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Morphological and Histo-Anatomical Study of *Bryonia alba* L. (Cucurbitaceae)

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Abstract

The purpose of this study consisted in the identification of the macroscopic and microscopic characters of the vegetative and reproductive organs of *Bryonia alba* L., by the analysis of vegetal material, both integral and as powder. Optical microscopy was used to reveal the anatomical structure of the vegetative (root, stem, tendrils, leaves) and reproductive (ovary, male flower petals) organs. Histo-anatomical details were highlighted by coloration with an original combination of reagents for the double coloration of cellulose and lignin. Scanning electronic microscopy (SEM) and stereomicroscopy led to the elucidation of the structure of tector and secretory trichomes on the inferior epidermis of the leaf. Micrographic analysis of the powder obtained from aerial parts revealed segments of each organ (e.g. stomata, trichomes) and confirmed furthermore the results obtained by the histo-anatomical studies. Sections achieved through vegetative organs reveal typical anatomical structures: a transition to the secondary structure for the root, a typical dicotyledons' structure with bicollateral vascular bundles for the stem, a bifacial structure of the leaf and stem-like structures for the tendrils and petioles, which prove they are metamorphosis of the stem. Anatomical structure of reproductive organs was performed hereby for the first time and revealed a typical anatomical structure for the 3-lodged ovary and a leaf-like structure of the male flower petal. Some of the results obtained confirm existing data from the scientific literature and additional information have been provided, outlining features that were not previously reported, such as SEM analysis of the leaf trichomes and histo-anatomical structure of the reproductive organs.

Keywords: anatomy, optical microscopy, scanning electronic microscopy, stereomicroscopy, vegetative and reproductive organs

Introduction

Family Cucurbitaceae (order Cucurbitales) comprises 90 genera, with about 700 species, distributed mostly in the tropical regions (Tutin *et al.*, 2010) and is one of the most important plant families over the world, especially from the economic point of view (Kocyan *et al.*, 2007). It comprises species as *Cucurbita pepo* L. (pumpkin), *Cucumis sativus* L. (cucumber), *Citrullus lanatus* (Thunb.) Mansf. (watermelon), which represent the basis of a large number of industries, including food and cosmetic industry (Ali and Al-Hemaid, 2011). Despite its economical importance, Cucurbitaceae family comprises less known species, but which were found to have promising importance, especially from the therapeutic point of view (Manvi and Ganesh, 2011).

Bryonia genus comprises a total of 10 species, which are distributed from the Mediterranean to Central European region (Tutin *et al.*, 2010), Northern Africa and Central Asia (Kocyan *et al.*, 2007; Schaefer and Renner, 2011).

Recent classification of the Cucurbitaceae family includes *Bryonia* genus in the Cucurbitoideae subfamily, Bryonieae tribe (Kocyan *et al.*, 2007). Phylogeny studies (Kocyan *et al.*, 2007; Volz and Renner, 2008) revealed that Bryonieae tribe comprises only the *Ecballium elaterium* (L.) A. Rich. species and the 10 *Bryonia* species, of which seven are dioecious and three are monoecious. *B. alba* L. is a normally monoecious species which, according to Volz and Renner (2008), ranges from Central Europe to Kazakhstan, while *B. cretica* L. and *B. dioica* Jacq. are dioecious species, difficult to separate but having different area distribution, especially Southern, Central and Eastern part of Europe, where *B. dioica* Jacq. appears to be spreading. Geographic distribution of the major chloroplast haplotypes of the *Bryonia* species (Volz and Renner, 2008, 2009) suggest that *B. alba* L. might be the only *Bryonia* species found in Romania and also the most widespread *Bryonia* species in the world.

B. alba L. is known especially for the use in homeopathy, for its antipyretic, anti-inflammatory, antibacterial, laxative-purgative and smooth muscle relaxant properties (Demarque *et al.*, 2007). At the same time, there is scientific evidence of other therapeutic activities

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of *B. alba* L. such as citotoxic (Baek Ni *et al.*, 1995; Konopa *et al.*, 1974), antiinflamatory (Park *et al.*, 2004), hepatoprotective (Manvi and Ganesh, 2011), anti-diabetic (Singh *et al.*, 2012), which have been shown to be promising for the treatment of various diseases.

Morphological and histo-anatomical studies have a great importance in the *Bryonia* genus since they are essential for the correct description of the species, of its vegetal products, for the identification of substitutions and adulterations with species without biological activity, but also for the correct localization of the active principles in different parts of the plants (Manvi and Ganesh, 2011).

