

REVIEW ARTICLE

Occurrence and significance of cell inclusions in Rubiaceae

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ABSTRACT

The present communication embodies the results of foliar anatomical studies in 40 species of the family Rubiaceae. Particularly, diverse crystal forms such as raphides, rosette crystals, druses, crystal-sand and granular matter have been recorded from various foliar tissues. All these types are totally absent in three species. In others, they either occur singly or in different combinations. The authors also discussed them taxonomically. Evidence has been also borrowed from past literature, besides author's own work. The present authors, however, cautioned their use in taxonomy and suggested their use only after critical investigations in the alliance.

Introduction

It was Bureau (1861) who applied anatomical features in plant classification for the first time. Bailey (1952), Metcalfe (1954, 1961), Dickison (1975) and few others laid down the guiding principles of systematic anatomy. Paliwal and Anand (1978) discussed the role of anatomical evidence in classifying angiosperms. However, cell inclusions observed commonly throughout a group of angiosperms have not critically studied. The present authors while investigating foliar anatomical features of some Rubiaceae came across their occurrence and endeavored to point out taxonomic significance. This forms the subject matter of this communication.

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 Botanic Garden, Ootacamund (Tamilnadu) and Lal Bag Botanic 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. Free hand transections of petioles and leaves of preserved and herbarium materials were stained in safranin (1%) and fast green (1%). They were mounted in D. P. X. after the customary method of dehydration. 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 some cases, Three Acid Treatment (TAT Method) was followed (Ramayya and Vanaja, 1979). 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. In case of petioles and leaves middle part was selected for sectioning. The sketches were drawn using prism type camera lucida. The sketches were inked by using Camligrath or Rotring isographs technical inking pens with 0.1, 0.2, 0.3 points. The observations have been provided in Table 1.

Table 1: Foliar cell inclusions in Rubiaceae

Sr. No.	Plant Studied	Tribe	Plant part	Raphides (R)	Crystal-sand (C s)	Rosette crystals (R c)	Granular matter (Gm)	Druses (Dru)
1	<i>Canthium coromandelicum</i> (Burm. f.) Alston	Vanguerieae	Petiole	A	A	A	A	A
			Leaf T. S.	Ground tissue	A	Ground tissue	A	A
2	<i>Canthium rheedei</i> DC.	Vanguerieae	Petiole	A	A	Ground tissue	A	A
			Leaf T. S.	A	A	Ground tissue	A	A
3	<i>Chassalia ophioxyloides</i> (Wall.) Craib	Psychotrieae	Petiole	Ground tissue	A	A	A	A
			Leaf T. S.	Ground tissue and Mesophyll cells	A	A	Ground tissue	A
4	<i>Coprosma baueri</i> Endl.	Anthospermeae	Petiole	Ground tissue	A	A	A	A
			Leaf T. S.	Ground tissue	A	A	A	A
5	<i>Coprosma lucida</i> J.R. et G. Forst.	Anthospermeae	Petiole	Ground tissue	A	Ground tissue	A	A
			Leaf T. S.	Ground tissue	A		A	A
6	<i>Gardenia gummifera</i> L. f.	Gardenieae	Petiole	A	A	Ground tissue & Pith	A	A
			Leaf T. S.	A	A	Ground tissue & Pith	A	A
7	<i>Gardenia jasminoides</i> Ellis	Gardenieae	Petiole	A	A	Ground tissue	A	A
			Leaf T. S.	A	A	Ground tissue & Pith	A	A
8	<i>Gardenia latifolia</i> Aiton	Gardenieae	Petiole	A	A	Ground tissue & Pith	A	A
			Leaf T. S.	A	A	Ground tissue & Pith	A	A
9	<i>Gardenia longistyla</i> (DC.) Hook.	Gardenieae	Petiole	A	A	Ground tissue	Ground tissue	A
			Leaf T. S.	A	A	Ground tissue, Pith & Mesophyll cells	A	A
10	<i>Geophila repens</i> (L.) Johnston	Psychotrieae	Petiole	A	A	A	A	A
			Leaf T. S.	A	A	A	A	A
Sr No.	Plant Studied	Tribe	Plant part	Raphides (R)	Crystal-sand (C s)	Rosette crystals (R c)	Granular matter (Gm)	Druses (Dru)
11	<i>Guettarda speciosa</i> L.	Guettardeae	Petiole	A	A	A	A	A
			Leaf T. S.	A	A	Ground tissue & Mesophyll cells	A	A
12	<i>Haldina cordifolia</i> (Roxb.) Ridsd.	Naucleae	Petiole	A	A	Ground tissue & Pith	A	A
			Leaf T. S.	A	A	Ground tissue & Pith	A	A
13	<i>Hedyotis stylosa</i> R. Br. ex Wight & Arn.	Hedyotideae	Petiole	Ground tissue	A	Ground tissue	A	A
			Leaf T. S.	Ground tissue	A	A	A	A

