HISTOMORPHOLOGY OF FOLIAR EPIDERMIS AND PHARMACOGNOSY IN ASCLEPIADACEAE

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Introduction

In Asclepiadaceae, a family rich in medicinal plants, it is the leaf that usually constitutes the exclusive or at least the major portion of the official part in a large number of species. As such, a detailed study of the morphology of the foliar epidermis is an important aspect of

the pharmacognosy of the family. In an earlier account of six species in this line (Krishnamurthy and Sundaram, 1967) it was pointed out that the real criterion of differentiation among the specimens lies more in a combination of isolated characters rather than in any one or two of them, however striking they may be. Consequently the description has to refer more to the relative configuration of the cell elements making up the general and total appearance or pattern of the epidermal surface. It will be of some use if we can specify consistent points of such description in a comparative manner. The present contribution attempts this by a description of six other species of the family and a discussion of all the plants studied so far.

Material and Methods

Leaves of the following species are studied: *Ceropegia juncea*, Roxb., *Cryptostegia grandiflora*, R. Br., *Cynanchum pauciflorum*, R. Br., *Heterostemma tanjorense*, W. & A., *Dregea volubilis* (Linn. f.) Benth ex Hook f. and *Tylophora indica* (Burm.) Merr. (*T. asthmatica*, W. & A.). All are climbers (Gamble, 1957) and find mention as medicinal plants in the literature excepting, probably, *Heterostemma tanjorense*. The leaves of the African counterparts of *Cynanchum* are known to cause a specific bovine and sheep disease called cynanchosis (Watt and Breyer-Brandwijk, 1962). Leaf is included in the official part in all except *Ceropegia* whose tubers are official and where, however, Ayurveda recognises three separate varieties (Nadkarni, 1954). The present study has been done throughout with the help of fresh preparations of epidermal peelings. Chloral hydrate boiling was done judiciously as this was found to damage the cuticular striation sometimes. Peels were taken from the mid-region of a lamina half and invariably included larger vascular strands.

