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Comparative morpho-micrometric analysis of some *Bauhinia* species (Leguminosae) from east coast region of Odisha, India

Pritipadma Panda[1], Sanat Kumar Bhuyan[2], Chandan Dash[3], Deepak Pradhan[3], Goutam Rath[3] and Goutam Ghosh[3]*

[1]Esthetic Insights Pvt. Ltd., Plot No: 631, Rd Number 1, KPHB Phase 2, Kukatpally, Hyderabad, Telangana 500072, India

[2]Institute of Dental Sciences, [3]School of Pharmaceutical Sciences, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar, Odisha 751003, India

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Bauhinia vahlii has been reported for several medicinal properties, such as tyrosinase inhibitory, immunomodulatory and free radical scavenging activities. *Bauhinia tomentosa* and *Bauhinia racemosa* also possess anti-diabetic, anticancer, antidiabetic, anti-obesity and antihyperlipidemic activities. Therefore, the correct identification of these plants is critically important. The aim was to investigate the comparative morpho-micrometric analysis of 3 species of *Bauhinia* belonging to the family Leguminosae (Fabaceae) by using conventional as well as scanning electron microscopy to support species identification. In *B. racemosa*, epidermal cells are polygonal with anticlinical walls; whereas wavy walled cells are found in *B. tomentosa* and *B. vahlii.* Anisocytic stomata are present in *B. racemosa,* while *B. tomentosa* shows the presence of paracytic stomata and anomocytic stomata in *B. vahlii*. Stomatal numbers and stomatal indices were found to be more in *B. vahlii* than *B. tomentosa* and *B. racemosa.* On the other hand, uniseriate, unicellular covering trichomes are found in *B. racemosa* and *B. tomentosa* but *B. vahlii* contains only uniseriate, multicellular covering trichomes. Based on these micromorphological features, a diagnostic key was developed for identification of the particular species which helps a lot in pharmaceutical botany, taxonomy and horticulture, in terms of species identification.

Keywords: *Bauhinia racemosa*, *Bauhinia tomentosa*, *Bauhinia vahlii*, SEM, Standardization

IPC code; Int. cl. (2015.01)- A61K 36/00, A61K 36/48

Introduction

The taxonomical status of angiosperms is very much complex, which is not only due to the enormous number of species distributed worldwide but also to the lack of distinguished morphological characters. Hence, micromorphological characters play a crucial role in the proper recognition of different species of medicinal plants[1].

The leaf epidermal cells are employed as one of the most noteworthy taxonomical characters to identify the systematic phylogeny of several major families[2]. Among all of the flowering families, Leguminosae (Fabaceae) is the third-largest family, mainly famous for its eye-catching ornamental flowers. It contains approximately 720 genera and more than 18,000 species distributed globally[3]. Besides other parameters, the micromorphological parameter serves as an important diagnostic tool for the recognition of various leguminous plants[4].

*Correspondent author
Email: goutamghosh@soa.ac.in
Mob.: 9853126440

The determination of shape, size, length, and width of various parts of the plant are also necessary to compare the similarities and dissimilarities, along with taxonomical relationships of different species within the same genus[5]. Trichomes and epidermal cells of leaves have also been used as diagnostic aids for the identification of a particular taxon[6,7]. On the other hand, size, the pattern of distribution, and types of stomata present in leaves have also taxonomic significance for a better understanding of phylogeny[8]. Fayed *et al.* studied trichomes and stomata using scanning electron microscopy (SEM) for a better understanding of the systematics of *Teucrium* L. (Lamiaceae)[9].

Bauhinia is an extremely auspicious genus of small trees, shrubs or climbers comprising more than 300 species belongs to the large flowering family Leguminosae, distributed throughout Africa, Asia, and South America[10]. The genus named after its discoverer Bauhin brothers is characterized by its special cow's hoof-like lobed leaves and showy flowers[11]. Though an important genus, *Bauhinia* has not been studied taxonomically worldwide. Therefore,

a detailed taxonomic revision of this genus is urgently required as it has a wide range of traditional claims for the treatment of various types of ailments, such as diabetes, skin infections, inflammation, wounds and diarrhea[12].

B.vahlii is the largest creeper of India commonly called as *siyali* (Odia), *malu* (Hindi), *madapu* (Telugu), and camel's foot creeper (English) and is frequently used by tribal sovereignty of Odisha, Andhra Pradesh, and Madhya Pradesh[13]. The leaf decoction of this species *Bauhinia* has been used for a long time in the folk traditions of Indian populations to cure diarrhea and dysentery. Moreover, the pods are cooked as vegetables, and leaves are used for fodder, making of mats, food plates and as tobacco wrappers[14]. Besides these holistic uses, methanolic extract of *B. vahlii* leaves possesses tyrosinase inhibitory, antioxidant, immunomodulatory, antimicrobial, and free radical scavenging activities[10].

B. racemosa L. (Caesalpiniaceae), a small crooked wild tree, commonly known as *Jhinjeri* in Odia and Sonapatta tree in English. It is widely distributed all over India, Ceylon, China, and Timor[15]. Traditional healers in India have used this species for many ailments including headache, fever, skin diseases, tumors, blood diseases, dysentery, and diarrhea. Recently, the leaf extract has also been reported to have antidiabetic, anti-obesity and antihyperlipidemic activities[16].

B. tomentosa is another ornamental medicinal species, commonly called as yellow bell orchid. It is also known as *haldiakanchan* (Odia), *adavimandaramu* (Telugu), *kanchini* (Tamil), and *phalgu* (Sanskrit). It is distributed along the coastal strip from southern Kwazulu-Natal to Maputoland, tropical Africa, India, and Sri Lanka[17]. Literature findings suggest that different indigenous systems of therapies for the treatment of skin abscesses, the paste of leaves have been used. The leaf buds and flowers are reported to have activity against dysentery and microbial infections[18]. The gold and silver nanoparticles of methanolic extract of leaves have also been validated in recent pharmacological studies such as anticancer and free radical scavenging properties[19].

Despite numerous pharmacological properties, studies on leaf anatomy, and micromorphology, only a few *Bauhinia* species have been investigated systematically so far. The Pharmacognostical and Physico-chemical studies on the bark of *B. tomentosa* were reported previously. Elbanna *et al.* standardized the leaves of *B.vahlii* of Egypt emphasizing their morphological and histological parameters[20]. Very recently, numerous research works have been carried out to standardize different species of *Bauhinia* of taxonomical importance. To the best of the author's knowledge, there has been no systematic information on the micro-morphology of the pantropical taxa of *Bauhinia*. Therefore, in this study, micro-morphology of the leaf of three *Bauhinia* species from the ecoregion of Odisha, India was investigated for the first time by the process of conventional microscopy as well as scanning electron microscopy. It is expected that the results of this study attribute a better understanding of interspecific and geographical variations as well as systematic study among these species.

Materials and Methods

Collection and authentication of plant sample

The fresh leaves of *B. racemosa*, *B. tomentosa* and *B. vahlii* were collected from the Chandaka Reserve Forest, Bhubaneswar in April 2015. The plants were identified by Dr P. C. Panda, Principal Scientist, and authenticated by comparison with the herbarium specimens at the herbarium of Regional Plant Resource Centre, Bhubaneswar, Odisha, India. The voucher specimens SPS/SOAU-06, SPS/SOAU-20, and SPS/SOAU-21 were deposited in the Department of Pharmacognosy, School of Pharmaceutical Sciences, Siksha 'O'Anusandhan University, Bhubaneswar, Odisha.

Micro-morphological studies

The detailed morphological studies of leaves of all these test species *B. racemosa*, *B. tomentosa* and *B. vahlii* were carried out according to the standard methods. The first-hand transverse section of leaves was taken, stained, and mounted following reported micro techniques[17]. The study of the stomatal morphology, venation pattern, and trichome distribution, peridermal section as well as clearing of the leaf was done with 5% sodium hydroxide or epidermal peeling by maceration employing Jeffrey's maceration fluid[21]. The diagrams were taken with the help of an inverted microscope. Photomicrographs of all the sections were taken with Nikon Lab photo 2 microscopic units at different magnifications.

Scanning electron microscopy

For scanning electron microscopy (SEM), the material was desiccated in a graded ethanolic series

and carbon (IV) oxide critical point apparatus called Balzers CPD-030. Finally, they were coated with gold by Balzers Sputtering SCD-030. The scanning microscope MIRA 3 TESCAN was used to capture electron micrographs[22].

