J. Phytol. Res. 2 (1) 1989

STRUCTURAL, DEVELOPMENTAL AND HISTOCHEMICAL STUDIES IN THE COLLETERS OF *CALOTROPIS* L. (ASCLEPIADACEAE)

P. M. KURIACHEN and YASH DAVE
Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar-388 120, Gujarat, India.

In *Calotropis procera* (Ait.) R. Br. colleters are present on the adaxial basal parts of petiole, lamina and calyx. They develop from epidermal and hypodermal initials. A mature colleter consists of a long head and a short stalk. The head region consists of a secretory epithelial layer enclosing elongated central cells. The colleters of *Calotropis gigantea* (L.) R. Br. and *C. procera* have the same structure. During senescence the epithelial and central cells show lignification. Large number of druses type crystals of calcium oxalate are found in the central cells. The secretion of colleters is a translucent yellow resinous substance.

Keywords : Histochemistry; Colleters; *Calotropis*; Asclepiadaceae.

Introduction

Glandular secretory hairs similar to the colleters described by Hanstein (1848; Fide Foster, 1949) have been studied in several dicot families like Rhizophoraceae (Lersten and Curtis, 1974), Rubiaceae (Metcalfe and Chalk, 1950; Lersten, 1974a, b; Dave *et al.*, 1988), Apocynaceae (Metcalfe and Chalk, 1950; Ramayya and Bahadur, 1968; Williams *et. al.*, 1982; Dave *et. al.*, 1987) and Asclepiadeceae (Frye, 1902; Metcalfe and Chalk, 1950; Arekal and Ramakrishna, 1980) and called these structures variously as colleters, shaggy hairs, squamellae, stipular glands, glandular trichomes, or extrafloral nectaries. These finger shaped colleters or secretary glands, with long heads and short stalks, produce a yellowish translucent viscous substence and are found to be structurally analogous to the resin glands of Salicaceae (Curtis and Lersten, 1974) and Passifloraceae (Durkee *et al.*, 1984). As these secretory structures of Ascelpiadaceae are found so very closely similar to those of Rhizophoraceae, Rubiaceae and Apocynaceae, that we have described them here as colleters. In the present paper, the detailed morphology, distribution, development and histochemistry of colleters in *Calotropis procera* are described and compared with those of *C. gigantea*.

Materials and Methods

Shoot tips, leaves and flowers of *Calotropis procera* and *Calotropis gigantea* at different developing stages were collected from the wildly growing plants and fixed in formalin acetic–alcohol mixture. Materials were dehydrated, embedded and microtomed (Johansen, 1940). For general staining haematoxylin–safranin or safranin –fast green combinations were used. Periodic acid—Schiff's reagent (Jensen, 1962) and Coomassie brilliant blue (C. B. B.) (Eklavya, 1979) were used to localize starch and proteins respectively. Lipid was localized using Sudan black IV (Jensen, 1962) and oil red 'O' (Lillie and Fullmer, 1976). The chemical nature of crystals was tested with capric acetate and ferric sulphate and phloroglucinol and HCl were used to detect lignification (Johansen, 1940). Fehlings solution was used to find out the presence of sugar in the secretion (Plummer, 1985). Fresh living colleters at its secretory phase were observed under phase contrast microscope to find out the mode of secretion. Micrographs were taken using Carl–Zesis tessovar, phase contrast and photomicroscope–1.

Results

Calotropis procera is an erect shrub bearing opposite decussate leaves and pale pink flowers in umbellate cymes. Colleters are present on the adaxial basal parts of petiole, leaf blade and calyx. In a single leaf about 40-50 colleters are seen: 15-20 at the base of petiole and 25–30 at the junction of petiole and leaf blade (Figs. 1, 2). The calyx is five partite and each sepal bears 5–7 colleters at its inner (adaxial) basal part (Fig. 3). The only contention between the calycine and leaf colleters is the size difference. The calycine colleters are shorter than the leaf colleters and some of them show bifurcating from the base to tip or at the terminal portion. A mature leaf colleter measures 1800-2200 mμ in length and 150-180 mμ in width at its broader basal to middle regions and a calycine colleter measures 1000-1200 mμ in length and 150–200 mμ in width at the broader region. The foliar colleters attain maturity and become secretory before leaf attains a length of 2 cm. The secretion is in the peak period before the opening and spreading of the leaves that cover the apical bud. The calyx colleters mature and secrete before the flower bud reaches a length of 5 mm. The secretion comes out of these finger shaped mature colleters, spreads at the lower part of the young leaves and corolla in their early stages of development.

