HISTO-ANATOMICAL RESEARCHES ON THE VEGETATIVE ORGANS OF *EUPATORIUM CANNABINUM* L. SPECIES

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Abstract

In this paper, there are presented the results of the histo-anatomical research on the vegetative organs (roots, rhizomes, aerial stems, leaves) of *Eupatorium cannabinum* L. species, from which the medicinal products *Eupatorii cannabini rhizoma cum radicibus* and *Eupatorii herba* are obtained.

Rezumat

În această lucrare, sunt prezentate rezultatele cercetării histo-anatomice asupra organelor vegetative (rădăcini, rizomi, tulpini aeriene, frunze) ale speciei *Eupatorium cannabinum* L., de la care se obţin produsele medicinale *Eupatorii cannabini rhizoma cum radicibus* şi *Eupatorii herba*.

Keywords: *Eupatorium cannabinum* L.; histo-anatomical researches.

Introduction

Eupatorium cannabinum L., hemp agrimony, from *Asteraceae* (*Compositae*) family, is an Eurasian perennial herb found in the plains, hills and mountains, through wet places, besides waters. The root is cylindrical, tuberised. The rhizomes are short and thick, having violet-blue colour. The stem is cylindrical, short hairy, simple or branched, up to 175 cm high. The leaves are opposite, petiolate, digitate-composite, with 3–5 lanceolate and serrate leaflets. The small anthodiums brought together in a dense corymbiform-composite raceme consist of 4–6 reddish flowers [14, 16].

The medicinal products *Eupatorii cannabini rhizoma cum radicibus* and *Eupatorii herba* contain: volatile oil; bitter sesquiterpene lactones with germacrane structure (eupatolid, eupatoriopicrin); hetero-glycans; flavonosides (heterosides of eupatorin, hispidulin, campherol, quercetin); polyacetylene compounds; a sweetening diterpene heteroside (stevioside), 300 times sweeter than sucrose; triterpenoids; polyphenols;

resins; benzofurans (cistifolin, euparin, euparone); pyrrolizidine alkaloids (supinidin, lycopsamine, intermedine); simple carbohydrates; organic acids; vitamins; mineral salts [3, 6, 7, 8, 12].

The above-mentioned vegetal products have the following actions: choleretic-cholagogue, hepatoprotective, diuretic, anti-inflammatory, laxative-purgative, antibiotic, antiviral, immunostimulatory, nematocide, cytotoxic *in vitro* against malignant cells. They are used to treat hepato-biliary diseases, acute gastritis, inflammation of the bladder, inflammations and skin irritations [2, 3, 6, 7, 8, 12].

The medicinal uses are limited because of the content of sesquiterpene lactones (allergenic, cardiotoxic) and pyrrolizidine alkaloids (hepatic, renal and pulmonary toxicity, mutagenic, teratogenic). Currently, homeopathic preparations obtained from *Eupatorium* extracts give favourable results in treating acute gastritis and bladder inflammation [3, 6, 7, 8].

The histo-anatomical researches on *E. cannabinum* species are fragmentary and general, mostly being relatively old [4, 5, 9, 10, 11, 13]. This fact and the therapeutic value of the plant have led us to perform the present research.

Materials and Methods

The vegetal material is represented by the roots, rhizomes, stems and leaves of *E. cannabinum* species, harvested in August 2009, from plants in blossom, from the edge of irrigation channels near Seaca de Câmp village, Dolj County. The identification of the species was made according to the Romanian Flora [16].

In terms of the histo-anatomical study, the material passed through the following steps:

(a) Fixation and preservation in 70% alcohol solution.

(b) Manually sectioning, using the hand microtome and the botanical razor, with elder pith as support.

(c) Removal of cell content, with sodium hypochlorite for 20–35 minutes (depending on the material), after which the cross sections were washed with distilled and acetic water.

(d) Staining of cross sections with iodine green and alum carmine red: conventional staining for the histo-anatomical studies of plants. Sections were first stained with iodine green (one minute), washed with 90% alcohol solution, and then stained with alum carmine red (20 minutes) and consecutively washed with distilled water.

(e) Making of permanent preparations: stained cross sections were mounted into glycerol-gelatine drops, added between the blade and slide.

(f) Valorisation of the preparations: drawings at MC1 photon microscope (Romania) with projection mirror (Projektionszeichenspiegel) and colour photographs using NOVEX photon microscope (Holland), with Canon A540 digital camera were made. Scale bar = 100 μm [1, 15].

Results and Discussion

The root structure (Figure 1)

Lateral roots, very thin, have typical primary structure (Figure 1a): the rhizodermis contains small cells with thickened external wall. Here and there are relatively long absorbent hairs.