Data about the histo-anatomical characterisation of *B. alba* L. were first published by Toma and Rugină (1998) and revealed the histo-anatomical structure of the vegetative organs (root, stem, leaves). Other histo-anatomical researches on the species were published by Manvi and Ganesh (2011), describing the anatomy of the root and localization of some active principles (starch grains). Existing data regard mostly the structure of root, which is the organ of the plant that contains the most important active principles, cucurbitacins (Konopa *et al.*, 1974). Anatomical structures of other organs are still poorly described.

The lack of a description of important morphological and histo-anatomical characters, important for both the taxonomy and for the species identity, was noticed. Cucurbitaceae family is widely known for the presence of trichomes on the inferior surface of the leaves, which represent an important taxonomic significance on the species belonging to this family (Ali and Al-Hemaid, 2011). At the same time, since *Bryonia* genus was proved to be an important model system for the study of plant sex chromosome evolution (Volz and Renner, 2008), the correct histo-anatomical description of the reproductive organs appears to be of great importance.

Therefore, the aim of the present study was to analyse the main morphological and histo-anatomical characters of *B. alba* L. by scanning electronic micoscopy on the leaf's inferior epidermis and optical microscopic analysis of the reproductive organs. Given the fact that this information is missing from the scientific literature, the present study may improve the knowledge in the ability of understanding the taxonomic importance of the *Bryonia* genus in the Cucurbitaceae family, but may also be very useful for the correct and complete determination of the identity of a species that proved promising for therapeutics.

Materials and methods

The vegetal material was harvested from the spontaneous flora of Cluj County, (North-Western Romania) and identified at the Department of Pharmaceutical Botany of "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, where voucher specimen nr. 105.3.1.1-7 was deposed.

Stereomicroscopy

For the identification of macroscopic characters, photos of the vegetative and reproductive organs were taken with a Motic K-500L stereomicroscope, connected to a MoticCam Pro 205A digital camera.

Optical microscopy – classical technique

Preserved fragments of vegetative organs (root, stem, petioles) were cross-sectioned using a Nahita 501 manual microtome. Sections were stained using alum carmine Alfa Aesar® (specific staining reagent for cellulose, which is stained purple-red) and malachite green Merck® (specific staining reagent for lignin, which is stained green), included in gelatin and studied using an Olympus CX31 optical microscope equipped with a digital camera. In order to better observe the trichomes on the inferior face of the leaves, superficial sections were performed (Crişan *et al.*, 2013).

Optical microscopy – histological tehnique

Fragments of the preserved vegetal material (leaf lamina, tendrils, ovary and male flower petals) were fixed and dehydrated. Vegetal material was then passed into xylene and poured in paraffin cubes, which were sectioned using a Microtec CUT 4050 microtome. Sections were stained with toluidin blue (staining reagent which stains cellulose in purple-blue and lignin in green) and studied with the optical microscope.

All vegetal material containing compounds that might interfered with the microscopic analysis (e.g. root sections containing a large amount of starch grains, leaves sections containing chlorophyll) were previously clarified, using a chloralhydrate solution, which, at the boiling temperature, allowed the precipitation of undesirable compounds.

Optical microscopy – study of the powder

Samples of vegetative organs and of aerial parts were dried and grinded using a Grindomix knife mill to obtain the powder, which was studied with the optical microscope, using chloralhydrate as inclusion medium (European Pharmacopoeia 8th edition, 2014).

Scanning electronic microscopy

Analysis were performed on fresh vegetal material, namely on the inferior epidermis of the leaves, in order to investigate the type of trichomes present on this part of the leaf. The samples were studied under vacuum, using a JEOL JSM 5510 LV Scanning Electronic Microscope, after a vacuum metalizing technique (Nagy *et al.*, 2014).

Results

Root

Transversal sections of the root had circular contour (Fig. 1.a) and presented, at the sectioned level, a transition towards a secondary anatomical structure, resulting from secondary meristems' activity. Phellogen (Fig. 1.b.1) was a single layer tissue, with cellulosic walls, colored in purple-red. It had generated the secondary cork (Fig. 1.b.2) on the outside and phelloderm (Fig. 1.b.3) on the inside. Primary cortex (Fig. 1.b.4) was less developed. Secondary cork had tangentially suberified cells, coloured in green-brown with the mixture of colorants used. Both cortexes had cells

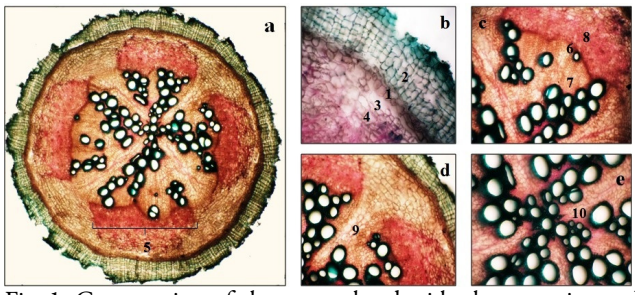

Fig. 1. Cross section of the root colored with alum carmine and malachite green – general view 40x (a) and structural details view 200x (b, c, d, e)

