LOCALIZATION AND DISTRIBUTION OF HYPERICIN IN THE VEGETATIVE AND FLORAL ORGANS OF THE *HYPERICUM* SPECIES

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Abstract

The following study presents the localization of hypericin in the secretory tissues of various plant organs (stem, leaves, sepals and petals) of the following Hypericum species found in the spontaneous flora of the Bihor county: H. hirsutum L., H. maculatum Crantz - variety typicum Frohlich, H. perforatum L.

We observed the influence of secretory tissues on the content of hypericin depending on their absence or presence in various vegetative and floral organs. Hypericin was identified in the different plant organs through thin layer chromatography, while quantitative determination was performed through the technique of spectrophotometry presented in the European Pharmacopoeia (2008).

Key words: *H. perforatum*, *H. maculatum*, *H. hirsutum*, hypericin, black glands, CSS.

INTRODUCTION

Hypericum species are characterized by the presence of several types of secretory structures: **transparent (or translucent) bags, secretory canals and black secretory glands (point shaped nodules).** Not all these structures are present in every *Hypericum* species, a fact that was noticed through both macroscopic and microscopic analysis (Gîtea D. et all., 2011). Of these secretory structures, the canals and the black secretory glands are characteristic to the *Hypericum* genus, these being the places where hypericins are synthesized (Gîtea D. 2010; Sipos M.).

Knowledge about the histological and anatomical structure of *Hypericum perforatum*'s secretory tissues are old and incomplete. More recently, Curtis and Lersten (1990) have supplied data about the t**ranslucent glands** and **black nodules** found on the leaves and petals of some *H. perforatum* varieties from northern America, while Baroni Fornasiero and collaborators (1998) have studied the black nodules found on the leaves of some Italian varieties (Curtis J.D., Lersten N.R., 1990; Zobaed S.M.A., et all, 2006; Baroni Fornasiero R., Bianchi A, 1998)

In a study performed on the secretory tissues of *H. perforatum*, D. Ciccarelli and collaborators (2001) distinguish between 3 types of secretory tissues in St. John's wort: **translucent glands and cavities,** an equivalent of secretory

bags, **three types of secretory canals – type A, B, C** and **black nodules** (Ciccarelli D. et all, 2001)

MATERIAL AND METHOD

The plant material was collected from the spontaneous flora of the Bihor county, during June and July of 2011, from various areas (Ciocârlan V., 2000)

The presence of some black secretory glands and canals in various organs of *Hypericum* species led to the localization of hypericin in these organs.

In the case of *H. hirsutum* L. a histological localization of hypericin was performed in the secretory glands found on sepals, for *H.maculatum* hypericin was localized in the black secretory canals found on petals, whereas for *H.perforatum* – in the black point-shaped secretory glands found on the edge of the leaves.

In order to observe the distribution of hypericin in various organs of the freshly harvested plants, we separated, with a pair of pincers, the sepals, petals, leaves and also the stems, which were sectioned into small fragments. The separated organs (about 1g) were extracted with 10 ml methyl alcohol, on water bath at 80ºC for 15 minutes, and the obtained methanol solutions were filtered.

The methanol solutions thus obtained confirmed the presence of hypericin through thin layer chromatography in the following experimental conditions (Tămaş M., 2001; Oniga Ilioara et all, 2008; Jork H., et all., 1990)

Stationary phase: - silica gel plates GF 254 (Merk), of 10x10 cm and 0,25 mm thickness.

Mobile phase: - formic acid: water : ethyl acetate in rapport of 10: 15: 85 V/V/V

Application: - 20µL of test solutions and 10µL of reference solution applied as linear spots of 15 x 3 mm.

Migration: 12 cm

Plate drying: 100-105ºC for 10 minutes.

Detection: - the plate is sprayed with a solution of 10g/l reactive substance NEU (diphenylboric acid 2-aminoethyl ester) in methanol and then with a solution of 50g/l of polyethylene glycol 4000 in methanol. After 30 minutes the plate is examined in UV light at 365nm, and afterward it is photographed in UV light as well as in daylight.

The organs analyzed for the localization of hypericin were examined with a stereo-microscope on filter paper and the secretory tissues were pressed with the very sharp tip of a spatula needle. Reddish-violet colored dots caused by hypericin subsequently appeared on the filter paper; the

shred filter paper was then subjected to hot extraction with 2 ml of methanol and the obtained solutions were referred to chromatographic analysis.

RESULTS AND DISSCUSIONS

In the case of the *H.hirsutum* species, the secretory glands are large, spherically shaped and dark colored; moreover, they are attached to a pedicel in the extension of the lobes (fimbriae) on the edge and tip of the sepals.