The cork is very thick, differentiated into three sub-areas: a single layer of exodermis (Figure 1b), composed of large polygonal-shape cells, with slightly suberized walls; cortical parenchyma (7–8 cell layers) of meatus-type, consisting of relatively large cells, some of them with moderately thickened and lignified wall; endodermis of the primary-type, with Caspary thickenings in the side walls of the cells (Figure 1c).

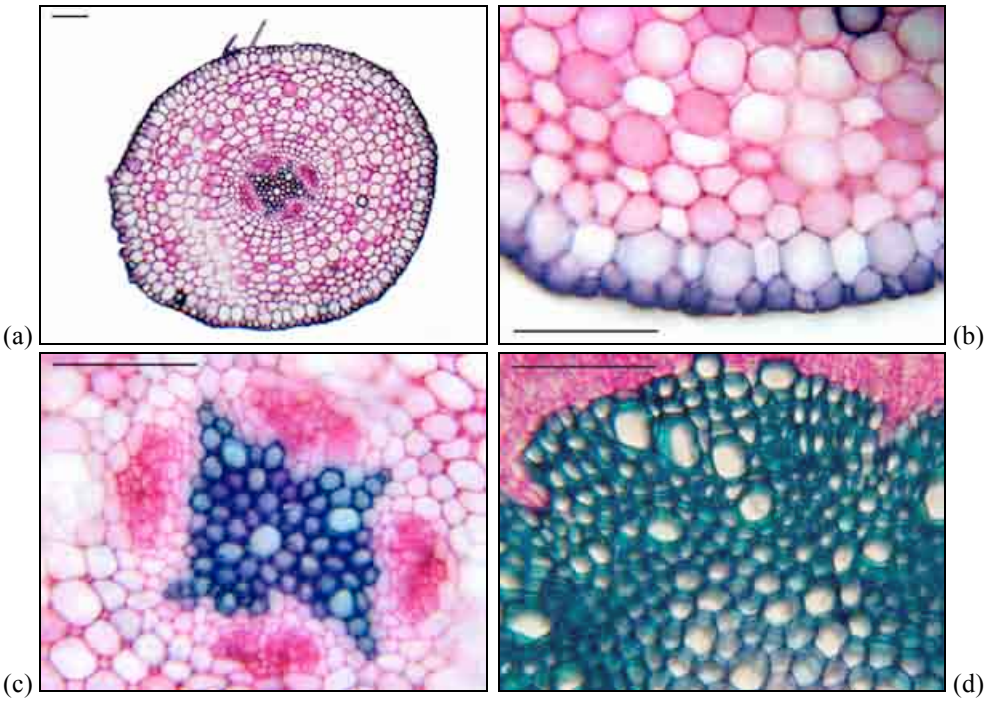


Figure 1 (a–d)
Cross sections through the root of *E. cannabinum*.

The central cylinder (Figure 1c) includes four xylem fascicles and as many of phloem, alternatively arranged. The phloem includes sieve-tubes and

annex cells, and the xylem has proto- and metaxylem vessels, the latter join to the centre replacing the marrow.

The middle third of the main root was already past to the secondary structure (Figure 1d), distinguishing: rhizodermis with thickened external walls; very few absorbent hairs; some cells of rhizodermis have a strongly-curved external wall or are noticeably radially-elongated. The exodermis is completely suberized. The cortical parenchyma is very thick (15–18 cell layers), but with cells smaller than those of the exodermis. Into the cortical parenchyma, cells with thickened and lignified walls similarly to the mechanic idioblasts are frequent. The endodermis remains of Casparian type.

The phloem tissue components are visibly collenchymatised. The xylem forms a compact lignified massif, with disorderly dispersed vessels of various diameters; the axial area is devoid of vessels, recalling a moderate sclerified and lignified pith. The phloem tissue forms a ring area, unevenly thick; in front of the former fascicles of the primary structure, the thickness is greater. Into the secondary xylem, only libriform elements are observed, the cellulosic parenchyma cells lacking.

The rhizome structure (Figure 2)

In the cross section, the rhizome has a circular shape (Figure 2a). The epidermis contains cells with slightly suberized walls (Figure 2b).

