Secretory structures in the capitula of *Santolina leucantha* Bertol. (Asteraceae). Morphology and histochemistry

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ABSTRACT. – In the capitula of *Santolina leucantha* Bertol. two distinct types of secretory structures have been examined: secretory ducts and glandular trichomes. The ducts are rather variable both in their morphology and size; the trichomes are pluricellular and biseriate. The two structures have a quite different distribution: the glandular trichomes are present on the corolla and on the scales, the ducts are present in the stigma, in the scales and in the receptacle.

The histochemical analyses of their secretion have revealed the presence of several compounds; although similar, the two secretions are partially different, in that they differ in the lipid composition; moreover peptic substances and tannins seem to be produced by glandular trichomes only.

KEY WORDS – *Santolina*, secretory structures, morphology, histochemistry

INTRODUCTION

In the genus *Santolina* (Asteraceae) some species are used as herbal remedies in folk medicine (Uphof, 1968; Negri, 1976). Their use is justified by the presence of secondary metabolites, several of which have been identified by chemical analysis.

For the genus as a whole, there is no data in relation to the structures responsible for the production of such compounds and the present research has the aim of investigating these structures.

The species under study, *Santolina leucantha* Bertol. (*), is an endemic of the Apuan Alps (NW Tuscany) and its local vernacular name is "camomilla apuana" because its dried capitula are used as a succedaneous for the true chamomile (Corsi *et al.*, 1980).

In spite of the use, the active substances present in the capitula of *S.*

(*) *S. leucantha* is the correct name for the well-known *S. pinnata* Viv. (Garbari and Bechi, 1992).

leucantha are largely unknown, with the exclusion of some phenolic compunds (Flamini *et al.*, 1994). Because of this situation, further to the morphological study of the secretory structures of the capitula by light and scanning electron microscopy, a histochemical investigation has been also performed in order to identify the principal components of the secretion and their site of production and/or storage.

Material and Methods

The plant samples used in this study have been collected near Campocecina (Apuan Alps, NW Tuscany), m 1320 on the sea level. *Exsiccata* in PI.

Light microscopy

The observations have been performed on fresh capitula at different stages of development, as follows:

a) longitudinal and trasversal sections of capitula (20 μm in thickness) obtained with a digital Cryostat-Leitz 1720 used at −8 °C.

b) sections (2 μm in thickness) of material fixed in FAA, dried and embedded in L.R. white resin and cut with an Autocut Automatic Microtome Leica 2055.

c) isolated flowers and involucral scales clarified in Javel's water prior to staining.

The following histochemical techniques have been used:

— Toluidine Blue (Sappa, 1959) as a generic stain;
— Ruthenium Red (Jensen, 1962) and Delafield's Haematoxylin (Faure, 1914) for pectin-like substances;
— Sudan III (Johansen 1940) and Alkanna tincture (Faure, 1914) for lipids;
— Nile blue (Cain, 1947) for neutral and acid lipids;
— Nadi's reaction (David and Carde, 1964) for terpenoids;
— Concentrated sulphuric acid (Geissman and Griffin, 1971) for sesquiterpene lactones;
— Coomassie Brilliant Blue R (Fairbanks et al., 1971) and Millon's reagent (Faure, 1914) for proteins;
— Potassium bichromate (Faure, 1914) for tannins;
— Wagner's reagent and Dittmar's reagent (Furr and Mahlberg, 1981) for alkaloids.

Controls were set up according to the methods suggested by the authors for each histochemical test.

To better identify the secretory ducts, the capitula have been macerate following the method by Kapoor and Kaul (1966).

Scanning electron microscopy

The material was fixed in glutaraldeide (2% in pH 7.4 phosphate buffer), dehydrated in increasing concentrations of alcohol and acetone, dried to the "critical point", metalizzed and examined at 15 KV with a Cambridge Stereoscan 90 microscope.

Results

Morphology

The capitula of *Santolina leucantha* are made up of tubular flowers surrounded by series of involucral scales. Each individual flower possesses a small interfloral scale. At least two types of secretory structures are visible in the capitula: glandular trichomes and secretory ducts.

Further, there is a strikingly visible papillose epidermis at the level of the corolla.