In spite of the difference between the size of leaf and calyx colleters, the mode of development follows the same pattern. Both epidermal and

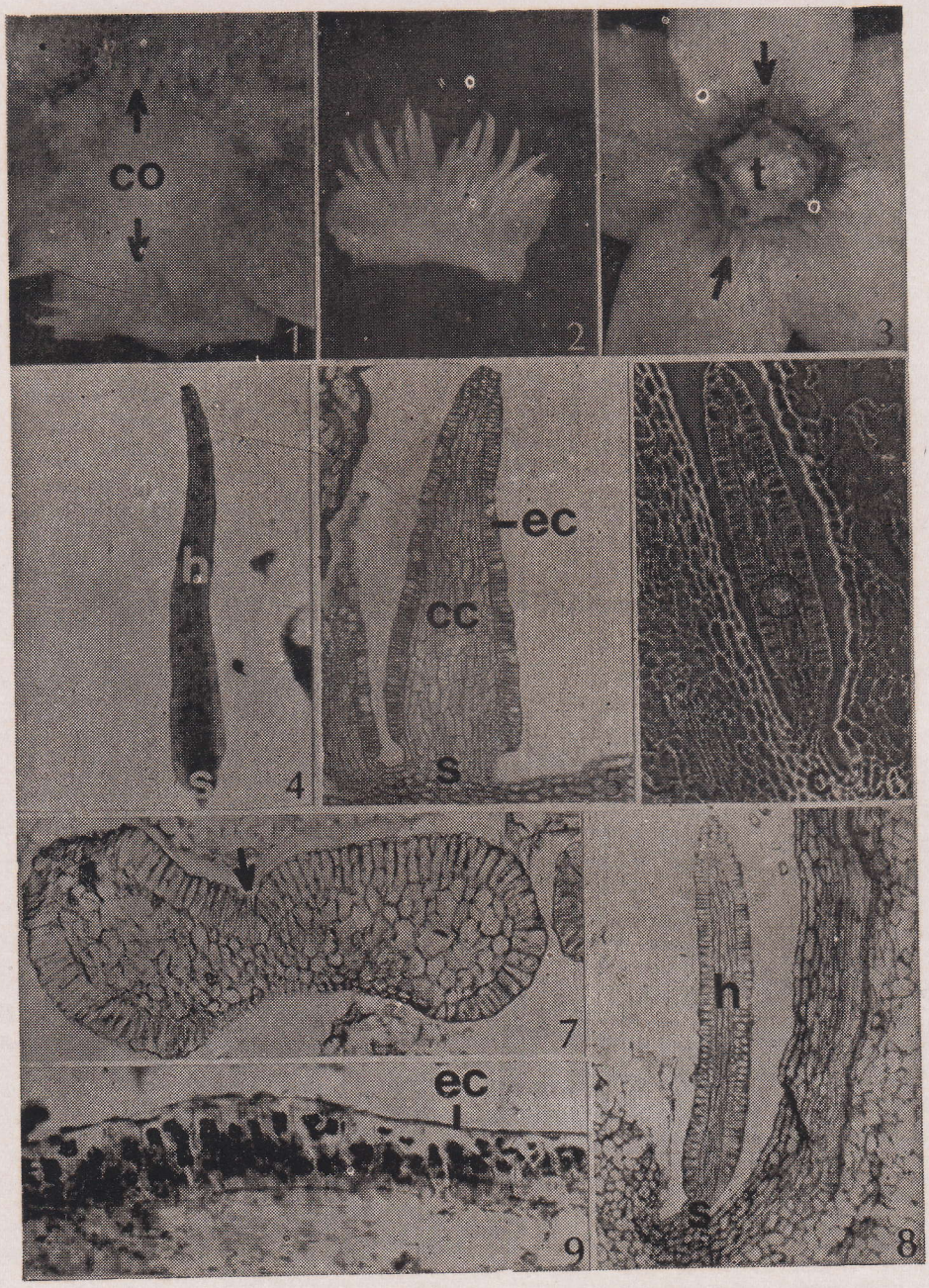
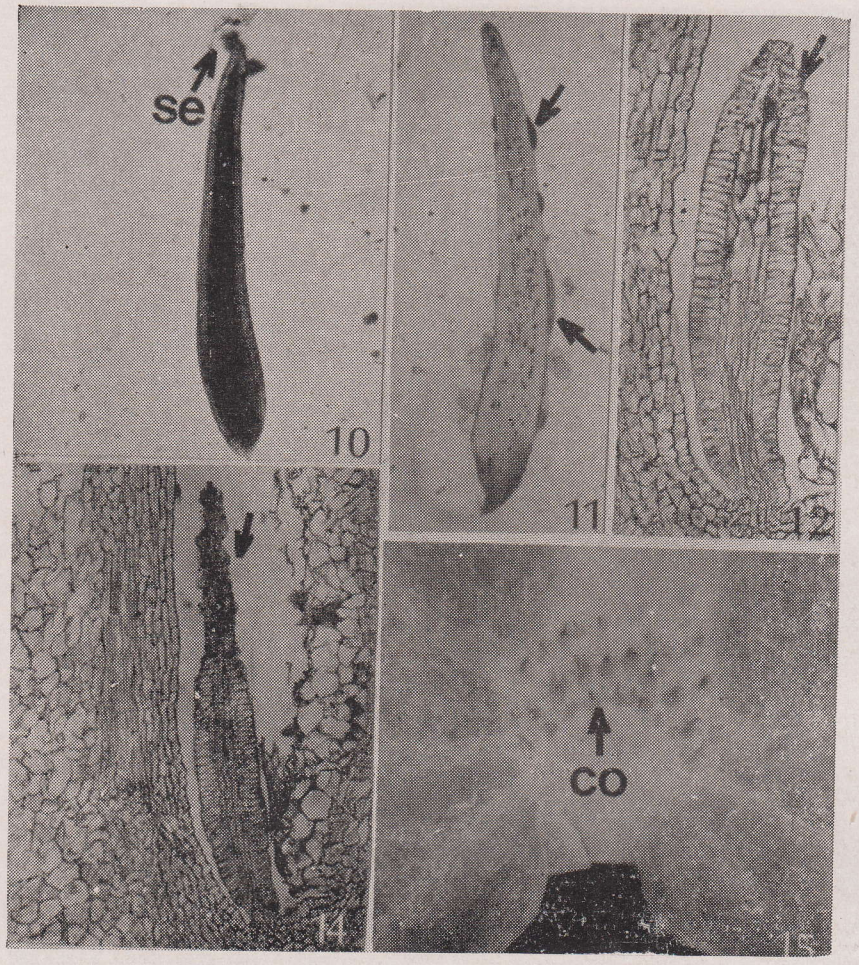
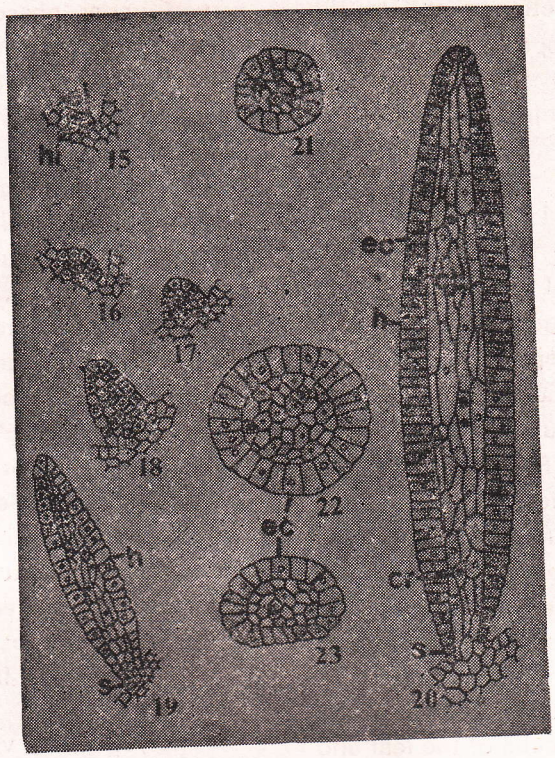