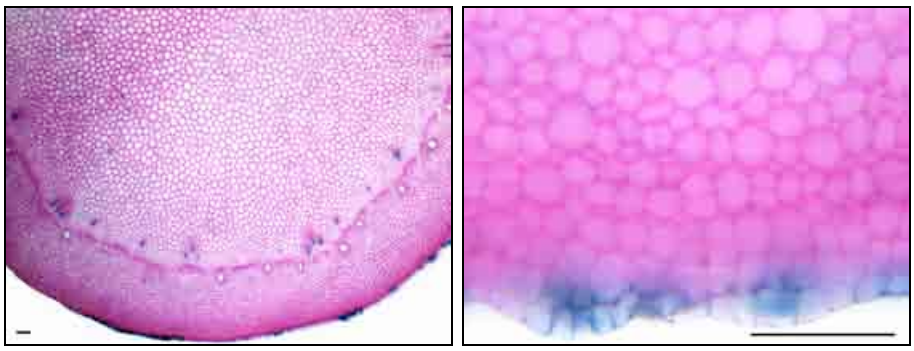
(a)  (b)

Figure 2 (a and b)
Cross sections through the rhizome of *E. cannabinum*.

The cork (Figure 2c) is thick (15–20 cell layers), parenchymatous cellulosic; the outside layers contain cells with collenchymatised walls. The conducting tissues form numerous fascicles of different sizes (Figure 2c): 12–15 large fascicles, and among them 2–3 small fascicles. The xylem comprises vessels with thickened and lignified walls arranged in radial rows (Figure 2d), and cells of xylem parenchyma with thin cellulosic walls. The phloem includes sieve-tubes, annex cells and fewer phloem parenchyma cells with thickened

cellulosic walls. The small fascicles (Figure 2e) contain very few xylem vessels or sometimes are missing. Between bundles, in front of the phloem tissue, secretory channels are present: one secretory channel (Figure 2f) between two fascicles of different sizes; they have the channel coated with flattened epithelial cells. The pith is parenchymatous cellulosic, of meatus-type.

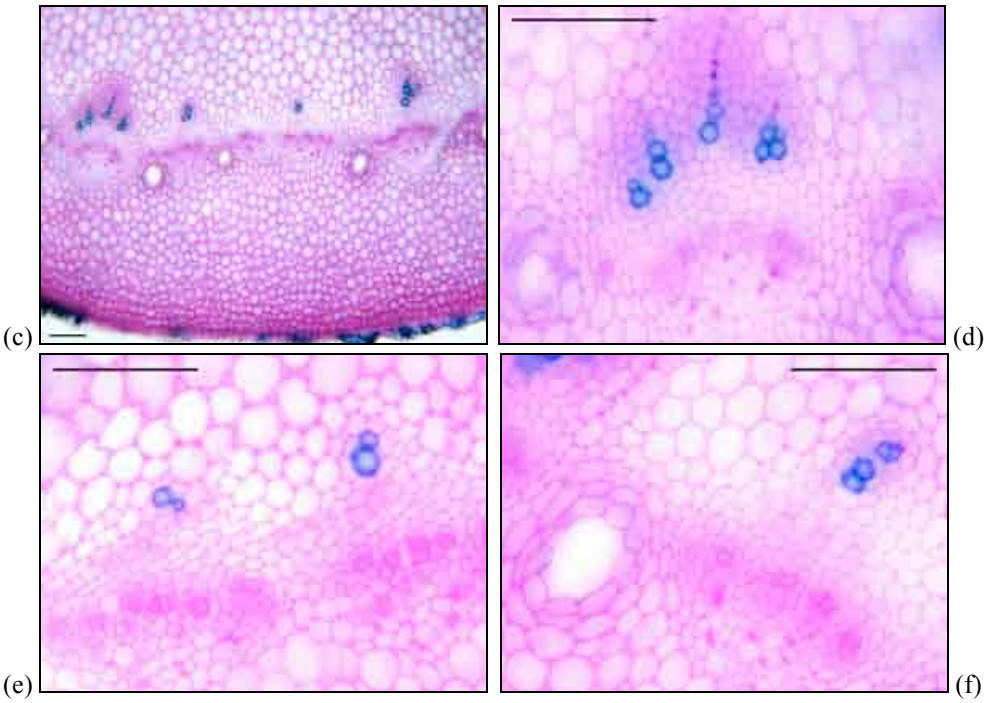


Figure 2 (c–f)
Cross sections through the rhizome of *E. cannabinum*.

The upper third stem structure (Figure 3)

The shape is irregular, elliptical (Figure 3a), with four ribs visibly attenuated. The epidermis contains isodiametric cells, often quadratic, with thickened external and internal walls. The external wall is covered with a striated cuticle. Here and there, pluricellular uniseriated relatively long tector hairs are observed (Figure 3b) and pluricellular secretory hairs (Figure 3c), with uni-cellular pedicle and uni- or pluricellular gland.

The bark is composed of angular collenchyma cords in the ribs (Figure 3d), which continue between them under the form of a single layer, rarely two. Moreover, the bark is parenchymatous-type, the cells having all the walls moderately thickened, but cellulosic. The bark does not end with a special type endodermis, nor the central cylinder with a pericycle.

