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# Rendiconti

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## Studies in plant morphology by scanning electron microscopy and applications to plant species of pharmaceutical interest. Inflorescences of Arnica montana L. subsp. montana

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# SEZIONE III

**(Botanica, zoologia, fisiologia e patologia)**

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**Botanica.** — *Studies in plant morphology by scanning electron microscopy and applications to plant species of pharmaceutical interest. Inflorescences of* Arnica montana *L. subsp.* montana. Nota di ELSA MARIELLA CAPPELLETTI (*), presentata (**) dal Socio C. CAPPELLETTI.

RIASSUNTO. — Vengono descritti i microcaratteri delle superfici epidermiche delle brattee involucrali, dei fiori ligulati e dei fiori tubulosi di *Arnica montana* L. subsp. *montana* ed è discussa la loro potenziale importanza ai fini tassonomici e diagnostici della droga.

## INTRODUCTION

Some surface features of the epidermal cells, namely cuticle ornamentations, have been regarded as valuable diagnostic characters for many vegetable drugs [1, 3, 7, 8, 9, 11, 13, 18, 19, 20, 25].

Previous investigations have shown that the microcharacters evidenced by surface scanning of the epidermises may actually be important diagnostic features not only for powdered leaves [17], but also in the case of powdered capitula of *Compositae* [4].

The flower heads of *Arnica montana* are reported to occur sometimes admixed with those of two adulterant species: *Calendula officinalis* L. and *Anthemis tinctoria* L. [5, 10, 25].

Since previous works [4, 17] have shown that only an accurate study of not shrunk epidermises allows a correct interpretation of the distorted structures occurring in air-dried drugs, the surface microcharacters of the epidermises as apparent in fixed and gradually dehydrated samples, have been first taken into account.

Therefore, in the present paper the microcharacters of the *Arnica* inflorescences have been investigated by SEM. In a subsequent work the flower heads of *Calendula* and *Anthemis* will be described, while in a third one the possibility of a botanical identification of *Arnica* from the two adulterant species in dried, powdered samples will be discussed.

## MATERIAL AND METHODS

Arnica inflorescences were collected from spontaneous specimens of *Arnica montana* L. subsp. *montana* growing on Mount Bondone (Trento, NE Italy).

The following parts of the flower heads have been considered: involucral bracts, ligulate ray-florets and tubulate disk-florets.

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(**) Nella seduta del 16 dicembre 1978.

A lot of freshly excised samples was fixed in 6% glutaraldehyde in 0.1 M cacodylate buffer (pH 6.9), gradually dehydrated in increasing concentrations of acetone and then processed by the technique of critical point drying in a Balzers Union Critical Point Dryer.

The samples, coated with carbon and gold in a Jeol JEE 4B vacuum evaporator were observed with a JSM-U3 Jeol Scanning Electron Microscope at an accelerating voltage of 25 kV, at the «Centro Universitario Grandi Apparecchiature Scientifiche» (CUGAS) of the University of Padua.

## Results

*Involucral bracts*

The abaxial epidermises of involucral bracts are characterized by sinuous epidermal cells with smooth cuticles, raised stomata with striated subsidiary cells (Pl. I, Fig. 1), uniseriate pluricellular covering trichomes, and glandular hairs (Pl. I, Fig. 2) made up of a long biseriate pluricellular stalk and a pluricellular globose head (Pl. I, Fig. 3). Less abundant glandular trichomes with a short stalk are also present.

On the adaxial bract surfaces, smooth rectangular cells are present at the bract basis. At the apex, through transitional stages, aspects quite similar to those observed on the abaxial page, are reached.

*Corolla of ray-florets*

The adaxial ligule is characterized by papillose, nearly isodiametric epidermal cells with cuticle striations running from a rugose area on the top of the cells towards its base (Pl. I, Fig. 4). Towards the tube-limb junction a few covering trichomes are present (Pl. I, Fig. 5).

In the abaxial ligule a very different cell pattern occurs, faintly striated subrectangular cells with globose glands (Pl. I, Fig. 6), a few stomata and uniseriate pluricellular covering trichomes being present.

In the lower tubular zone of the ray-floret corolla, quite smooth cells and a great amount of covering trichomes and small globose glands can be observed (Pl. II, Fig. 7 and Pl. II, Fig. 8).