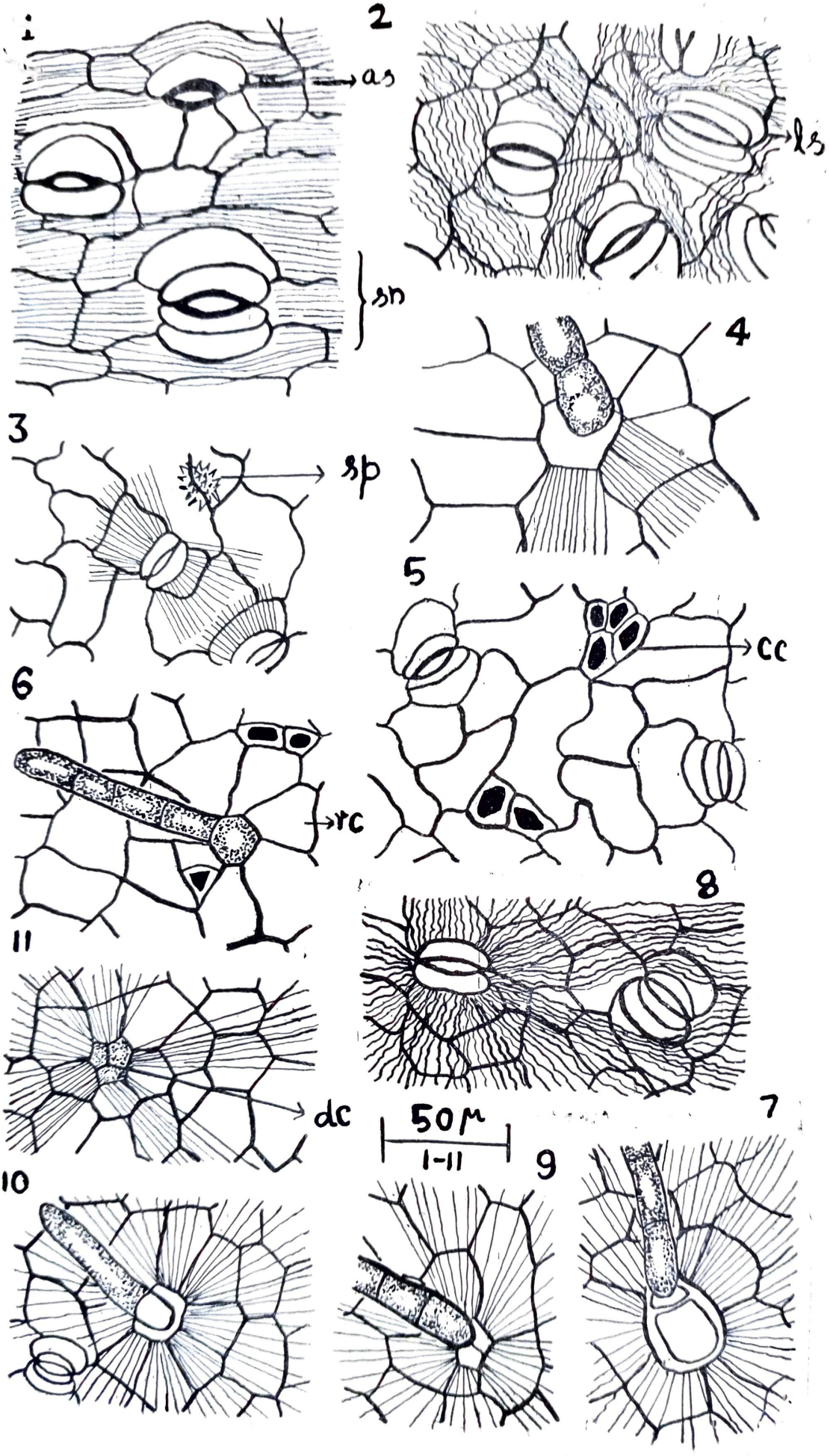
Observations

Salient, chiefly quantitative, features are given as a comparative table (Table I). The relative configuration of the cell elements of the epidermal surface is described as follows:

Ceropegia juncea: Lower Epidermis.—*Intercostal area*: Majority of the cells are longer than broad, the longer axes consistently lying in the axial direction of the leaf. In the greater part of the area the cells lie in, end to end, continuous, uniseriate rows giving an almost 'parallel' appearance (Fig. 1). Around the stomata, which are quite frequent, the cells are columnar and curved, with the result the stomata appear in the general parallel run of the *i.c.* cells (intercostal cells) to form 'nodes' along which the intercostals seem to 'flow' past. Individual cells are usually 4-sided, straight-walled and clear, *i.e.*, devoid of contents. *Stomata*: Long axes uniformly lie in the axial direction of the leaf. Hence, the stomata also appear to lie in parallel 'series'; the series themselves are 2–3 *i.c.* cell 'layers' apart

TABLE I

Name of the plant	Stomatal count (mm^2)		Stomatal measurement (μ)				Type of stomata	Stomatal index		Palisade ratio
			Lower		Upper			Lower	Upper	
	Lower	Upper	Length	Breadth	Length	Breadth				
1. *Ceropegia juncea*, Roxb.	270	292	40·30	29·40	39·50	31·60	Paracytic	9	11	3
2. *Cryptostegia grandiflora*, R. Br.	241	..	31·00	21·00	..	..	do.	23	..	12
3. *Cynanchum pausiflorum*, R. Br.	214	..	25·20	16·50	..	..	do.	9	..	7
4. *Heterostemma tanjorense*, W. & A.	156	..	24·00	18·00	..	..	do. and anomocytic	7	..	2
5. *Dregea volubilis* (Linn. f.) Benth ex Hook f.	340	..	28·30	17·50	..	..	Paracytic	13·5	..	10
6. *Tylophora indica* (Burm.) Merr.	404	..	21·70	15·30	..	..	do. and anomocytic	15	..	4