The central cylinder is very thick and contains numerous (24–26)

libero-ligneous fascicles of different sizes, large and medium-sized being of collateral-open type; the largest fascicles (Figure 3, d and e) are in front of the ribs, separated by the angular collenchyma cords through only 1–2 layers of cortical cells.

The phloem includes sieve-tubes and annex cells, and the xylem contains vessels placed in radial rows separated by cellulosic parenchyma cells. The medullary rays between the fascicles are parenchymatous cellulosic of different width. The pith is thick, parenchymatous cellulosic, meatus-type; many cells in the axial area are disorganised, resulting a small aeriferous cavity with irregular contour.

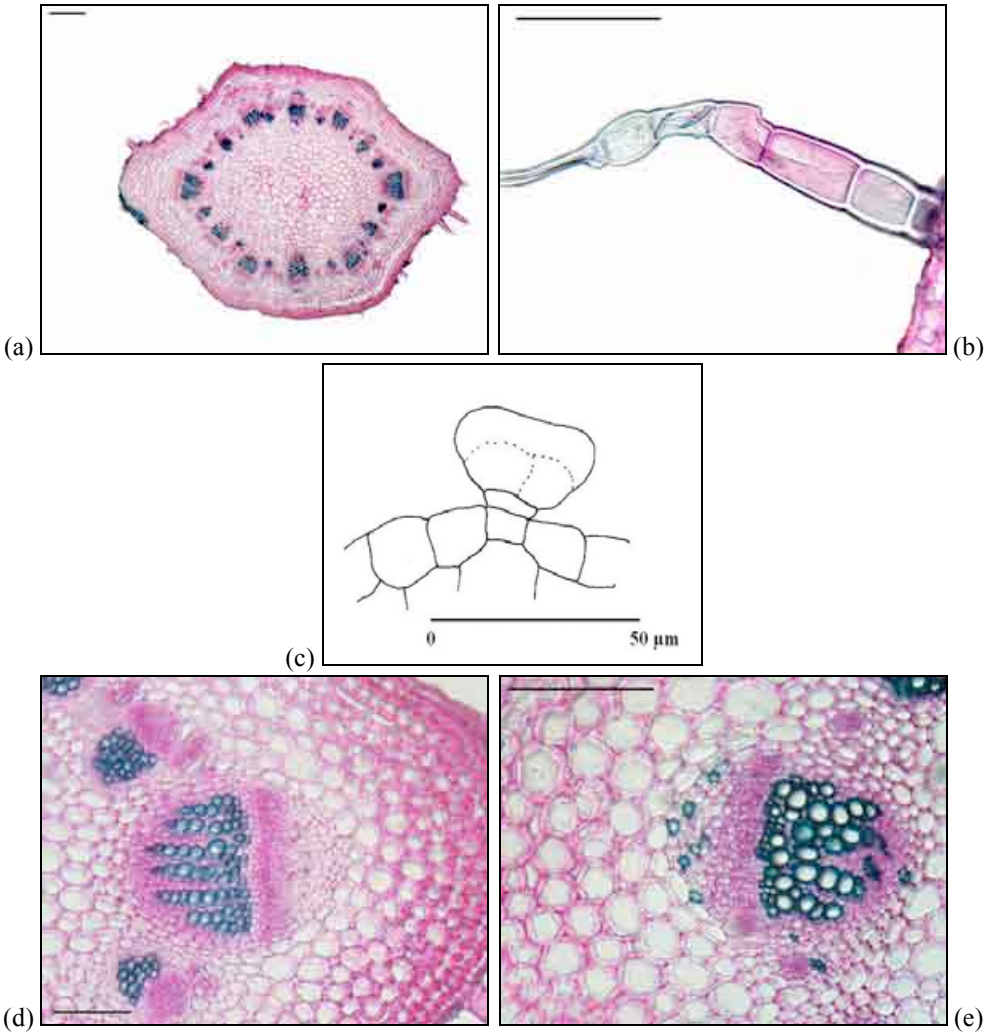


Figure 3 (a–e)
Cross sections through the upper third stem of *E. cannabinum*.

The middle third stem structure (Figure 4)

In cross section, the shape is circular (Figure 4a) with small protrusions from place to place. The bark consists of two sub-areas: an external, collenchymatous (with angular collenchyma one) and an internal, parenchymatous, meatus-type one (Figure 4b).

In the central cylinder, almost all the conducting fascicles, the large number (37–40) have one sclerenchyma cord with different thickness (Figure 4, c–f) composed of cells with thick and heavy lignified walls. All fascicles have elements of secondary origin, especially in the xylem; in the latter, the vessels from the primary origin are separated of cellulosic parenchyma cells, the cells with cambial origin (secondary) have libriform fibres between them (ligneous).