			Leaf Epidermis	A	A	A	A	Leaf Epidermis
14	<i>Hymenodictyon orixense</i> (Roxb.) Mabb.	Cinchoneae	Petiole	A	Ground tissue & Pith	Ground tissue & Pith	A	A
			Leaf T. S.	A	A		A	A
15	<i>Ixora alba</i> L.	Ixoreae	Petiole	A	A	Ground tissue & Pith	A	A
			Leaf T. S.	A	A	Ground tissue & Pith	A	A
16	<i>Ixora brachiata</i> Roxb. ex DC.	Ixoreae	Petiole	A	Ground tissue & Pith	Ground tissue & Pith	A	A
			Leaf T. S.	A	A	Ground tissue & Mesophyll cells	A	A
17	<i>Ixora finlaysoniana</i> Wall. ex Don	Ixoreae	Petiole	A	A	Ground tissue & Pith	A	A
			Leaf T. S.	A	A	Ground tissue & Pith	A	A
18	<i>Ixora javanica</i> DC.	Ixoreae	Petiole	A	A	Ground tissue	A	A
			Leaf T. S.	A	A	Ground tissue & Pith	A	A
19	<i>Ixora johnsonii</i> Hook. f.	Ixoreae	Petiole	A	A	Ground tissue & Pith	A	A
			Leaf T. S.	A	A	Ground tissue & Mesophyll cells	A	A
20	* <i>Ixora lanceolaria</i> Colebr.	Ixoreae	Petiole	A	A	Ground tissue	A	A
			Leaf T. S.	A	A	Ground tissue & Pith	A	A

Sr No	Plant Studied	Tribe	Plant part	Raphides (R)	Crystal-sand (C s)	Rosette crystals (R c)	Granular matter (Gm)	Druses (Dru)
21	<i>Ixora malabarica</i> (Dennst.) Maberley	Ixoreae	Petiole	A	A	Ground tissue	A	A
			Leaf T. S.	A	A	Ground tissue & Mesophyll cells	A	A
22	<i>Ixora monticola</i> Gamble	Ixoreae	Petiole	A	A	Ground tissue & Pith	A	A
			Leaf T. S.	A	A	A	A	A
23	<i>Ixora nigricans</i> R. Br. ex Wight & Arn.	Ixoreae	Petiole	A	A	A	A	A
			Leaf T. S.	A	A	Ground tissue	A	A
24	<i>Ixora polyantha</i> Wight	Ixoreae	Petiole	A	A	Ground tissue	A	A
			Leaf T. S.	A	A	Ground tissue & Pith	A	A
25	<i>Ixora singaporensis</i> Linn.	Ixoreae	Petiole	A	A	Ground tissue	A	A
			Leaf T. S.	A	A	Ground tissue, Pith & Mesophyll cells	A	A
26	<i>Ixora thwaitesii</i> Hook. f.	Ixoreae	Petiole	A	A	Ground tissue	A	A
			Leaf T. S.	A	A	Ground tissue & Mesophyll cells	A	A

