

## Investigations on foliar epidermis in some Rubiaceae

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### Abstract

The paper reports foliar epidermal features of 20 unstudied species belonging 12 genera of the family Rubiaceae. The foliar stomates although paracytic generally in the taxa investigated, other types are also noticed rarely or occasionally on the same surface. The other epidermal features such as stomatal index (S.I) and frequency (S.F), stomatal abnormalities, cell wall contours, cell inclusions, etc. have been described in detail. Significance of various epidermal features in taxonomy of the taxa investigated is discussed.

**Keywords:** Foliar epidermis, Stomata, Cell wall contours, Rubiaceae.

### INTRODUCTION

The foliar epidermal features of Rubiaceae have been documented by Solereder (1908), Metcalfe and Chalk (1950). They are further studied by different workers (Pant and Mehra, 1965; Bahadur, Rajagopal and Ramayya, 1971; Patel and Zaveri, 1975; Singh, Jain and Sharma, 1975; Goursat and Guignard, 1975; Bhatt and Inamdar, 1977; Vales, 1983, 1984; Bhatt, 1985; Mathew and Sivarajan, 1987; ; Darok 1997, 2000; Darok and Borhidi 1997; Darok, Borhidi and Kaposvari, 1995, 2000; Darok, Kocsis and Borhidi 2000, Vieira *et al.* 2001; Rathnakumari *et al.* 2002, Tarsila *et al.* 2009, Patil and Patil, 2009). The present study deals with 20 unstudied species belonging to 12 genera of the Rubiaceae.

### MATERIALS AND METHODS

The plant materials were collected from Tropical Botanic Garden and Research Institute, Palode, Thiruvanthapuram District (Kerala); Forest Research Institute, Peechi, Trichur (Kerala); Calicut University, Botanical Garden (Kerala); Malbar Botanical Garden, Kozhikode (Kerala); Government Batanic Garden, Ootacamund (Tamilnadu) and Lal Bag Batanic Garden, Bangalore (Karnataka). The collected materials were fixed in F.A.A. solution, and then permanently preserved in (70%) alcohol. Healthy herbarium materials were also obtained from Calicut University Herbarium (Kerala). Herbarium materials whenever used were first boiled in water for 5-10 minutes. Few drops of acetic acid were added to soften and to help recovery of tissue to natural state with a gap of few minutes after boiling. The materials were washed in water and kept ready for next stage of operation. For the study of the paradermal view of the epidermis small rectangular area of

epidermal were removed from the middle portion of the leaf blade. The chemical method was followed for the separation of peels. Diluted nitric acid and chromic acid (5-10%) were used in different proportions. In case of some leaves, the chemical method is not suitable. The mechanical scratching method was used for obtaining the peels. Epidermal peels were stained in safranin (1%). They were mounted in (50%) glycerin and ringed with nail paints. The sketches were drawn using prism type camera lucida. The sketches were inked by using Camligraph or Rotring isographs technical inking pens with 0.1, 0.2, 0.3 points. The observations have been provided in table – I.

The stomatal index was decided as defined by Salisbury (1932). Stomatal frequency was calculated as by Ghosh and Davis (1973). The term used for describing stomata are as those of Metcalfe and Chalk (1950) Dilcher (1974) and Van-cotthem (1970). The typification of subsidiary cells is after Ramayya and Rajagopal (1980).

### Abbreviations

F c : Foot cell, G s : Giant stomata, P : Papillae, Str : Striations, A : Absent., S.I: Stomatal index, S.F: Stomatal frequency.

### OBSERVATIONS

***Canthium coromandelicum*** ( Burm. f.) Alston : Leaves hypostomatic.

Leaf Adaxial: Epidermal cells chlorophyllous, sides 4-8, walls undulate or arched, foot cells of trichomes present (Fig. 1a).