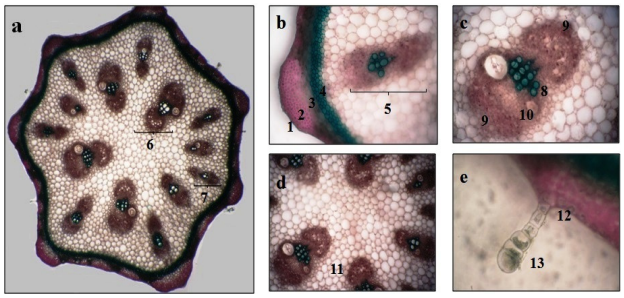

Fig. 2. Cross section of the stem colored with alum carmine and malachite green – general view 40x (a) and structural details (b, d – 200x; c, e – 400x)

with cellulose walls. Secondary conductive tissues (Fig.1.a.5) were formed by simple vascular bundles (xylem and phloem) and arranged in a star shape. Cambium (Fig. 1.c.6) (single-layer tissue, cells with cellulosic walls) separated the xylem (inside) (Fig. 1.c.7) from the phloem (outside) (Fig. 1.c.8). The mixture of colorants stained the xylemian tissue (vessels with lignified walls) in green and the phloemian tissue (vessels with cellulose walls) in purple-red. Secondary medullary rays (Fig. 1.d.9) had cellulosic walls and separated the vascular bundles. The centre of the section was occupied by the secondary xylem (Fig. 1.e.10).

Stem

Transversal section of the stem was polygonal-shaped (Fig. 2.a), representing a primary anatomical structure. Epidermis (Fig. 2.b.1) had isodiametric cells, with cellulosic walls, colored in purple-red. Below the epidermis, the collenchymatic cortex (Fig. 2.b.2) appeared as a multi-layer tissue, with cellulose thickenings, colored in purple-red. Separated by strings of parenchymatous cells (Fig. 2.b.3), colored in purple-red, the sclerenchymatic cortex (Fig. 2.b.4) was colored in green, due to the lignin that thickens the cell walls. Vascular bundles (Fig. 2.b.5) were bicollateral and open, disposed regularly in the fundamental parenchyma, which had cellulose walls, colored in purple-red. There were two types of vascular bundles: larger ones (Fig. 2.a.6), disposed towards the centre of the section and smaller ones (Fig. 2.a.7), disposed towards the exterior of the section. Xylem (Fig. 2.c.8) had lignin thickenings on vessels and was colored in green. It appeared in the centre of the bundle, being situated between two strings of phloem (Fig. 2.c.9), which had cellulose walls and was colored in purple-red. Between the external phloem and the xylem, the cambium (Fig. 2.c.10) was found as a single-layer tissue, colored in purple-red. Vascular bundles were separated by medularry rays (Fig. 2.d.11), colored in purple-red. Secretory multi-string trichomes appeared on the epidermis, having a pluricellular basis (Fig. 2.e.12) and 2-3 secretory cells on the top (Fig. 2.e.12).

Tendrils

Tendrils had a stem-like structure (Fig. 3.a), being a stem metamorphosis. Almost the same parts of the anatomical structure of the stem were observed on the section. These zones and their coloration with the toluidine blue reagent were: epidermis (purple-blue) (Fig. 3.a.1), collenchymatic cortex (purple-blue) (Fig. 3.a.2), fundamental parenchyma (purple-blue) (Fig. 3.b.3), medullary rays (purple-blue) (Fig. 3.b.4) and medullary parenchyma (purple-blue) (Fig. 3.a.5). Same type of bicollateral and open vascular bundles (Fig. 3.c.6) were present, similar to the ones found in the structure of the stem: xylem (Fig. 3.c.7), colored in green, in the centre of the bundle, being situated between two strings of phloem (Fig.

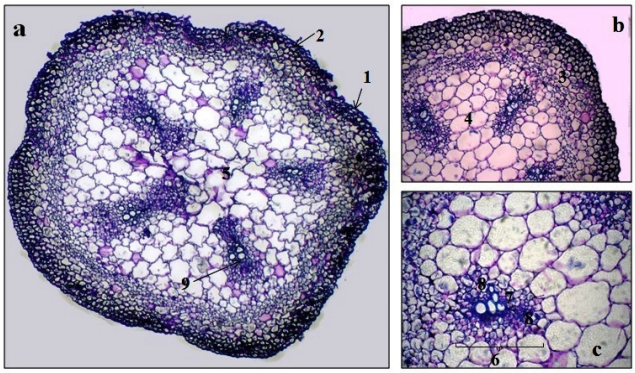

Fig. 3. Cross section of the tendril colored with toluidine blue – general view 40x (a) and structural details (b – 200x; c – 400x)