Physico-chemical study

Physico-chemical parameters such as ash values, extractive values, and loss on drying were carried out according to the standard procedure mentioned in the WHO guidelines. The Powder behavior and fluorescence study of powder materials were performed by following standard methods[23].

Preliminary phytochemical screening

Preliminary phytochemical analyses of methanol extract of leaves of all these plant species were carried out using standard procedures[23].

Results

Morpho-micrometric analysis

Qualitative and quantitative macromorphological features such as shape, size, stomata, trichomes, venation pattern, vein termination, vein-islet, palisade cell of all the species of *Bauhinia* leaves are shown in Table 1.

The leaves of all the species are pulvinus bifoliate in shape. The lamina has an entire margin with a cordate base and emarginated apex which is found to be the same in all the three species. The length and width of the leaves of *B. racemosa* were found to be 5-7 cm in length and 7-9 cm in width, while the length of *B. tomentosa* leaf was recorded 5-9 cm and width was found 7-10 cm. The length and width of the leaves of *B. vahlii* were 14-48 and 12-45 cm, which is found quite larger than that of these species. Leaf surfaces of *B. racemosa* and *B. tomentosa* were less hairy with prominent protruding midribs (7-9 in number per leaf) whereas the leaf surface of *B. vahlii* was found to be densely hairy with a greater number of midribs (11-13) as mentioned in (Fig. 1 a-o).

In *B. racemosa*, epidermal cells are polygonal with anticlinical walls, whereas wavy walled cells are found in *B. tomentosa* and *B. vahlii*. Epidermal cell number was found comparatively more in *B. racemosa* than that of *B. vahlii* and *B. tomentosa* (Fig. 2 a-c).

On the other hand, uniseriate, unicellular covering trichomes are found in *B. racemosa* and *B. tomentosa* but *B. vahlii* contains only uniseriate, multicellular covering trichomes. The length and width of trichomes are found to be increasing in these species

Table 1 — Leaf features that allow separation of the studied *Bauhunia* species

Leaf features	*B. racemosa*	*B. tomentosa*	*B. vahlii*
External	Pulvinous bifoliate	Pulvinous bifoliate	Pulvinous bifoliate
Morphology	Entire	Entire	Entire
Margin	Emarginate	*Emarginate*	Deeply lobed emarginate
Apex	Cordate	Cordate	Cordate
Base	5-7 cm	5-9 cm	14-48 cm
Size (Length & width)	7-9 cm	6-10 cm	12-45 cm
Number of protruding midribs	9	7	13
Epidermal cell walls	Straight walled polygonal cells	Wavy walled cells	Wavy walled cells
Surface (Adaxial & abaxial)	Less hairy, Uniseriate, unicellular	Less hairy, uniseriate, unicellular	Densely hairy, uniseriate,
Trichomes	coveringtrichomes	covering trichomes	multicellular covering trichomes
Length of entire trichomes	42.18 µm-74.51 µm-154.66 µm	246.72 µm-382. 22 µm-432.25 µm	281.2 µm-372.12 µm-463.98 µm
Width of trichomes	3.56 µm-6.43 µm	8.45 µm-12.32 µm	10.56 µm-11.73 µm-14.08 µm
Stomata type	Anisocytic	Paracytic	Anomocytic
Stomata frequency	20/mm^2	76.66/mm^2	288.33/mm^2
Epidermal cell frequency	823.33/mm^2	503.33/mm^2	526.66/mm^2
Stomatal index	1.25-2.35-3.33	8.77-13.16-15.38	25.67-30.20-33.33
Length of stomata	17.62 µm-19.59 µm-24. 64 µm	14.08 µm-18.89 µm-21.12 µm	17.6 µm-18.53 µm-21.12 µm
Width of stomata	14.82 µm-14.43 µm-15.84 µm	7.56 µm-8.11 µm-10.56 µm	10.56 µm-11.73 µm-14.08 µm
Palisade cell	3.75-6.25-5.12	5-5.47-7	1.75-2.35-2.75
Veination pattern	Reticulate	Reticulate	Palmate reticulate
Vein termination	350-450-540	150-193.33-230	40-55-80
Vein-islet	290-392-430	150-218.33-260	320-383.33-440
Lamina	Dorsiventral	Dorsiventral	Dorsiventral
Midrib	Plano-convex	Heart shape	Heart shape
Vascular bundle	Conjoint and collateral	Conjoint and collateral	Conjoint and collateral
Xylem	Centrifugal	Centrifugal	Centrifugal

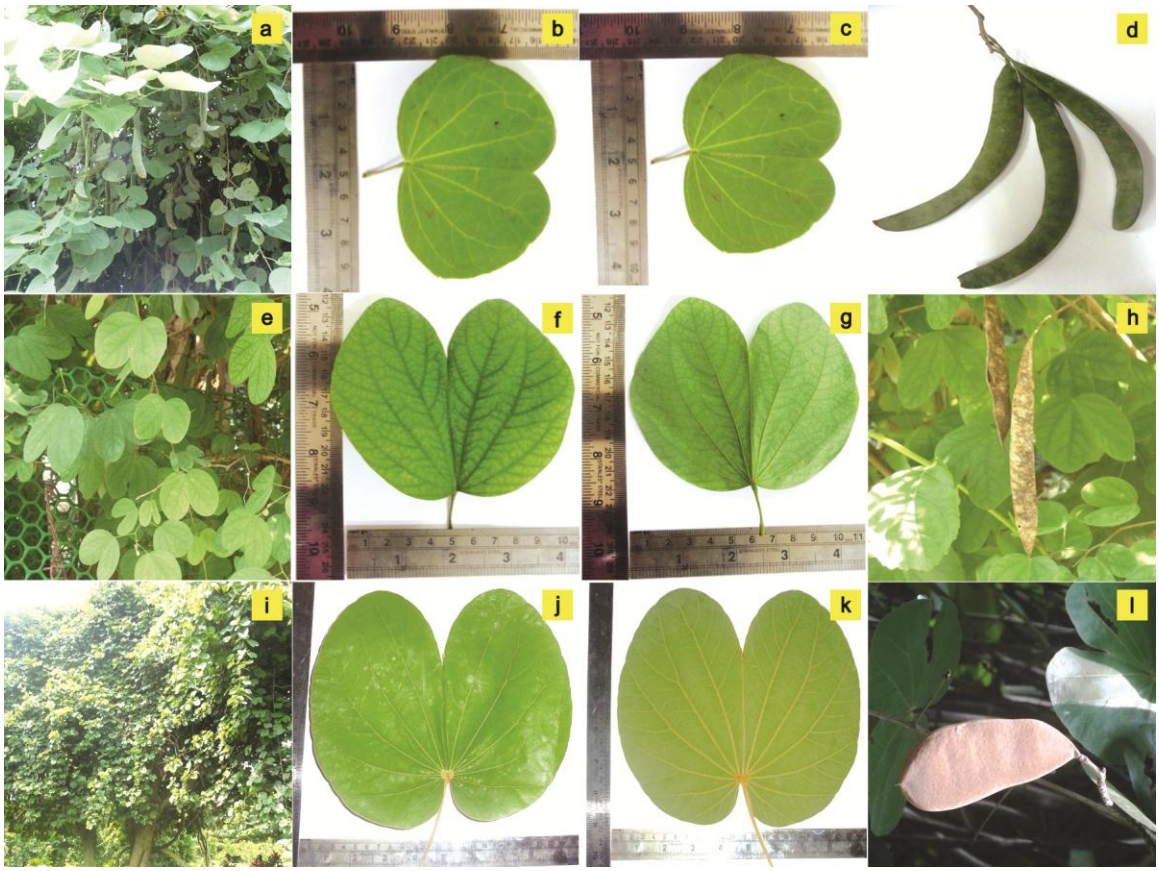

Fig. 1 — Macroscopic characteristic of *B. racemosa*, *B. tomentosa* and *B. vahlii*. (a)-plant of *B. racemosa*, (b,c)-adaxial epidermis and abaxial epidermis of *B. racemosa*, (d)-fruit of *B. racemosa*, (e)-plant of *B. tomentosa*, (f,g)-adaxial epidermis and abaxial epidermis of *B. tomentosa*, (h)-fruit of *B. tomentosa*, (i)-plant of *B. vahlii*, (j, k)-adaxial epidermis and abaxial epidermis of *B. vahlii*, (l)-fruit of *B. vahlii*.

in the order of *B. racemosa* <*B. tomentosa* <*B. vahlii* (Fig. 2 d-f).