*Corolla of disk-florets*

The adaxial epidermis shows the same morphological features found in the adaxial ligule.

The abaxial epidermis does not differ from that found in the tubular zone of the ray-floret corolla, in regard to both cell microcharacters and gland and covering trichome distribution.

*Calyx*

In both ray- and disk-florets of *Arnica*, the calyx is represented by a pappus consisting of bristles (Pl. II, Fig. 8), each made up of many elongated smooth cells (Pl. II, Fig. 9).

*Ovaries*

The ovaries of both ligulate and tubulate florets are covered by smooth-cuticled rectangular epidermal cells and are characterized by abundant glandular trichomes and twin-hairs (Pl. II, Fig. 10, Pl. II, Fig. 11 and Pl. II, Fig. 12).

## DISCUSSION

The surface scanning of *Arnica* inflorescences failed to reveal the presence of special cell features. The only surfaces showing peculiar shape and ornamentation patterns, i.e. the adaxial ligules, are covered by an epidermis of the helianthoid type, which is a feature very widespread among the *Compositae*, being typical of the *Anthemideae*, *Heliantheae* (*sensu* Stuessy), *Tageteae* (*sensu* Strother), and the subtribes *Eriophyllinae* and *Arnicinae* [2].

The same helianthoid type epidermis has been found in the adaxial ligules of both Pyrethrum and the adulterant species *Leucanthemum vulgare* [4]. Therefore the adaxial ligules assume no diagnostic value.

In the involucral bracts, which more clearly denote their foliar origin, the sinuous cell shape and the presence of stomata showing faint striations, which run concentrically on the guard cells, and perpendicular to the major axes of the stomata on the subsidiary ones (Pl. I, Fig. 1), are features not uncommon in foliage leaves, having been observed for instance in *Atropa bella-donna* [16, 17].

In the *Arnica* flower heads, although peculiar shape and ornamentation patterns of the epidermal cells are lacking, a series of structures, such as trichomes, glands and pappus bristles are present, the morphology and distribution of which are important for the botanical identification of the drug.

The present work, in which the microcharacters revealed by surface scanning of the *Arnica* flower heads are described, represents the basis for comparative researches on the adulterant species and at the same time a contribution to the knowlegde of epidermis features, which in some instances can assume taxonomic value. In fact characters involving epidermal structures, i.e. the presence of adaxial ligules of the helianthoid (and not senecioid) type, of corolla hairs restricted to the tube-limb junction and of achene hairs with divergent pointed tips, are reasons for the exclusion of *Arnica* from the tribe *Senecioneae* [15], a statement which is in agreement with chemical evidences, the chemical pattern of *Arnica* species (absence of pyrrolizidine alkaloids and production of polyacetylenes, melanins and sesquiterpene lactones of the pseudoguaianolide class) being much more characteristic of other tribes of the *Compositae*, particularly the *Heliantheae* [21, 23].

Among the different flower parts, we feel that the greatest importance must be attached to the fruit surface, the achene morphology having been

utilized for the classification at the generic and sometimes at the specific level in some tribes of both *Umbelliferae* [12] and *Compositae* [14]. The achene in fact shows ecological [22], functional and structural [6] analogies with the seed, the taxonomic value of which is today generally recognized [24].

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Fig. 1.

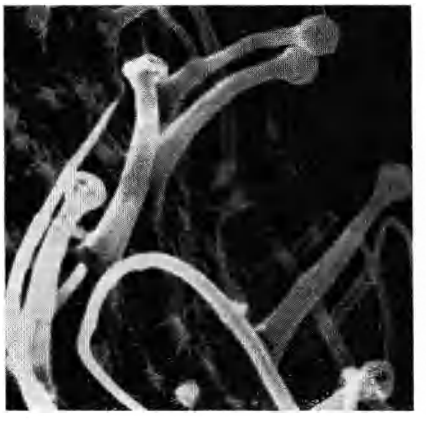


Fig. 2.

Fig. 3.

