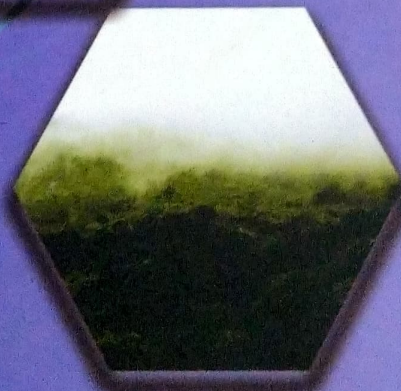
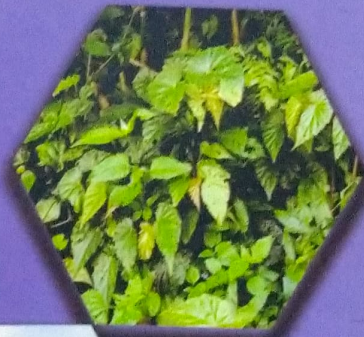


TRENDS IN PLANT SYSTEMATICS

M.B. VISWANATHAN



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Comparative Anatomy of Two Species of *Phyllanthus* L. (Euphorbiaceae s.l.) From West Bengal with Special Reference to Venation Pattern and Seed Anatomy

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Phyllanthus amarus Schum. & Thonn. and *Phyllanthus fraternus* Webster, are very much similar with each other morphologically. These two species have been in use as herbal medicine and are referred as 'Bhumyamlaki' since ancient times for jaundice and other liver-related disorders. However, *Phyllanthus amarus* is medicinally much more important than the latter one due to its higher content of active constituents like phyllanthine and hypophyllanthine. Therefore, it is of great importance if we can separate out these two species even from fragmented conditions as found in herbal drugs often, using anatomical characters. In the present study, the anatomical studies including venation pattern, seed anatomy, epidermal anatomy and stomatal complex were undertaken for the two species of *Phyllanthus*.

Key words: *Phyllanthus amarus*; *Phyllanthus fraternus*; anatomy; venation; West Bengal.

INTRODUCTION

The genus *Phyllanthus* L. was first described by Linnaeus in *Species Plantarum* in 1753. The genus contains c. 833 species distributed all over the world (Govaerts *et al.*, 2000). However, Kathriarachchi *et al.* (2006) recommended inclusion of four more genera to make it a large monophyletic genus *Phyllanthus* s.l. which would comprise c. 1269 species (Govaerts *et al.*, 2000). In Indian flora, the genus is represented by 53 species (Gangopadhyay *et al.*, 2007). Out of these 53, there are 12 herbaceous species which form an herbaceous complex group (*Phyllanthus* Herbaceous Complex). Due to morphological similarities among them, they are often misidentified. *Phyllanthus amarus* Schum. & Thonn. and *Phyllanthus fraternus* Webster, are morphologically very much similar with each other. These two species are also referred as 'Bhumyamlaki' and have been in use as herbal medicine since ancient times for jaundice and other liver-related disorders. However, *Phyllanthus amarus* is medicinally much more important than the latter species due to higher content of active constituents like phyllanthine and

hypophyllanthine. Therefore, it is of great importance if we can separate out these two species even from fragmented conditions as often found in herbal drugs, using anatomical characters. Furthermore, the anatomical characterization can be used for betterment of the present classification.

In the present study, the anatomical studies including foliar venation pattern, seed anatomy, epidermal anatomy, stomatal complex were undertaken for the two species of *Phyllanthus*.

MATERIALS AND METHODS

Materials were collected from different parts of Nadia district of West Bengal, always in 3-4 sets and the voucher specimens were preserved in the Herbarium of Department of Botany, University of Kalyani as listed in Table 1. All the specimens were properly identified by consulting standard floras of Indian regions after dissecting the floral parts (Webster, 1970; Henry & Santapau, 1973; Mitra & Jain, 1985; Lodh & Mukherjee, 2014). The Herbarium of National Botanical Research Institute, Lucknow (LWG) and Central National Herbarium (CAL), Botanical Survey of India, Sibpur, Howrah, were also consulted for that purpose.