Anisocytic stomata are present in *B. racemosa*, while *B. tomentosa* shows the presence of paracytic and anomocytic stomata in *B.vahlii.* Stomatal numbers, as well as stomatal indices, were found to be more in *B. vahlii* than *B. tomentosa* and *B. racemosa*. No significant variation in length and width of stomata was found in these three species (Fig. 2 g-i).

In this micrometric analysis, it is also found that palisade cells are varied from 3.75–6.25–5.12 in *B. racemosa*, 5–5.47–7 in *B. tomentosa* and 1.75–2.35–2.75 in *B. vahlii*. The highest number of palisade cells are found in *B. tomentosa* (Fig. 2 j-l).

Reticulate venation pattern was found in all these three species. Mid veins and veinlets are thick in *B. racemosa* in comparison to other species. The vein-termination number was found to be less (40–55–80) but a greater number of vein-islet (320–383.33–440) were recorded in *B. vahlii* than that of two other species (Fig. 2. m-o).

Transverse sections of leaf lamina

In the transverse section of the lamina, epidermal layers are found to be composed of polygonal parenchymatous cells and more numbers of palisade cells are found on the adaxial surface as mentioned in (Fig. 3 a-i). The epidermal surface of the lower epidermis of the lamina in *B. racemosa* showed the presence of anisocytic stomata, while *B. vahlii* and *B. tomentosa* showed anomocytic stomata and paracytic stomata, respectively. The covering trichomes of uniseriate, unicellular are found in *B. racemosa* and *B. tomentosa* but *B. vahlii* consists of uniseriate, multicellular covering trichomes followed by the presence of mesophyll region containing two-layered palisade cell at the adaxial side and embedded by spongy parenchyma. Conjoint and collateral vascular bundles embedded by lignified sclerenchymatous tissues are common in all these three species as shown in Fig. 4(a-f), 5(a-h), and 6(a-j).

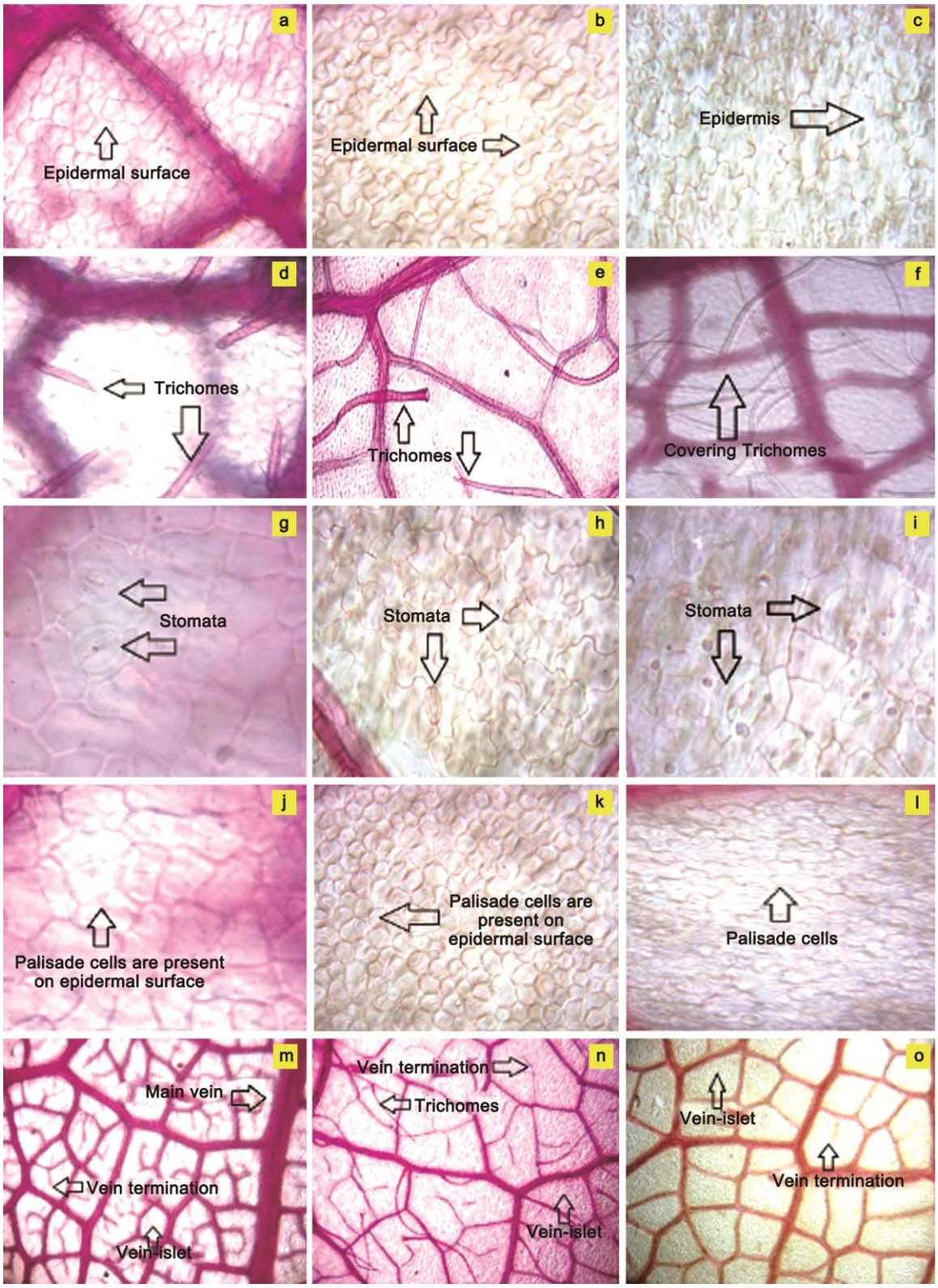

Fig. 2 — a) Epidermal surface of *B. racemosa*, b) epidermal surface of *B.tomentosa* c) epidermal surface of *B. vahlii*, d) trichomes of *B. racemosa*, e) trichomes of *B. tomentosa*, f) trichomes of *B. vahlii*, g) stomata of *B. racemosa*, h) stomata of *B. tomentosa*, i) stomata of *B. vahlii*, j) palisade cell of *B. racemosa*, k) palisade cell of *B. tomentosa*, l) palisade cell of *B. vahlii*, m) venation pattern of *B. racemosa*, n) venation pattern of *B. tomentosa*, o) venation pattern of *B. vahlii*.