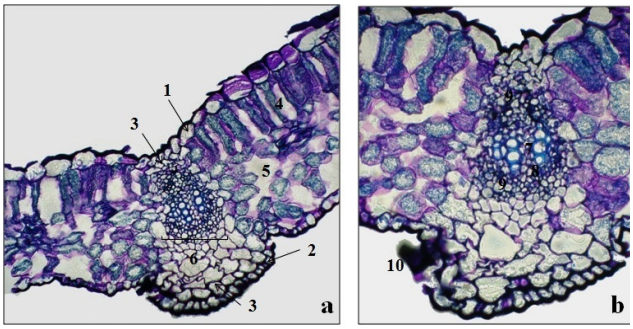

Fig. 4. Cross section of the leaf lamina colored with toluidine blue – general view 40x (a) and structural detail of the central vascular bundle 200x (b)

3.c.8), colored in purple-blue. Cambium (Fig. 3.a.9), colored in purple-blue, separated the exterior phloem from the xylem. The number of vascular bundles was reduced compared to the stem and schlerenchymatic cortex was absent.

Leaf

Sections revealed the bifacial anatomical structure of the leaf lamina (Fig. 4.a), delimited by an upper epidermis (Fig. 4.a.1) and a lower one (Fig. 4.a.2), with 2 protuberances, corresponding to the central vein, more proeminent on the inferior face. Each of these protuberances contained collenchymatic tissue (Fig. 4.a.3), with cellulose thickenings, colored in purple-blue. Both epidermises were colored in purple-blue with toluidin blue. Under the upper epidermis, palisade parenchyma (Fig. 4.a.4) was found, colored in purple-blue and lacunar parenchyma (Fig. 4.a.5) was found above the lower epidermis, similarly colored. Vascular bundles (Fig. 4.a.6) were collateral and closed, with xylem (Fig .4.b.7), colored in green, situated towards the upper epidermis and phloem (Fig. 4.b.8), colored in purple-blue, situated towards the lower epidermis. Vascular bundles were protected by 2 sclerenchyma arches (Fig. 4.b.9), colored in green due to their lignin thickenings. On the lower epidermis, both secretory and tector trichomes appeared (Fig. 4.b.10).

Stereomicroscopy (Fig. 5.a) and superficial sections (Fig. 5.b,c) allowed a better observation of the different types of trichomes on the inferior epidermis of the leaf. Tector pluricellular trichomes (Fig. 5.b) and secretory trichomes (Fig. 5.c) were noticed.

Furthermore, in order to elucidate the structure of each type of trichome, SEM analysis was performed on the inferior surface of the leaf. Images (Fig. 6.a-f) revealed stomata (Fig. 6.b) and the structure of each type of trichome (Fig. 6.c-f). Tector trichomes had a pluricellurar body and a cup-like basis, also formed by

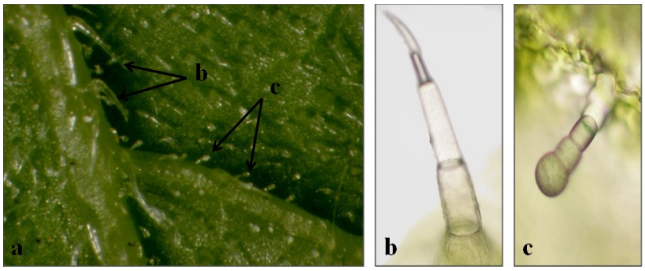

Fig. 5. View of the principal types of trichomes by stereomicroscopy 300x (a) and by optical microscopy – pluricellular trichome 400x (b) and secretory multi-seried trichome 400x (c)