The phloem contains sieve-tubes, annex cells and few cells of phloem parenchyma, all easily collenchymatised. The medullary rays are moderately sclerified and lignified at the xylem level, and parenchymatous cellulosic at the phloem level. Between the cords of periphloem sclerenchyma and in front of the phloem attached to these cords, secretory channels are visible (Figure 4d), relatively large, with the "sewer" surrounded by a layer of epithelial cells.

The pith is parenchymatous cellulosic, without cell disruption.

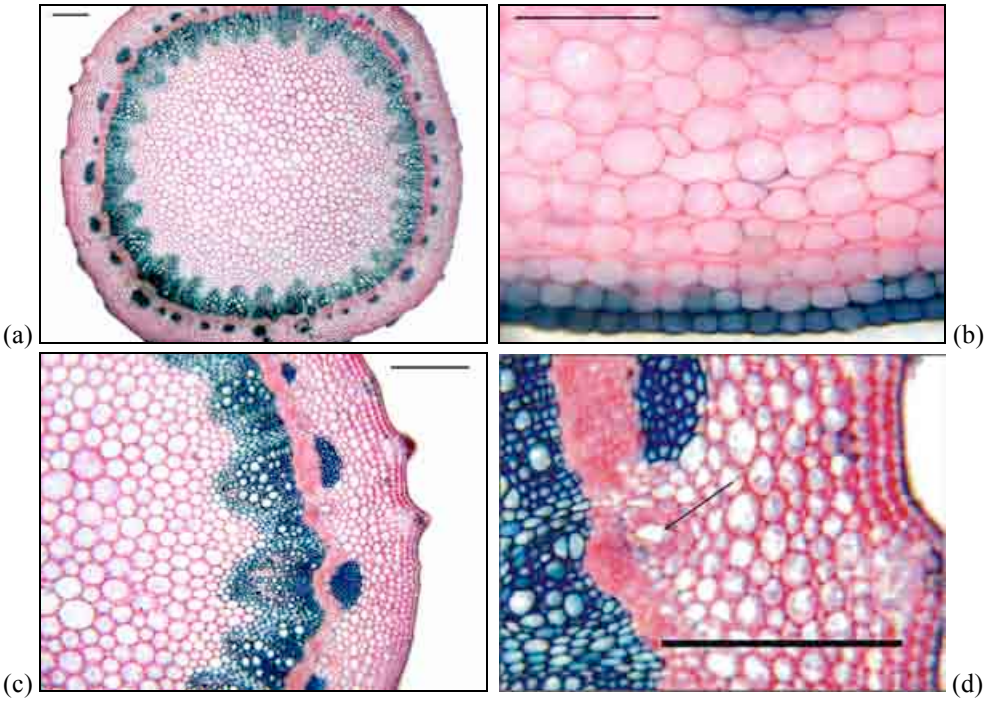


Figure 4 (a–d)

Cross sections through the middle third stem of *E. cannabinum*.

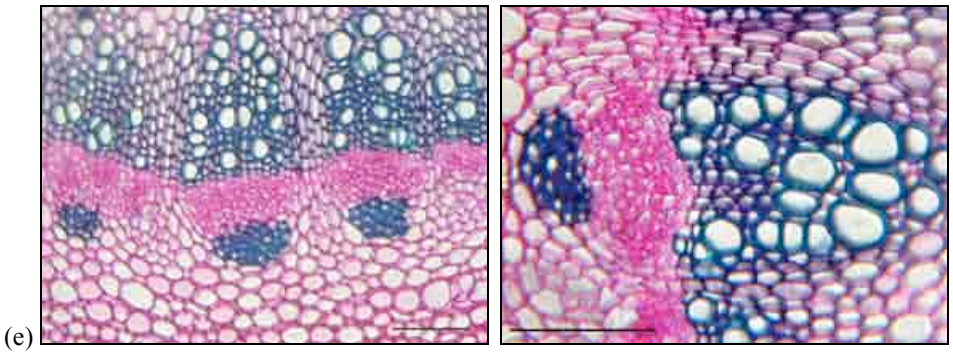


Figure 4 (e and f)
Cross sections through the middle third stem of *E. cannabinum*.

The lower third stem structure (Figure 5)

The structure differs from hypodermic collenchymatic tissue reduced to 1–2 layers (Figure 5, a and b), with flattened radial secretory channels. The xylem very thick and with medullar rays (sclerified and lignified) forms a thick ring, with visible invaginations in the pith (Figure 5, c and d), which is often the primary xylem. Into the xylem, dispersed irregular vessels are present and the libriform dominate between them.

