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Studies in plant morphology by scanning electron microscopy and applications to plant species of pharmaceutical interest. Comparative research on differential microcharacters of the dried, powdered flower heads of Arnica montana L. and of two adulterant species

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Botanica. — *Studies in plant morphology by scanning electron microscopy and applications to plant species of pharmaceutical interest. Comparative research on differential microcharacters of the dried, powdered flower heads of* Arnica montana *L. and of two adulterant species.* Nota di ELSA MARIELLA CAPPELLETTI (*), presentata (**) dal Socio C. CAPPELLETTI.

RIASSUNTO. — Un esame comparativo dei microcaratteri delle infiorescenze di *Arnica montana, Calendula officinalis* ed *Anthemis tinctoria* per mezzo del microscopio elettronico a scansione, ha permesso di stabilire che a livello delle superfici epidermiche abassiali di brattee involucrali e ligule, nonché a livello dell'epicarpo degli acheni, sono presenti particolarità che consentono l'identificazione delle tre specie anche disponendo di campioni di droga polverizzata.

INTRODUCTION

In previous papers [2, 3] the microcharacters evidenced by surface scanning of the epidermises of the flower heads of *Arnica montana* and of the adulterant species *Calendula officinalis* and *Anthemis tinctoria* have been described.

The above-mentioned observations were made on samples fixed and processed in such a way as to avoid shrinking phenomena, which is always necessary for a correct interpretation of distortions occurring during tissue air-drying [4, 10].

The air-dried and powdered capitula are now comparatively examined, in order to ascertain if a botanical identification may be possible even on very small drug fragments.

MATERIAL AND METHODS

Arnica inflorescences were collected from spontaneous specimens of *Arnica montana* L. subsp. *montana* growing on Mount Bondone (Trento, NE-Italy); the flower heads of *Calendula officinalis* L. and *Anthemis tinctoria* L. came from plants cultivated in the Botanical Gardens of the University of Padua.

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(**) Nella seduta del 16 dicembre 1978.

The inflorescences were neither fixed nor acetone dehydrated, but only air-dried, powdered, and then metallized and observed by a SEM Jeol JSM-U_3, at the accelerating voltage of 25 kV, at the «Centro Universitario Grandi Apparecchiature Scientifiche» (CUGAS) of the University of Padua.

RESULTS

The abaxial epidermises of the involucral bracts of the three species taken into account, thoroughly described in previous papers [2, 3], appear to be quite different, the differences consisting both in cell morphology and in the type of trichomes occurring. The cell cuticle striations found in *Anthemis* abaxial bracts, clearly recognizable in dried samples (Pl. I, Fig. 1), represent an excellent character, while the possibility to differentiate the abaxial bracts of *Arnica* (Pl. I, Fig. 2 and Pl. I, Fig. 3) from those of *Calendula* (Pl. I, Fig. 4) is assured by the presence on the bract body of *Arnica* of long covering trichomes. The different morphology of short- and long-stalked glandular trichomes is also recognizable in the dried *Arnica* drug, whereas the cell shape and the risen stomata situation are not detectable (Pl. I, Fig. 2 and Pl. I, Fig. 3).

The adaxial bracts assume scarce diagnostic importance, those of *Anthemis* being quite similar to the proximal bract segments of both *Arnica* and *Calendula*, and the distal segments of adaxial bracts of the latter two species being easily mistaken for the abaxial ones.

The adaxial ligules fail to represent a sure character for differentiating *Arnica* from *Anthemis*, both showing the helianthoid type of epidermis described by Baagøe [1]. The helianthoid type having a quite different appearance, even in dried samples, from the mutisioid one, (compare Pl. I, Fig. 5 with Pl. I, Fig. 6), the adaxial ligules can be on the contrary utilized to differentiate *Calendula* from the other two species.

The abaxial corolla of disk-florets does not even allow discrimination between *Arnica* and *Anthemis*, rectangular cells with smooth outer walls being present in both species.

The abaxial ligules are on the contrary excellent diagnostic characters, making the identification of the three species possible even on fragments made up of few cells. The cuticle striations of *Anthemis*, the lenticular shape of *Calendula* cells and the smooth appearance of *Arnica* cells are in fact easily recognizable even in dried samples (Pl. II, Fig. 7 and Pl. II, Fig. 8).

The ovaries too can be easily identified in the powdered drug, not so much by the microcharacters of the epidermal cells (all with smooth cuticles), as by the presence of abundant twin-hairs and glandular trichomes in *Arnica* (Pl. II, Fig. 9), of abundant glandular trichomes in ray-floret and inner disk-floret ovaries of *Calendula* (Pl. II, Fig. 10) and by the absence of trichomes in *Anthemis* (Pl. II, Fig. 11).

