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CONTRIBUTIONS TO THE ANATOMY OF *RUSCUS ACULEATUS* L. (*LILIACEAE*)

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Abstract: The paper deals with the anatomical characters of the vegetative organs in *Ruscus aculeatus* L. (butcher's broom). The investigations aimed to carry out a comparative study on roots, stems and phylloclade of mature and young plants obtained from aseptically *in vitro* germinated seeds.

R. aculeatus L. is one of the protected species, included into the "Red List" elaborated for the Romanian flora. In the same time it is valuable for medical purposes having important phytotherapeutic properties. Therefore, it is desirable to obtain *in vitro* cell cultures of the plant in order to provide raw material for the pharmaceutical industry.

Introduction

Ruscus aculeatus L. (Fam. Liliaceae), butcher's broom, is a perennial species, native in the Mediterranean part of Europe, but it also grows in the Southern and Western part of Romania. The plant occurs usually in forests, meadows, bushes or open rocky places [14].

Butcher's broom is a small evergreen shrub, with horizontal rhizomes and tough leaf-like branches having assimilative role, known as phylloclade. Leaves are reduced to membranous scales. The white-greenish solitary flowers appear on the lower side of the phylloclade. The fruit is reddish berry, each with 1-2 spherical seeds [14, 8].

Ruscus aculeatus L. is considered protected species and included in the Red List elaborated from the Romanian flora [2, 4, 5]. In the same time it is a medicinal plant with important pharmaceutical properties like anti-inflammatory, vein-tonic, anti-haemorrhoidal, anti-thrombotic, diuretic, laxative and febrifuge actions [3, 8]. Rhizomes and roots (*Rusci rhizoma cum radicibus*) are used for preparation of some pharmaceutical products such as: Venelbin, (Biocur), Ruscoven (Aboca), Varicosin (Phyto Pharmica), and Butcher's Broom Root (Nature's Way) [1].

As alternative to the exploitation of the spontaneous flora, are the in *vitro cultures* of the species in order to obtain biomass containing steroidal saponins, with anti-oedema, vein-tonic and anti-haemorrhoidal effects.

The present paper deals with the comparative histoanatomical study of the vegetative organs of adult *versus* young plants obtained by *in vitro* germination of *R. aculeatus* seeds. There are no Romanian records concerning the histoanatomical structure of *R. aculeatus.* On the other hand, neither in the pharmacological treaties nor in the anatomic atlases of medicinal plants no records are present concerning the microscopic structure of *Rusci rhizoma cum radicibus* [3, 9, 10, 11, 12, 13].

Materials and Methods

The samples of mature plants (roots, rhizomes, stems and phylloclade), have been collected in September 2004 from "Dealurile Lipovei" area (Arad county). The investigated material has been supplemented with fragments of young shoots (at rhizomes level) collected in

May 2005 from the same area. All these pieces were fixed in a mixture of acetic acid and ethyl alcohol (1:3) and preserved at 4°C (refrigerator) [6, 7].

In order to obtain callus and cellular suspensions, plantlets were grown by aseptic *in vitro* germination of *R. aculeatus* seeds, sampled from mature wild plants, at the Institute of Biological Research Cluj-Napoca. These young plants (37 days old) were also used for this study.

In order to perform studies on the anatomical features, cross-sections were performed by both microtome and manual techniques (scalpel, thin blades). The sections were stained with alum-carmine, iodine green or Toluidine Blue (0.2%) and subsequently embedded in Canada balsam and glycerin jelly [6, 7]. The observations were performed with light microscopy connected to digital image extensions.

Results and Discussions

Cross-sections of the root (Fig. 1) reveal the rhizodermis with many hair roots followed by an exodermis, the later one colored in green with Toluidine Blue. The root cortex is well-developed, with parenchymatous cells enclosing intercellular spaces, some of them having a very dense content (presumably mucilage). The stele is very well contoured, with differentiated endodermis and consists of cells with "U" shaped lignin thickenings and it is interrupted by passage cells. The vascular system consists of alternating xylem and phloem bundles (approx. 30). The xylem comprises 3-4 vessels with the metaxylem towards the center and the protoxylem facing the pericycle. The central part of the root is occupied by the pith, becoming lignified with the plant growth and maturation (Fig. 2).