The glandular trichomes are of a single type; they are very frequent on the external surface of the corollas, often distributed in rows on the corolla tube, but they are rather rare on the involucral and interfloral scales.

These trichomes are pluricellular and biseriate. When fully developed, they consist of two basal cells and of a body made up of a variable number of cell pairs (3 to 5) of which the inferiors (one or two pairs) make up the peduncle and the others the secretory head (Figs. 1a, b). The basal cells are always at a higher level with regards to the adjacent epidermic cells. Their walls are thick and covered by a striated cuticle (Fig. 1a). The peduncle cells are generally smaller than those of the head and have thick walls and cuticle. The cells of the head, instead, have a thin wall and cuticle. The force of the secretion flow causes the cuticle to swell producing a large sub-cuticular space (Fig. 1c).

The trichomes appear quite early in the development of the capitulum. They are formed on the external surface of the petals and on the bracts primordia. Some already differentiated and secreting glandular trichomes are present on the capitula at the very early stage of their development ($1 \div 2$ mm in diameter). Glandular trichomes are therefore completely developed well before the end of the differentiation of the corolla.

The secretional activity is continuous during the period of flowering. The material accumulated in the sub-cuticular space is extruded through breaks in the cuticle (Fig. 1d) caused by the pressure of secretion.

The secretory activity slows down with the aging of the trichomes. Old trichomes appear to be made up of collapsed cells which degenerate in time.

The secretory ducts are present in the involucral and interfloral scales, in the receptacle, in the peduncle of the capitulum and, at the level of the flower itself, in the stigma, but are not present in the corolla. Like the trichomes, they are already visible at the early stages of the development of the capitulum. Initially, the duct lumen is small and its surrounding cells differ from the adjacent cells for the presence of the oleoresin which later flows through the duct.

When the differentiation is complete, the duct lumen is of variable size, full of secretion and surrounded by a layer of quite flattened cells (Fig. 1e). The morphology of the ducts, their occurrence and distribution are variable