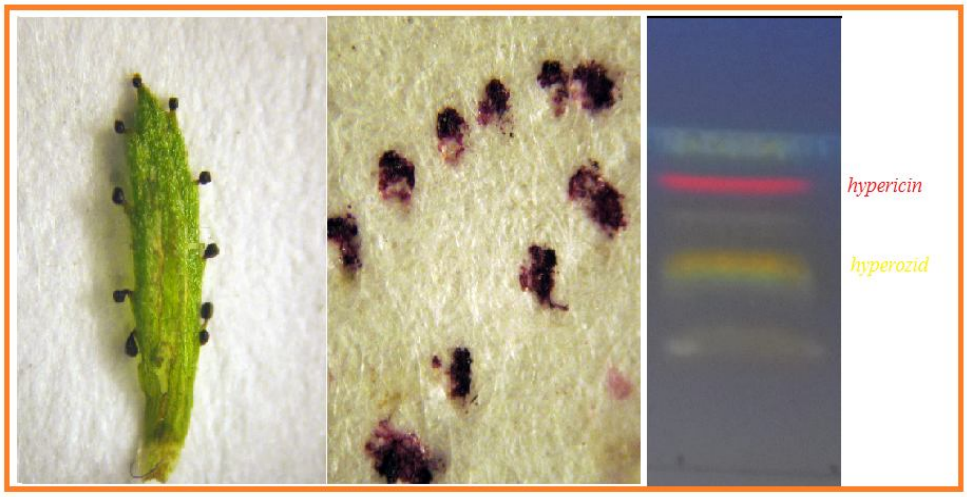

Fig.1. Detail of sepal with secretory glands on its edges (*H. hirsutum)*; red-violet hypericin dots on filter paper; CSS hypericin in UV light: filter paper with pressed glands

The examination of the chromatogram regarding the content analysis of *H.hirsutum* L. secretory glands revealed a red fluorescence at Rf=0,80, characteristic of hypericin, but also the presence of flavonoids (hyperoside), with yellow fluorescence and phenylpropanoid compounds with blue-gray fluorescence (Fig.1). Hypericin was similarly identified in the case of chromatograms obtained from extractive solutions resulted from pressing the secretory canals of *H.maculatum* petals and the point-shaped glands found on the edges of *H.perforatum petals*.

As for the study regarding the distribution of hypericin in the vegetative and floral organs of *H. hirsutum* L., CSS analysis was performed for the 4 extractive alcoholic solutions from stem, leaves, sepals and petals of this species. The obtained chromatogram is presented in fig. 2.

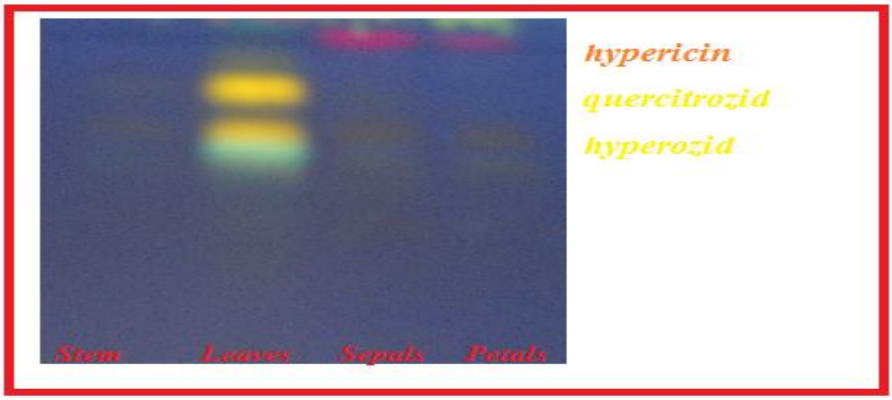

Fig.2. CSS UV hypericin: methanol extract from *H. hirsutum* L. stems, leaves, sepals and petals

Concerning *H. hirsutum* L., the highest quantity of hypericin is present in the sepals, due to the secretory glands found on their edges, while the flovonoid compounds are present in the leaves. Hypericin is present in the petals too, but in a much smaller quantity, and it cannot be found in stems and leaves. This is the first histological localization of hypericin for the *H. hirsutum* L. species.

As for the *H. maculatum* Crantz species, the black secretory canals are distributed on the entire surface of the petals as dots or strips and less on leaves and sepals, whereas in the case of *H. perforatum* L. the black secretory glands can only be found on the edge of the petals and less on leaves and sepals.

Fig. 3. Sepals and petals of *H. maculatum;* enlarged petals of *H. perforatum* with black secretory glands on edges

The identification of hypericin by CSS was performed through methanol extracts obtained from the stem, leaves, sepals and petals of the *H. maculatum* Crantz and *H. perforatum* L. species. It must be mentioned that the highest quantity of hypericin is present in the petals. The

chromatography plate obtained from methanol extracts from the stem, leaves, sepals and petals of *H. maculatum* Crantz is presented in fig. 4.

