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A Quantitative Morphological Analysis of Some *Hypericum* Species

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Abstract

Hypericum perforatum L. (Hypericaceae) is a medicinal plant of considerable interest for the therapeutic potentialities of its biologically active compounds. Due to the presence of hybrids and frequent adulterants from other species of *Hypericum*, the identification of the drug obtained of this species is difficult. Therefore, a quantitative morphological analysis of the leaf epidermises of *H. hircinum* L. and *H. perfoliatum* L. compared with *H. perforatum* L., carried out by means of scanning electron microscopy and image analysis, was performed to identify phytognostic markers useful for the characterization of these different *Hypericum* species. Size and shape parameters of the leaf surface cells have permitted a comparative study of the cogeneric species examined, providing a key factor in their recognition and/or selection. Unlike the methods employed so far, the results obtained by means of this innovative kind of analysis supply a valid criterion, not only for the morphological differentiation of the Italian *Hypericum* species studied, but also for an accurate and reproducible quality control of the commercial samples, often made up of drugs obtained from different species, subspecies and varieties.

Keywords: *Hypericum perforatum* L., *Hypericum hircinum* L., *Hypericum perfoliatum* L., commercial samples, quality control, leaf epidermises, micromorphometric analysis.

Introduction

Hypericum perforatum L. (Hypericaceae) is a medicinal plant of considerable interest for the therapeutic potentialities of its biologically active compounds, hypericin and pseudohypericin being the most prominent ones. Extracts from St. John's wort (*H. perforatum*) show anti-depressive activity that justifies their clinical use (Linde et al., 1996).

Chemical investigations carried out on different *Hypericum* species have pointed out a wide range of phytoconstituents such as flavonoids (Berghöfer & Hölzl, 1987; Sakar et al., 1991), hypericins (Gray et al., 2000), the content of which shows intraspecific extreme variability, xanthones (Ishiguro et al., 1995ab, 1996), phloroglucinol derivatives (Rocha et al., 1995, 1996) among which hyperforin regardes as responsable for the antidepressive activity (Chatterjee et al., 1998).

As the identification of the drug obtained of this species is difficult due to the presence of hybrids and frequent adulterants from other species of *Hypericum*, a quantitative morphological analysis of the leaf epidermises of *H. hircinum* L. and *H. perfoliatum* L. compared with *H. perforatum* L., carried out by means of scanning electron microscopy and image analysis, was performed to identify phytognostic markers useful for the characterization of these different *Hypericum* species; these markers often prove to be indispensable for the identification and quality control of the vegetable drugs (Rapisarda et al., 1996ab, 1998).

Materials and methods

Leaves of *Hypericum hircinum* L, *H. perfoliatum* L. and *H. perforatum* L., were collected in the neighbourhood of Messina in March, 1998. The plants were botanically confirmed using available literature (Pignatti, 1982) by Prof. G. Gramuglio, plant taxonomist. Voucher specimens of the plants were deposited in the herbarium of the Pharmaco-Biological Department of the University of Messina in Italy.

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For each species we examined 10 samples of fully developed large fresh leaves, all picked from the main branches. Fragments about 5 mm square of the freshly-picked leaves were taken from the vein-islet of the central area, the vein-islet near the margin and from the vein-islet near the midrib of the lamina. The samples were fixed by infiltration with 5% glutaraldehyde, buffered with 0.1 M Na-cacodylate at pH 7.2 for 12 h, washed with the same buffer, postfixed in 1% osmium tetraoxide in the same buffer at room temperature for 2 h, washed with the same buffer and dehydrated in ethyl alcohol. They were critical-point dried with liquid CO_2 and then coated with gold in a Polaron E5100 SEM Coating Unit. Observations were carried out with a SEM Philips mod. 500. The micrographs obtained at magnification ×320 were investigated with an Image Analyzer (IA) VIDAS 2.1 Zeiss.

Preliminary tests have indicated that 100 microscopic fields in all, constituted of 70 taken from the vein-islet of the central area, 15 from the vein-islet near the margin and 15 from the vein-islet near the midrib of the lamina, are sufficient to statistically represent the cell distribution of both leaf surfaces of each sample examined (Rapisarda et al., 1996a,b).