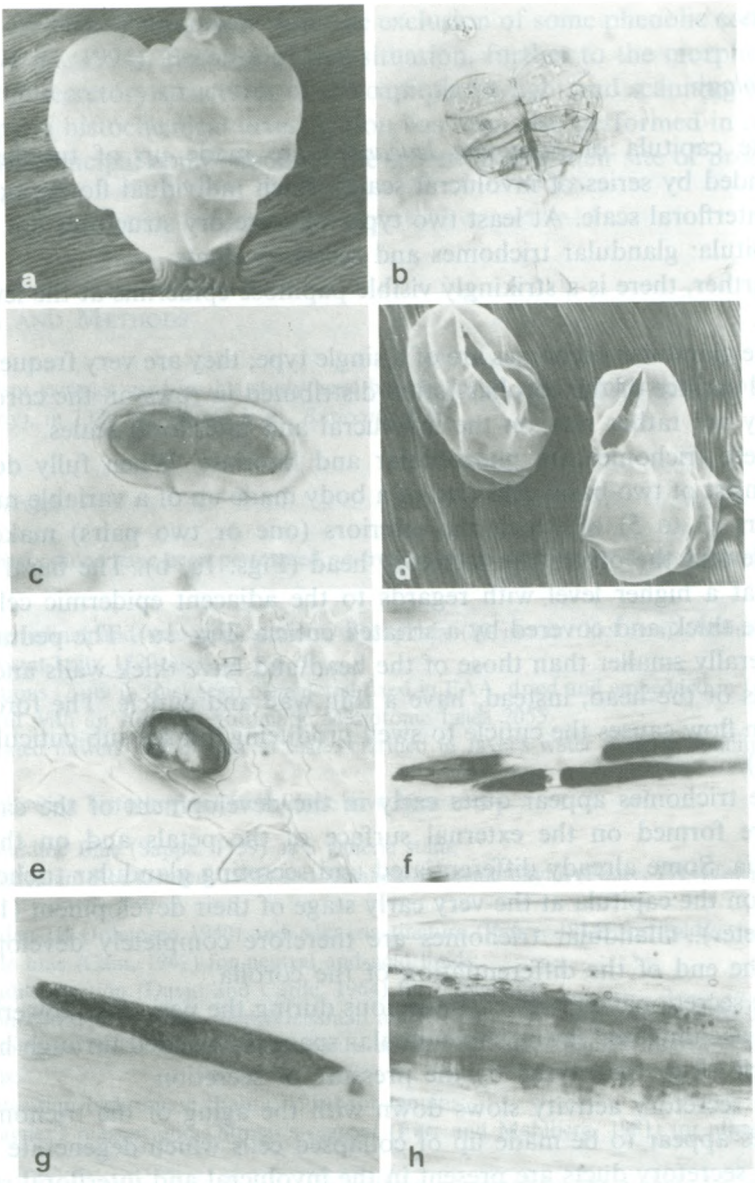

Fig. 1 – Secretory ducts and glandular trichomes in *Santolina leucantha* Bertol.: morphology
a S.E.M. micrography of the glandular trichome (625 ×).
b Glandular trichome in which the head is distinguishible from the peduncle (400 ×).
c Glandular trichome, seen from above, showing the subcuticular space (400 ×).
d S.E.M. micrography of two glandular trichomes; note the ruptured cuticle (420 ×).
e Secretory duct (receptable cross-section. Alkanna tincture, 350 ×).
f Elongated, spindle shaped secretory ducts (50 ×).
g isolated duct (maceration in KOH, 80 ×).
h Drops of secretion in the cells near a duct (scale, Alkanna tincture, 250 ×).

inside the capitulum. In the scales, they are elongated and spindle shaped (Fig. 1f) and are found in a median position parallel to the nervation and often appear septate. After maceration in KOH, they are easily isolated (Fig. 1g). This may be thought to be caused by a thickening of their cellular walls, but this has not been observed. In the scales only one duct is usually present, but sometimes two, of equal size and placed side by side, are observed as well. In the developing scales, the cells very close to the duct show drops of secretion (Fig. 1h) and probably contribute to the elaboration of the material which is then accumulated in the lumen.

The receptacle presents several small secretory ducts of an ellipsoidal form (Fig. 2a), distributed in the parenchyma around the cribro-vascular bundles; they never anastomose.

In the peduncle of the capitulum, instead, the ducts are elongated, rather thin and septate (Fig. 2b).

In the stigma there are two ducts one in each lobe (Fig. 2c). They are elongated and have a lumen of variable size but always large and sometimes ampulla-like (Fig. 2d). Also in this case the ducts often appear septate (Fig. 2e). Semithin transversal sections of the stigma show very clearly that the lumen of the duct is very large in comparison with the entire structure (Fig. 2f).

The glandular epidermis is found at the apex of each of the corolla lobes on the inner side where it appears in the form of large papillae rich in secretion (Fig. 2g). Preliminary observations by S.E.M. show that the papillae have folds and striations on their cuticle (Fig. 2h). This type of secretory structure is still under study.

Histochemistry

The glandular trichomes contain an almost colourless oleoresin; in the secretory ducts, instead, the oleoresin is of various shades of amber-yellow, viscous and at times distributed in a discontinuous way.

The secretions react positively to the Alkanna tincture and to the stain Sudan III (Figs. 3a, b). With the Nile Blue stain, the secretion of trichomes takes a blue colouration and shows a few dispersed red droplets; the product of the ducts is a red secretion (Fig. 3c, d). Nadi's test shows drops of varying colours from blue to pale violet in the trichomes and a red secretion in the ducts (Figs. 3e, f). The reaction to the concentrated sulphuric acid is positive at the level of the trichomes head (Fig. 3g) and also at the level of the ducts (Fig. 3h). The histochemical tests for proteins give negative results in both structures. The reaction of the stain Ruthenium Red is positive, though weak, in the trichomes (Fig. 3i) but negative in the ducts. Potassium bichromate gives weak positive results in the distal cells of the trichomes, while in the peduncle and in the basal cells of the trichomes the results are rather positive (Fig. 3l). The reaction in the ducts is uncertain due to their natural amber