For stem, hand sections were from fresh specimens, stained by aqueous saffranin (1%), mounted in glycerine jelly and studied under the Microscope. For venation pattern, leaves were first soaked in hot water, transferred to 2.5% KOH solution and left for overnight in a saturated solution of chloral hydrate. Then, leaves were stained in 1% aqueous saffranin solution and through gradual dehydration. Permanent slides were prepared (Trease & Evans, 1989). Description was followed after Hickey (1972) and Dilcher (1974). For epidermal anatomy, leaves were taken from fresh plant, oxidized in Schulze's reagent (Potassium Chlorate and Conc. HNO_3) and heated over a spirit lamp for 2-3 minutes. The reagent was decanted and the materials were repeatedly washed with water. Then 1% aqueous solution of saffranin was added. The leaf cuticle was then cleared using a fine brush and mounted in 70% glycerine and studied under the Microscope. For anatomical study of seed walls, correctly oriented hand sections were made using modified Hand Microtome to represent the cellular composition as observed in transverse section (Arnott, 1959).

OBSERVATIONS

A comparative account of the anatomical characters of the stem, venation pattern, seed anatomy, stomatal complex, epidermal anatomy of the studied species of *Phyllanthus* are provided (Tables 2-5; Figs. 1-3.). Some of the artificial keys prepared for the studied species are provided.

Key to the species of *Phyllanthus* based on anatomical characters

- 1a. T.S of stem round in outline; hypodermal sclerenchyma cells of seed coat more or less tangentially oriented, with swollen walls...
.....*Phyllanthus amarus*
- 1b. T.S of stem with 5 wing-like protrusions; hypodermal sclerenchyma cells of seed coat radially oriented, with straight walls, not swollen
.....*Phyllanthus fraternus*

Key to the species of *Phyllanthus* based on foliar venation pattern

- 1a. Leaves with 4-5 pairs of secondaries; highest order of venation is to 5th or 6th order; ultimate areoles are formed by the joining of mostly 3rd, 4th and 5th order of veins; free vein tips are mostly with 2 or 2-3 tracheids, mostly with broader tracheids
.....*Phyllanthus amarus*
- 1b. Leaves with 5-7 pairs of secondaries; highest order of venation is to 4th or 5th order; ultimate areoles are formed by the joining mostly of 3rd and 4th order of veins; free vein tips are mostly with 1-2 tracheids with medium width, not much broader
.....*Phyllanthus fraternus*

DISCUSSION AND CONCLUSION

The present study of *P. amarus* Schum. & Thonn. and *P. fraternus* Webster) concludes that these two species are clearly distinguishable by anatomical parameters (Khatoon *et al.*, 2006). Epidermal cells have more pronounced undulations in *P. fraternus* Webster. In T.S. of seed, hypodermal sclerenchyma cells are more swollen and more or less tangentially oriented in *P. amarus* Schum. & Thonn. but in *P. fraternus* Webster, it is radially oriented with straight walls (not swollen). Number of secondaries in leaves is 4-5 pairs in *P. amarus* Schum. & Thonn. instead of 5-7 pairs in *P. fraternus* Webster. Nature of free vein endings is also slightly different in these two species. From this aforesaid-discussion, this study has significant value for characterization of these two species of *Phyllanthus*. Therefore, not only the floral and vegetative characters are useful for taxonomic study but also the histological characters play a major role for isolation of any taxon even when flowering stage is not available in our hand. Therefore, it can be concluded that all the anatomical characters are useful for isolation and betterment of existing classification.

Options for sustainable agriculture due to their stimulating effects on plant growth and their potential are necessary to increase plant production. In the present study, we found an efficient biocontrol agent, *Enterobacter* strains for plant pathogenic fungi (*R. solani*). Hence, it is suggested that *Enterobacter* sp. can be incorporated as integrated management of disease, where the strain may be used as biocontrol agent as well as biofertilizer.

