

SECRETORY STRUCTURES AT SPECIES OF *HYPERICUM* GENERA FROM BIHOR COUNTY, ROMANIA. NOTE I. VEGETATIVE ORGANS

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Abstract

The article is part of a complex experimental study which comprises morphology, anatomy and phytochemical research studies of several species of the *Hypericum* genera from Bihor district, Romania. By means of histo-anatomy techniques there were studied the secretory structures, namely the translucent glands (secretory pockets) and the black glands, the secretory canals type A and B, in the leaves and stems of four species of the *Hypericum* genera from Bihor county, Romania.

Rezumat

Articolul face parte dintr-un studiu experimental complex ce cuprinde cercetări de morfologie, anatomie și fitochimie ale mai multor specii ale genului *Hypericum* din județul Bihor, Romania. Prin tehnici de histo-anatomie s-au studiat structurile secretoare, și anume; glandele translucide (buzunare secretoare) și glandele negre, canalele secretoare de tip A și B, în frunzele și tulpinile a patru specii ale genului *Hypericum* din județul Bihor. În Farmacopeea Română Ediția a X-a (1993), pentru monografia *Hyperici herba* este menționată numai specia *H. perforatum* L., dar este posibil să se recolteze și alte specii din flora spontană din zona de deal și de munte, în primul rând *H. maculatum* Crantz. Aceste caractere histo-anatomice ar putea constitui criterii de identificare a substituirilor.

Keywords: secretory structures, stem, leaf, *Hypericum perforatum* L., *H. tetrapterum* Fries, *H. maculatum* Crantz, *H. hirsutum* L.

Introduction

In the „Flora of Romania” (1956) there are cited 13 spontaneous species belonging to the *Hypericum* genera and one cultivated [17], while Ciocârlan (2000) in „Romania’s Illustrated Flora” mentioned only 11 spontaneous species and one cultivated [5].

In the spontaneous flora of Bihor district, Romania there are specified the following species of *Hypericum*: *H. perforatum*, *H. tetrapterum*, *H. maculatum*, *H. montanum*, *H. humifusum*, *H. hirsutum* [2, 6, 15, 16].

Various species of the *Hypericum* genera were studied from a morphological [5, 8, 17], anatomical [4, 7, 8, 13, 21], phytotherapeutic [11, 12, 19, 20] and phytochemical point of view [1, 8, 9, 10, 12, 14, 18].

The present article is part of a complex experimental study in which there are comparatively studied certain aspects regarding the secretory structures and the secretion products [9, 10] of several species of the *Hypericum* genera from Bihor district, Romania. In the Romanian Pharmacopoeia 10th edition (1993), in the monograph *Hyperici herba* it is mentioned only the *H. perforatum* L species. However, it is possible to be harvest other species from the spontaneous flora from the hills and mountain area, as well, mostly *H. maculatum* Crantz. Their histo-anatomical characteristics could constitute criteria of identifying the substitutions [22].

Materials and methods

The aerial part from 4 species of *Hypericum* was harvested during the blooming period in June-July 2008 (Table I).

Table I

Species of the *Hypericum* genera from Bihor district, Romania and the areas of harvesting

No.	Species	Harvesting area	Date
1.	<i>Hypericum perforatum</i> L.	Tileagd	06.06.2008
2.	<i>Hypericum tetrapterum</i> Fries (<i>H. acutum</i> Munch., <i>H. quadrangulum</i> Crantz)	Pietroasa	17.07.2008
3.	<i>Hypericum maculatum</i> Crantz (<i>H. quadrangulum</i> L., <i>H. commulatum</i> Schur)	Stâna de Vale	17.07.2008
4.	<i>Hypericum hirsutum</i> L. (<i>H. villosum</i>)	Vadul Crișului	01.07.2008

For the histo-anatomic analysis fresh fragments of stems and leaves were fixed in the alcohol mixture: acetic acid (3:1) and preserved at 4⁰C. The cross-sections through stem and lamina were colored with Congo red and iodine green and were analysed with various sets of ocular-objective (10x and 4x; 10x and 10x; 10x and 40x), photographed with a Canon A550 camera, attached at the ocular of the microscope with an adapter. The photographs were processed using ACDSee Photo Manager software.

Results and discussion

The species of *Hypericum* from Romania's spontaneous flora are herbaceous plants, with ovate-lanceolate opposed leaves, which through transparency present several translucent zones corresponding to the secretory pockets [15].

Metcalf and Chalk (1957) studied the anatomic structure of the stem of *H. androsaemum* L. and *H. elodes* L. [13], while Toma and Rugină (2000) at *H. perforatum* L. [21]. According to the research of the authors above mentioned and of those achieved by ourselves, the structure of the stem is a secondary one due to the activity of the *cambium* which produces a consistent ring of secondary xilem towards the interior and a developed area of secondary *phloem* towards the exterior. The anatomy of the stem is similar to the species of the *Hypericum* genera taken into study by us (Fig.1).