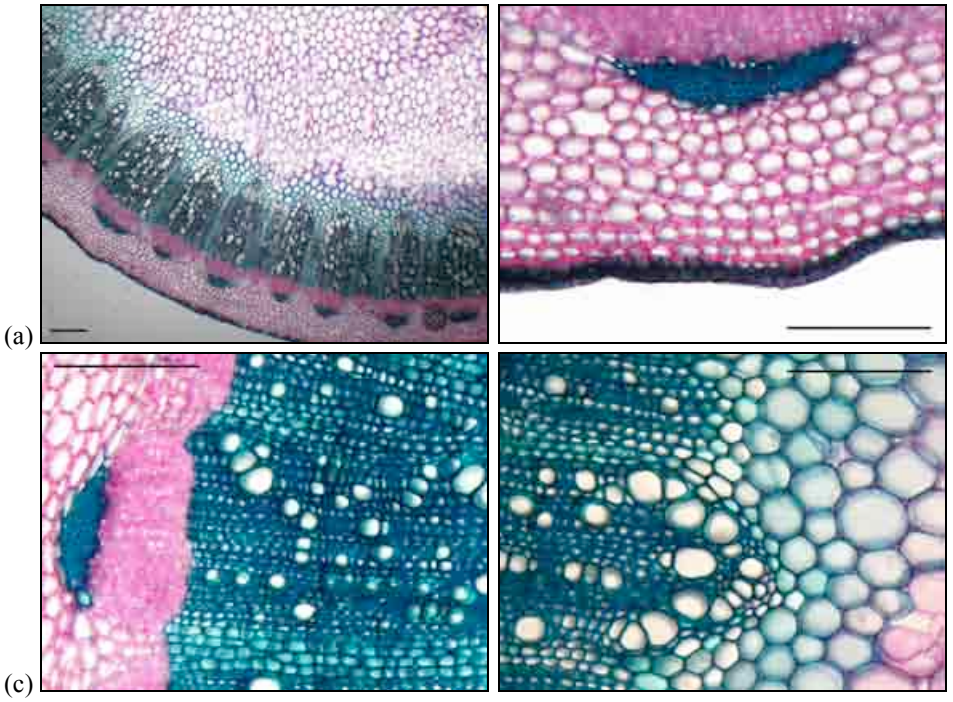


Figure 5 (a–d)
Cross sections through the lower third stem of *E. cannabinum*.

The leaf structure (Figures 6–8)

The petiole structure (Figure 6)

In cross section, the petiole is broad-wing with approximately half-moon shape around the central part (Figure 6a). The epidermis has very small isodiametric cells with thickened internal and external walls; the external wall is covered with a finely striated cuticle. At the level of epidermis, there are many tector and secretory hairs of the form and structure as those observed in stem.

The cortical parenchyma is collenchymatised on both sides and with cells having thinner walls in the middle, between the conducting fascicles. The conducting tissue (Figure 6, b–d) formed more (18–20) libero-ligneous fascicles with different sizes, larger ones (5–7) alternating with other small or very small, all of collateral type and with primary structure. The phloem includes sieve-tubes and annex cells, and the xylem contains vessels ordered in radial rows separated by cells of ligneous cellulosic parenchyma.

At the level of the wings, very wide, but thin, the mesophyll is homogeneous with numerous thin conducting fascicles. At the level of both epidermises, there are many tector hairs and very rare secretory hairs. The stomata are present only in the lower epidermis, so the leaf's limb is hypostomatic.