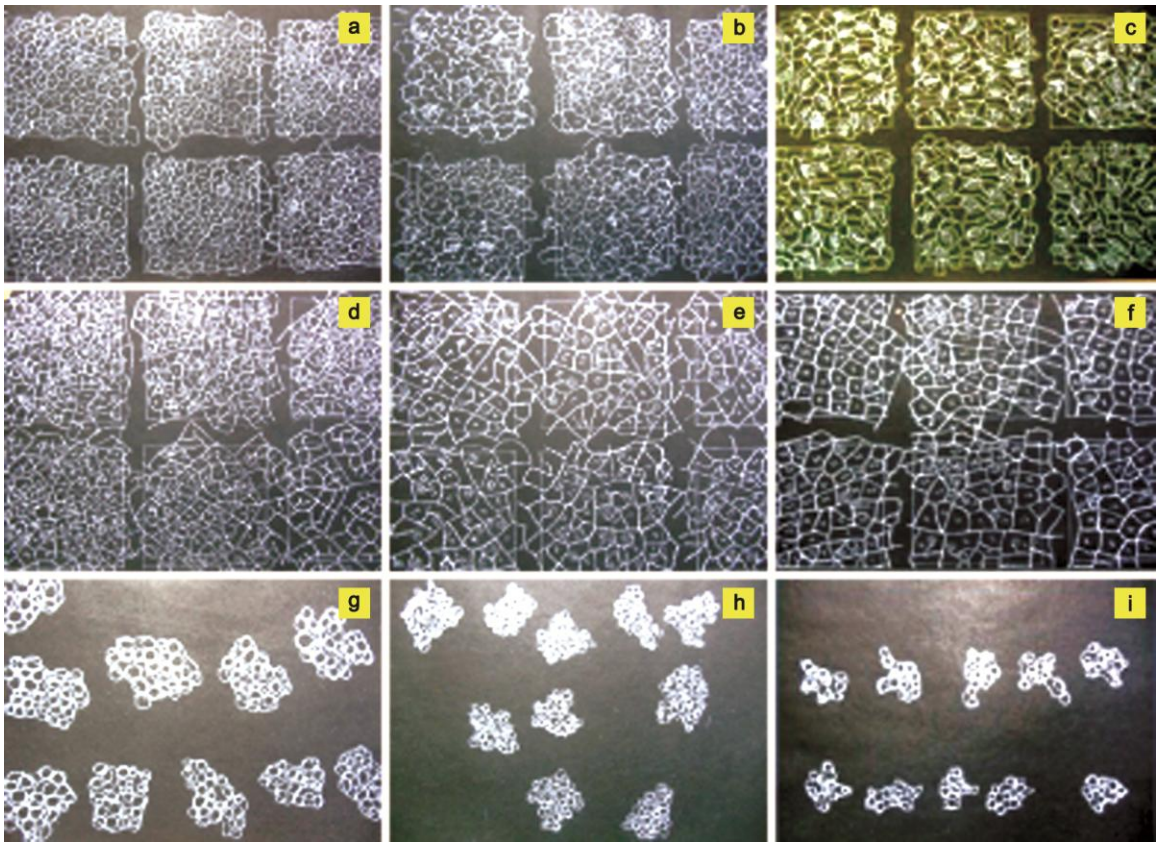

Fig. 3 — Schematic diagram of leaf constant of three *Bauhinia* species. a) stomata of *B. racemosa*, b) stomata of *B. tomentosa*, c) stomata of *B. vahlii*, d) venation pattern of *B. racemosa*, e) venation pattern of *B. tomentosa*, f) venation pattern of *B. vahlii*, g) palisade cell of *B. racemosa*, h) palisade cell of *B. tomentosa*, i) palisade cell of *B. vahlii*.

Transverse section of leaf passing through the midrib

The transverse section of the midrib of all these investigated species showed cuticle, epidermis, ground tissue, endodermis, pericyclic fiber, centrifugal xylem, and conjoint type of collateral vascular bundles. But it was found that the shape of the midrib varies from species to species, i.e., plano-convex outline on the abaxial side in *B. racemosa*, and heart shape in *B. tomentosa* and *B. vahlii*. The epidermis of *B. racemosa* consists of a single layer of rectangular cells covered with a thick cuticle. Below the upper and lower epidermis few layers of parenchymatous cells are present. A single-layer of endodermis is covering the vascular bundle. The vascular bundle is present at the center, whereas the phloem is more developed towards the dorsal side. Phloem tissue consists of sieve tubes, companion cells and phloem parenchyma. Xylem comprises vessels, tracheids and thin-walled parenchyma. Growth of xylem is centrifugal as protoxylem lies towards center and metaxylem towards the periphery. Vascular bundle is an arc-shaped, conjoint, and collateral as phloem is present outside of the xylem. There are no significant differences in distribution patterns and types of vascular bundles in *B. tomentosa* and *B. vahlii* except a distinctive pith and few calcium oxalate crystals in the phloem region of *B. vahlii*. Apart from these vascular bundle characters, numerous numbers of uniseriate and multicellular covering trichomes are present on the lower epidermis of the midrib of *B. vahlii* than that of two other species (Fig. 4-6).

Powder microscopy

The detailed powder microscopic characteristics of *B. racemosa* leaves revealed the presence of uniseriate, unicellular covering trichomes, fragments of anisocytic stomata, fragment of straight-walled polygonal epidermal cells, prismatic calcium oxalate crystals, non-lignified phloem fibres, and yellow coloured pollen grains (Fig. 7). The powder characteristics are evaluated against different chemical reagents as shown in Table 2, and other experimental data such as fluorescent nature,

Physico-chemical characteristic, and phytochemical investigation data are being elaborated in the feature are shown in Table 3-5. *B. tomentosa* leaf powder showed the presence offragments of palisade cells, elongated uniseriate, unicellular covering trichomes, fragments of numerous paracytic stomata and non-lignified phloem fibers (Fig. 8). Similarly, the anomocytic stomata, elongated multicellular covering trichomes, non-lignified phloem fibers, and both types of xylem vessels, i.e., pitted and spiral types are found to be scattered throughout leaf powder of *B. vahlii* (Fig. 9).

Scanning electron microscopic study

Scanning electron microscopy mainly works under higher magnifications. Hence, it may be used as a gold standard for whole or surface screening of any plant part. In the leaf surface study, reticulate venation pattern, vein-islets, and vein terminations are demarcated in the species level. The SEM study of the powdered leaf showed uniseriate, unicellular covering trichomes in *B. racemosa* and *B. tomentosa*, while a huge number of multicellular covering trichomes were observed in *B. vahlii*. Besides this, there are three different types of stomata like anisocytic, paracytic and anomocytic stomata are visible in *B. racemosa*, *B. tomentosa*, and *B. vahlii*, respectively (Fig. 10).

UPGMA statistical analysis

UPGMA statistical analysis showed the micro-morphological similarities among these investigated species ranged from 0.10 to 0.80 (Fig. 11).

Discussion

In the present research work, micromorphological, physicochemical, and fluorescence analysis of all these three species were performed, as well as scanning

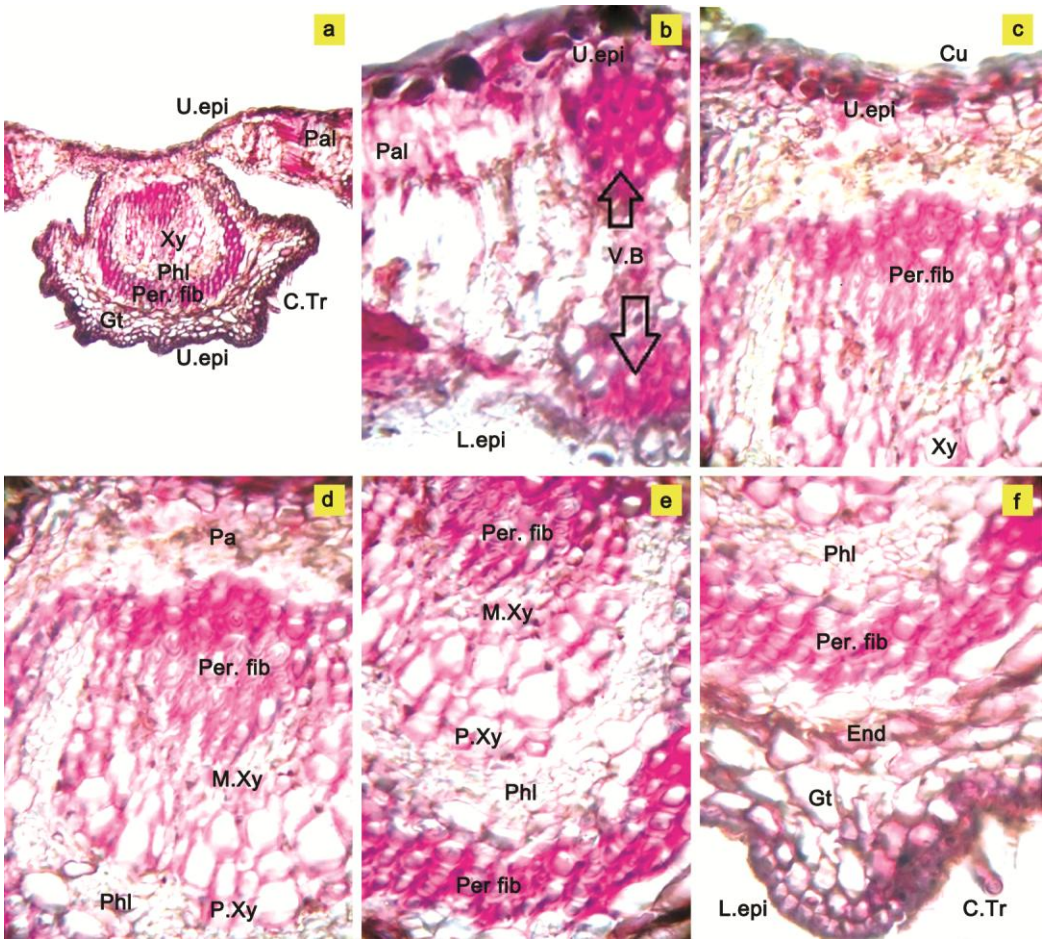

Fig. 4 — *B. racemosa* Transverse (a)-transverse section of midrib at magnification 5X, 5X, (b)-transverse section of lamina at magnification 5X, 10X, (c, d, e, f)-transverse section of midrib at magnification 5X, 40X.
Abbreviation-U. epi-Upper epidermis, L. epi-Lower epidermis, Per. fib-Pericyclic fibre, Gt-Ground tissue, Pal-Palisade cell, Xy-Xylem, Phl-Phloem, C. Tr-Covering trichomes, V.B-Vascular bundle, Pa-Parenchyma, M. Xy-Meta xylem, P. Xy-Proto xylem.