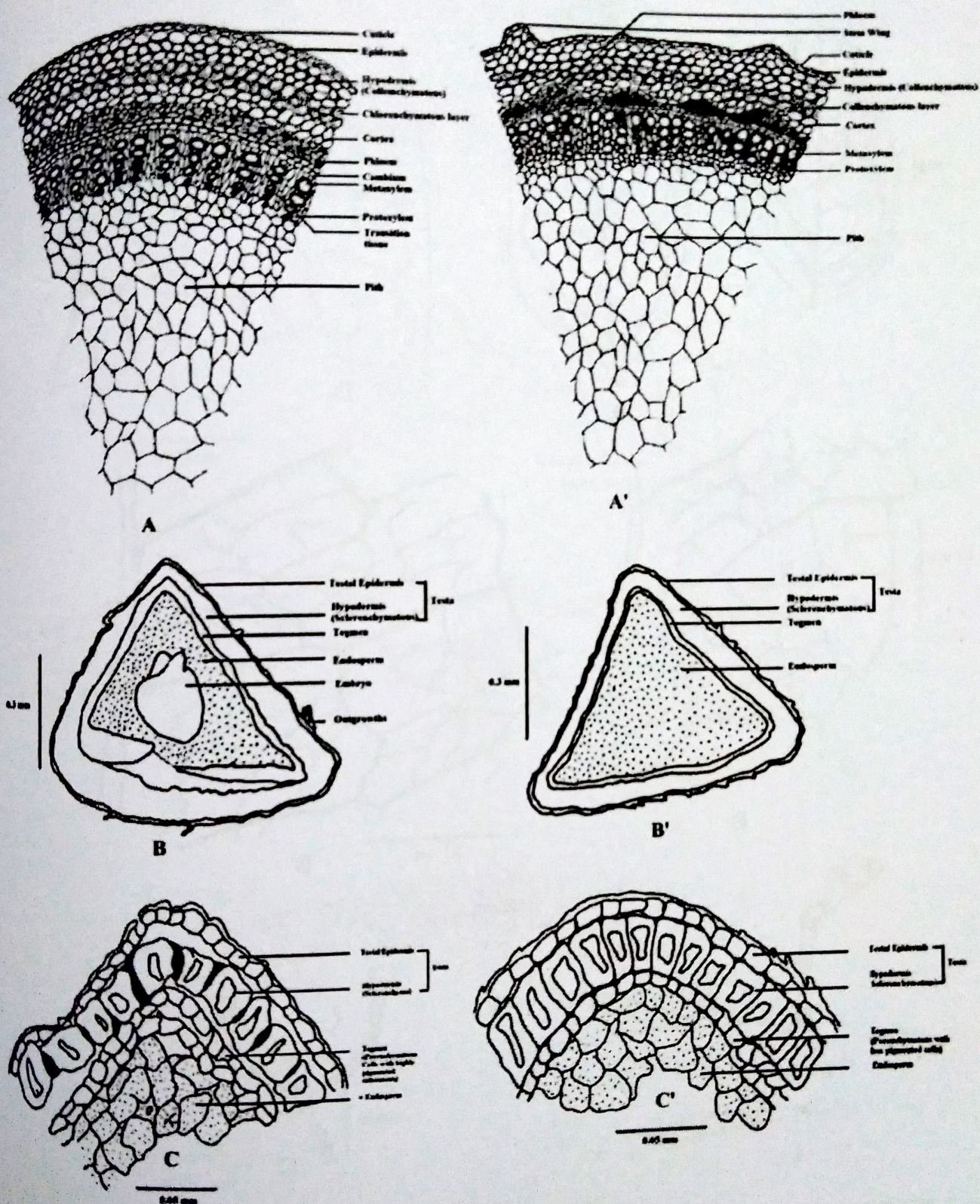


Fig. 1. Stem anatomy and seed anatomy of *Phyllanthus amarus* and *Phyllanthus fraternus*: A-C. *Phyllanthus amarus*: A. Cellular representation of T.S. of tender stem; B. T.S. of seed (Diagrammatic); C. Cellular representation of T.S. of seed. A'-C': *Phyllanthus fraternus*: A'. Cellular representation of T.S. of tender stem; B'. T.S. of seed (Diagrammatic); C'. Cellular representation of T.S. of seed.