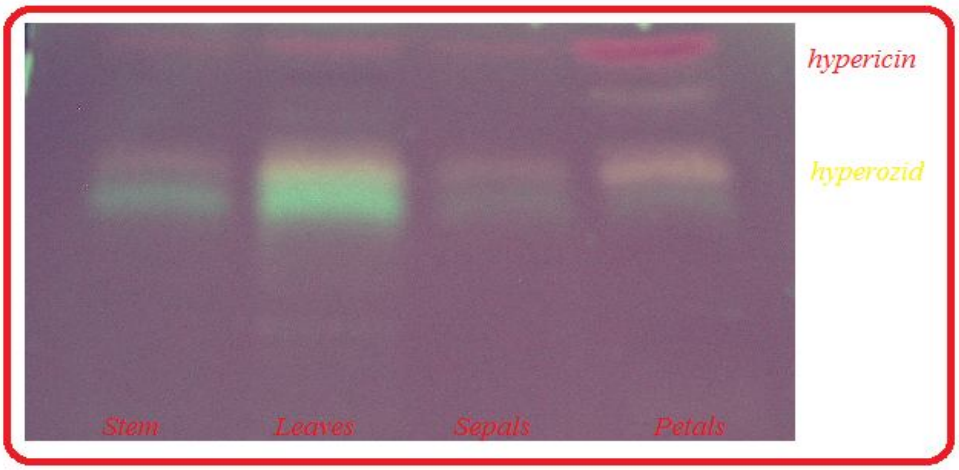

Fig.4. CSS UV hypericin: methanol extract from the stem, leaves, sepals and petals of *H. maculatum* Crantz

In the case of *H. perforatum* L., hypericin is present in all the analyzed plant organs, but the highest concentration is in the petals, followed by sepals, leaves and stems.

CONCLUSIONS

After observing the distribution of hypericin, the conclusion is that in the case of the *H. hirsutum* L. species, the highest quantity of hypericin is present in the sepals, whereas the highest quantity of hypericin for *H. maculatum* Crantz and *H. perforatum* L. is in the petals.

The leaves and stem of *H. maculatum* Crantz and *H. perforatum* L contain a small quantity of hypericin. The stem and leaves of *H. hirsutum* L. do not contain hypericin, as they do not have black glands, containing only flavones (Tab. 1).

Table 1

Distribution of hypericin in vegetative and floral organs

Species	**Stems**	**Leaves**	**Sepals**	**Petals**
H. perforatum L.	+	++	++	+++
H. maculatum Crantz	+	++	+	++++
H. hirsutum L.	-	-	+++	++

The analyzed species of *Hypericum* show various distributions of hypericin, in correlation with the presence of secretory structures; this also constitutes one of the main criteria of distinguishing between species.

REFERENCES

1. Gîtea D., Şipoş M., Tămaş M., Paşca B., Secretory structures at species of *Hypericum* genera from Bihor county, Romania. Note I. Vegetative organs, *Farmacia*, 2011, 59(3), 424-431.
2. Gîtea Daniela, Teză de doctorat: Cercetări farmacobotanice comparative asupra unor specii de *Hypericum* din județul Bihor - UMF „Iuliu Haţieganu" Cluj-Napoca, 15.06.2010, îndrumător prof. Dr. Tămaş Mircea.
3. Monica Şipoş, Daniela Gîtea, Secretory structures in species of the *Hypericum* genus from Bihor county. Note II. Flowers, *Farmacia*, sub tipar.
4. Curtis J.D., Lersten N.R.: Internal secretory structures in *Hypericum* (Clusiaceae): *H. perforatum* L., and *H.balearicum* L.*, New Phytologist,* 114, 1990, p.571-580;
5. Zobaed S.M.A., Afreen F., Goto E., Kozai T. Plant-evironment interactions: accumulation of hypericin in dark glands of *Hypericum perforatum*, *Annals of Botany*, 2006, 98, 793-804.
6. Baroni Fornasiero R., Bianchi A.: Anatomical and ultrastructural observations in *Hypericum perforatum* L. leaves, *Journal of Herbs, Species and Medical Plants,*5, 1998, p.21-33;
7. Ciccarelli D., Andreucci A.C., Pagini A.M., Translucent glands and secretory canals in *Hypericum perforatum* L.(*Hypericaceae*): morphological, anatomical and histochemical studies during the course of ontogenesis, *Annals of Botany*, 2001, 88, 637-644.
8. Ciocârlan V., Flora ilustrată a României, Editura Ceres, Bucureşti, 2000, 498-501.
9. Tămaş M., Drăgulescu C., Oniga Ilioara, Gliga Florina: Comparative phytochemical research on some species of *Hypericum* and populations of *H. perforatum* L. in România*,* Acta oecologica, vol. VIII, 2001, p.25-33;
10. Oniga Ilioara, Tămaş M., Vlase L., Toiu A., Benedec Daniela, Jula R.: Botanical and phytochimical studies of *Hypericum perforatum* L. and *H. maculatum* Crantz from Romania, Proreding of the Vth Conf. AMAPSEEC, Bruno 2008, p.1-6;
11. Jork H., Funk W., Fischer W., Wimer H.: Thin Layer Cromatography, Reagents and Detection Methods, vol. I, 1990, p.277-280;
12. *** European Pharmacopoeia ed. VI, vol. 2, Council of Europe Strasbourg, 2008, p.2958-2959;