Figs. 1–11

FIGS. 1–11. Fig. 1. Lower epidermis of *Ceropegia juncea*. Fig. 2. Lower epidermis of *Cryptostegia grandiflora*. Figs. 3–4. *Cynanchum pauciflorum*. Fig. 3. Lower epidermis. Fig. 4. Upper epidermis. Figs. 5–7. *Heterostemma tanjorense*. Fig. 5. Lower epidermis. Fig. 6. Hair of lower epidermis. Fig. 7. Upper epidermis. Figs. 8–9. *Dregea volubilis*. Fig. 8. Lower epidermis. Fig. 9. Upper epidermis. Figs. 10–11. *Tylophora indica*. Fig 10. Lower epidermis. Fig. 11. Upper epidermis.

as., abnormal stoma of single guard cell; *cc.*, crystal cell; *dc.*, degenerated cell; *ls.*, large stoma; *rc.*, radiating cell at the hair base; *sn.*, 'stomatal node'; *sp.*, sphaeride.

while the adjacent stomata in a series are 4–5 *i.c.* cells apart. In size, the stomata are the same as that of the intercostals or even larger and as such look rather pronounced. The distinctness is heightened by the clustered 'flowing' around of the columnar intercostals. Abnormal stomata of single guard cells are sometimes seen (Fig. 1). *Striations*, profuse and show a simple pattern of continuous running lines extending the full length of the 'parallel' cell series of the intercostals and the 'flowing' around of the 'stomatal nodes'.

Upper Epidermis.—*Intercostal area*: The parallel row-like appearance of the cells is not present; individual cells, straight-walled, 5–8-sided, polygonal, often rhomboidal, slightly longer or as long as broad. The longer axes are disposed in all directions and consequently the 'stomatal nodes' are absent. *Stomata*, similar in size to the *i.c.* cells, many, as frequent as on the lower epidermis but not showing the 'parallel' or any other regular distribution. *Striation*: Apart from a slight circling around the stomata this shows a uniform distribution as in the lower epidermis *irrespective* of the direction of the orientation of the long axes of the individual cells.

Cryptostegia grandiflora: Lower Epidermis.—*General surface* is cut up into many separate large islets by means of broad 'reticulum'-like branching sheet of costal strands, each islet studded richly with stomata. *Intercostal area*: Cells are straight-walled, 4–5–6-sided, *slightly* longer than broad. *Stomata*, as large as or even slightly larger than the *i.c.* cells, very closely arranged, separated by 2–3 *i.c.* cells only and merging in the faintness of outline with them. At irregular intervals there appear in each of the islets one or two very large-sized stomata, the vestibular slit being nearly one and a half or double that of the other stomata (Fig. 2). *Striations*, very prominent and made up of irregularly curved lines strongly covering the whole surface of the islet giving it a wrinkled appearance excluding only the guard and often the subsidiary cells (Fig. 2). No particular centred of radiation is traceable.

Upper Epidermis.—*General surface*: The reticular formation into islets is present as in the lower epidermis but neither the costal branches are so broad (usually of 2–3 cell thickness only even in the branches immediately near the midrib) and pronounced nor the intercostal islets show the prominent features of stomatal profusion or the

wrinkled striae of the lower epidermis. *Intercostal area*: Cells, straight-walled, 5–8-sided, polygonal, isodiametric. *Stomata*, absent. *Striations*, prominent; pattern as in the lower epidermis.