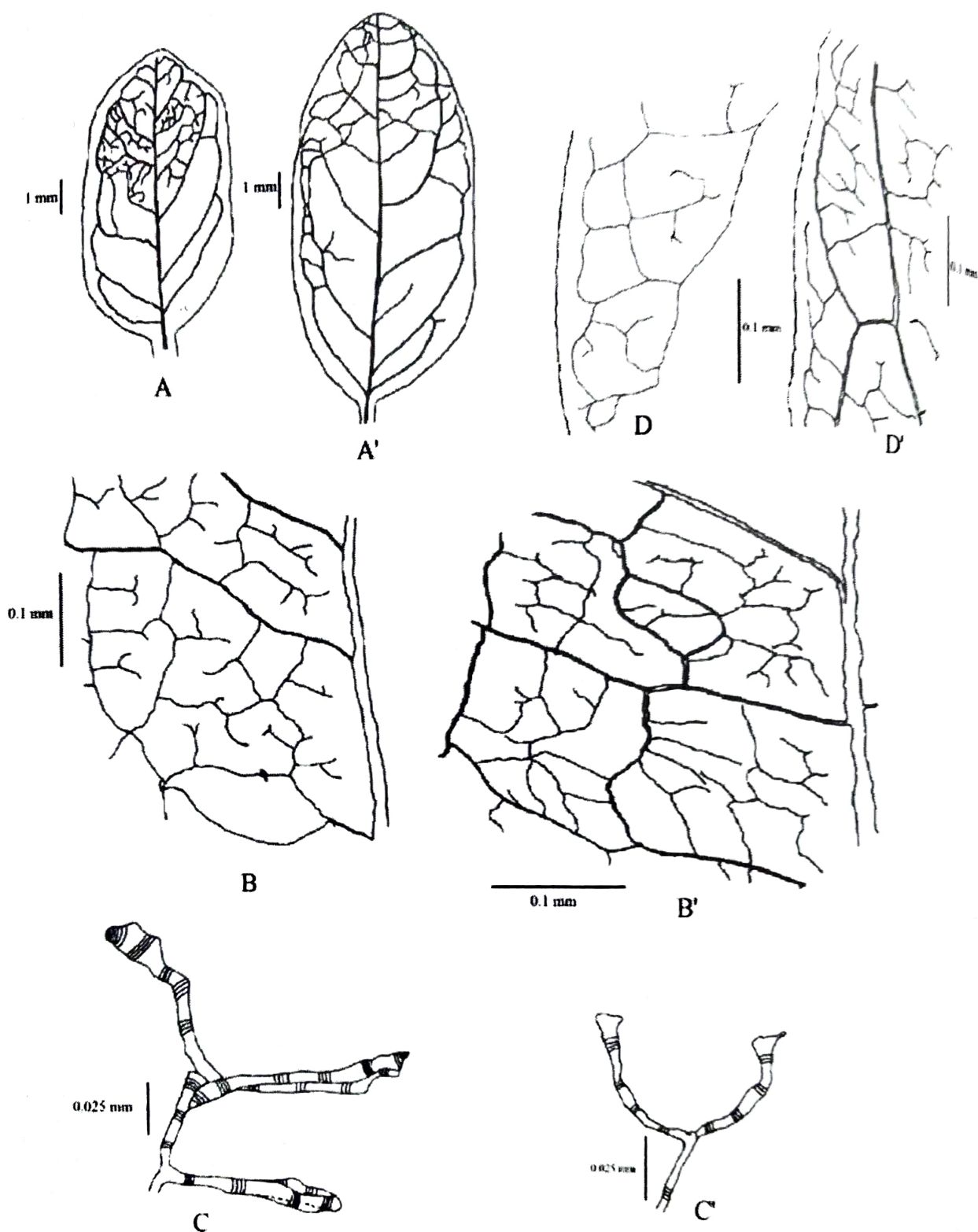


Fig. 2. Venation pattern, marginal venation and free vein endings of *Phyllanthus amarus* and *Phyllanthus fraternus*: A-C. : *P. amarus* : A. Leaf; B. Venation pattern; C. Free vein endings; D. Marginal venation. A'-D': *P. fraternus*: A'. Leaf; B'. Venation pattern; C'. Free vein endings ; D'. Marginal venation.