Leaf Abaxial: Stomata usually paracytic, superficial, orientation random, distribution diffuse, juxtaposed and superimposed contiguous stomata present, S.I. 21.10 and S.F. 210. Subsidiaries cells 2, sides 2-5, walls arched and undulate, mostly F-type, rarely A and C-type. Guard cells elliptical, chlorophyllous. Epidermal cells chlorophyllous, sides 4-9, walls straight, arched or undulate, sinuses U-shaped (Fig. 1b).

***Canthium rheedei*** DC. : Leaves hypostomatic.

Leaf Adaxial: Epidermal cells chlorophyllous, side 4-8, walls

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arched and undulate, sinuses U-shaped (Fig. 2a).

Leaf Abaxial: Stomata mostly paracytic, rarely parahexacytic-dipolar, superficial, orientation random, distribution diffuse, rarely juxtaposed contiguous stomata present, S.I. 24.08 and S.F. 250. Subsidiaries cells 2-4, sides 2-6, walls arched, mostly F-type, rarely C-type. Guard cells elliptical, chlorophyllous, Epidermal cells chlorophyllous, sides 5-9, straight, walls arched and undulate, sinuses U-shaped (Fig. 2b).

**Chassalia ophioxylodes** (Wall.) Craib : Leaves hypostomatic.

Leaf Adaxial: Epidermal cells chlorophyllous, sides 4-8, walls straight, isodimetric, hexagonal or polygonal, striations and foot cell of trichome present (Fig. 3a).

Leaf Abaxial: Stomata mostly paracytic, rarely pericytic, superficial, orientation random, distribution diffuse, S.I. 22.15 and S.F. 245. Subsidiaries cells 1-2, sides 3-4, walls arched, F-type. Guard cells elliptical, chlorophyllous. Epidermal cells chlorophyllous, sides 4-7, walls straight and slightly arched, or undulate, sinuses U-shaped (Fig. 3b).

**Coprosma lucida** J. R. et. G. Forst. : Leaves hypostomatic.

Leaf Adaxial: Epidermal cells chlorophyllous, sides 4-8, walls straight and arched, polygonal (Fig. 4a).

Leaf Abaxial: Stomata mostly paracytic, rarely anisocytic, superficial, orientation random, distribution diffuse, superimposed contiguous stomata present rarely, S.I. 22.43, S.F. 265. Subsidiaries cells 2-4, sides 5-8, walls arched and undulate, sinuses U-shaped, mostly F-type, rarely A- and C-type. Guard cells elliptical, chlorophyllous. Epidermal cells chlorophyllous, sides 5-8, walls arched or undulate, sinuses U and V-shaped (Fig. 4b).

**Gardenia gummifera** L. f. : Leaves hypostomatic.

Leaf Adaxial: Epidermal cells chlorophyllous, sides 4-8, walls arched and undulate, sinuses U-shaped, rarely V-shaped, foot cell of trichome present (Fig. 5a).

Leaf Abaxial: Stomata mostly paracytic, rarely anomocytic superficial, orientation random, distribution diffuse, juxtaposed, superimposed and obliquely oriented contiguous stomata present, stomata with single guard cells and giant stomata also present. S.I. 19.38, S.F. 195. Subsidiaries cells 2-4, sides 3-5, walls arched, mostly F-type, rarely A- and C-type. Guard cells elliptical, chlorophyllous. Epidermal cells chlorophyllous, sides 4-8, walls arched and undulate, sinuses U-shaped, foot cells of trichomes present (Fig. 5b, 5c, 5d).

**Gardenia jasminoides** Ellis: Leaves hypostomatic.

Leaf Adaxial: Epidermal cells chlorophyllous, sides 5-7, walls arched and undulate, sinuses U-shaped (Fig. 6a).