27	<i>Luculia gratissima</i> (Wall.) Sweet	Cinchone ae	Petiole Leaf T. S.	A A A	A Ground tissue A	A A Ground tissue	A A A	A A A
28	<i>Morinda reticulata</i> Gamble	Morindea e	Petiole Leaf T. S.	A A A	A A Ground tissue	A A Ground tissue & Pith	A A A	A A A
29	<i>Nauclea orientalis</i> L.	Naucleae	Petiole Leaf T. S.	A A A	A A Ground tissue	A A Ground tissue & Pith	A A A	A A A
30	<i>Nauclea parvifolia</i> Roxb.	Naucleae	Petiole Leaf T. S.	A A A	A A Ground tissue	A A Ground tissue & Pith	A A A	A A A
Sr No.	Plant Studied	Tribe	Plant part	Raphides (R)	Crystal-sand (C s)	Rosette crystals (R c)	Granular matter (Gm)	Druses (Dru)
31	<i>Ophiorrhiza mungos</i> L.	Hedyotide ae	Petiole Leaf T. S.	Ground tissue Ground tissue	A A	A A	A A	A A
32	<i>Ophiorrhiza pectinata</i> Arn.	Hedyotide ae	Petiole Leaf T. S.	Ground tissue Ground tissue	A A	Ground tissue A	A Ground tissue	A A
33	<i>Ophiorrhiza tirunelvelica</i> Henry & Subrum.	Hedyotide ae	Petiole Leaf T. S.	Ground tissue Ground tissue	A A	A A	A A	A A
34	<i>Pavetta breviflora</i> DC.	Ixoreae	Petiole Leaf T. S.	A A	A A	Ground tissue Ground tissue	A A	A A
35	<i>Pavetta calophylla</i> Bremek.	Ixoreae	Petiole Leaf T. S.	A A	A A	A A	A A	A A
36	<i>Pavetta tomentosa</i> Roxb. ex J. E. Smith	Ixoreae	Petiole Leaf T. S.	A A	A A	Ground tissue Ground tissue	A Ground tissue & Pith	A A
37	<i>Pentas carnea</i> Benth.	Hedyotide ae	Petiole Leaf T. S.	A Ground tissue	A A	A Ground tissue	A A	A A
38	<i>Psilanthus travancorensis</i> (Wight & Arn.) Leroy	Ixoreae	Petiole Leaf T. S.	A A	A A	Ground tissue Ground tissue	A A	A A
39	<i>Psychotria nudiflora</i> Wight & Arn.	Psychotrie ae	Petiole Leaf T. S.	A A	A A	A A	A A	A A
40	<i>Rondeletia amoena</i> (Planch.) Hemsl.	Rondeletie ae	Petiole Leaf T. S.	A A	A A	Ground tissue Ground tissue	A A	A A

Discussion

The present authors investigated foliar anatomy of 40 species belonging to 19 genera and the eleven tribes viz., Vanguerieae, Psychotrieae, Anthosperemeae, Gardenieae, Guettardeae, Naucleae, Hedyotideae, Cinchoneae, Ixoreae, Morindeae and Rondeletieae. The observations pertaining especially to cells inclusions are given in Table 1. It is to be noted that three species viz., *Geophilar repens*, *Pavetta calophylla* and *Psychotria nudiflora* are devoid of crystals. The present authors noticed cells inclusions in the form of raphides, rosette crystals, druses, crystal-sand and granular matter. Druses are observed only in case of foliar epidermal cells of *Hedyotis stylosa* but in petiole and veins total absence of crystals is also noted in *Limnosipahea* and *Retiniphyllum* (Metcalfe and Chalk 1950). Raphides are noted in ground tissue of petioles, in the cells of

veins and rarely in mesophyll cells of the ten species belonging to the tribe viz., Vanguerieae, Psychotrieae, Anthosperemeae, Hedyotideae and Cinchoneae. Rosette crystals are observed in ground tissue and pith of petioles and mesophyll cells of 33 species out of the total 40 species investigated. It thus appears more common than other crystal forms and lacks only in the tribe Psychotrieae. Crystal-sand is present in the ground tissue and pith cells in only four species viz., *Hymenodictyon orixense*, *Ixora brachiata*, *Nauclea orientalis* and *N. parvifolia*. It thus appears of rare occurrence. Likewise, granular matter is rarely observed in petiolar ground tissue in case of only four species viz., *Chassalia ophioxylodes*, *Gardenia longistyla*, *Ophiorrhiza pectinata* and *Pentas carnea*.