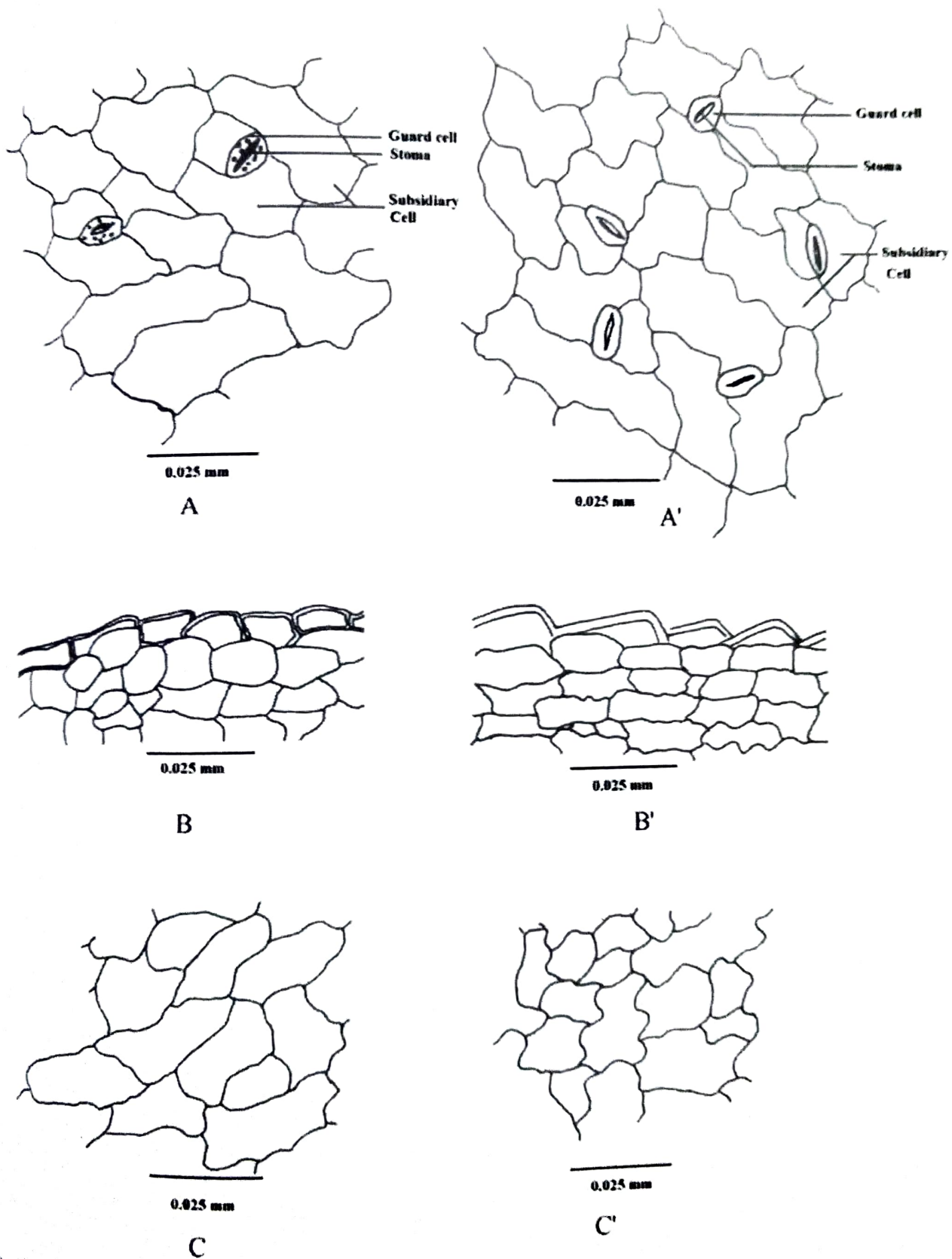


Fig. 3. Epidermal Anatomy : A-C. *Phyllanthus amarum*: A. Stomatal complex; B. Marginal epidermal cells; C. Epidermal cells. A'-C'. *Phyllanthus fraternus*: A. Stomatal complex; B'. Marginal epidermal cells; C'. Epidermal cells.