Leaf Abaxial: Stomata mostly paracytic, rarely pericytic, superficial, orientation random, distribution diffuse, rarely superimposed contiguous stomata present, stomata with single guard cells and giant stomata also present. S.I. 23.8, S.F. 218. Subsidiaries cells 2, sides 5-7, walls arched and undulate, sinuses U-shaped, mostly F-type, rarely A-type. Guard cell elliptical, chlorophyllous. Epidermal cells chlorophyllous, sides 5-11, walls arched and undulate, sinuses U-shaped (Fig. 6b, 6c, 6d).

**Gardenia longistyla** (DC.) Hook : Leaves amphistomatic.

Leaf Adaxial: Stomata paracytic, superficial, orientation random, distribution diffuse, S.I. 1.50, S.F. 11. Subsidiaries cells 2, sides 2-3, walls arched, F-type. Guard cells elliptical, Chlorophyllous. Epidermal cells chlorophyllous, sides 5-7, walls undulate, sinuses U-shaped (Fig. 7a).

Leaf Abaxial: Stomata mostly paracytic, rarely pericytic, superficial, orientation random, distribution diffuse. S.I. 21.25, S.F. 205. Subsidiaries cells 2, sides 2-8, walls arched and undulate, sinuses U-shaped, F-type. Epidermal cells chlorophyllous, sides 5-9, walls undulate, sinuses U-shaped rarely V-shaped (Fig. 7b).

**Geophila repens** (L.) Johnston : Leaves hypostomatic.

Leaf Adaxial: Epidermal cells chlorophyllous, sides 5-8, walls straight, isodiametric, penta to polygonal (Fig. 8a).

Leaf Abaxial: Stomata mostly paracytic and rarely amphiparacytic, superficial, orientation random, distribution diffuse, stomata with single guard cell and arresting development present, S.I. 15.35, S.F. 140. Subsidiaries cells 2-4, sides 3-4, walls arched, F-type. Guard cells elliptical. Chlorophyllous, papillate, Epidermal cells chlorophyllous, sides 4-7, walls straight and slightly arched or undulate, sinuses U-shaped (Fig. 8b, 8c, 8d).

**Haldina cordifolia** (Roxb.) Ridsd. : Leaves hypostomatic.

Leaf Adaxial: Epidermal cells chlorophyllous, sides 4-8, walls arched and undulate, sinuses U-shaped (Fig. 9a).

Leaf Abaxial: Stomata mostly paracytic, rarely pericytic, superficial, orientation random, distribution diffuse, rarely superimposed contiguous stomata present, S.I. 20.47, S.F. 235. Guard cells elliptical, chlorophyllous. Subsidiaries cells 2, sides 3-6, walls arched, mostly F-type, rarely A and C-type. Epidermal cells chlorophyllous, sides 5-8, walls arched and undulate, sinuses U-shaped (Fig. 9b).

**Hymenodictyon orixense** (Roxb.) Mabb. : Leaves hypostomatic.

Leaf Adaxial: Epidermal cells chlorophyllous, sides 5-7, walls arched and undulate, sinuses U-shaped, foot cell of trichome present (Fig. 10a).

Leaf Abaxial: Stomata usually paracytic, superficial, orientation random, distribution diffuse, juxtaposed contiguous stomata present, S.I. 17.80, S.F. 179. Guard cells elliptical, chlorophyllous. Subsidiaries cells 2, sides 4-10, walls undulate, sinuses U-shaped, A, C and F-type. Epidermal cells chlorophyllous, sides 4-8, walls undulate, and sinuses U-shaped, foot cell of trichome present (Fig. 10b).

**Ixora finlaysoniana** Wall. ex Don. : Leaves hypostomatic.

Leaf Adaxial: Epidermal cells chlorophyllous, sides 4-8, walls straight, slightly arched, n hexa to polygonal, striations present (Fig. 11a).