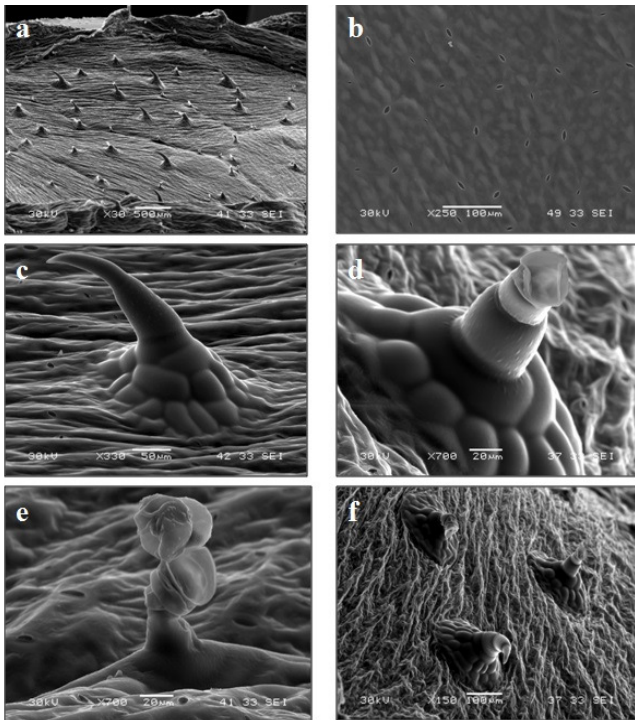

Fig. 6. View of the inferior epidermis of the leaf – general view (a), stomata (b), pluricellular tector trichome (c), multi-seried secretory trichome with pluricellular basis (d), multi-seried secretory trichome with unicellular basis (e), the 3 types of trichomes (f)

multiple cells (Fig. 6.c). Secretory trichomes (Fig. 6.d-f) revealed 2 types of structures: first one was represented by multi-seried trichomes with unicellular basis (Fig. 6.e), and the second one was represented by multi-seried trichomes with a pluri-cellular basis (Fig. 6.d).

Petiole

Sections revealed a semicircular structure of the petiole, laterally compressed (Fig. 7.a). The stem-like structure revealed the relationship between the two structures. Differences appeared on the outer part of the structure, which was protected by a collenchymatic arch (Fig. 7.b.1), colored in purple-red. Vascular bundles (Fig. 7.c.2) were bicollateral and open, disposed regularly in the fundamental parechyma. Larger vascular bundles (Fig. 7.a.3) were situated towards the opposite part of the lateral compression, whereas smaller ones (Fig. 7.a.4) were situated near the compression. Xylem (Fig. 7.c.5) was delimited by phloem on both sides (Fig. 7.c.6) and, for the mature petioles, sclerenchyma arches may appear (Fig. 7.c.8) in order to protect the bundles. The cambium (Fig. 7.c.7) was a single-layer tissue, colored in purple-red,

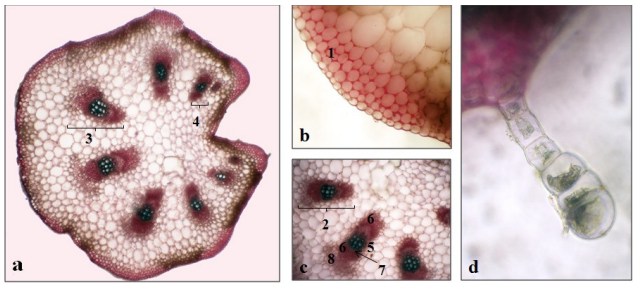

Fig. 7. Cross section of the petiole colored with alum carmine and malachite green – general view 40x (a) and structural details (b – 200x, c – 100x, d – 400x)

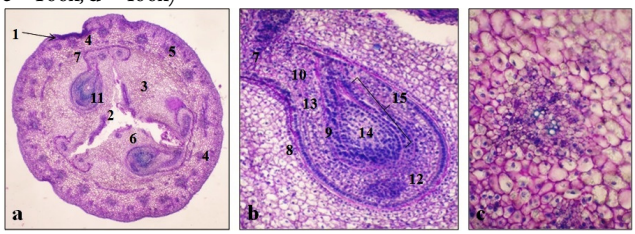

Fig. 8. Cross section of the ovary colored with toluidine blue – general view 40x (a) and detail of a median vascular bundle (c – 400x); cross section of the ovule colored with toluidine blue – general view 400x (b)

between the phloem situated towards the exterior of the section and the xylem. Secretory multi-seried trychomes (Fig. 7.d) appeared on the epidermis.

Reproductive organs

The structure of the ovary was a typical one. It revealed a 3-carpelar ovary (Fig. 8.a), each carpel containing 2 ovules (Fig. 8.b), more or less developed. The anatomical structure was surrounded by the external epidermis (Fig. 8.a.1). Internal epidermis is not well marked, but the carpel welding line (Fig.8.a.2) can be easily observed. Fundamental parenchyma (Fig. 8.a.3) contained one type of vascular bundle (Fig.8.a.4,5,6), placentation being parietal, as for the vast majority of the species of the Cucurbitaceae family. Ovules (Fig. 8.b) were anatrope and were sustained by the funiculus (Fig. 8.a.b.7) on the ovarian cavity. External (Fig. 8.b.8) and internal (Fig. 8.b.9) integuments delimitated the structure. Hilum (Fig. 8.b.10) was the part that connected the funiculus to the ovary. Part of the funiculus was united with the ovule and formed the raphe (Fig. 8.a.11). The vascular bundle that crosseed the funiculus formed a branch-like structure, the chalaza (Fig. 8.b.12). At the bottom, the ovule presented the micropyle (Fig. 8.b.13). In the fundamental parenchyma (nucellus) (Fig. 8.b.14), the embryo sac (Fig. 8.b.15) was found. Toluidine blue colored most of the structure in purple-blue, excepting the xylem vessels from the vascular bundles, which were coloured in green.