Table 1. Details of a list of voucher specimens

Species name	Collection localities & numbers	Collection time	GPS location
<i>P. amarus</i> Schum. & Thonn.	1. Kalyani University Campus, Nadia, W.B. (P. Lodh 003, 004, 005)	Sept., 2013	22°59'18.60'' N 88°26'57.32'' E
	2. Searampur, Near Ghat Hoogly, W.B. (P. Lodh 006, 007)	May, 2013	22°45'33.03'' N 88°20'31.80'' E
<i>P. fraternus</i> Webster	1. Birohi, Nadia, W.B. (P. Lodh 008, 019, 011)	Sept., 2013	22°59'02.28'' N 88°33'25.07'' E
	2. B.C.K.V. Mohonpur Campus, Nadia, W.B. (P. Lodh 009, 010)	Sept., 2013	22°57'49.25'' N 88°31'27.68'' E

Table 2. Venation pattern of two species of *Phyllanthus*

Name of the Species		<i>Phyllanthus amarus</i> Schum. & Thonn.	<i>Phyllanthus fraternus</i> Webster
	Characters		
1.	Venation	Pinnate and brochidodromous	Pinnate and brochidodromous
2.	Secondary vein	Alternate, basal pairs often opposite; moderately thick.	Alternate, basal pairs often opposite; moderately thick
3.	No. of secondaries	4-5 pairs	5-7 pairs
4.	Angle of divergence of secondaries	Acute, basal 1-2 pairs of secondary veins more obtuse than the upper ones	Acute, basal 1-2 pairs of secondary veins more acute than the upper ones
5.	Avrg. Angle of divergence of middle secondaries	$\pm 47^\circ$	$\pm 56.67^\circ$
6.	Inter-secondary vein	Present, straight, unbranched	Present, straight, unbranched
7.	Tertiary veins	Tertiary veins forming random reticulations	Tertiary veins forming random reticulations
8.	Higher order venation	Venation is to 5° or 6° order. The ratio of 3° order and last order is 3:1. Quaternary veins (4°) are thin, orthogonal. Quaternary veins (5°) are thick, orthogonal. Highest order of vein showing excurrent branching is of 4° and 5°; veins of 4° order onwards are more or less same thickness. Reticulation is with the formation of areoles and the ultimate areoles are formed by the joining of mostly 3°, 4° and 5° order of veins.	Venation is to 4° or 5° order. The ratio of 3° order and last order is 5:3. Quaternary veins (4°) are thin, orthogonal. Quaternary veins (5°) are thick orthogonal. Highest order of venation showing excurrent branching is of 4° order; veins of 4° onwards are more or less of same thickness. Reticulation is with the formation of areoles and the ultimate areoles are formed by the joining mostly of 3° and 4° order of veins.
9.	Areoles	Areoles are well-developed, meshes of irregular shape, more or less variable in size, randomly arranged, showing no preferred orientation; shape quadrangular or pentangular mostly, sometimes polygonal; size small.	Areoles are well-developed, meshes of irregular shape, more or less variable in size, randomly arranged with no preferred orientation; shape triangular, quadrangular or polygonal sometimes; size small.
10.	Free Vein endings	Free vein endings present within the ultimate areoles as mostly one, sometimes 2 or even may be absent, both branched and unbranched, branched once mostly in equal dichotomy or sometimes in unequal dichotomy, straight or curved, mostly medium in size, very few short, consisting of 2-3 spirally thickened elongated tracheids and both traverse 40-70% of length of areolar space, and all are devoid of any sheath cells. The very tips are mostly with 2 or 2-3 tracheids, mostly with broader tracheids.	Free vein endings mostly absent within the ultimate areoles, or when present mostly one in number, rarely branched, straight usually, sometimes curved, medium in size, consisting of 1-2 spirally thickened elongated tracheids, traverse 50-70% of length of areolar space and all devoid of any type of sheath cells. The very tips are mostly with 1-2 tracheids with medium width, not much broader.