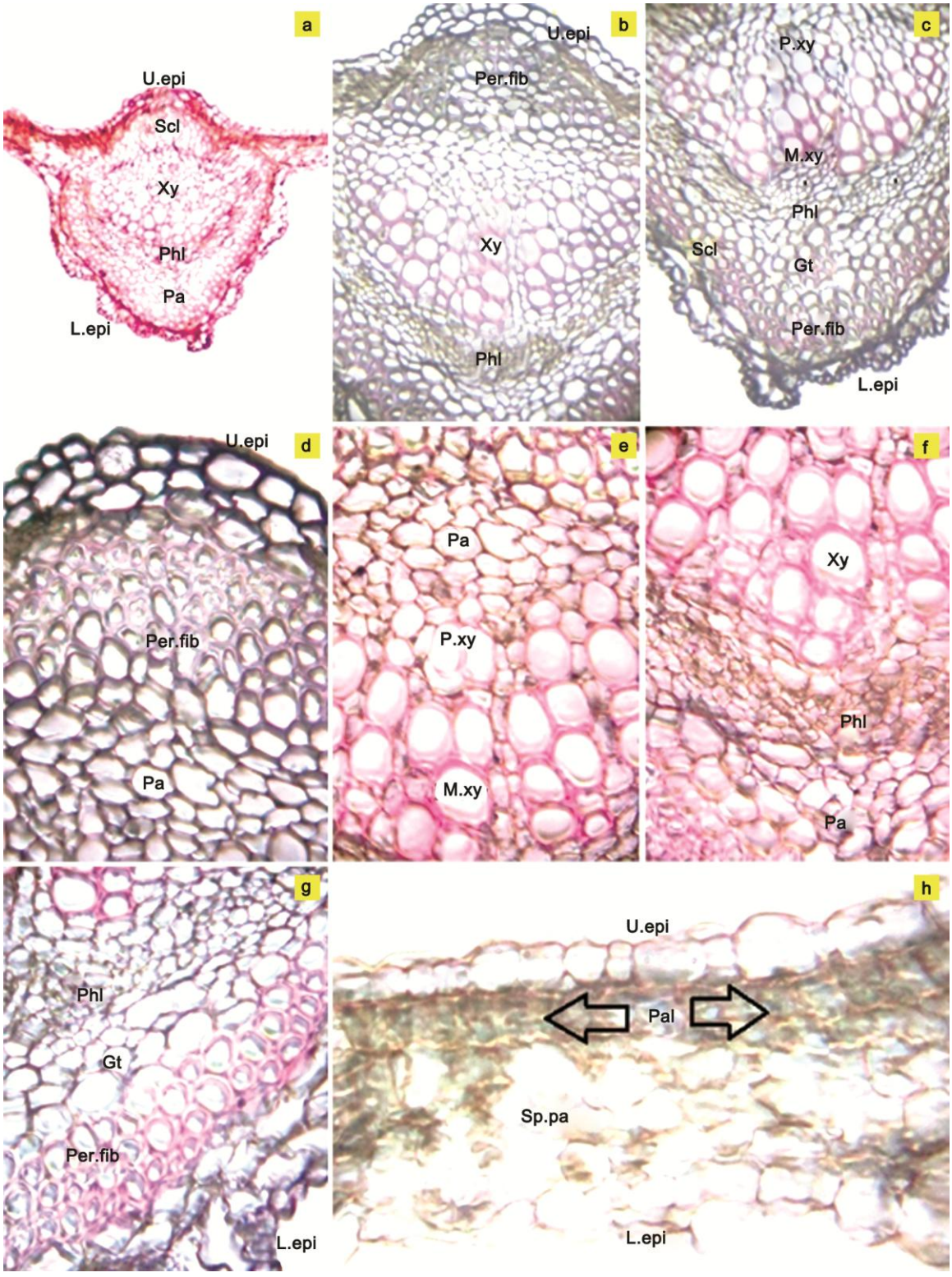

Fig. 5 — *B. tomentosa* (a) transverse section of midrib at magnification 5X, 5X, (b, c)-transverse section of midrib at magnification 5X,10X, (d-g)-transverse section of midrib at magnification 5X, 40X, (h) transverse section of lamina 5X, 10X.
Abbreviation-U. epi-Upper epidermis, L. epi-Lower epidermis, Per. fib-Pericyclic fibre, Gt-Ground tissue, Pal-Palisade cell, Xy-Xylem, Phl-Phloem, Scl-sclerenchymatous, V. B-Vascular bundle, Pa-Parenchyma, M. Xy-Meta xylem, P. Xy-Proto xylem, Sp.pa-Spongy parenchyma.

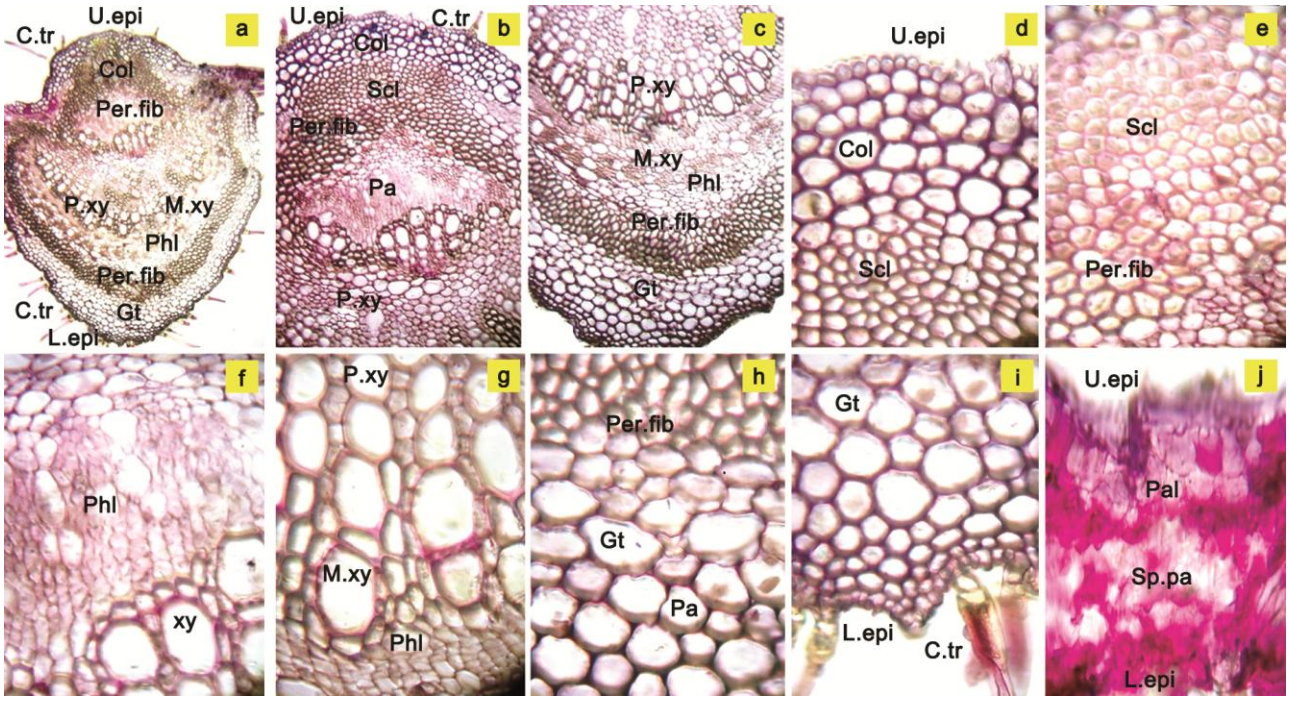

Fig. 6 — *B. vahlii* (a)-transverse section of midrib at magnification 5X, 5X, (b, c)-transverse section of midrib at magnification 5X, 10X, (d-i)-transverse section of midrib at magnification 5X, 40X, (j) transverse section of lamina 5X,10X.
Abbreviation-U. epi-Upper epidermis, L. epi-Lower epidermis, Per. fib-Pericyclic fibre, Gt-Ground tissue, Pal-Palisade cell, Xy-Xylem, Phl-Phloem, C. Tr-Covering trichomes, Scl-sclerenchymatous, P-Pith, Col-Collenchyma, V.B-Vascular bundle, Pa-Parenchyma, M. Xy-Meta xylem, P. Xy-Proto xylem, Sp.pa-Spongy parenchyma.