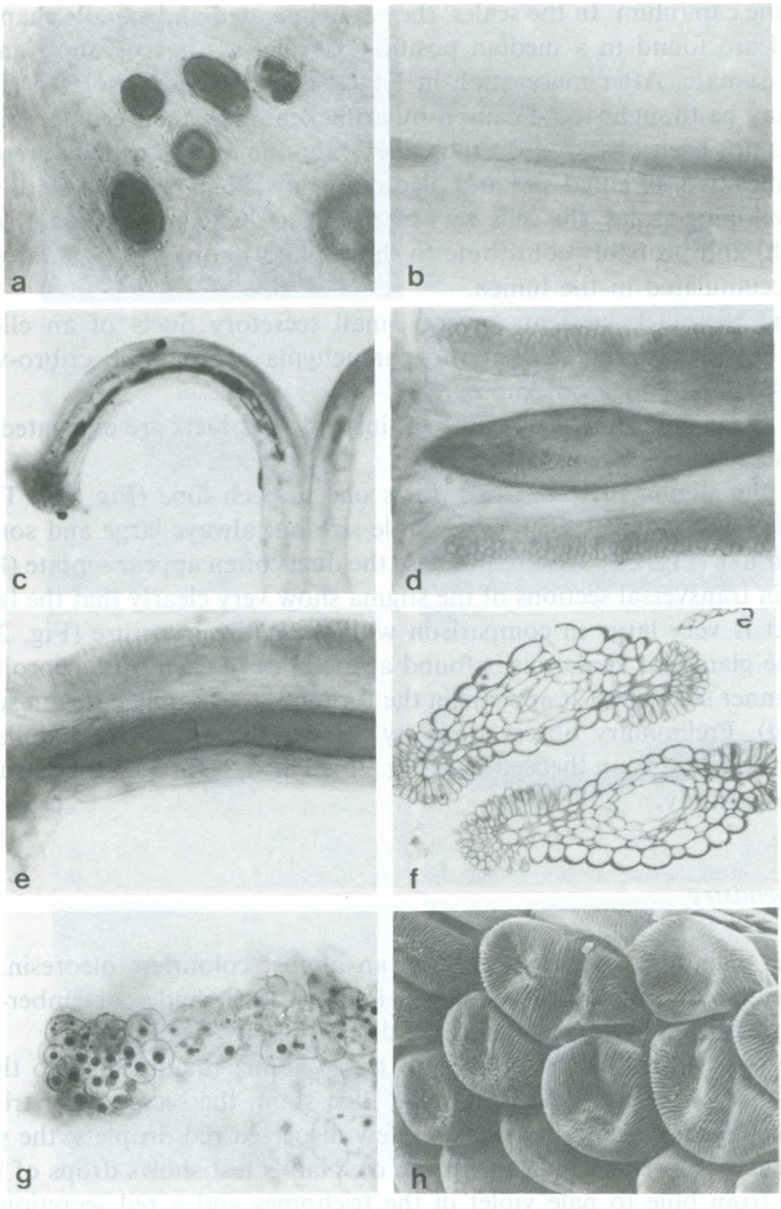

Fig. 2 – Secretory ducts and glandular epidermis in *Santolina leucantha* Bertol.: morphology
a Ellipsoidal secretory ducts (receptable, 160 ×).
b Elongated septate duct (peduncle of the capitulum, 350 ×).
c Stigma showing the duct present in each lobe (40 ×).
d Ampulla-like duct of the stigma (190 ×).
e Duct in the stigma showing a septum (160 ×).
f Cross section of the stigma showing the large secretory canal (200 ×).
g Glandular epidermis at the apex of the corolla lobes (200 ×).
h Papillae as seen by S.E.M.; note the striated cuticle (440 ×).