Results and discussion

In this study, we examined the external tangential wall of the epidermal cells of both leaf surfaces of *H. hircinum* L, *H. perfoliatum* L. and *H. perforatum* L., obtaining with the IA parameters that can be divided into two groups; the first covers size measurement, the second various aspects of shape (Fig. 1). The most obvious size measurement (Russ, 1989, 1990) is the area (a) of a feature, the external tangential wall of the cells in our case, converted to the appropriate real units of measure (square microns); by this value the equivalent circular diameter (D_C) of all the examined cells was obtained, calculated simply as $\sqrt{(4 \cdot a)/\pi}$, because a linear measure of size is often more useful than the area and it is common to convert the measured area to the equivalent circular diameter. Other parameters, like the linear perimeter (lp), the

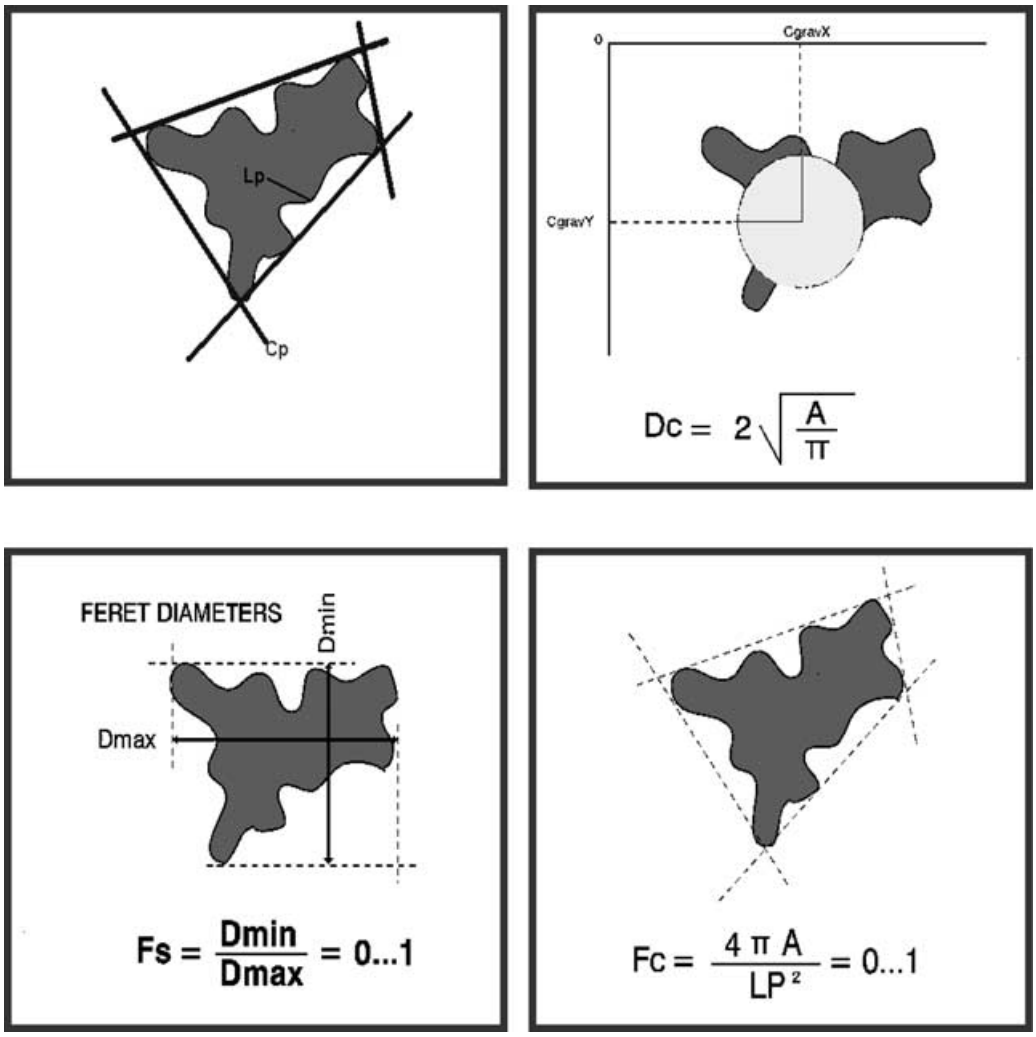

Figure 1. Main shape (*Fs, Fc*) and size (*Dc*) parameters of external tangential wall of the leaf epidermal cells.