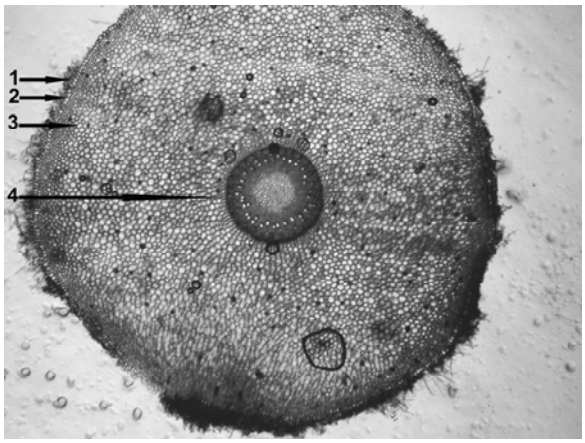


Fig. 1: Cross-section of the root. General view (X 15). 1 - rhizodermis; 2 - cutis; 3 - cortex; 4 - stele.

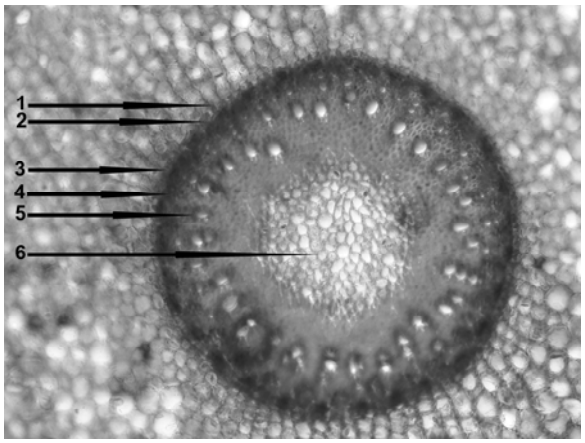


Fig. 2: Structure of the stele (X 52). 1 - endodermis; 2 - pericycle; 3 - passage cells; 4 -phloem; 5 - xylem; 6 - pith.

Thin sections cut through the root of the young plant obtained by *in vitro* germination of seeds (Fig. 3) exhibit the rhizodermis with adsorbent unicellular root hairs containing cytoplasm. The differentiation of the exodermis is initiated. The cortical parenchyma is well developed and consists of cells with thin cellulose walls. It is twice thicker than the diameter of stele. The endoderm cells display "U" shaped lignin thickenings. The number of the xylem and phloem bundles is much lower (about 11) in this stage compared with the adult plant. The phloem bundles are better differentiated as the alternating xylem ones. The central part is occupied by lignified pith.

Cross-sections of the rhizome disclose (Fig. 4) a secondary cork followed by cortical parenchyma separated from the stele by lignified endodermis, interrupted by passage cells (green-bluish colored with Toluidin Blue). There are leptocentric vascular bundles each having its own endoderm, all of them being embedded into the basic parenchyma (Fig. 5).

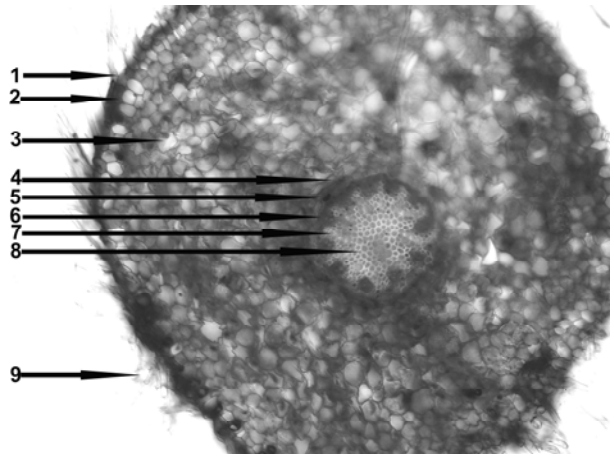


Fig. 3: Cross-section of plantlet root. General view (X 62). 1 - rhizodermis; 2 - cutis; 3 - cortex; 4 - endodermis; 5 -pericycle;.6 - phloem; 7 - xylem; 8 -pith; 9 - hair roots.

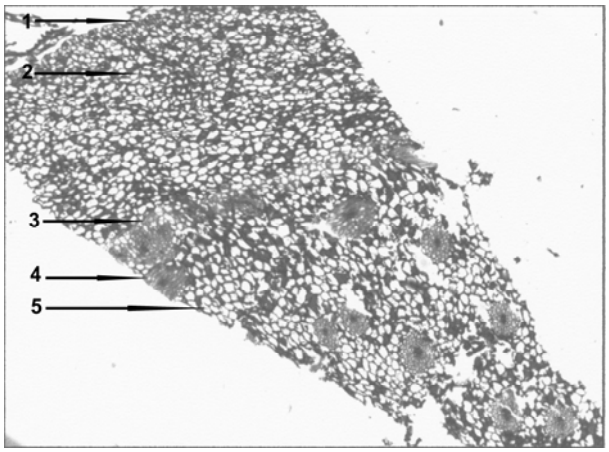


Fig. 4: Cross-section of the rhizome. General view. (X 53). 1 - cork; 2 - cortex; 3 - endodermis; 4 -vascular bundle; 5 - fundamental tissue.