The structure of the male flower petals was similar to the structure of leaves (Fig. 9.a). Superior (Fig. 9.b.1) and inferior (Fig. 9.b.2) epidermis surrounded the structure, which contained several vascular bundles (Fig. 9.b.3), spread in the fundamental parenchyma. Each vascular bundle was collateral and closed, being protected by a sclerenchyma arch (Fig. 9.b.4). Secretory multi-seried trichomes were present (Fig. 9.c).

Powder

Analysis of the powder confirmed the morfological and histo-anatomical structure of the vegetative and reproductory organs. Parts of the organs could be found in the powder of the root, leaves, fruit and in the aerial part of the species. Powder of the roots

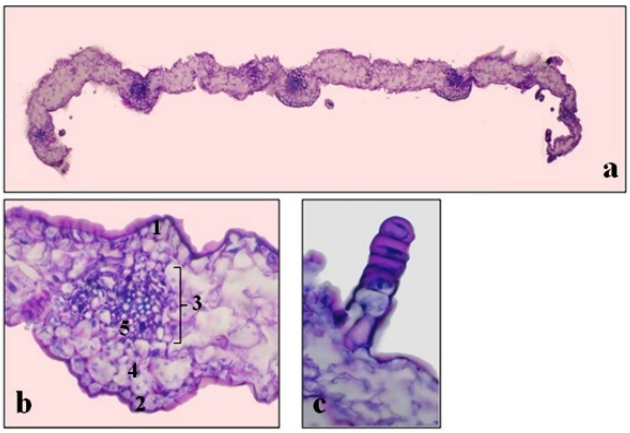

Fig. 9. Transversal section of the male flower petal colored with toluidine blue – general view 40x (a) and structural details (b – vascular bundle 200x; c – secretory trichome 400x)

showed the presence of cortex and cork cells (Fig. 10.A.) and xylem vessels (Fig. 10.B). In the leaf powder, parts of tector trichomes (Fig. 10.C-D.) and of epidermis with stomata (Fig. 10.E) were found. The powder of the fruit showed parts of fundamental parenchyma (Fig. 11.I-J) and the powder of the aerial parts (Fig. 11.F-H) contained each of the parts of the analysed structures (tector trichomes, parenchyma cells etc.).

Discussions

Microscopic analysis allowed the identification of the anatomical structures of the vegetative and reproductive organs: secondary anatomical structure of the root, primary anatomical structure of the stem, petiole and tendril anatomical structure, bifacial anatomical structure of the leaf, anatomical structure of the ovary and male flower petals.

Scientific approaches on the morpho-anatomic study of *B. alba* L. species (Toma and Ruginã, 1998) provide fragmentary and relatively old data concerning morpho-anatomy of the vegetative organs. Few other approaches on the topic exist, but most of them concerning the anatomy and morphology of the root, which has proved to be the most important part of the species, containing the largest amount of active principles, cucurbitacins, which exibited cytotoxic activity (Baek Ni *et al.*, 1995; Konopa *et al.*, 1974). Manvi and Ganesh (2011) revealed the anatomical structure of the root of the species and the localization of the strach grains in the fundamental parenchyma of the root. Same results were obtained in the present study, both concerning the morpho-anatomical structure of the root and the large amount of starch grains, which was removed in order to observe better the structure. In the same direction, the studies of Burrows and Shaik (2014) on the anatomy of the taproot of *Citrullus colocynthis* (L.) Schrad., *Citrullus lanatus* (Thunb.) Mansf. and *Cucumis myriocarpus* E. Mey. ex Naud. proved a similar structure of the root to the one found for *B. alba* L. in this study, supporting the relationship between the species of the same family and representing, at the same time, one more argument in sustaining the taxonomic position of *B. alba* L. In the same direction of sustaining the relationship between the species of the Cucurbitaceae family, comes the study of Christodoulakis *et al.* (2011), which revealed similar leaf structure of *Ecballium elaterium* (L.) A. Rich., the closest species to *B. alba* L. in taxonomy. Other existing data on the morpho-anatomy vegetative organs of the species were not found.