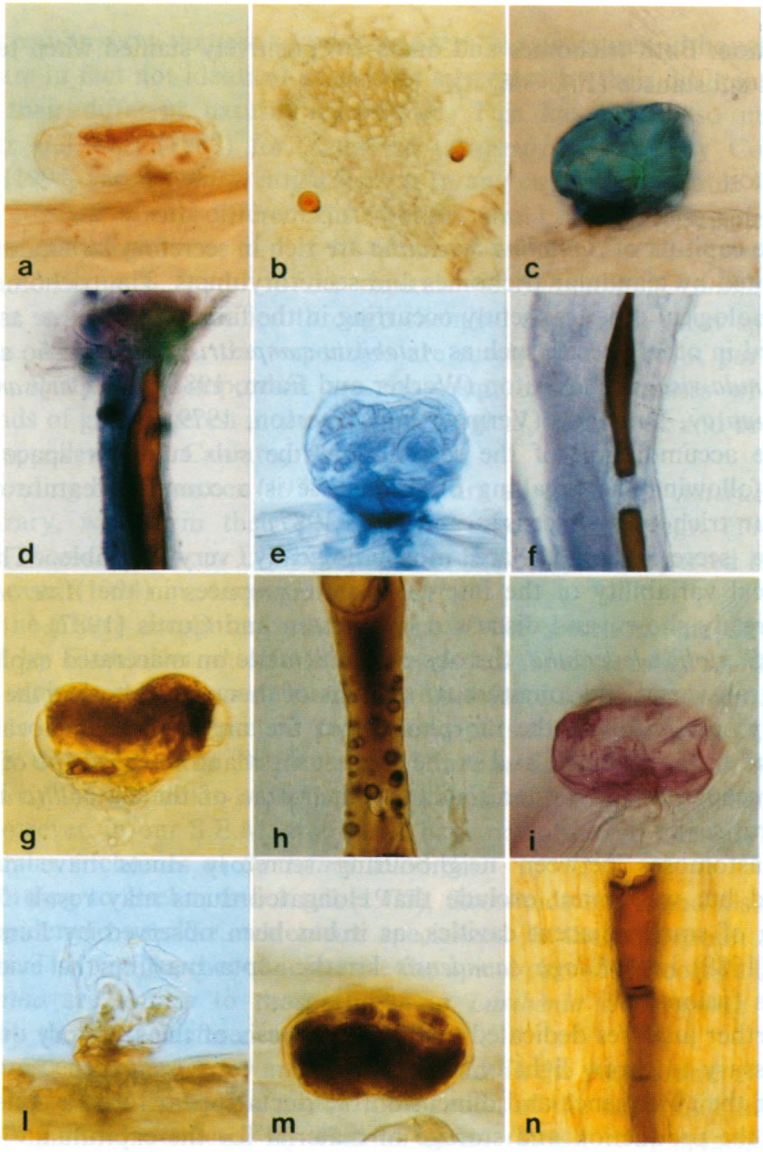

Fig. 3 – Secretory ducts and glanular trichomes in *Santolina leucantha* Bertol.: histochemistry
a A trichome coloured in red with Alkanna (400 ×).
b Receptable duct coloured in red with Alkanna (200 ×).
c Glandular trichome stained blue with Nile blue (400 ×).
d Secretory duct stained red with Nile blue (150 ×).
e Glandular trichome with light blue spots (Nadi's reagent, 400 ×).
f Duct secretion stained red (Nadi's reagent, 180 ×).
g Dark red staining of the glandular head with H_2SO_4 (400 ×).
h Duct stained dark red with H_2SO_4 (120 ×).
i Glandular trichome weakly coloured with rhutenium red (400 ×).
l Trichome stained with potassium bicromate (400 ×).
m Glandular trichomes stained brown-red with Dittmar's reagent (200 ×).
n Brown-red staining of the duct with Dittmar's reagent (250 ×).

colouration. Both trichomes and ducts are positively stained when tested for alkaloid substances (Fig. 3m, n).

Discussion

The capitula of *Santolina leucantha* are rich in secretory structures mainly represented by glandular trichomes and secretory ducts. The trichomes are of a morphological type frequently occurring in the family *Asteraceae* as already described in other species such as *Artemisia campestris* L. (Ascensão and Pais, 1987), *Inula viscosa* (L.) Aiton (Werker and Fahn, 1981) and *Chrysanthemum morifolium* cv. Dramatic (Vermeer and Peterson, 1979).

The accumulation of the secretion in the sub cuticular space and its release following the breaking of the cuticle is a common feature of many glandular trichomes (Ascensão and Pais, 1987).

The secretory ducts are morphologically very variable. The morphological variability of the internal secretory spaces in the *Asteraceae* has been already shown and discussed by Lersten and Curtis (1987).

In *Santolina leucantha*, the observations made on macerated capitula and on the transversal and longitudinal sections of them indicate that the internal secretory spaces follow the morphology of the organ they are localized in: elongated in the peduncle and in the stigma, short and in the shape of pockets in the receptable and rather similar to the vittae of the *Umbelliferae* in the involucral scales.