Fig. 1 Young leaf base, see colleters at the base of leaf blade and petiole x 4;
Fig, 2 Colleters at the base of leaf blade (Petiole and leaf blade removed)



x 10; Fig. 3 Calyx with colleters (at arrows) x 5; Fig. 4 Whole mount of a mature leaf colleter x 38; Fig. 5 L. S. of a mature calycine colleter x 86; Fig. 6 Phase contrast micrograph showing crystals In the colleter x 82; Fig. 7 T. S. of a bifurcated calycine colleter, see the lobation at arrow x 115; Fig 8 Calycine collcter of *C. gigantea* x 90; Fig. 9 Lipid particles in the epithelial cells x 345 (c-collenchyma; cc-central cell; co-colleter; ec-epithelial cell; h–head; p–petiole; s-stalk; t–thalamus)

Fig. 10 Whole mount of colleter showing the secretion coming out through the terminal part x 36; Fig. 11 Whole mount of colleter showing the accumulation of secretion at the sides x 44; Fig. 12 Senescing colleter with empty cells and crushed terminal tissue x 120; Fig, 13 A senescing colleter showing completely disorganized terminal tissue x 98; Fig. 14 Mature leaf base showing senescent colleters x 1 (co–colleter; se-secretion)



Figs. 15—19 Developing stages of colleter in L. S. 15–18 x 360; 19 x 300; Fig. 20 A mature leaf colleter in L. S. before secretion x 310; Figs. 21—23 T.S. of colleter through terminal middle and basal parts respectively 21—23 x 310 (cc-central cell; cr-crystal; ec-epithelial cell: ei-epidermal initial; h-head; hi-hypodermal initial; s-stalk)

hypodermal cells take part in the development of colleter. Two to three epidermal and hypodermal cells become prominent by the presence of dense cytoplasm and larger nuclei and these cells appear slightly raised over the surface of the organ (Fig.15). The epidermal initials divide only anticlinally, but the hypodermal cells divide both anticlinally and periclinally. The repeated divisions of these initials result in the formation of a mound of meristematic tissue (Figs. 16,17). Later, by further divisions and enlargement of the cells this mound becomes differentiated into an elongated colleter having a short stalk and a long head (Figs. 4, 18-20). The epidermal derivatives transform into the palisade like secretory epithelial cells and the hypodermal derivatives produce the elongated central cells

(Fig. 5). In transection of colleter the epithelial cells appear as they are in longisection and the central cells appear polygonal in shape (Figs. 21-23).

The epithelial cells of the stalk region are smaller and non-secretory, The elongation of the central cells is parallel to the long axis of the colleter (Fig. 5). The epithelial cells are denser during the secretory phase due to the accumulation of the secretory substance (Figs. 5, 21), and is covered by a cuticle. Large number of druses of calcium oxalate are seen in the central cells (Fig. 6). Rarely crystals are also seen in the epithelial cells. The bifurcated calycine colleters appear bilobed in transection (Fig. 7). Vascular strands are not seen in the colleters, but the cells of the leaf and calyx just below the colleter seem to be thick walled collenchymatous (Fig. 6). The calycine colleter of *Calotropis gigantea* is also observed to have the same structure as that of *C. procera* (Fig. 8). Histochemical analysis shows that starch or protein is not deposited in the colleter tissue during secretory or presecretory phase. However, lipid particles are found to be accumulated in large quantities in the epithelial cells during the secretory phase of the colleter (Fig. 9).