Cynanchum pauciflorum: Lower Epidermis.—*Intercostal area*: Cells near the larger costal strands (of 10–12 cells in breadth) are slightly longer or mostly as long as broad, polygonal, 5–6-sided and straight-walled. From here onwards they become gradually isodiametric, the walls becoming more bent or irregularly outlined (Fig. 3) and almost amoeboid as the cells reach a greater distance from the strands. In the vicinity of the smaller veinlets they are completely amoeboid (Fig. 3). The stomata are distributed uniformly among all these four types of cells. *Stomata*, not prominent, the guard cells having a faint outline and the stomata themselves being much smaller than the *i.c.* cells; usually 2–3 cells apart. Abnormal stomata of single guard cells are rarely seen. *Sphaerides*, many and prominent, sparse in the neighbourhood of the bigger strands where however their size is large. Near the smaller veinlets, *i.e.*, among the amoeboid *i.c.* cells mostly, they are smaller but occur more closely. *Striations*, not very prominent; the striae flowing out from the stomata in two lateral groups to short distances only (Fig. 3).

Upper Epidermis.—*General surface*: Appearance quite distinct from the lower epidermis because the cells are throughout of the same shape and do not show either the irregular outline or the amoeboid form (Fig. 4). *Intercostal area*: Cells are straight-walled, 6–8-sided, polygonal, large, isodiametric and devoid of contents. *Sphaerides*, large, few, very spharsely distributed. *Striation*, almost absent. *Hairs*, very sparse each hair made up of an uniseriate unbranched row of 5-6 cells; persistent on a base of radiating group of intercostals (Fig. 4) which do not differ in size or otherwise from the other *i.c.* cells. Some striation is seen on these radiating cells.

Heterostemma tanjorense: Lower Epidermis.—Histomorphologically most highly differentiated of all the investigated species and hence included in the present study. *Intercostal area*: Cells do not show any gradual change in form as in *C. pauciflorum*; instead, all the cells are uniformly amoeboid or rather irregularly outlined. Individual cells are of different shapes, elongated and almost columnar, curved or amoeboid but with the number of the arms few unlike as in the typically amoeboid cells of *C. pauciflorum*. Most of the cells have one long axis but the long axes of these cells are disposed in all directions with the result the cells show a characteristic dove-tailed or mosaic alignment (Fig. 5). The mosaic further shows at intervals characteristic crystal containing cells (Fig. 5). *Stomata*, far apart (6–10 cells apart), not prominent, much smaller than the other *i.c.* cells, guard cells have faint outline and get lodged in the curves of the intercostals, their long axes disposed in all directions. *Striation*, absent. *Crystal cells*, usually in a group of 2–3 or 4 cells, often in a pair, at frequent intervals in the *i.c.* area. Each individual crystal cell is

very small compared to the *i.c.* cells, cuboidal, its lumen occupied almost completely by a single large diamond-shaped crystal. *Hairs*, (Fig. 6), very sparse; made up of a uniseriate unbranched row of 4–6 columnar cells.

Upper Epidermis.—*General surface*: Appearance is markedly different from the lower epidermis because of the complete absence of the long dove-tailed prominently amoeboid cells. *Intercostal area*: Cells 6–8-sided, polygonal, walls not straight but very frequently bent or even curved, large and mostly isodiametric (Fig. 7). *Stomata*, absent. *Striation*, faint, hair bases acting as radiating centres. *Crystal cells*, very infrequent. *Hairs*, more frequent than in the lower epidermis.

Dregea volubilis: Lower Epidermis.—*General surface*: Large prominent branching costal strands made up of 4-walled, narrow axially elongated cells divide the area into very large (in comparison to *C. grandiflora*) islands. *Intercostal area*: Individual cells, isodiametric or slightly longer than broad, walls bent. *Stomata*, prominent, very close, 2–3 cells apart, as large as or slightly smaller than the other *i.c.* cells, guard cells with a sharp outline. At frequent intervals some of the stomata form radiating centres for striations while the other stomata merely lie in the run of these striae (Fig. 8). *Sphaerides*, large, distributed closely specially in the region of the smaller vein islets. *Striation*, very profuse, covering the whole surface commencing from the outer walls of the guard cells. Striae extend in all directions, showing the radiating centres around the stomata at frequent intervals.