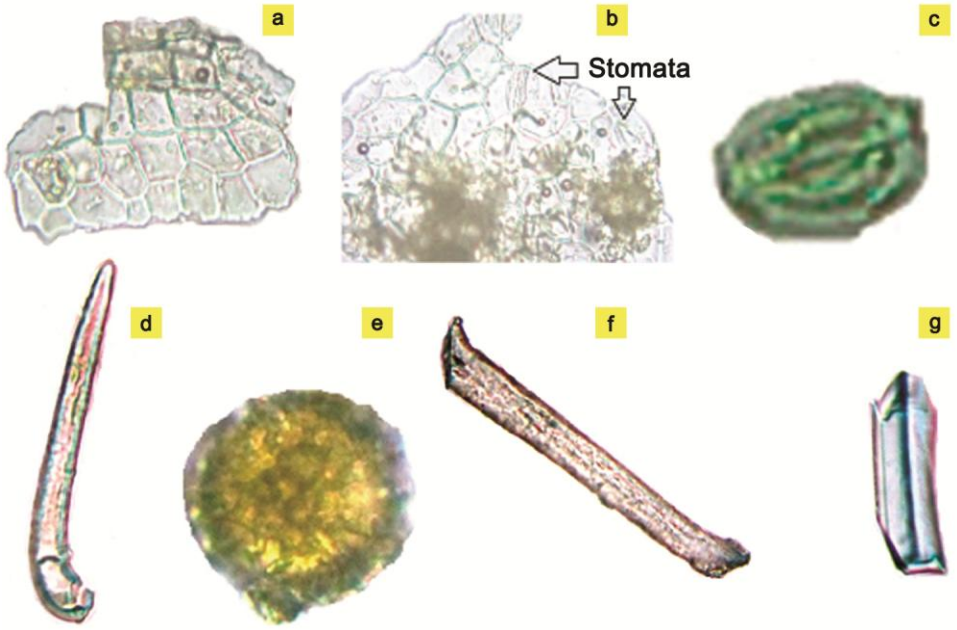

Fig. 7 — Diagnostic characteristic of *B. racemosa* leaf powder (a) epidermal surface, (b,c) stomata, (d) trichomes, (e) pollen grains, (f) lignified phloem fibres, (g) crystals.

Table 2 — Behavior of powder with different chemical and reagents

Chemicals/Reagent	*B. vahlii*	*B. racemosa*	*B. tomentosa*
Powder as such	Green	Green	Green
Powder+ Picric acid	Light yellow	Light yellow	Light yellow
Powder + Conc. HNO_3	Orange	Light orange	Light orange
Powder + Conc. HCL	Brown	Greenish brown	Dark green
Powder + Conc.H_2SO_4	Dark brown	Dark brown	Dark brown
Powder +Glacial acetic acid	Faint green	Green	Green
Powder + 5% $FeCL_3$	Dark green	Dark green	Dark green
Powder + NaOH (5N)	Faint yellow	Yellow	Faint yellow
Powder + KOH (5%)	Dull green	Green	Dull green
Powder + Iodine/20	Light blue	Greenish blue	Greenish blue

Table 3 — Fluorescence analysis of powder drug

	B. vahlii		*B. racemosa*		*B. tomentosa*	
Chemicals/Reagent	Day light	Short wave	Day light	Short wave	Day light	Short wave
1N NaOH in methanol	Deep green	Green	Light green	Green	Deep green	Deep green
1N NaOH	Brown	Light brown	Reddish brown	Greenish brown	Greenish brown	Green
Ethanol	Light green	Green	Deep green	Green	Green	Green
HNO_3+NH_3	Light orange	Whitish green	Orange	Green	Greyish brown	Faint Green
50 % HNO_3	Light brown	Dull green	Deep grey	Greenish grey	Light brown	Faint Green
I N HCL	Watery	Dull green	Watery	Dull green	Deep brown	Deep green
H_2SO_4	Dark brown	Brown	Dark brown	Dark brown	Dark brown	Green
50% H_2SO_4	Light green	Green	Dull green	Brownish green	Yellowish brown	Green
Glacial acetic acid	Green	Faint green	Light brown	Light green	Greenish brown	Light green
HNO_3	Light orange	Green	Brownish green	Green	Green	Green
Powder as such	Green	Green	Green	Green	Green	Green

Table 4 — Physico-chemical properties of powder of *Bauhinia* species

Plant name	Ash value (%w/w)			Extractive values (% w/w)		Loss on drying (% w/w)
	Total ash	Water-soluble ash	Acid-insoluble ash	Alcohol-soluble	Water-soluble	
B. vahlii	7.53±0.98	2.78±0.11	1.19±0.06	19.07±0.14	22.75±0.03	9.31±0.12
B. racemosa	6.34±0.44	2.35±0.12	0.98±0.13	17.00±0.32	20.31±0.07	8.31±0.34
B. tomentosa	4.93±0.30	2.01±0.45	0.82±0.26	15.91±0.42	17.42±0.04	7.21±0.09

electron microscopy for further confirmative studies of micro-morphological characters. Physico-chemical studies of crude drugs are helpful to find out the genuineness of the crude drug by detecting adulterants[24]. The ash values of the drug usually provide information about the earthy materials, whereas acid insoluble ash is a part of total ash which measures the presence of silica especially sand or siliceous earth. Similarly, water-soluble ash predicts the amount of water-soluble portion of total ash. Thus, all these ash values are thought to be important quantitative standards for the evaluation of any type of crude drug. Extractive values are also very useful for the determination of chemical constituent of the drug along with the determination of exhausted drugs. The moisture content should not be more than the permissible range as per WHO guidelines. Few plant species seem to be very complicated to be distinguished from each other. In that case, fluorescence analysis might be used as a rapid diagnostic and useful tool for the resolution of doubtful specimens to differentiate the adulterants[25]. Except for the limited type of chemical constituents of plants do not exhibit fluorescence in daylight in the visible range. The non-fluorescent compounds may often be converted into fluorescent derivatives or decomposition products by applying different chemical reagents. Hence, fluorescence analysis may contribute to its incredible role to identify crude drugs qualitatively and to enhance its credibility in the indigenous system of medicine.

Table 5 — Preliminary phytochemical screening of methanolic extract of leaves of different *Bauhinia* species

Test for	*B. vahlii*	*B. racemosa*	*B. tomentosa*
Carbohydrates (Molish's test)	+	+	+
Protiens (Biuret test)	–	–	– –
Steroid	+ +	+	+
1. Salkowski reaction			
2. Liebermann-Burchard reaction			
Cardiac Glycoside (Keller-killiani test)	+ +	+	+
Flavonoids	+ +	++	+ +
1. Shinoda test			
2. Lead acetate test			
Phenolic	+ +	+ +	+ +
1. $Fecl_3$			
2. Dil. iodine			
Alkaloid	+	+ -	+
1. Dragendorff's reagent			
2. Mayer's reagent			
3. Hager's reagent			
4. Wagner's reagent			
Terpenoids	+ + +	+ +	+ +

-Absent, + Mild, ++ Moderate, +++ Frequent

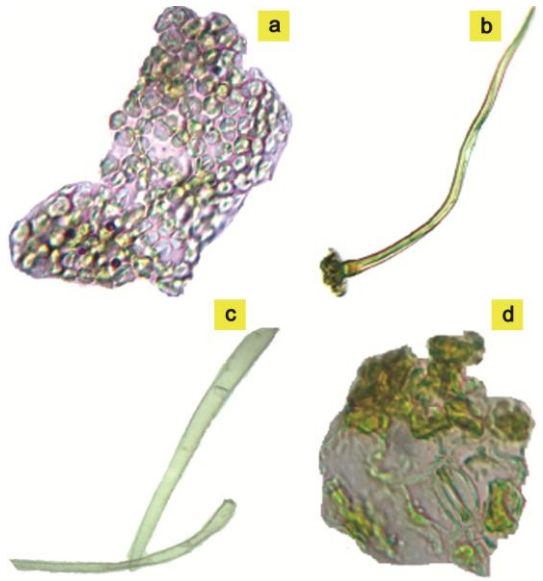

Fig. 8 — Diagnostic powder characteristic of *B. tomentosa* leaf powder (a) palisade cells (b) trichomes (c) fibres (d) stomata.