The 4 species are distinguished by the number and the position of the longitudinal edges: 2 opposed ones at *H. perforatum* L. (Fig.1, a), 4 at *H. tetrapterum* Fries, these are with wings, longer and thinner and equal among themselves (Fig.1, b), at *H. maculatum* Crantz of which 2 opposed ones, more prominent and 2 more attenuated (Fig.1, c), while at *H. hirsutum* L. the stem is circular with 2 prominences slightly perceivable, with the epidermis with numerous nonramified bicellular tector trihomes (the basis and the body), uniformly disposed (Fig.1, d). This type of bicellular trihomes which cover the stem and leaves of *H. hirsutum* L. were discovered at other species as well [3].

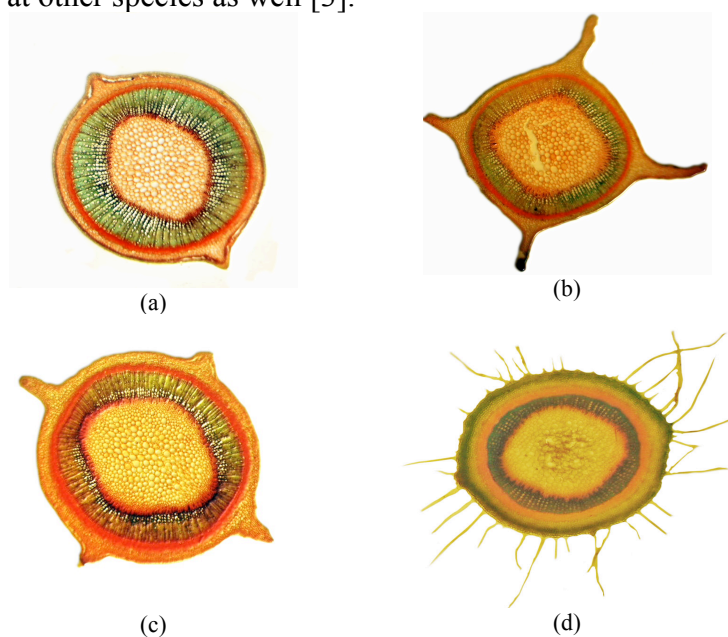


Figure 1

Anatomy of the stem (40x) and longitudinal edges: a) *H. perforatum* L., b) *H. tetrapterum* Fries, c) *H. maculatum* Crantz, d) *H. hirsutum* L.

In the histological structure of the stem there are present secretory structures. Thus, the type B secretory canals, from the subepidermic area are present at *H. perforatum* L. (Fig.2) and also at *H. tetrapterum* Fries, but there are not present at *H. maculatum* Crantz as well as at *H. hirsutum* L. Ciccarelli *et al.* (2001) performed research studies on the *H. perforatum* L. species, the composition, the ontogenetic development and the secretion products (resines, lipides, alkaloids, volatile oil and tannins) of this type of canals [4].

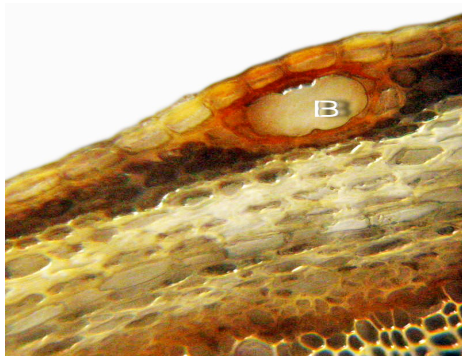


Figure 2

Type B canal in the stem of *H. perforatum* L.(400x)

In the secondary phloem of the stems of all 4 species studied by us, type A secretory canals are present (according to the description and the classification of Ciccarelli *et al.*, 2001) [4]. In figure 3, type A canals can be observed in the secondary phloem of the stem of *H. hirsutum* L. The first mentioning of the presence of some secretory structures in the secondary phloem of the stem of *H. elodes* L. were made by Metcalfe and Chalk (1957) [13].

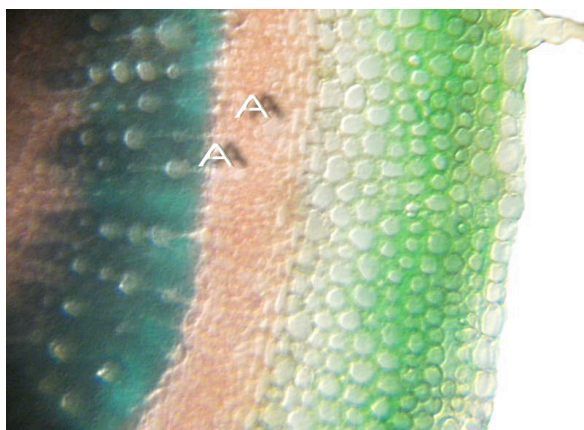


Figure 3

Type A canals in the secondary phloem of *H.hirsutum* L. stem (400x).