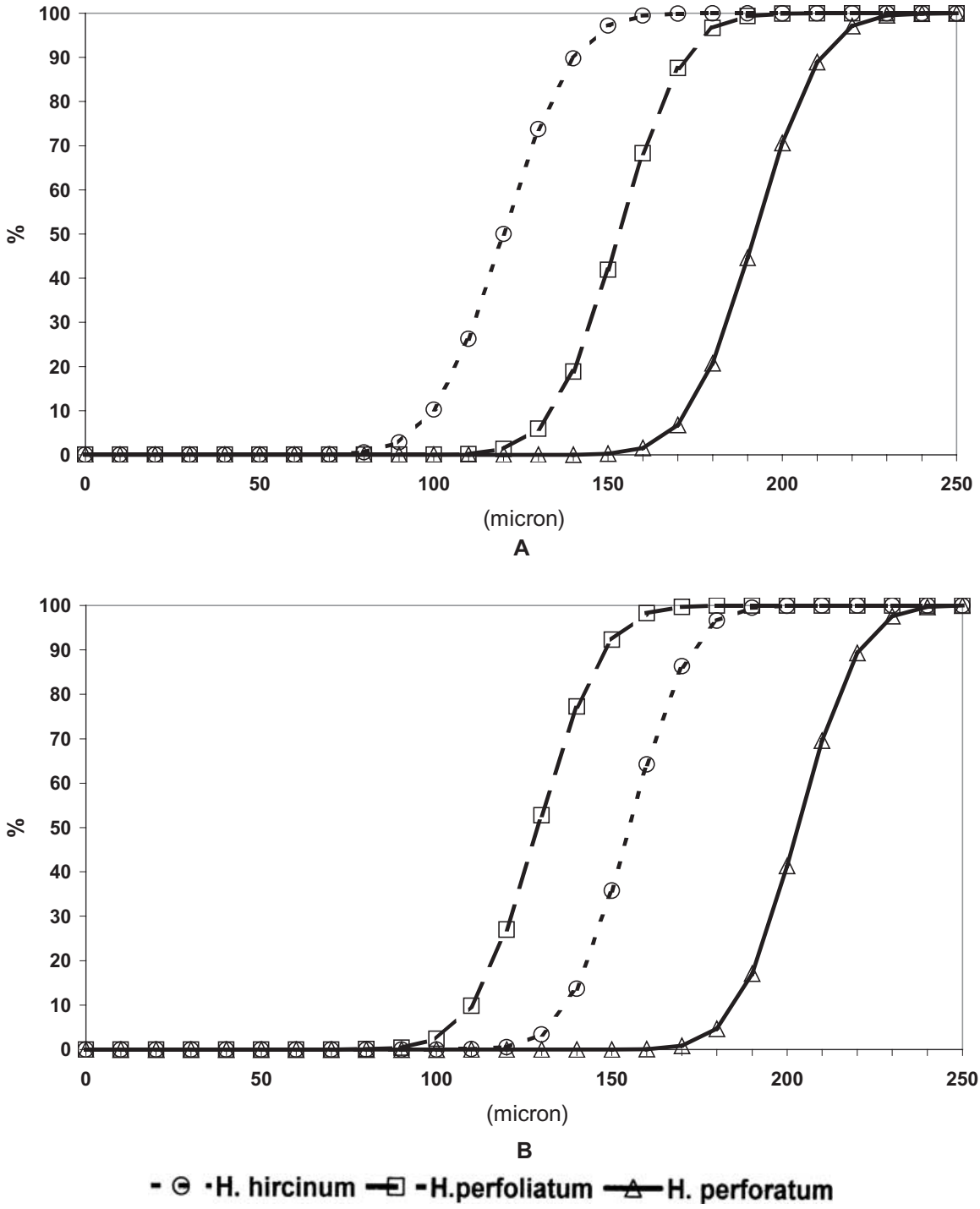

Figure 2. Leaf epidermal cells. Dc of external tangential wall (A: upper epidermis; B: lower epidermis).

longest (d_{max}) and the shortest (d_{min}) of the Feret diameters (Fd) measured in 32 different directions at an angular resolution of 5.7° were obtained mostly to be used in the calculation of the shape factors.

The shape factors (Russ, 1989, 1990), like the aspect ratio (F_S) of the cells defined as d_{min}/d_{max}, the circularity shape factor (F_C) defined as ($4\pi a/lp^2$) the values of which range between close to 0 for very elongated or rough cells and 1 for circular cells, were then also calculated. These latter parameters are generally undimensionless numbers, and are usually generated by combining size parameters in various ways.