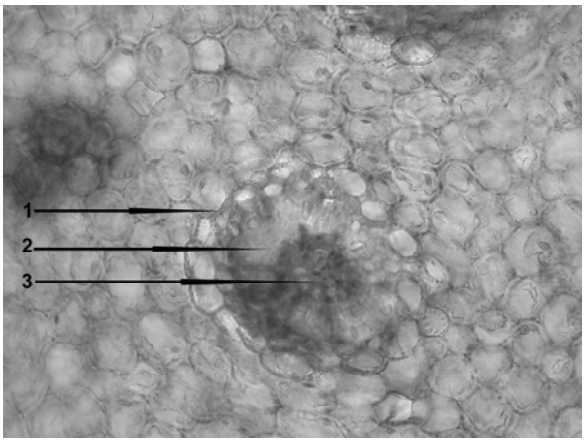


Fig. 5: Leptocentric vascular bundle (X 316). 1 - endodermis; 2 - xylem; 3 - phloem.

Analyzing the adult *R. aculeatus* stem (Fig. 6) one can notice a strong cutinized epidermis followed by the chlorenchyma layer, consisting of two chloroplasts rich cell strata in the crest zone, 3-4 strata in the vallecular zone and a lacunar parenchyma in its inner portion. The bark is delimited towards the center by the endoderm and sclerenchymatous pericycle. In the central parenchyma numerous collateral bundles are located; those situated towards the periphery are smaller. The vascular bundles are completely surrounded by sclerenchyma sheaths which are more developed when lining the phloem. The xylem oriented towards the interior of stem consists of several elements (12-14); the phloem formed towards the periphery of the stem comprises sieved tubes and companion cells. The central parenchyma exhibits dotted cells too (Fig. 7).

The young *R. aculeatus* shoots have the same structure as the stem of adult plants, except that the crests just start to differentiate in the young ones. The cortex consists of cellulose walled cells leaving large intercellular spaces.

Cross-sections through the stem of *in vitro* young plants exhibit no major differences as compared with the mature stems, except for the much reduced sizes of the structural elements.

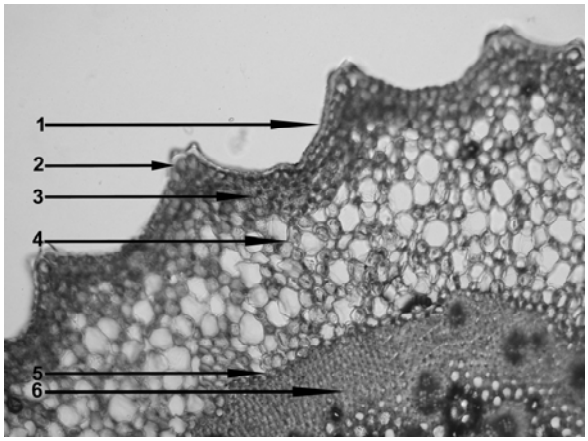


Fig. 6:. Cross-section of the stem. General view (X 100). 1 - cuticle; 2 - epidermis; 3 - chlorenchyma; 4 -lacunar parenchyma; 5 - endodermis; 6 - sclerenchymatous pericycle.

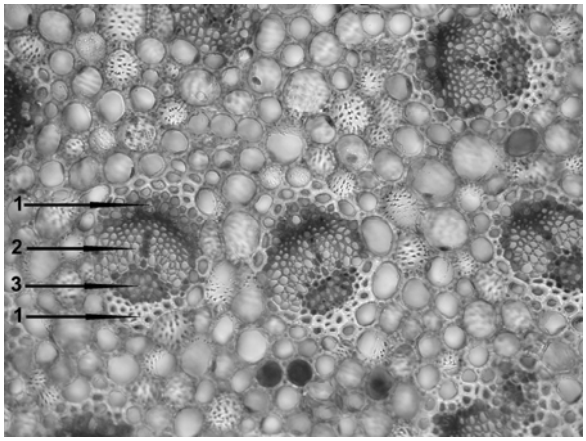


Fig. 7: Detail of the inner part of steam showing the collateral bundles. 1 - sclerenchyma; 2 - xylem; 3 –phloem.