According to Bass *et al.*, some species of *Cassia* show significant differences in leaf epidermis based on the shape of the cells or structure of the cell walls[26]. This matches with our results, in which the leaf epidermal cells are polygonal and straight in

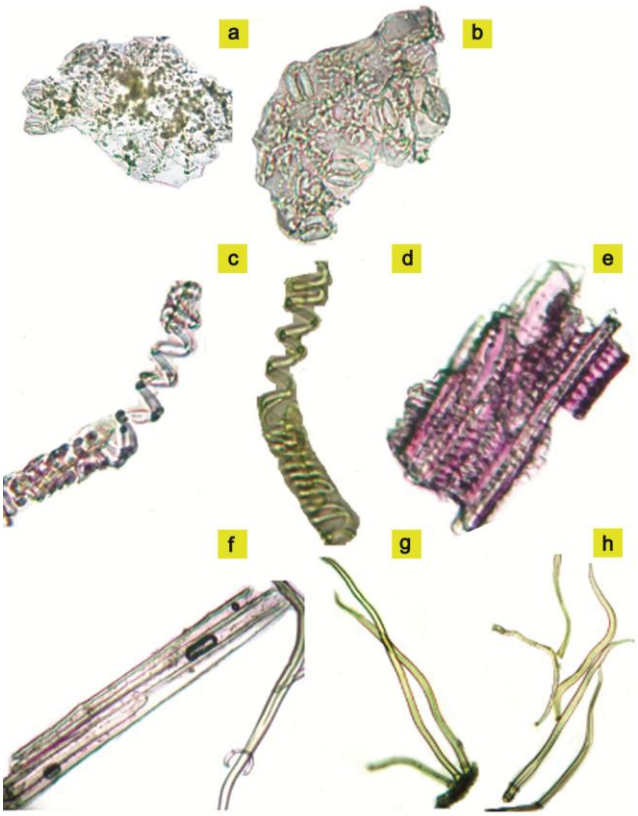

Fig. 9 — Diagnostic characters of *B. vahlii* are (a,b) stomata, (c,d,e) vessels, (f) fibres, (g,h) trichomes.

shape in *B. racemosa* while wavy shaped epidermal cells are found in *B. tomentosa* and *B. vahlii*. Additionally, epidermal cell frequency was found to be higher in *B. racemosa* than that of *B. tomentosa* and *B.vahlii*.

On the other hand, the qualitative and quantitative characteristics of the stomata are considered as a highly informative parameter of the leaf epidermis. The stomatal shape, size, frequency and indices have great taxonomic significance for the recognition of different species in the same taxa[27].

According to Carpenter and Smith, variations in stomatal frequencies offer a valuable diagnostic clue for the identification of particular species in generic level[28]. In the present study, *B. vahlii*, *B. tomentosa*, and *B. racemosa* showed huge differences in stomatal frequencies.

The Stomatal index is also considered an important diagnostic parameter for species delimitation[25, 27]. The wide range of stomatal size in these three species is in agreement with the observations of Wilkinson in 1971[29].

Albert and Sharma reported the presence of anisocytic and anomocytic stomata in *B. racemosa*

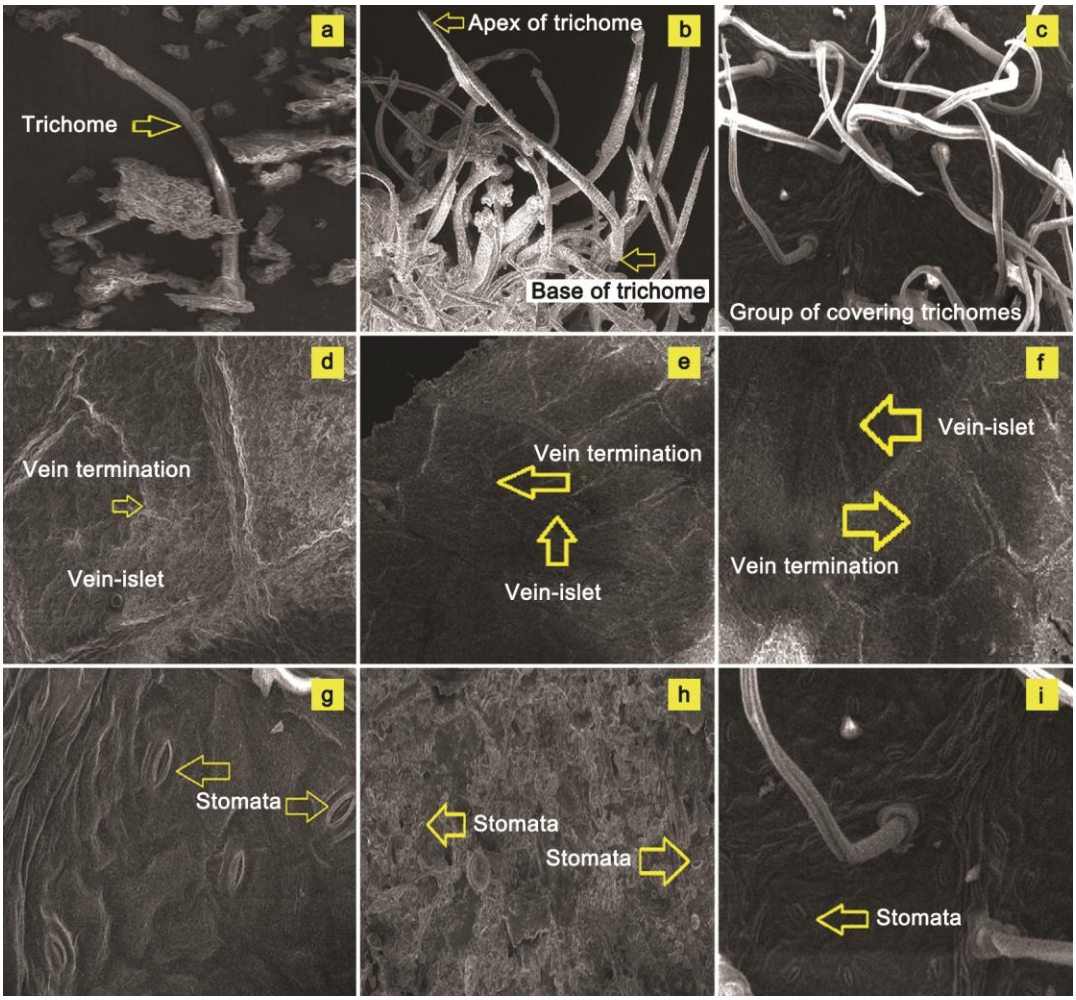

Fig. 10 — Scanning Electron Microscopy photography of leaf surface of three *Bauhinia* species. a) Trichomes of *B. racemosa*, b) Trichomes of *B. tomentosa*, c) Trichomes of *B. vahlii*, d) Venation pattern of *B. racemosa*, e) Venation pattern of *B. tomentosa*, f) Venation pattern of *B. vahlii*, g) Stomata of *B. racemosa*, h) Stomata of *B. tomentosa*, i) Stomata of *B. vahlii*.

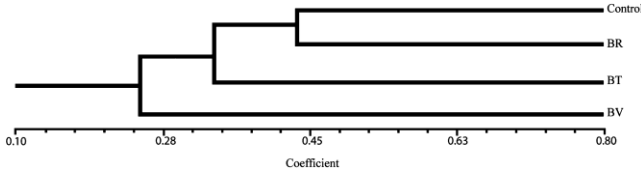

Fig. 11 — UPGMA dendrogram of studied taxa based on simple-matching similarity coefficients derived from morpho micrometric results. B R (*B. racemosa*), B T (*B. tomentosa*), B V (*B. vahlii*).

and paracytic stomata in *B. tomentosa* leaves, which were collected from Gujarat, western India[30]. Recently, Elbanna *et al.*, published his research article on Egyptian species of *B. vahlii* mentioning that this leaf is devoid of stomata[20]. In our present study, we recorded anisocytic stomata present in *B. racemosa*, paracytic type of stomata in *B. tomentosa* and anomocytic stomata in *B. vahlii* leaves, collected from eastern Odisha, India, which is not in agreement with the above report on this plant.