All the conventional statistical data, like mean, median, variance, standard deviation, skewness and kurtosis were provided by the IA. The Kolmogorov-Smirnov test (Russ, 1990), was applied to the curves of distribution of the main

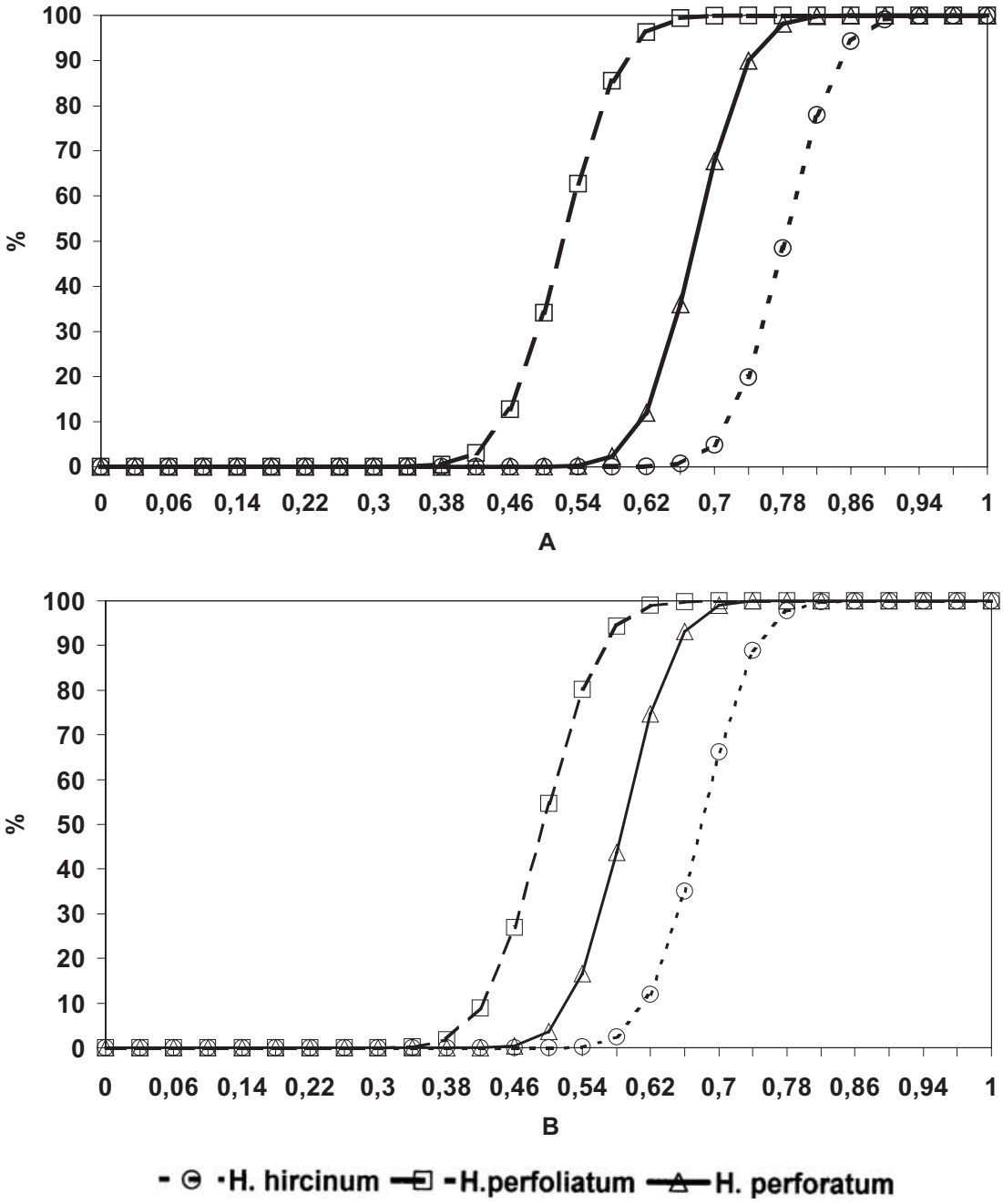

Figure 3. Leaf epidermal cells. Fs of external tangential wall (A: upper epidermis; B: lower epidermis).

shape and size parameters of the epidermal cells of the *Hypericum* leaves investigated.

For the derived morphometric parameters, the results obtained (Figs. 2, 3 and 4) show significant differences of the main shape (*Fs, Fc*) and size (*Dc*) parameters of the epidermal cells among *Hypericum* leaves investigated by means of the Kolmogorov-Smirnov test. The cumulative curves show, not only the accurate interpolation of the different values of 2000 cells as provided by IA, but above all the shape parameters that can easily be used to characterize the cells both of the upper and of the lower epidermises of the leaves of *H. hircinum, H. perfoliatum* in comparison with the leaves of *H. perforatum*.

These results permitted a comparative study of the cogeneric species examined, providing a key factor in their recognition and/or selection. Unlike the methods employed so far, this innovative kind of analysis represents a fast method that supply a valid criterion not only for the morphological differentiation of the *Hypericum* species studied, but also for an accurate and reproducible quality control of the commercial samples, often made up of drugs obtained from different species, subspecies and varieties.