The phylloclade in adult plants and plantlets (Figs. 8, 9) differs in size (0.46 mm, respectively 0.28 mm diameter), but shows the same arrangement of the elements: epidermis (upper and lower), homogeneous assimilatory parenchyma consisting of four chloroplasts rich cell strata located both parts of epidermis, and finally, in its central part an aggregate of large parenchyma cells lacking chloroplasts. The epidermis of the phylloclade in adult plants is covered by a thick cuticle interrupted by well differentiated stomata. The cuticle of *in vitro* grown young plants is thinner and stomata are not yet differentiated. The vascular bundles are leptocentric. The main bundle is larger than those located towards the phylloclade surface and which tend to become progressively smaller.

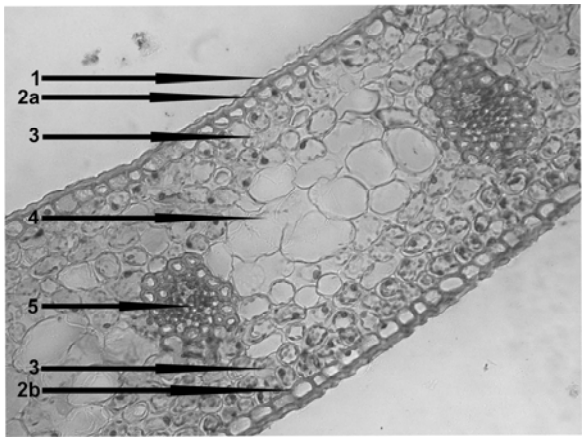


Fig. 8: Cross-section of the adult plant phylloclade. General view (X 116). 1- cuticle; 2 - epidermis: a -upper; b - lower; 3 - assimilatory parenchyma; 4 -parenchyma cells; 5 - leptocentric vascular bundle.

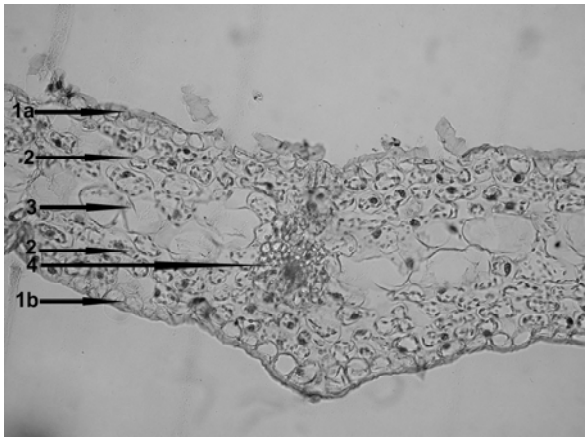


Fig. 9: Cross-section of the plantlet phylloclade. General view (X 116). 1 - epidermis: a - upper; b - lower; 2 - assimilatory parenchyma; 3 - parenchymatous cells; 4 - leptocentric vascular bundle.

Conclusions

The authors carried out a comparative histoanatomical study concerning the root, rhizome, stem and phylloclade structure of both adult and young ones, the later obtained from aseptic *in vitro* germinated seeds of *R. aculeatus* L.

Both adult and young plants obtained by *in vitro* technique exhibit the same vegetative organs except the rhizomes which are absent in plantlets. Moreover, these vegetative organs (root, stem, phylloclade), exhibit the same histological organization, except that the structural elements are smaller for the young plantlets.

In the stem of *R. aculeatus* the vascular bundles are of collateral type. The rhizome and phylloclade exhibit leptocentric vascular bundles, with the phloem in the centre, with the xylem round it.

These findings define more accurately the anatomic structure of the medicinal plant biomass, *Rusci rhizoma cum radicibus*, used to obtain products of phytotherapeutic interest.

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CONTRIBUŢII LA STUDIUL ANATOMIC AL SPECIEI *RUSCUS ACULEATUS* L. (*LILIACEAE*)

(Rezumat)

Ruscus aculeatus L. (ghimpe) este o specie spontană în flora României, răspândită sporadic în pădurile de foioase termofile din sud şi vest, fiind considerată o specie periclitată, aflată în pericol de extincţie. În acelaşi timp este şi o valoroasă plantă medicinală prin rizomii săi care conţin saponine steroidice cu acţiune antiinflamatoare şi antireumatică.

Pentru protejarea speciei în flora spontană se preconizează introducerea ei în cultura de suspensii celulare în vederea extragerii principiilor farmaceutic active.

Se prezintă structura rădăcinilor, rizomilor, tulpinilor aeriene şi filocladiilor comparativ cu cele provenite din plantule obţinute prin germinarea seminţelor *in vitro*. Structura organelor plantulelor obţinute prin tehnica *in vitro* este asemănătoare cu a celor provenite din planta matură, elementele mecanice şi conducătoare fiind mai puţin dezvoltate pentru prima categorie.