The cell inclusions crystallize out in diverse forms in the Rubiaceae. Solereder (1908) recognized three different forms like

crystal-sand, raphides and clustered crystals. However, Metcalfe and Chalk (1950) reviewed them and recognized the types such as crystal-sand, raphides, clustered crystals, small acicular crystals and sphero-crystals. Welle *et al.* (1983) reported rhombic crystals in ray and parenchyma cells woods of the tribe Guettardeae. Rathna Kumari *et al.* (2002) observed prismatic crystals, apart from acicular, rosette and usual raphide types in *Morinda pubescens*. Prismatic crystals are also recorded by Tarsil *et al.* (2009) in *Simira*. Seven kinds of crystals were isolated from the root of *Morinda officinalis* by Huoz, *et al.* (1986). The present authors observed either singly e.g. rosette type in *Canthium rheedei*, *Gardenia gummifera*, *G. jasminoides*, *G. latifolia*, *Guettardia speciosa*, *Morinda reticulata*, *Pavetta breviflora*, *Psilanthus travancorensis* and *Rondeletia amoena* or along with other types in few species. Raphides also occur as single type e. g. in *Coprosma baueri*, *Luculia gratissima* and *Ophiorrhiza mungos*. They are found in combinations with other types as well. Druses although recorded only in *Hedyotis stylosa* but they are associated with other types as well. The past studies (*cf.* Solereeder, 1908; Metcalfe and Chalk, 1950; Tarsil *et al.*, 2009; Welle *et al.*, 1983; Dessein, *et al.*, 2001; Aiello, 1979; Darwin, 1977; Rathna Kumari *et al.*, 2002; Gibbs, 1974) indicated

their taxonomic significance at different levels such as variety of a species, generic, tribal and subfamilial ones. The present authors also lend support for their utility in taxonomy of the rubiaceae taxa. Their total absence or presence in different combinations or singly in the same organs/tissues or in different organs/tissues of taxon is valuable. It is, however, important that these should be studied critically before their employment in taxonomic considerations.

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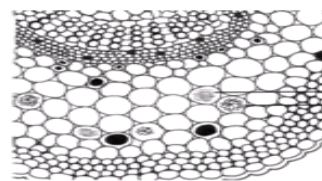


Fig 1. Leaf
Canthium coromandelicum

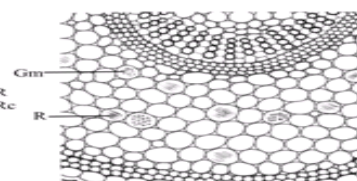


Fig. 2. Leaf
Chassalia ophioxylodes

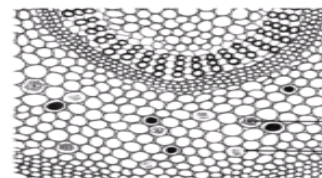


Fig 3. Petiole
Coprosma lucida

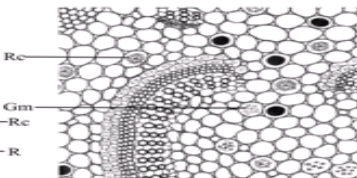


Fig. 4. Petiole
Gardenia longistyla

0.5 mm.

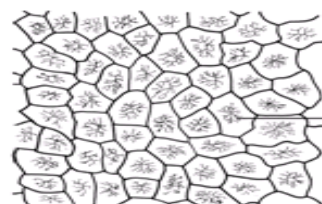


Fig 5. Leaf Epidermis
Hedyotis stylosa

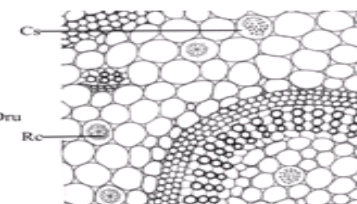


Fig. 6. Petiole
Hymenodictyon orixense

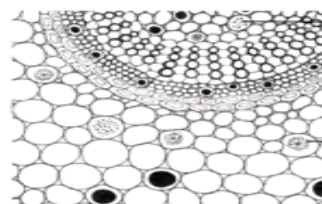


Fig 7. Petiole
Exora brachiata

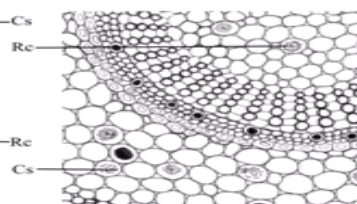


Fig. 8. Petiole
Nauclea orientalis

0.5 mm.

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*Original not consulted.