Leaf Abaxial: Stomata mostly paracytic, rarely amphiparacytic and desmocytic, superficial, orientation random, distribution diffuse, rarely stomata with single guard cells present. S.I. 16.56, S.F. 175. Guard cells elliptical, chlorophyllous. Subsidiaries cells 2-4, sides 2-6,

walls arched, mostly F-Type, rarely C-type. Epidermal cells chlorophyllous, sides 6-7, walls arched and undulated, sinuses U-shaped, striations present (Fig. 11b, 11c).

***Ixora javanica*** DC. : Leaves amphistomatic.

Leaf Adaxial: Stomata usually paracytic, superficial, orientation random, distribution diffuse, S.I. 2.75, S.F. 22. Guard cells elliptical, chlorophyllous. Subsidiaries cells 2, sides 4-6, walls arched F-Type. Epidermal cells chlorophyllous, sides 5-8, walls arched and undulate, sinuses U-shaped, striations present (Fig. 12a). Leaf Abaxial: Stomata mostly paracytic, rarely amphiparacytic and anomocytic, superficial, orientation random, distribution diffuse, superimposed contiguous stomata present. S.I. 18.88, S.F.195. Guard cells elliptical, chlorophyllous. Subsidiaries cells 2-4, sides 3-5, walls arched, mostly F-type, rarely A-type. Epidermal cells chlorophyllous, sides 5-9, walls undulate, sinuses U-shaped, striations present (Fig.12b, 12c).

***Ixora lanceolaria*** Colebr. : Leaves hypostomatic.

Leaf Adaxial: Epidermal cells chlorophyllous, sides 4-8, walls straight and slightly arched, striations present (Fig. 13a).

Leaf Abaxial: Stomata usually paracytic, superficial, orientation random, distribution diffuse, rarely juxtaposed and superimposed contiguous stomata and rarely stomata with single guard cells present, S.I. 19.25, S.F.216. Guard cells elliptical, chlorophyllous. Subsidiaries cells 2, sides 4-7, walls arched, mostly F-type, rarely A and C-type. Epidermal cells chlorophyllous, sides 6-10, walls arched and undulated, sinuses U-shaped, striations present (Fig.13b,13c, 13d).

***Ixora monticola*** Gamble : Leaves hypostomatic.

Leaf Adaxial: Epidermal cells chlorophyllous, sides 5-8, walls straight, slightly arched isodimetric, hexagonal, striations present (Fig. 14a).

Leaf Abaxial: Stomata usually paracytic, superficial, orientation random, distribution diffuse, juxtaposed and superimposed contiguous stomata present, S.I. 18.90, S.F.198. Guard cells elliptical, chlorophyllous. Subsidiaries cells 2, sides 4-7, walls arched, mostly F-type, rarely A-type. Epidermal cells chlorophyllous, sides 4-10, walls arched and undulate, sinuses U-shaped, striations present (Fig. 14b, 14c).

***Ixora thwaitesii*** Hook. f. : Leaves hypostomatic.

Leaf Adaxial: Epidermal cells chlorophyllous, sides 5-8, walls straight, slightly arched, isodiametric, hexa to polygonal, striations present (Fig.15a).

Leaf Abaxial: Stomata mostly paracytic, rarely amphiparacytic, superficial, orientation random, distribution diffuse, rarely stomata with single guard cells and arresting development present. S.I. 20.58, S.F. 208. Guard cells elliptical, chlorophyllous. Subsidiaries cells 2-4, sides 4-6, walls arched, mostly F-type, rarely A and C-type. Epidermal cells chlorophyllous, sides 5-9, walls arched and undulate, sinuses U-shaped, striations present (Fig.15b).

***Luculia gratissima*** (Wall.) Sweet : Leaves hypostomatic.

Leaf Adaxial: Epidermal cells chlorophyllous, sides 4-7, walls straight, slightly arched, hexagonal, isodiametric, foot cells of trichome present (Fig. 16a).