Upper Epidermis.—*Intercostal area*: Cells large, 6–8-walled, sides mostly straight, isodiametric. *Stomata*, absent. *Sphaerides*, large, distributed closely specially in the region of the smaller vein islets. *Striation*, very profuse, covering the whole surface irregularly, striae running in all directions; hair bases often form centres of radiation (Fig. 9). *Hairs*, very sparse; long, simple, bent, made up of a uniseriate unbranched row of much elongated, columnar cells.

Tylophora indica: Lower Epidermis.—*General surface*: Studded at regular intervals with many sphaerides and hairs. *Intercostal area*: Cells, 5–6-sided and polygonal with straight sometimes slightly bent walls. Since the hairs are placed throughout the general surface at regular intervals, they form focal structures with reference to which the other cell elements get aligned (Fig. 10). *Hairs*, simple, unbranched, elongated, bent, tapering and made up of a uniseriate row of elongated, columnar cells. They arise from a basal, single, more or less round cell surrounded by a ring of 6–8 polygonal clear cells, usually similar in appearance and size to the *i.c.* cells but sometimes slightly larger. *Stomata* distributed uniformly over the area closely; 1–2 *i.c.* cells apart; in size, almost the same as the other *i.c.* cells. *Sphaerides*, only slightly larger than the basal cell of the hairs, numerous, closely arranged. *Striation*, almost absent in the general surface excepting

of the cells of the hair base where radiating arrangement is seen (Fig. 10).

Upper Epidermis.—*Intercostal area*: Cells, polygonal, isodiametric, 6-sided, walls straight, at distant intervals close groups of small cells occur (Fig. 11), probably representing the degenerated hair bases. *Hairs*, at very infrequent intervals, similar in size and structure to the *i.c.* area. *Sphaerides*, present, not so many as in the lower epidermis. *Striations*, simple, not very pronounced.

Discussion

The following are some of the general features important for sketching a description of the relative configuration of the cells of the epidermal surface consistently: (*a*) *Intercostal area* : (1) The dimensions of the cells, *viz.*, whether they are isodiametric or elongated and if latter, whether any consistency is seen in the disposition of the long axes of the cells (parallel alignment in the lower epidermis of *Ceropegia juncea*) or not, as in all the other species studied. (2) The shape of the cells, *viz.*, straight or bent-walled, columnar or amoeboid (*Cynanchum pauciflorum* and *Heterostemma tanjorense*). In the cases where a single leaf shows cells of many shapes, the regions where the latter occur with reference to costal strands is a feature to be noted. This will be of critical pharmacognostic value as a portion of the costal strand is usually available even in small broken samples of leaf drugs (*Cynanchum pauciflorum*). (3) Special features, if any, such as stomatal 'nodes' of *Ceropegia juncea* and close groups of narrow cells of *Tylophora indica*. (*b*) *Stomata* : (1) Direction of the vestibular slit. As we are concerned with the appearance of the stomata on the general surface of the foliar epidermis, the vestibular slit becomes an important criterion for description. This usually exists in the form of a narrow ellipse whose long axis can be considered as the long axis of the stoma itself. This axis is usually disposed in all directions on the epidermal surface with the result the stomata themselves appear tilted in all directions. Sometimes only, the long axes show a consistent relative distribution, *e.g.*, the parallel alignment of lower epidermis in *Ceropegia juncea*. (2) The density of stomatal distribution. Since the distribution is usually uniform, a *ready reckoner* of the stomatal density is given by the number of the intercostals that intervene the adjacent stomata (1–2 *i.c.* cells apart in *Tylophora indica*, 6–10 cells apart in *Heterostemma tanjorense*). (3) Size and prominence in comparison with the *i.c.* cells; smaller in *Cynanchum pauciflorum*, larger in *Ceropegia juncea—see* Table I; faint in *Cynanchum pauciflorum*, prominent in *Dregea volubilis*. (4) Special features, if any, *e.g.*, unusually large stomata at intervals along with the usual sized ones as in *Cryptostegia grandiflora*, some stomata forming radiating centres for striations while others merely lie in the run of the striae as in *Dregea volubilis*. (*c*) *Striation*: Presence (*Dregea volubilis*) or absence (*Cryptostegia grandiflora*) of radiating foci. (*d*) *Costal region*: By costal region we refer to the epidermal cells covering the vascular strands only and as such the presence or absence of vascular traces underneath the costal cells is a question of no importance here. In fact the