The rows of mucilaginous cells found on the epicarp surface of *Anthemis* represent the structures that undergo the greatest changes during drying; they are only barely recognizable in powdered samples (Pl. II, Fig. 11 and Pl. II, Fig. 12), especially those present on ray-floret ovaries, made up of few cells.

DISCUSSION

The comparative investigations by SEM on capitulum microcharacters allow definition of the surfaces that can assume importance in order to differentiate the flower heads of *Arnica montana* from those of *Calendula officinalis* and *Anthemis tinctoria*.

Previous researches [4, 10] have shown that cuticle ornamentation is generally recognizable in powdered air-dried samples, striations, for instance, being easily differentiated from ridges originated from shrinking phenomena [10]. On the contrary the differences in cell outline in most cases can hardly be distinguished in dried samples.

Scarce diagnostic importance can moreover be attached to general features not involving single cell morphology, such as density or distribution of stomata, hairs or glands, since to recognize these characters fairly large fragments of tissues are needed, which is not always the case in powdered drugs.

As far as the possibility of discriminating between *Arnica* and the two adulterant species is concerned, scarce diagnostic importance must be attributed both to the adaxial bract epidermises and to the adaxial ligules, which fail to differentiate the two species (*Arnica* and *Anthemis*) showing epidermises of the helianthoid type. The same is true for Pyrethrum and *Leucanthemum vulgare* [4].

Greater diagnostic value is assumed by the abaxial bracts and particularly by the abaxial ligules and the ovaries.

The importance of the epicarp microcharacters for the botanical identification of *Arnica*, as well as of Pyrethrum [4] adulterations, fits the previously suggested [2] taxonomic reliability of achene morphology.

The twin-hairs found on *Arnica* ovaries and listed by Nordenstam [9] among the reasons for the exclusion of this genus from the tribe *Senecioneae*, are considered diagnostic characters by many authors [5, 8, 11, 12, 13] and are easily recognizable in drug fragments. This is not equally true for the rows of mucilaginous cells of *Anthemis* epicarp, in contrast with that found in *Leucanthemum vulgare* [4] in which the mucilaginous cells, confined to the ribs [6], represent a more conspicuous feature.

SEM observations have moreover evidenced, as previously pointed out [10], that very often the descriptions of microcharacters involving cuticle ornamentation features which are available in the literature, are largely inaccurate and misleading: compare for instance the pictures of abaxial and adaxial ligules of *Calendula* with the characters listed by Jackson and Snowdon [7].

The surface scanning of flower heads of *Arnica montana*, *Anthemis tinctoria* and *Calendula officinalis* proved its usefulness in defining the diagnostic microcharacters, the receptacles and involucres of *Arnica* being often removed from the drug because of their liability to attack by insects [13]. The most important surfaces for a comparative characterization of the three species are therefore represented by abaxial ligules and achene epicarps.

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Fig. 1.

Fig. 2.

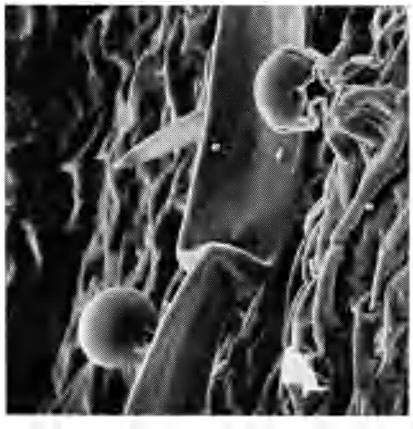


Fig. 3.

Fig. 4.

Fig. 5.

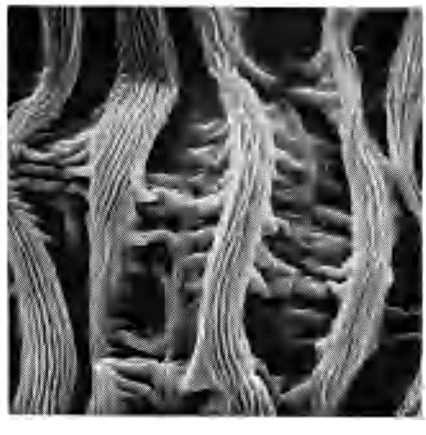
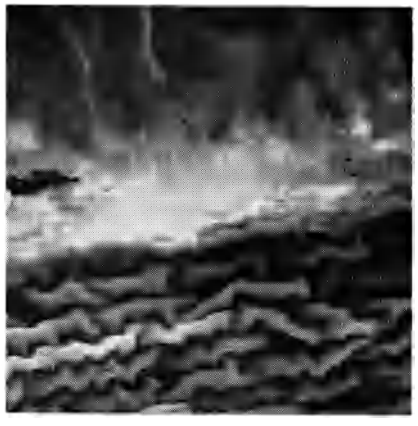


Fig. 6.