Leaf Abaxial: Stomata mostly paracytic, rarely anisocytic, superficial, orientation random, distribution diffuse, S.I. 15.49, S.F. 160. Guard cells elliptical, chlorophyllous. Subsidiaries cells 2-3, sides 4-8, walls undulate, sinuses U-shaped, F-type. Epidermal cells chlorophyllous, sides 4-8, walls undulate, sinuses U-shaped (Fig. 16b).

***Nauclea orientalis*** : (L.) L. Leaves hypostomatic.

Leaf Adaxial: Epidermal cells chlorophyllous, sides 5-8, walls straight, isodiametric, penta to polygonal, foot cells of trichomes and striations present (Fig.17a).

Leaf Abaxial: Stomata usually paracytic superficial, orientation random, distribution diffuse, stomata with arresting development present, S.I. 16.35, S.F. 178. Guard cells elliptical, chlorophyllous. Subsidiaries cells 2, sides 3-7, walls arched, F-type. Epidermal cells chlorophyllous, sides 5-8, walls straight and arched (Fig.17b).

***Pavetta breviflora*** DC. : Leaves hypostomatic.

Leaf Adaxial: Epidermal cells chlorophyllous, sides 5-7, walls undulate, sinuses U-shaped, (Fig.19a).

Leaf Abaxial: Stomata mostly paracytic, rarely pericytic, superficial, orientation random, distribution diffuse, S.I. 17.40, S.F. 180. Guard cells elliptical, chlorophyllous. Subsidiaries cells 2, sides 4-7; walls undulate, sinuses U-shaped, F-type. Epidermal cells are, chlorophyllous, sides 5-8, walls undulate, sinuses U-shaped (Fig.18b).

***Pavetta tomentosa*** Roxb. ex J. E. Smith : Leaves hypostomatic.

Leaf Adaxial: Epidermal cells chlorophyllous, sides 5-8, walls undulate, sinuses U and V-shaped, foot cells of trichome is present (Fig. 19a).

Leaf Abaxial: Stomata mostly paracytic, rarely pericytic, superficial, orientation random, distribution diffuse, S.I. 15.90, S.F. 170. Guard cells elliptical, chlorophyllous. Subsidiaries cells 2, sides 2-8, walls undulate, sinuses U-shaped, F-type. Epidermal cells are, chlorophyllous, sides 5-8, walls undulate, sinuses U and V-shaped (Fig. 19b).