typical features of the costal cells are seen better in fragments where the vascular tissues beneath do not mask them. (1) The usual extent of the costal area can be readily measured by the number of the cells making up its breadth (the larger strands of 10-12 cells extent in *Cynanchum pauciflorum*). (2) Special features, if any, such as the 'reticulum' of branching strands in *Cryptostegia grandiflora* and *Dregea volubilis*. (e) *Presence of sphaerides* (*Cynanchum pauciflorum*, *Dregea volubilis*), *crystal cells* (*Heterostemma tanjorense*) and *hairs* (*Tylophora indica*).

All the above features generally differ between the upper and lower epidermis.

The following histomorphological features are noteworthy in comparison with the species studied earlier, *viz.*, *Calotropis gigantea*, R. Br. purple and white variety, *Leptadenia reticulata*, W & A, *Daemia extensa*, R. Br., *Hemidesmus indicus* R. Br., and *Telosma minor*, Craib (Krishnamurthy and Sundaram, 1967). Both the upper and lower epidermis have a similar appearance in the purple and white varieties of *Calotropis gigantea* and *Daemia extensa* while in *Ceropegia juncea* though the stomata are found on both the epidermis, the latter's appearance on either side is otherwise quite different. In all the other species stomata are confined to lower epidermis only. Hairs are profuse and distributed at regular intervals in the two varieties of *Calotropis* as well as *Tylophora indica* while they also occur, though not in such profusion, in many other species, *viz.*, *Leptadenia reticulata*, *Daemia extensa*, *Hemidesmus indicus*, *Telosma minor* and *Dregea volubilis*. In all of them, the type of the trichome is multicellular, uniseriate and simple, in conformity with what is usual in the family (Metcalfe and Chalk, 1957). Cuticular striation whose importance is well stressed recently (Stace, 1961) shows a number of interesting patterns in the series of twelve plants so far studied. Striation is very profuse in the two varieties of *Calotropis gigantea*, covers the entire surface and shows radiating foci centering around the stomata. This type of radiating pattern where the striae flank the stomatal foci is a very common occurrence in the family and is found in many other species of our study: *Dregea volubilis*, *Leptadenia reticulata*, *Telosma minor* and *Cynanchum pauciflorum*. *Dregea volubilis* exhibits an interesting variation where only some stomata form such radiating centres for striation while the others merely lie in the general run of the striae. Hairs act as such radiating centres for the faint striation in *Tylophora indica* and *Heterostemma tanjorense*. In *Cryptostegia grandiflora*, the striation is very profuse and made up of irregularly curved lines giving the whole surface a wrinkled appearance leaving out only the guard cells and often the subsidiary cells; no particular centre of radiation is traceable in this species. The striation may occur in the form of groups of simple straight lines extending over a shorter (*Telosma minor* and *Cynanchum pauciflorum*) or greater distance (the full length of the inter-costal cell series in *Ceropegia juncea*) with no radiating centres traceable anywhere.

SUMMARY

The detailed study of the histomorphology of the foliar epidermis is an important aspect of the pharmacognosy of leaf drugs. In an earlier study of six medicinal plants of Asclepiadaceae in this line, it was pointed out that the real criterion of differentiation here lies more in a combination of isolated characters rather than in any one or two of them however striking they may be. Accordingly the description has to refer mainly to the relative configuration of all the cell elements making up the general pattern of the epidermal surface. By a study of six more species of Asclepiadaceae, an attempt is made to specify consistent points of such description so as to facilitate the study of all the features comparatively in any further investigation.

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