In what concerns the black glands, which produce the hypericin, described by Curtis and Lersten (1990), these are placed under the epidermis at the level of the stems [7]. They are present at the first 3 species taken into study, but they are missing at *H. hirsutum* L. At the species *H. maculatum* Crantz there were noticed, besides the subepidermic glands described until now (Fig.4, A), a new type of black glands, not specified until the present date in the specialty literature, which covers the edge like a glove (Fig.4, B).

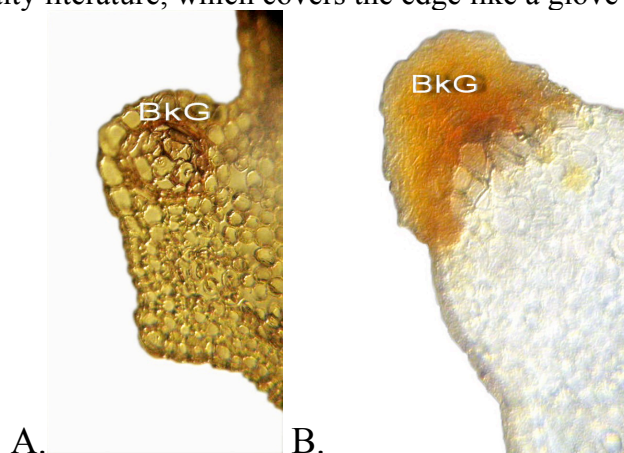


Figure 4

Black glands (BkG) in *H. maculatum* Crantz stem (400x)

From the analysis of the structure of the leaves in cross-section it was noticed that all the 4 species present a lamina with a bifacial structure, with a palisadic tissue towards the upper part, formed of 1-2 layers of cells and a lacunous one, more reduced towards the inferior part, and the epidermic cells are large, having the cuticula well developed. The secretory pockets are of higher dimensions and they extend all over the bast thickness (Fig.5). To be noted that such secretory formations are found only in the *lamina*, between the two *epidermae*.

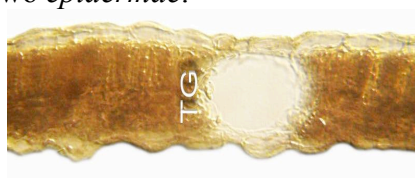


Figure 5

Translucent gland in the *lamina* of *H. tetrapterum* Fries (100x)

The secretory glands of hypericin are found in the mezophyll on the margins of the leaves, most of them being placed towards the top of the *lamina*. In the primary phloem from the conducting fascicles of the nervures

in the *lamina*, at all species taken into study, there was observed the presence of the secretory canals type A (Fig.6).

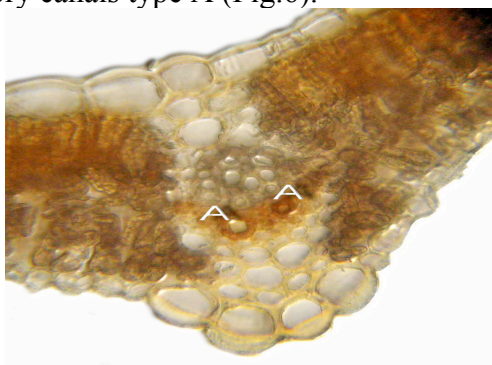


Figure 6

Type A canals in the primary phloem from the vascular bundles from the *lamina* nervation at *H. perforatum* L.(100x).

Table II centralizes the data referring to the secretory structures present in the stems and leaves of the species of the *Hypericum* genera taken into study.

Table II

Secretory structures at 4 species of *Hypericum* from Bihor county, Romania

Vegetative organs	Species				
	Secretory structures	<i>H.perforatum</i> L.	<i>H.tetrapterum</i> Fries	<i>H. maculatum</i> Crantz	<i>H. hirsutum</i> L.
Stem	Type A canal	YES	YES	YES	YES
	Type B canal	YES	YES	NO	NO
	Black glands	YES	YES	YES	NO
Leaf	Type A canal	YES	YES	YES	YES
	Translucent glands	YES	YES	YES	YES
	Black glands	YES	YES	YES	YES

Conclusions

According to the anatomic structure of the stem, the species of *Hypericum* studied can be differentiated according to the number, the position and the dimension of the longitudinal edges.

In the subepidermic area of the stem, only at *H. perforatum* L. and *H. tetrapterum* Fries, there are secretory canals type B and in the area of the

secondary phloem, at all species taken into study, the type A secretory canals are present.

The black glands, which produce the hypericina, are placed at the level of the stems under the epidermis. They are present at the first 3 species taken into study, but they are missing at *H. hirsutum* L.

The secretory glands of hypericina are to be found at all species in the mezophyll on the margins of the leaves, most of them being placed towards the peak of the *lamina*.

At all species taken into study, in the primary *phloem* within the conducting fascicles from the nervures of the *lamina*, type A secretory canals are present.

In the foliar mezophyll of *lamina*, secretory pockets can be found in all species taken into study.

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