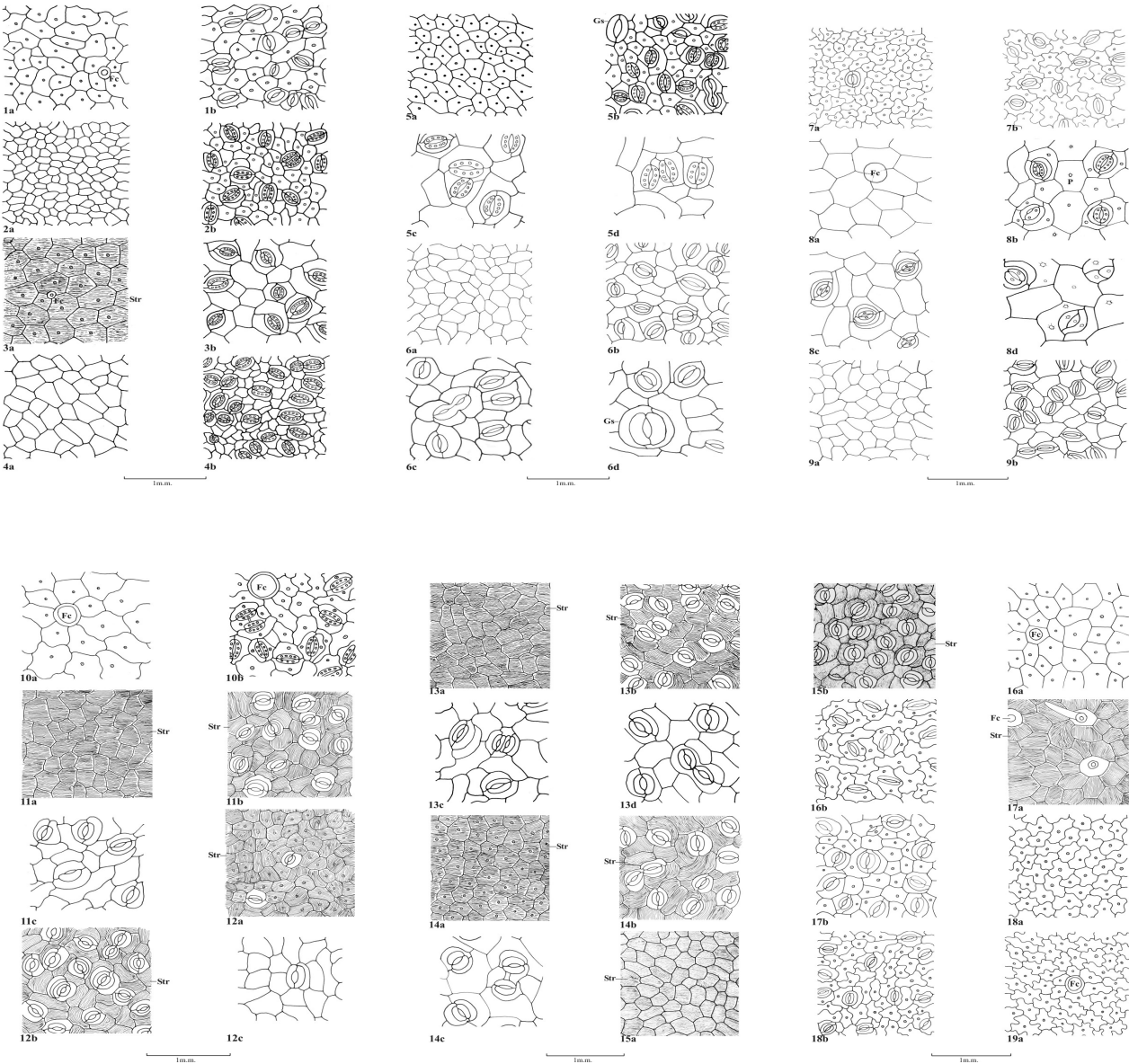
***Pentas carnea*** Benth. : Leaves hypostomatic.

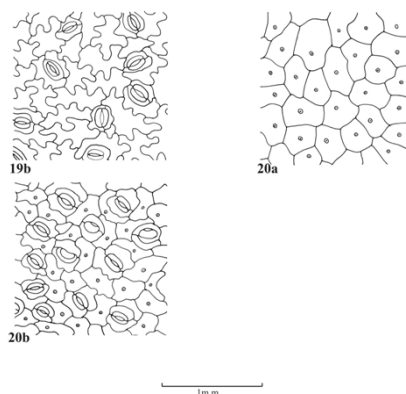
Leaf Adaxial: Epidermal cells chlorophyllous, sides 4-7, walls arched and undulated, sinuses U-shaped (Fig. 20a).

Leaf Abaxial: Stomata usually paracytic, superficial, orientation random, distribution diffuse, rarely stomata with single guard cells present, S.I. 19.48, S.F. 205. Guard cells elliptical, chlorophyllous. Subsidiaries cells 2, sides 3-5, walls undulate, sinuses U-shaped, F-type. Epidermal cells chlorophyllous, sides 4-10, walls undulate, sinuses U-shaped (Fig. 20b).

Table 1. Stomatal Index and Stomatal Frequency (Per sq.cm.)