Table 3. Stem anatomy of two species of *Phyllanthus*

Name of the Species		<i>Phyllanthus amarus</i> Schum. & Thonn.	<i>Phyllanthus fraternus</i> Webster
Characters			
		STEM	
1.	Epidermis	Uniseriate, consists of barrel-shaped cells, highly cutinized on outer walls	Uniseriate, consists of barrel-shaped cells, highly cutinized on outer walls
2.	Hypodermis	Consists of 2-3 layers of collenchyma cells, followed by 2-3 layers of chlorenchymatous cell	Consists of 2-3 layers of collenchyma cells, followed by 2-3 layers of chlorenchymatous cell
3.	Cortex	Consists of 2-3 layers of thin-walled, parenchymatous cells	Consists of 2-3 layers of thin-walled, parenchymatous cells
4.	Xylem	Occurs as a broad zone, with prominent protoxylem and metaxylem	Occurs as a broad zone, with prominent protoxylem and metaxylem
5.	Phloem	Occurs as a narrow zone, as a continuous layer towards the periphery	Occurs as a narrow zone, with prominent patches of phloem tissue towards the periphery
6.	Pith	Massive, Parenchymatous	Massive, parenchymatous
		NODE	
7.	No of lacuna and traces	Unilacunar, 1-3 traced	Unilacunar, 1-3 traced

Table 4. Seed anatomy of two species of *Phyllanthus*

Name of the Species		<i>Phyllanthus amarus</i> Schum. & Thonn.	<i>Phyllanthus fraternus</i> Webster
Characters			
1.	Morphology Shape	Triangular	Triangular
2.	Seed length (L) : (in mm)	0.9	1.0
3.	Seed breadth (B) : (in mm)	6.5	0.7
4.	Ratio (L/B)	1.38	1.42
5.	Seed coat colour	Brown, slightly darker than that of <i>P. fraternus</i>	Yellowish brown, slightly lighter than that of <i>P. amarus</i>
6.	Hilum	Round in outline	Elliptic in outline
7.	Surface Longitudinal ribs Concentric ribs-	No of longitudinal ribs on back 5-6. No. of concentric ribs on sides 6-7	No of longitudinal ribs on back 7-9. No. of concentric ribs on sides 7-9.
8.	Trichomes	Absent	Absent
1.	Anatomy Epidermis	Epidermis made up of barrel-shaped cells in uniseriate layer	Epidermis made up of barrel-shaped cells in uniseriate layer
2.	Hypodermis	Hypodermal sclerenchyma cells with swollen walls	Hypodermal sclerenchyma cells with straight walls, i.e. not swollen
3.	Inner Seed coat	Inner seed coat layer with parenchyma cells filled with dense pigmented substances	Inner seed coat layer with parenchyma cells are not densely pigmented like <i>P. amarus</i>

Table 5. Stomatal complex of two species of *Phyllanthus*

Name of the Species		<i>Phyllanthus amarus</i> Schum. & Thonn.	<i>Phyllanthus fraternus</i> Webster
	Characters		
1.	Type	Both anomocytic and anisocytic.	Both anomocytic and anisocytic.
2.	Length of Stomata (in μm)	6.73	6.76
3.	Width of Stomata (in μm)	3.72	3.57
4.	L/W Ratio	1.81	1.89
5.	Length of Stoma (in average)	6.35	6.5
6.	Stomatal Index	18.38	21.53

Table 6. Epidermal anatomy of two species of *Phyllanthus*

Name of the Species		<i>Phyllanthus amarus</i> Schum. & Thonn.	<i>Phyllanthus fraternus</i> Webster
	Characters		
	I. Cuticular Thickness	Thick	Thick
	II. Upper epidermal cells		
1.	Shape	Isodiametric to irregular	Isodiametric to irregular
2.	Size	ca. 15-20 μm in diam.	ca. 13-16 μm in diam.
3.	Arrangement	Random	Random
4.	Anticlinal Wall	Undulate	Undulate, undulation more
5.	Surface Ornamentation	Absent	Absent
	III. Leaf marginal cells		
1.	Shape	Rectangular	Rectangular
2.	Size	13-15x8-10 μm	13-15x5-6 μm
3.	Arrangement	Non-random	Non-random
4.	Anticlinal Wall	Straight to slightly rounded	Undulate
5.	Surface Ornamentation	Absent	Absent
	IV. Lower epidermal cells		
1.	Shape	Isodiametric to irregular	Isodiametric to irregular
2.	Size	Ca. 15-17 μm in diam.	Ca. 13-16 μm in diam.
3.	Arrangement	Random	Random
4.	Anticlinal Wall	Undulate	Undulate, undulation more compared to that of <i>P. amarus</i>
5.	Surface Ornamentation	Absent	Absent

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