. 6. Robinia pseudoacacia L., cross section through the stem (Congo red and chrysoidin stain, x100) – original

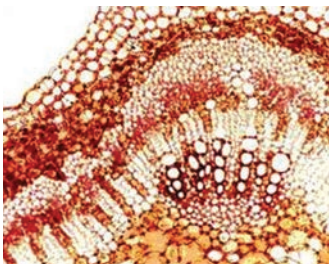

Fig. 7. Robinia pseudoacacia L., cross section through the stem (Congo red and chrysoidin stain, x100) – original

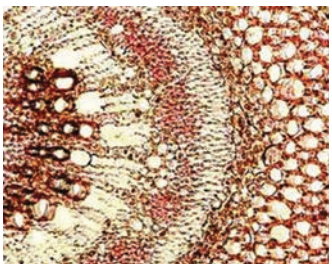

Fig. 8. Robinia pseudoacacia L., cross section through the stem (Congo red and chrysoidin stain, x100) – original

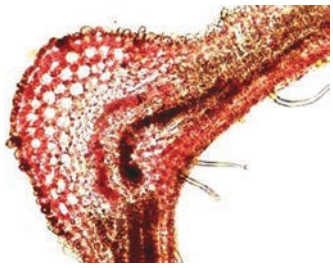

Fig. 9. Robinia pseudoacacia L., cross section through the leaf (Congo red and chrysoidin stain, x100) – original

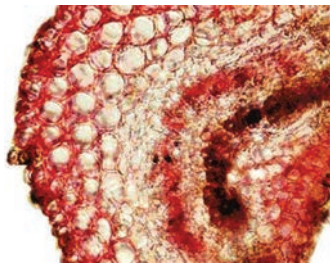

Fig. 10. Robinia pseudoacacia L., cross section through the leaf (Congo red and chrysoidin stain, x100) – original

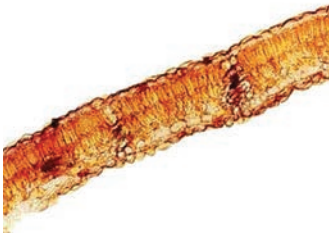

Fig. 11. Robinia pseudoacacia L., cross section through the leaf (Congo red and chrysoidin stain, x100) – original

Fig. 12. Robinia pseudoacacia L., cross section through the leaf (Congo red and chrysoidin stain, x100) – original

be distinguished cellulosic parenchyma cells containing starch. Both xylem and phloem are crossed by many parenchymatous cellulosic uniseriate rays. At the periphery of the phloem ring there are many belts of sclerenchymatic fiber separated by parenchymatous cellulosic cells containing starch [2–4,6,7] (Figures 1–4).

2. Anatomical structure of *Robinia pseudoacacia* L. stem

In cross section, one year old stems show secondary structure only to the central cylinder due to libero-ligneous cambium activity. The aerian stem has circular-ribbed contour. The epidermis has heteromorphic cells with external and internal walls thicker than the other. A thin cuticle covers the external walls. Here and there, there are multicellular, uniseriate trichomes often with a very long terminal cell. The bark is colenchymatic under the epidermis and parenchymatic to the inside. Just on the line between these two subareas the cells begin to differentiate into the phellogen. Some of the cortical cells, especially those closed to the central cylinder contain simple calcium oxalate crystals. The secondary origin conducting tissues form two concentric rings, one thinner, the phloem to the outside and one thicker, the xylem to the inside.

The phloem is made up of sieve tubes, annex cells and phloem parenchyma. The phloem cells contain simple calcium oxalate crystals. The xylem is made up of wide vessels dispersed in ground tissue represented by libriform fiber; in the primary xylem cellulosic parenchyma cells can be distinguished. Both xylem and phloem are crossed by many parenchymatous cellulosic uniseriate rays. At the periphery of the phloem ring there are many belts of sclerenchymatic fiber separed by parenchymatous cellulosic cells, some of them containing simple calcium oxalate crystals. In the centre of the stem there is the parenchymatic pith and with some cells containing calcium oxalate crystals [2–4,6,7] (Figures 5–8).

3. Anatomical structure of *Robinia pseudoacacia* L. leaf

The petiole. In cross section, the petiole shows an irregular- ribbed contour, with reduced ribs. The epidermis consists of cells with external and internal walls slightly thicker than the others and covered by a thin cuticle. The ground parenchyma from the external region is slightly collenchymatised in hypodermic position and the internal region has intercellular spaces. The cells from central area are very large with very thin walls. The conducting tissues forming many libero-ligneous fascicles disposed in a circle interrupted from place to place by many sclerenchyma elements connecting periphloem mechanical cords and moderately sclerified-lignified medullary rays. On the adaxial side there are some (2–5) smaller fascicles included into the fundamental parenchyma. The structure of all conducting fascicles remind of those from the stem [2–4,6,7].The foliar limb. In front side view, the epidermis displays polygonal cells, with curved lateral walls at the superior side of the limb. Stomata of anomocytic type are only in the lower epidermis, so the foliar limb is hypostomatic. Both epidermis displays uni- or three-cellular trichomes of different length. On transparency there could be observed simple prismatic or rhomboid calcium oxalate crystals. In cross section of the foliar limb, the middle vein is slightly prominent at the lower side and consists of hypodermic colenchyma and only one conducting fascicle. Both epidermis have thin cell walls and here and there have uni- or three-cellular trichomes. In the lower epidermis there are stomata with visible suprastomatic chamber. The mesophyll is differentiated in two layers of compact palisade tissue on the upper side (the hypodermic cells are higher) and multi layers of lacunate tissue on the lower side. Therefore, the leaf's limb has bifacial dorso-ventral structure. Some cells from the mesophyll, especially from palisade tissue have calcium oxalate crystals [2–4,6,7].

Conclusions

1. In an extensive pharmacognostic research we have made cross sections of the root, stem and leaf of *Robinia pseudoacacia* L. Each of the microscopic investigated plant organs shows structural features. Microscopic images of cross sections are showed for the first time using photomicrography technique.
2. Microscopic cross-sections of root, stem and leaf were examined and interpreted in order to specify the anatomical features within the pharmacognostical expertise required for herbal medicinal products.
3. The root has a secondary structure, both in the cortex and central cylinder. At the periphery of the phloem ring there are many belts of sclerenchymatic fiber separated by parenchymatous cellulosic cells containing starch.
4. The aerial stem has a secondary structure. The secondary origin conducting tissues form two concentric rings, one thinner, the phloem to the outside and one thicker, the xylem to the inside. At the periphery of the phloem ring there are many belts of sclerenchymatic fiber separated by parenchymatous cellulosic cells, some of them containing simple calcium oxalate crystals.
5. The leaf's limb has a bifacial dorso-ventral structure. Both epidermis have thin cell walls and here and there have uni- or three-cellular trichomes. The inflorescence peduncle structure is similar to stem structure but much more simpler and has only primary origin tissues.

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