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Secretory Structures in Vegetative and Floral Organs of *Hypericum perfoliatum*

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Hypericum L, the largest genus of Hypericaceae comprising c*a*. 484 species of shrubs and perennial and annual herbs, is worldwide in a large variety of habitats in subtropical and temperate areas [1]. *Hypericum* species, namely *H. perforatum* (St. John's wort) the most representative species of the genus, have been used in folk medicine thought the centuries for a large number of ailments. Nowadays, it is well known the therapeutic potentialities of their main compounds, hypericin, pseudohypericin and hyperforin, which justify its clinical use [2]. Despite the intense phytochemical and pharmacological research conducted in *Hypericum* species during the last decades, morpho-anatomical studies on the glands that produce the bioactive compounds are scarce and fragmented, only *H. perforatum* was studied in detail [3, 4, 5, 6].As part as an ongoing project on *Hypericum* glands, the present research aims to provide information about the morphology, anatomy and histochemistry of the secretory structures present on the aerial organs of *H. perfoliatum,* one of the seventeen species of *Hypericum* that occur wild in Portugal.

The types of glandular structures and their pattern of distribution on the leaves and flowers were studied by light (MO) and scanning electron microscopy (SEM). Samples for SEM were fixed with glutaraldehyde, dehydrated in a graded acetone series, critical-point dried and coated with gold. For general anatomy samples were fixed in the same fixative and embedded in Leica historesin®. Histochemical tests and standard control procedures were carried out in fresh material to localize *in situ* the main chemical classes of compounds present in the secretion. Observations were carried out Observations were carried out on a JEOL T220 scanning electron microscope and with a Leica DM-2500 microscope.

The aerial organs of *H. perfoliatum* present four different types of secretory structures (idioblasts, translucent glands, ducts and black nodules), that can occur exclusively in a specific organ or in more than one organ. Tanniniferous secretory cells are frequent in the epidermis, as well as in the ground parenchyma of all organs, where they are scattered together with crystal idioblasts containing druses of calcium oxalate. Translucent glands are spheroidal subepidermical glandular pockets delimited by two or three cell layers of fattened and densely-stained cells (Fig. 1A). They are typically found in the leaves, giving them a perforated appearance. Two types of secretory ducts, cavities that differ from translucent glands in the length, are present in the vegetative and floral organs. Type A ducts have a narrow lumen delimited by four secretory epithelial cells and occur associated to the phloem in all aerial organs with exception of stamens (Fig. 1B, arrow). Type B ducts have a wider lumen, are generally limited by ten thin-walled secretory cells surrounding by a sheath of thick-walled cells and are located in the parenchyma of sepals, petals and ovary. Black nodules are clusters of cells lacking a central intercellular space (lumen), surrounded by one or two-layers of flat cells of a delimiting sheath (Fig. 1C). The inner cells are large, irregular, tightly packed and filled with a dark red stained content. Spheroidal black nodules are found punctuating the leaf margins and in the connective tissue of the stamen (Fig. 1D), whereas long-shaped black nodules are distributed across the lamina of bracts, sepals and petals. Peculiar glandular emergences, which look like peduncular black nodules, are present along the margin of the bracts and sepals. They consist of a multicellular peduncle and a dark-red multicellular secretory head-a black nodule (Fig. 1E). Histochemical tests showed that translucent glands secreted essential oils rich in phenolic compounds (flavonolic aglycones), ducts produce oleoresins and nodules contain essentially hypericin. In mature organs, the disorganization of the inner cells of the nodules seems to form a large intercellular space, a lumen.

All these secretory structures were also found in *H. perforatum* with exception of peduncular black nodules [4, 5], that was only reported in *H. elodes*, but not studied in detail [7]. The obtained results allow as speculating

that nodules may be primitive multicellular structures, relics of an evolutionary process, that give rise to cavities, internal secretory structures that stores secretion material in intercellular spaces.

References

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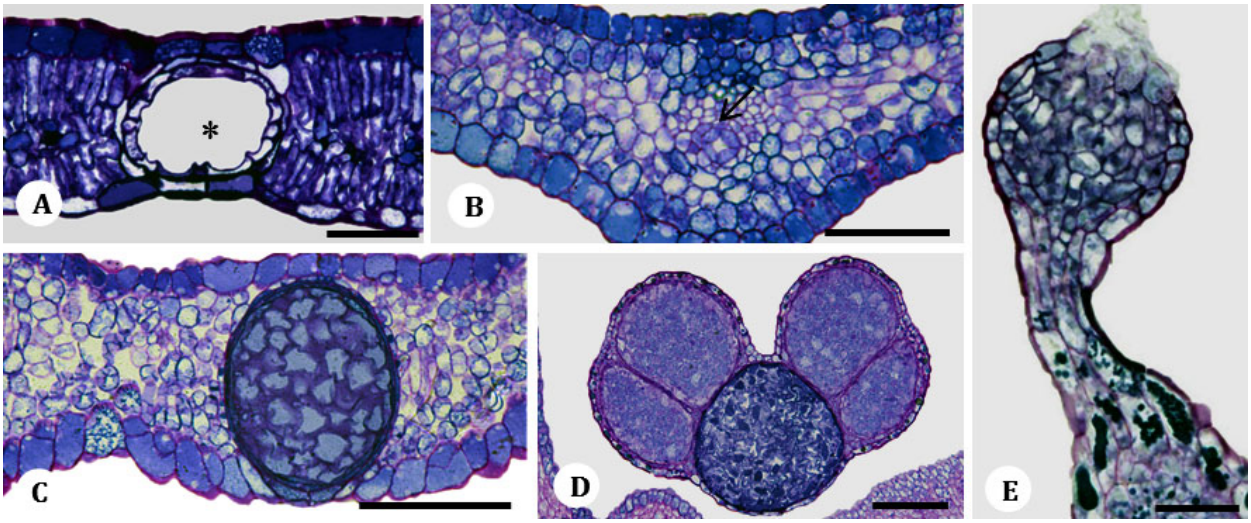

Figure 1. Secretory structures in *H. perfoliatum* organs. Light micrographs of historesin sections stained with PAS/Toluidine Blue *O.*A-B**,** Cross sections of leaves. A, Translucent gland with a large lumen (asterisk). B, A narrow type A duct associated to the phloem of the leaf main vein (arrow).C, Laminar black nodule on a leaf longitudinal section. D**,** Cross section of an anther showing a black nodule in the connective tissue. E**,** Detail of a nodular emergence (a peduncular black nodule) on the sepal margin. Bars = 80μm (A-D); Bars = 40μm (E).

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