Sr. No.	Plant Species Studied	Stomatal Index		Stomatal Frequency	
		Leaf Adaxial	Leaf Adaxial	Leaf Abaxial	Leaf Abaxial
1	<i>Canthium coromandelicum</i>	A	21.10	A	210
2	<i>Canthium rheedei</i>	A	24.08	A	250
3	<i>Chassalia ophioxylodes</i>	A	22.15	A	245
4	<i>Coprosma lucida</i>	A	22.43	A	260
5	<i>Gardenia gummifera</i>	A	19.38	A	195
6	<i>Gardenia jasminoides</i>	A	23.80	A	218
7	<i>Gardenia longistyla</i>	1.50	21.25	11	205
8	<i>Geophila repens</i>	A	15.35	A	140
9	<i>Haldina cordifolia</i>	A	20.47	A	235
10	<i>Hymenodictyon orixense</i>	A	17.47	A	179
11	<i>Ixora finlaysonianana</i>	A	16.56	A	175
12	<i>Ixora javanica</i>	2.75	18.88	22	195
13	<i>Ixora lanceolaria</i>	A	19.25	A	216
14	<i>Ixora monticola</i>	A	18.90	A	198
15	<i>Ixora thwaitesii</i>	A	20.58	A	208
16	<i>Luculia gratissima</i>	A	15.49	A	160
17	<i>Nauclea orientalis</i>	A	16.35	A	178
18	<i>Pavetta breviflora</i>	A	17.40	A	180
19	<i>Pavetta tomentosa</i>	A	15.90	A	170
20	<i>Pentas carnea</i>	A	19.48	A	205





## DISCUSSION

The paracytic stomatal type (*sensu* Metcalfe and Chalk, 1950) has been typified as Rubiaceous type by Vesque (1889). This type is widely documented in the family as stated earlier. The present authors also recorded it in 20 species belonging 12 genera of the Rubiaceae. Rare occurrence of other types e.g. anisocytic, anomocytic, pericytic, desmocytic, parahexacytic-dipolar and amphiparacytic are also observed rarely in few taxa investigated. Few stomatal abnormalities such as contiguous stomata – (i) superimposed, (ii) juxtaposed, (iii) obliquely oriented, and giant stomata are occasionally noted. The leaves are hypostomatic except *Gardenia longistyla* and *Ixora javanica*. The comparative distribution of stomata in these three taxa needs a mention. The stomatal index or frequency of adaxial foliar surface is far lower in comparison to the abaxial one. Such a condition of leaves is considered as functionally hypostomatic. The highest stomatal index (24.08) is noted abaxially in *Canthium rheedei*, whereas if the lowest (15.35) in *Geophila repens*. Three types of subsidiaries are recognized in angiosperms (Ramayya and Rajgopal, 1980). Of these, F-type subsidiaries occur predominantly, while other types viz., A and C are rarely observed on the same surface. All the three types on the same surface are noted in *Canthium coromandelicum*, *Coprosma lucida*, *Gardenia gummiifera*, *Haldina cordifolia*, *Hymenodictyon orixense*, *Ixora lanceolaria* and *Ixora thwaitesii*.

Epidermal cell walls are either undulate or straight. The former case is observed on both surfaces in *Canthium rheedei*, *Gardenia gummiifera*, *Gardenia jasminoides*, *Gardenia longistyla*, *Haldina cordifolia*, *Hymenodictyon orixense*, *Ixora javanica*, *Pavetta breviflora*, *Pavetta tomentosa* and *Pentas carnea*. The cell walls are straight on both surfaces in *Chassalia ophioclyoides* and *Geophila repens*. They are either straight or undulate abaxially and adaxially in some others. The foliar surfaces are striated on either side in eight species, whereas in rest others leaves are want of striations.

Although the members of Rubiaceae show uniform stomates, other epidermal characters appear to be of taxonomic significance as they occur differently and constantly in various species studied. Such characters are : (i) distribution of stomata, (ii) stomatal index and frequency, (iii) cell wall contours, (iv) types of subsidiaries and their distribution, (v) presence of crystals and, (vi) striations, etc. These can be conveniently employed to distinguish the rubiaceous taxa.

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