On the other hand, new important data is brought to support taxonomic position of the species. Microscopic and SEM analysis of the leaf epidermis, in order to elucidate the structure of the tector and secretory trichomes, have not been carried out before. Results obtained hereby are consistent to the one existing for other related species or for the Cucurbitaceae family. Christodoulakis *et al.* (2011) revealed similar structure of the tector trichomes for *E. elaterium* (L.) A. Rich. with the ones found for *B. alba* L.: pluricellurar body and a cup-like basis, also formed by multiple cells, less developed for *E. elaterium* (L.) A. Rich. Secretory trichomes appear to be more frequent for *B. alba* L. and have two

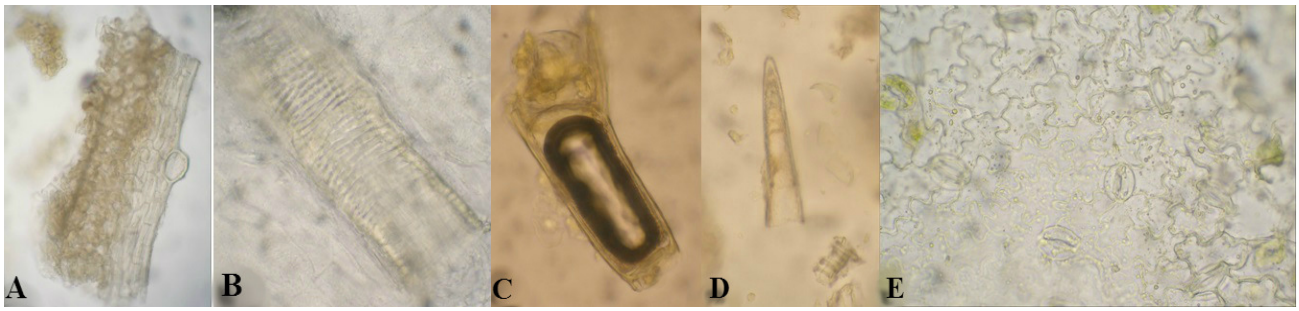

Fig. 10. Histological elements of the powder of root (A-B) and of leaves (C-E)

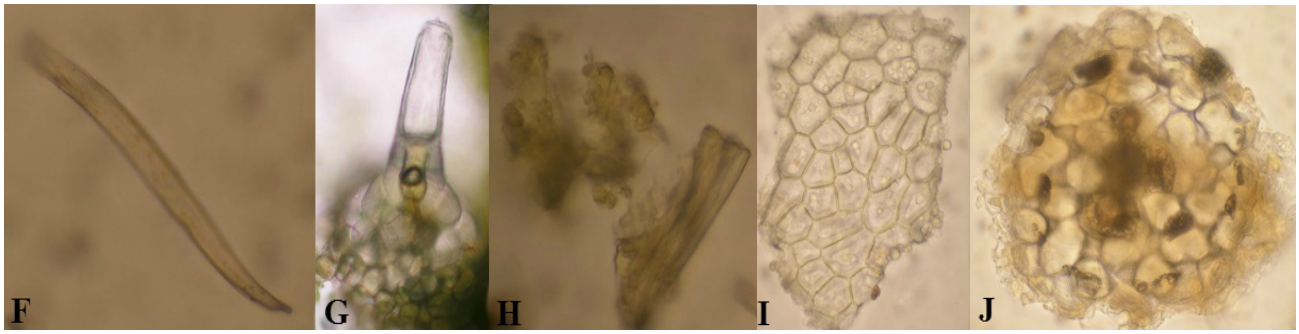

Fig. 11. Histological elements of the powder of aerial part (F-H) and of fruit (I-J)

different types, depending on their basis (multi-seried trichomes with one secretory cell on top, having unicellular basis and pluricellular basis), while the ones in *E. elaterium* (L.) A. Rich. have unicellular basis and a number of four secretory cells on top. Other SEM analysis confirming the relationship of *B. alba* L. with species in the same family were provided by Kolb and Müller (2004), which revealed the four types of trichomes in *Cucurbita pepo* L. subsp. *pepo* var. *styriaca*, of which type II resembles most with one type of secretory trichomes of *B. alba* L., multi-seried trichomes with one secretory cell on top, having unicellular basis. Furthermore, Ali and Al-Hemaid (2011) performed the analysis on the trichomes of some species of the family and revealed similarities with the ones found for *B. alba* L., highlighting the taxonomical importance of the trichomes in authentication of medical cucurbits.

Micrographic analysis gave supplementary arguments to confirm the results obtained in this study, which is the first that approaches this analysis for the species.

Not least, another element of novelty included in the study is the mixture of colorants used for the coloration, which is an original recipe and has not been used before for the double coloration of cellulose and lignin on the walls of the cells.

Conclusions

This study is bringing scientific evidence to support existing data on the morpho-anatomical structure of the root, stem and leaves belonging to the *B. alba* species, but also provides new data on the structure of reproductory organs (male flower petals, ovary). Also, by the SEM analysis, the study is bringing, for the first time, scientific evidence which might be helpful for the correct and complete identification of the tector and secretory trichomes found on the inferior surface of the leaf. The obtained data are important criteria for the macroscopic and microscopic characterization of the species. The results represent a first step towards the localization of the main active substances of this species. At the same time, data in this study represent important arguments to sustain taxonomic position of the *B. alba* L. species in the Cucurbitaceae family and can help establishing the relationships between species inside the family.