The fresh colleter at the secretory phase (i.e. before the unfolding of leaf or opening of flower) when observed under phase contrast microscope showed the secretion coming out from its terminal part. The secretory substance, which consists of a viscous fluid and abundant granular bodies, first discharges into the space between the epithelial cells and cuticle and flows from the basal to terminal part from where it oozes out (Fig. 10). The secretion also comes out through the sides of the colleter when the terminal portion is blocked by the senescence of tissue and accumulation of secretion. When it comes out through the sides it accumulates in between the epithelial cells and the cuticle bulges out (Fig.11). When living colleter is treated with oil red 'O' the granular substance in the colleter is found to be lipoid in nature.

The yellowish viscous secretion of the colleters of both *C.procera* and *C. gigantea* is insoluble or partially soluble in water and completely soluble in alcohol, xylol and acetone. The Fehling's test for reducing and non-reducing sugars gives negative result with the colleter secretions of *C. procera* and *C. gigantea.*

After the secretion stage (i. e. after the unfolding of the leaf and opening of the flower) the colleter shows the sign of senescence. The senescence of the colleter starts from the terminal part towards the base. The process of senescence is commenced by the emptying of central cells and epithelial cells

(Fig. 12), followed by an increment in the number of crystals. First the cells of the extreme terminal part appear shrunken (Fig. 12) followed by the lignification of central and epithelial cells. The disorganization and shrinkage of the cells proceed in a basipetal direction (Fig. 13) and finally the whole colleter becomes dead and shrunken. These senescent colleters are seen in the calyx at the base of young fruits and on mature leaf base as dark brown structures (Fig. 14).

Discussion

In *Calotropis procera* colleters are petiolar, laminar and calycine. These colleters have their origin from the epidermal and hypodermal cells of the respective parts of the organs. The colleters of Rubiaceae (Patel and Zaveri, 1975; Dave *et al.* 1988) and Apocynaceae (Ramayya and Bahadur 1968; Dave *et al.*, 1987; Thomas *et al.*, 1988) also have the same type of origin. The epidermal and hypodermal initials after several divisions elongate and form a 'standard type' colleter as described by Lersten (1974a) in Rubiaceae. The mature finger shaped colleter that consists of a short stalk and long head is very similar to that in *Asclepia* and *C. gigantea.* But these colleters are described as calyx gland in *Asclepias* by Frye (1902) and as extrafloral nectaries in *C. gigantea* by Arekal and Ramakrishna (1980). The colleters in *Plumeria alba* of Apocynaceae are also described as extrafloral nectaries by Mohan and Inamdar (1986). But Ramayya and Bahadur (1968) and Dave *et al.* (1987) have described these structures as squamellae or colleters on the basis of developmental, structural and secretional nature in certain Apocynaceae members.

Arekal and Ramakrishna (1980) noticed these structures at the basal part of lamina only, but in our observation they are also revealed at the basal part of petiole and calyx. The secretion of both *C. procera* and *C. gigantea* is translucent yeliowish sticky substance which comes out of the colleters before the spreading of leaves and opening of the flowers. It is found that starch or protein is absent in the secretory or presecretory phase, but lipid particles are abundant. Thus it can be concluded that this yellowish substance which is partially soluble in water and completely soluble in alcohol, xylol and acetone may be a resinous substance and is not nectar as Arekal and Ramakrishna (1980) have reported.

Even though the colleter of *Calotropis* is a well organized structure, a vascular supply as found in such structure of *Wattakaka volubilis* (Arekal and Ramakrishna, 1980 and *Aganosoma caryophyllota* (Dave, *et al.*, 1987) is not found here. The senescence of colleters starts by the destortion of the terminal cells from

where the secretion comes out and the senescence advances in a basipetal direction as in the case of *Aganosoma* and *Gardenia* (Dave *et al.* 1987, 1988).

Acknowledgement

We gratefully acknowledge the financial assistance from University Grants Commission, New Delhi.

Accepted March, 1989

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