Anastomoses between neighbouring secretory ducts have not been observed but we cannot exclude that elongated ducts may result from the merging of small adjacent cavities, as it has been observed by Lersten and Curtis (1989) in *Solidago canadensis* L.: the septa may be the evidence of previous fusions.

Further analyses dedicated to the ontogenesis of the secretory ducts may be necessary to throw light on this aspect.

For their frequence and dimension the ducts appear to be a conspicuous site for the production and storage of material for the capitulum.

Among the ducts, of particular interest and deserving further studies are the style ducts for the great quantity of the accumulated material and for the role they may have particularly as regards the reproductive process.

The principal constituents of the two structures are of a lipophilous nature, as seen for the positive reaction of the specific reagents. The produced lipids, on the basis of the Nile Blue stain reaction appear to be of various types: acidic and nonacidic. Nadi's test indicates that resiniferous acids are present in the ducts, while, in the trichomes, there is a mixture of resiniferous acids and essential oils.

Sesquiterpene lactones are present in both structures. Peptic substances and tannins seem to be produced exclusively by the glandular trichomes.

Our results show that the secretions of the two structures, although rather similar, are in fact not identical as already suggested by their different colour and by their different natural consistence. This has been also noted by Ascensão and Pais (1988) for *Artemisia campestris* L. and by Corsi and Necioni (1994) for *Artemisia nitida* Bertol. In any case it is probable that two different structures with different distribution, could have precise roles and a different metabolism.

The compounds found in the capitula of *Santolina leucantha* are in agreement with those identified through chemical analysis in other species of the genus. Among them, the terpenoids are surely an important part of the secretion. Particularly important are the sesquiterpene lactones which are compounds of great interest in the pharmacological, taxonomic and ecological fields (Cappelletti and Caniato, 1985).

According to Hegnauer (1977), tannins are absent in the *Asteraceae*; on the contrary, we affirm their presence, as reported by Negri (1976) for *Santolina chamaecyparissus* L. Their presence has been also reported by Corsi and Nencioni (1994) in *Artemisia nitida* Bertol.

To the above recognized constituents of the secretion, the flavonoids identified by Flamini *et al.* (1994) can be added. Their localization has not been the object of the present study and therefore, for the moment, is not possible to indicate the site of production of such compounds. Anyway, according to the existent literature, the flavonoids of the *Asteraceae* are produced by the glandular trichomes and deposited externally (Wollenweber, 1984). However, in our S.E.M. studies we have not observed deposits around the glandular trichomes of *Santolina leucantha*.

According to Brehm and Krell (1975), flavonoids are found also in the tips of epidermic papillae, which are specialized in the absorption of u.V. radiations. The papillae that characterize the apical part of the corolla lobes of *S. leucantha* are similar to those shown in *Lasthenia chrysostoma* Greene lingules by the above referenced authors.

Fluorescence microscopy analyses, which are in the programme of further studies, may give information on the localization of the flavonoids in *Santolina*. Among those extracted by Flamini *et al.* (1994), two, apigenine and luteoline, are found also in the true chamomile and are in part responsible for its antispasmodic and antiflogistic action. Their presence in *Santolina*, together with that of the various compounds observed in the secretion, shows that this species is very interesting as regards the production of secondary metabolites which justify its use in folk medicine.

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RIASSUNTO

Vengono riportati i risultati di uno studio morfologico ed istochimico riguardante le strutture secernenti dei capolini di *Santolina leucantha* Bertol. (Asteraceae), un'endemica delle Alpi Apuane utilizzata dalle popolazioni locali come succedaneo della camomilla. Sono stati individuati due tipi di strutture secernenti: canali secretori e peli ghiandolari.

I canali sono per morfologia e dimensioni molto variabili; i peli sono pluricellulari e biseriati. Le due strutture hanno una distribuzione alquanto diversa: i peli sono presenti sulla corolla e sulle squame, i dotti sono presenti nello stimma, nelle squame e nel ricettacolo.

L'indagine istochimica ha evidenziato nell'oleresina prodotta da esse la presenza di numerosi composti; anmche se simili, le due secrezioni in parte si differenziano nella composizione dei lipidi; inoltre le sostanze peptiche ed i tannini sembrano prodotti esclusivamente dai peli ghiandolari.

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