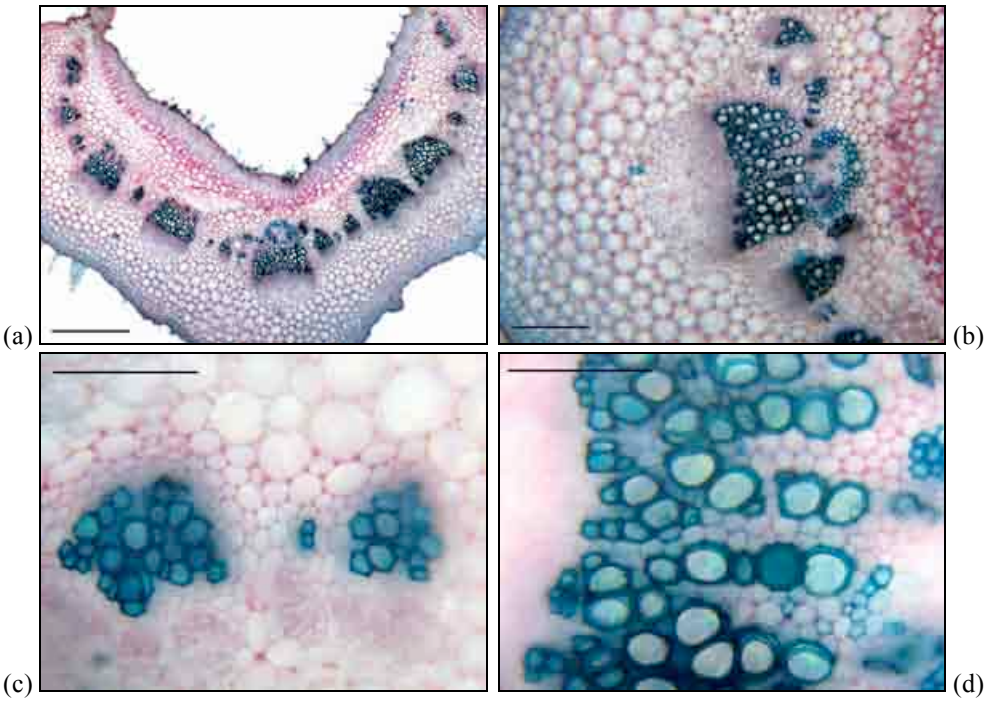


Figure 6 (a–d)
Cross sections through the petiole of *E. cannabinum*.

The leaf's limb structure (Figures 7 and 8)

The epidermis, seen from the front, is formed by cells with irregular contour, with curled sidewalls. Here and there, very long tector and secretory hairs are present. Around the base of the latter, epidermal cells are radially elongated (Figure 7, a and b), with straight sidewalls. The anomocytic-type stomata, are located only in the lower epidermis (Figure 7, c and d), so the leaf's limb is hypostomatic. On the same surface, the secretory hairs are more numerous.

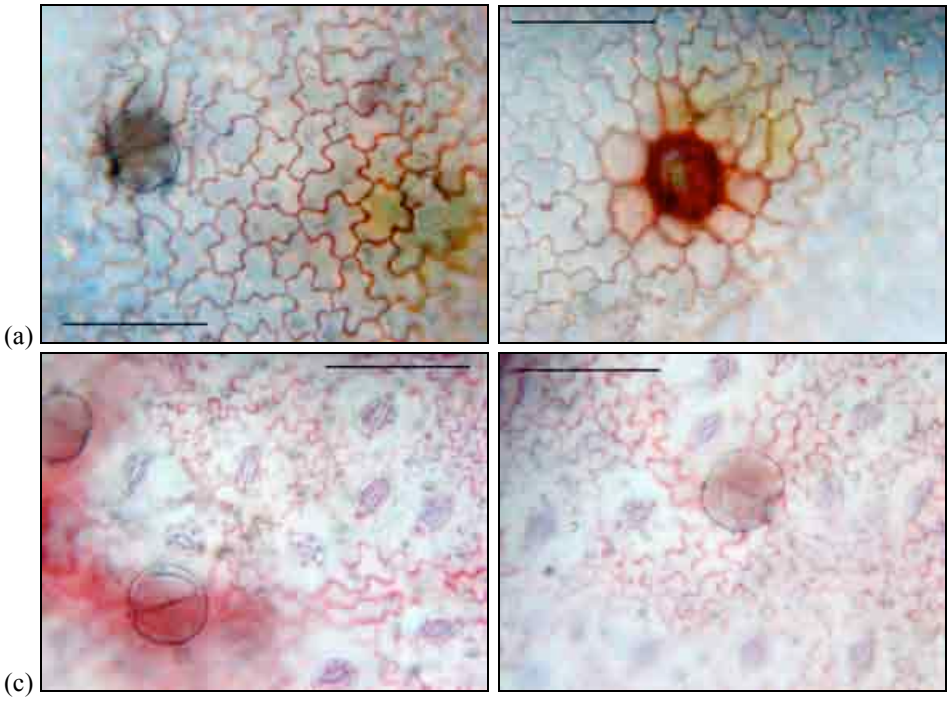


Figure 7 (a–d)
Epidermises of the leaf's limb of *E. cannabinum*.

In cross section, the median rib is very evident to the underside of the leaf's limb, taking a nearly circular form, and just the adaxial surface is flat (Figure 8a). Both epidermises have numerous tector and secretory hairs (Figure 8, d and e), the first being very long, with 5–8 cells (Figure 8f) and with a sharp top. In the fundamental parenchyma of the median rib there are 5–9 libero-ligneous fascicles of different sizes, arranged in an arch (Figure 8b), and having the structure of those from the petiole.

Between the lateral nerves (those of first order are evident also on the underside), the mesophyll is homogeneous (Figure 8c), with round cells, leaving small gaps or aeriferous meatuses between them; those in the middle of

the mesophyll are radially flattened. Thus, the leaf's limb has a bifacial isofacial structure. Both epidermises are composed of tangentially elongated cells (particularly at the adaxial surface) with thickened external wall.