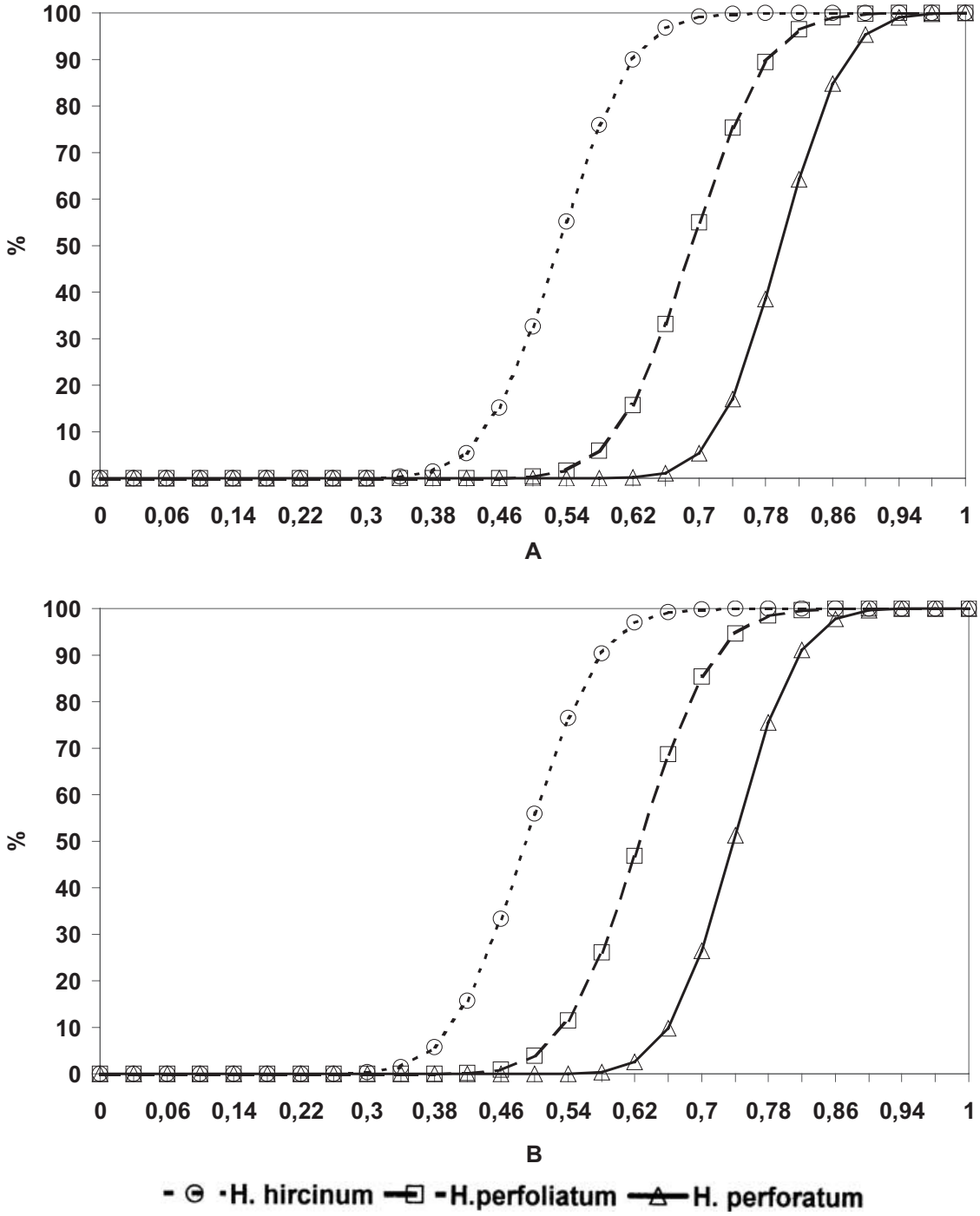

Figure 4. Leaf epidermal cells. Fc of external tangential wall (A: upper epidermis; B: lower epidermis).

In conclusion, we think that the quantitative morphological analysis of external tangential wall of the epidermal cells of both leaf surfaces could accompany the chemical analytical method utilized for *Hypericum* identification, because this depends on the presence of naphtodianthrones as marker compounds, the concentrations of which, as well as the other constituents, can vary depending on the growth environment, harvest time, drying procedures, storage conditions, and so on (Constantine & Karchesy, 1998).

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