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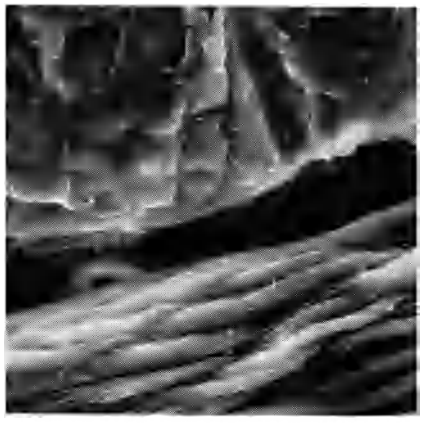

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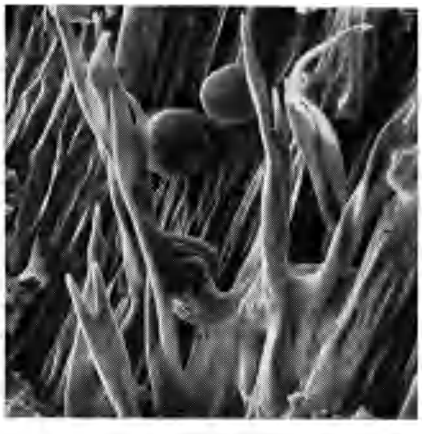

Fig. 9.

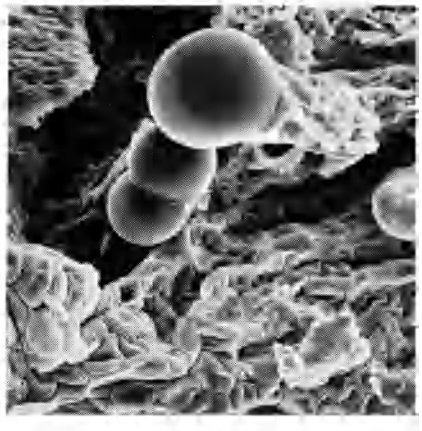

Fig. 10.

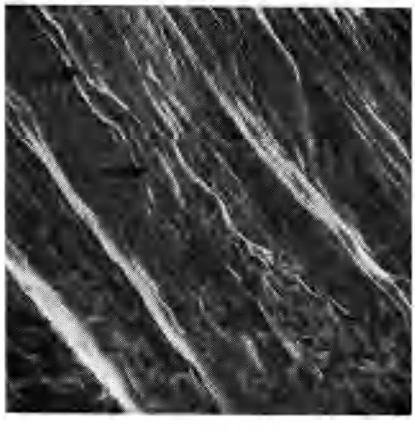

Fig. 11.

Fig. 12.

EXPLANATIONS OF PLATES I–II

PLATE I

Fig. 1. - *Anthemis tinctoria.* Involucral bract, abaxial surface: striated epidermal cells, stomata and covering trichomes. Air-dried sample (×400).

Fig. 2. – *Arnica montana.* Involucral bract, abaxial surface: epidermal cells, covering and glandular trichomes. Air-dried sample (×80).

Fig. 3. – *Arnica montana.* Involucral bract, abaxial surface: covering trichome and short-stalked glandular trichomes (×200).

Fig. 4. – *Calendula officinalis.* Involucral bract, abaxial surface: glandular trichomes. Air-dried sample (×200).

Fig. 5. – *Anthemis tinctoria.* Ray-floret, adaxial ligule: striated, papillose epidermal cells. Air-dried sample (×1,250).

Fig. 6. – *Calendula officinalis.* Ray-floret, adaxial ligule: epidermal cells as seen in air-dried samples (×1,250).

PLATE II

Fig. 7. – Abaxial ligules of *Arnica montana* (above) and *Anthemis tinctoria* (below). Air-dried and powdered samples (×350).

Fig. 8. – Abaxial ligules of *Arnica montana* (above) and *Calendula officinalis* (below). Air-dried and powdered samples (×200).

Fig. 9. – *Arnica montana.* Ray-floret, ovary: glands and twin-hairs with divergent pointed tips. Air-dried sample (×200).

Fig. 10. – *Calendula officinalis.* Ray-floret, ovary: smooth epidermal cells and glandular trichomes as seen in an air-dried and powdered sample (×650).

Fig. 11. – *Anthemis tinctoria.* Ray-floret, ovary: strongly distorted epidermal cells and hardly recognizable rows of mucilaginous cells (arrows). Air-dried sample (×200).

Fig. 12. – *Anthemis tinctoria.* Disk-floret, ovary: strongly distorted epidermal cells and a row of mucilaginous cells (arrow). Air-dried sample (×2,000).