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References

Ali MA, Al-Hemaid FMA (2011). Taxonomic significance of trichomes micromorphology in cucurbits. Saudi J Biol Sci 18:87-92.

Baek NI, Dong WL, YOU HL, Shin II K, Goorgen VA (1995).. Cytotoxic constituents from the roots of *Bryonia alba* L. Natural Product Sciences 1(1):43-49.

Burrows GE, Shaik RS (2014). Comparative developmental anatomy of the taproot of the cucurbitaceous vines *Citrullus colocynthis* (perennial), *Citrullus lanatus* (annual) and *Cucumis myriocarpus* (annual). Aust J Bot 62:537-545.

Christodoulakis NS, Kollia K, Fasseas C (2011). Leaf structure and histochemistry of *Echallium elaterium* (L.) A.Rich. (squirting cucumber). Flora 206:191-197.

Crişan G, Ştefănescu C, Ielciu I, Mocan A (2013). Pharmaceutical botany: micro- and macroscopic identification of vegetal species. UMF "Iuliu Haţieganu" Press, Cluj-Napoca, pp 5-19.

Demarque D, Jouanny J, Poitevin B, Saint Jean Y (2007). Pharmacologie et matière médicale homeopathique. 3ième edition, France, CEDH Press, pp163-167.

European Pharmacopoeia, 8th edition (2014). Methods of analysis– Microscopic examination of herbal drugs.

Kocyan A, Zhang LB, Schaefer H, Renner SS (2007). A multi-locus chloroplast phylogeny for the Cucurbitaceae and its implications for character evolution and classification. Mol Phylogenet Evol 44:553-577.

Kolb D, Müller M (2004). Light, conventional and environmental scanning electron microscopy of the trichomes of *Cucurbita pepo* subsp. *pepo* var. *styriaca* and histochemistry of glandular secretory products. Ann Bot-London 94:515-526.

Konopa J, Zielinski J, Matuszkiewicz A (1974a). Cucurbitacins, cytotoxic and antitumor substances from *Bryonia alba* L. I: Isolation and identification. Arzneimittel-forsch 24:1554-1557.

Konopa J, Matuszkiewicz A, Hrabowska M, Onoszka K (1974b). Cucurbitacines, cytotoxic and antitumor substances from *Bryonia alba* L. Part II: Biological studies. Arzneimittel-forsch 24:1741-1743.

Manvi, Ganesh PG (2011). Evaluation of Pharmacognostical parameters and Hepatoprotective activity in *Bryonia alba* Linn. J Chem Pharm Res 3(6):99-109.

Nagy B, Mânzatu C, Măicăneanu A, Indolean C, Barbu-Tudoran L, Majdik C (2014). Linear and nonlinear regression analysis for heavy metals removal using *Agaricus bisporus* macrofungus. Arabian Journal of Chemistry Open Acces doi:10.1016/j.arabjc.2014.03.004

Park CS, Lim H, Han KJ, Baek SH, Sohn HO, Lee DW, Kim YG, Yun HY, Baek KJ, Kwon NS (2004). Inhibition of nitric oxide generation by 23,24-dihydrocucurbitacin D in mouse peritoneal macrophages. J Pharmacol Exp Ther 309(2):705-710.

Schaefer H, Renner SS (2011). Phylogenetic relationships in the order Cucurbitales and a new classification of the gourd family (Cucurbitaceae). Taxon 60(1):122-138.

Singh R, Rajasree PH, Sankar C (2012). Screening for anti-diabetic activity of the ethanolic extract of *Bryonia alba* roots. International Journal of Pharmacy and Biological Science 2(3):210-215.

Toma C, Rugină R (1998). Medicinal plants anatomy. Ed Academiei Române, Bucureşti, pp126-128 (in Romanian).

Tutin TG, Burges NA, Chater AO, Edmondson JR, Heywood VH, Moore DM, Valentine DH, Walters SM, Webb DA (2010). Flora Europea, vol. 3. Cambridge Univ Press. Cambridge London-New York Melbourne, pp 297-299.

Volz SM, Renner SS (2008). Hybridization, polyploidy and evolutionary transitions between monoecy and dioecy in *Bryonia* (Cucurbitaceae). Am J Bot 95(10):1297-1306.

Volz SM, Renner SS (2009). Phylogeography of the ancient Eurasian medicinal plant genus *Bryonia* (Cucurbitaceae) inferred from nuclear and chloroplast sequences. Taxon 58(2):550-560.