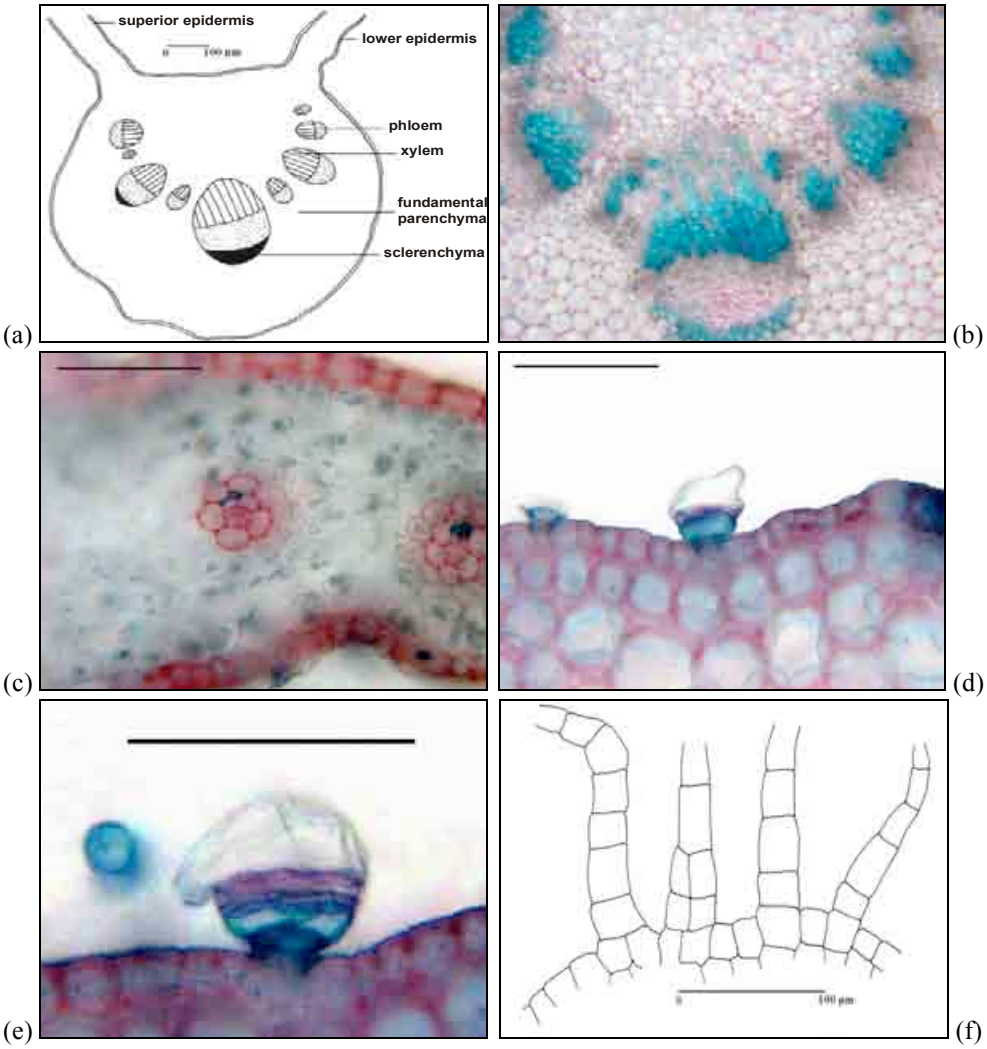


Figure 8 (a–f)
Cross sections through the leaf's limb of *E. cannabinum* (a–f).

Conclusions

Lateral roots and the lower main root have primary-type structure. In the main root, starting from the middle third is observed a pass to the secondary structure through the appearance of secondary phloem and xylem, due to the action of cambium.

Regarding the rhizome it is also seen a slight pass from the primary to the secondary structure. The conducting tissues of the rhizome are represented by 12–15 large conducting fascicles, including 2–3 small bundles. Between each fascicle there is a secretory channel.

At the top, the stem has a primary-type structure. Starting with the middle third, a secondary structure begins to reveal. In the stem structure are obvious: the massive cords of angular collenchyma in the bark, the large number of conducting bundles of collateral-open type, the presence of sclerenchyma cordons on the phloem' periphery, the existence of the secretory channels with large diameter, in front of the phloem bundles.

On the surface of stem and leaves there are relatively long uniseriate pluricellular tector hairs and pluricellular secretory hairs with unicellular pedicle and uni- or pluricellular gland.

The leaf's limb is hypostomatic, and the stomates are of anomocytic type. The limb structure has a bifacial isofacial type.

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