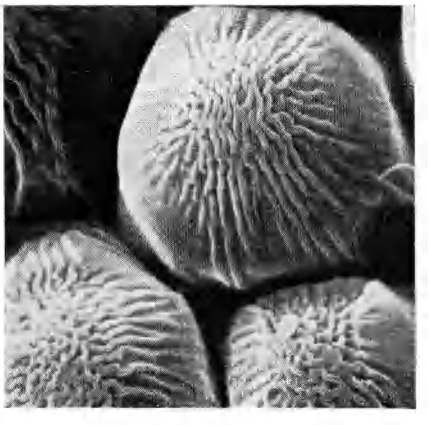
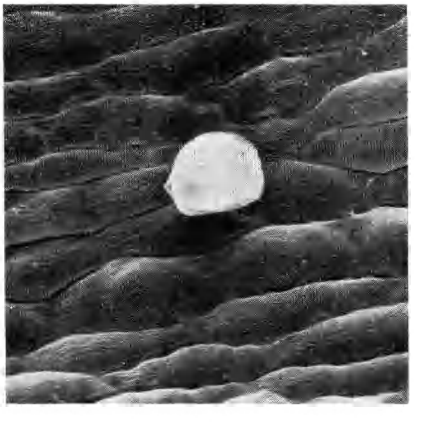


Fig. 4.

Fig. 5.



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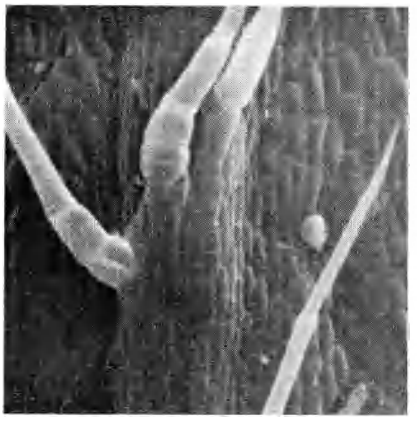

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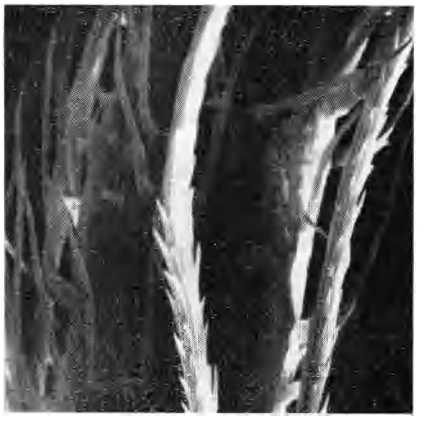

Fig. 8.

Fig. 9.

Fig. 10.

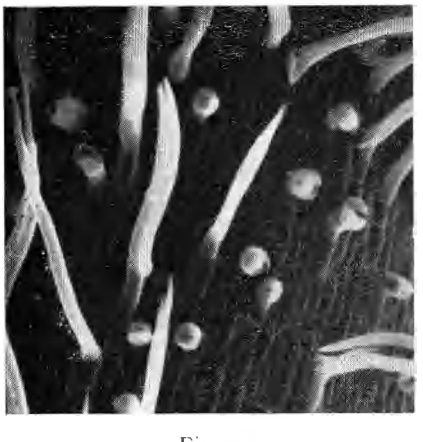

Fig. 11.

Fig. 12.

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## EXPLANATIONS OF PLATES I–II

### PLATE I

Fig. 1. – Involucral bract, abaxial surface: sinuous epidermal cells and stomata (×350).

Fig. 2. – Involucral bract, abaxial surface: covering trichomes and glandular hairs (×90).

Fig. 3. – Involucral bract, abaxial surface: pluricellular globose heads of glandular trichomes (×450).

Fig. 4. – Ray-floret, adaxial ligule: striated, papillose epidermal cells (×1,150).

Fig. 5. – Ray-floret, adaxial ligule: striated, papillose epidermal cells and covering trichomes towards the tube-limb junction (×500).

Fig. 6. – Ray-floret, abaxial ligule (×350).

### PLATE II

Fig. 7. – Ray-floret, abaxial surface of the tubular zone of corolla (×150).

Fig. 8. – Ray-floret, lower zone of the corolla: covering trichomes and two bristles of the pappus (×70).

Fig. 9. – Ray-floret: bristle of the pappus (×600).

Fig. 10. – Ray-floret, ovary (×70).

Fig. 11. – Disk-floret, ovary: glandular trichomes and twin-hairs (×150).

Fig. 12. – Ray-floret, ovary: glandular trichomes and twin-hair (×600).