Different environmental conditions like light, temperature, and habitats greatly affect the shape size and types stomata[31]. Further, it was found that the stomatal distribution, frequency, index, shape, and sizes in the leaves of different species can be used for the discrimination of different taxa within the genus[32].

Trichomes are epidermal hairs that are highly variable in plants. According to Bano *et al.*, in the morphological diversity and distribution of trichomes in the particular plant species affect taxonomic identification of plant[33]. Types of trichome varied from species to species, which is agreed with the report of Albert and Sharma, who found that both unicellular and multicellular covering trichomes

are present in *Bauhinia blakeana* and *Bauhinia malabarica*, while only multicellular covering trichomes are present in *Bauhinia purpurea*[34]. The presence or absence of trichomes can also play a crucial role in taxonomic studies.

In the present study, trichomes of all these three species of *Bauhinia* possess both long and short hairs, but the size and the morphology of the hair differ from each other. Uniseriate, unicellular covering trichomes are detected in both *B. racemosa* and *B. tomentosa*, but uniseriate, multicellular covering trichomes are restricted to *B. vahlii*. Thus, different species of the same genus may also be identified by their distinct trichome characters.

Scanning electron microscopy (SEM) is a powerful diagnostic method for the investigation of surface structures of herbal drugs, i.e., leaves, pollen grains and seeds. On the other hand, SEM has also the advantage over the compound microscope that the range of magnification is relatively more to easily focus the specimen and for three-dimensional image views. Moreover, SEM also plays an important role to abolish taxonomic conflicts within various species of particular genus[35].

Three different types of stomata like anisocytic, paracytic and anomocytic, were found in *B. racemosa*, *B. tomentosa* and *B. vahlii*, respectively. For more confirmation of the types of these stomata, scanning electron microscopy was thought to be carried out. Various types of trichomes, patterns of venation, and frequency of venation were also confirmed using SEM.

In the present study, SEM showed excellent morphological and structural details of the trichomes, stomata and venation of leaves of these species. Hence, the observation of leaf surface by using SEM could be considered to measure the significant separation of taxonomic units.

High similarity in the form of trichomes, shape, texture, margin, venation pattern, lamina, vascular bundle, and xylem between *B. racemosa* and *B. tomentosa* leaves. was seen from the comparative morpho-micrometric study of these investigated taxa (Table 1). But *B. vahlii* differs from both of these two species mainly by having larger leaves, multicellular trichomes, and anomocytic stomata. It was found to agree with UPGMA cluster analysis in which *B. racemosa* and *B. tomentosa* are more closed with a similarity coefficient value of 0.23 whereas *B. vahlii* having a similarity coefficient value of 0.18. (Fig. 11). Further, the matrix of the Leaf features that allows separation of the studied *Bauhinia* species collected from the East coast region, Odisha, India is mentioned in Table 6. Thus, the findings of this study showed significant taxonomic similarities among the species.

Table 6 — Presence (1) and absence (0) matrix of Leaf features that allows separation of the studied *Bauhinia* species collected from East coast region, Odisha, India

S. No.	Analyzed characteristics	*B. racemosa*	*B. tomentosa*	*B. vahlii*
1	Pulvinous bifoliate leaves	1	1	1
2	Entire margin	1	1	1
3	Emarginate apex	1	1	0
4	Deeply lobed emarginated apex	0	0	1
5	Cordate base	1	1	1
6	Length of leaves between 5-7 cm	1	0	0
7	Length of leaves between 5-9cm	0	1	0
8	Length of leaves between 14-48 cm	0	0	1
9	Width of leaves between 7-9 cm	1	0	0
10	Width of leaves between 6-10 cm	0	1	0
11	Width of leaves between 12-45 cm	0	0	1
12	Number of protruding midribs 9	1	0	1
13	Number of protruding midribs 7	0	1	0
14	Number of protruding midribs 13	0	0	1
15	Straight walled polygonal epidermal cell walls	1	0	0
16	Wavy walled epidermal cell walls	0	1	1
17	Less hairy surface	1	1	0
18	Densely hairy surface	0	0	1
19	Uniseriate, unicellular covering trichomes	1	1	0
20	Uniseriate, multicellular covering trichomes	0	0	1
21	Length of entire trichomes from 42.18 µm-154.66 µm	1	0	0

(*Contd*.)

Table 6 — Presence (1) and absence (0) matrix of Leaf features that allows separation of the studied *Bauhinia* species collected from East coast region, Odisha, India (*Contd*.)

S. No.	Analyzed characteristics	*B. racemosa*	*B. tomentosa*	*B. vahlii*
22	Length of entire trichomes from 246.72 µm-432.25 µm	0	1	0
23	Length of entire trichomes 281.2 µm-463.98 µm	0	0	1
24	Width of trichomes 3.56 µm-6.43 µm	1	0	0
25	Width of trichomes 8.45 µm-12.32 µm	0	1	0
26	Width of trichomes 10.56 µm-14.08 µm	0	0	1
27	Anisocytic stomata	1	0	0
28	Paracytic stomata	0	1	0
29	Anomocytic stomata	0	0	1
30	Stomata frequency 20/mm^2	1	0	0
31	Stomata frequency 76.66/mm^2	0	1	0
32	Stomata frequency 288.33/mm^2	0	0	1
33	Epidermal cell frequency 823.33/mm^2	1	0	0
34	Epidermal cell frequency 503.33/mm^2	0	1	0
35	Epidermal cell frequency 526.66/mm^2	0	0	1
36	Stomatal index 1.25-3.33	1	0	0
37	Stomatal index 8.77-15.38	0	1	0
38	Stomatal index 25.67-33.33	0	0	1
39	Length of stomata 17.6 µm-24. 64 µm	1	0	0
40	Length of stomata 14.08 µm-21.12 µm	0	1	0
41	Length of stomata 17.6 µm-21.12 µm	0	0	1
42	Width of stomata 14.8 µm-15.84 µm	1	0	0
43	Width of stomata 7.56 µm-10.56 µm	0	1	0
44	Width of stomata 10.56 µm-14.08 µm	0	0	1
45	Palisade cell 3.75-5.12	1	0	0
46	Palisade cell 5-7	0	1	0
47	Palisade cell 1.75-2.75	0	0	1
48	Reticulate veination pattern	1	1	0
49	Palmate reticulate	0	0	1
50	Vein termination 350-540	1	0	0
51	Vein termination 150-230	0	1	0
52	Vein termination 40-80	0	0	1
53	Vein-islet 290-430	1	0	0
54	Vein-islet 150-260	0	1	0
55	Vein-islet 320-440	0	0	1
56	Dorsiventral lamina	1	1	1
57	Plano-convex midrib	1	0	0
58	Heart shape midrib	0	1	1
59	Conjoint and collateral vascular bundle	1	1	1
60	Centrifugal xylem	1	1	1

Conclusion

In conclusion, all the investigated plants of *Bauhinia* genus are medicinally important and attributed to the traditional drugs in the Indigenous system of medicines. Different species of *Bauhinia* are locally known by the name Kanchan in Odisha. In comparison, the leaves of both species look similar (camel's foot or cow's hoof), which tends to be misleading for herbal formulas and various testing purposes during the processing of these leaves. Since it is a problematic genus in terms of its local name and morphological characteristics, especially in form, well-established quality control is therefore extremely necessary. The macroscopic and microscopic observations in the present correspondence will set the criteria that will be useful for detecting the identity and validity of various species of *Bauhinia*. In this analysis for the taxonomic differentiation of Bauhinia species, the leaf features including the scale, the number of protruding midribs, epidermal cell walls, styles of stomata and trichomes, along with the arrangement of vein-islets and vein terminations are carried out. Thus, at the species level of the genus *Bauhinia* in India, these characteristics might serve as strong diagnostic characteristics.

Conflict of interest

There are no conflicts of interest associated with this publication and we confirm that the manuscript has been read and approved by all named authors.

References

1 Dahlgren R, General aspects of angiosperm evolution and macrosystematics, *Nord J Bot*, 1983, **3**(1), 119-149.

2 Bhatia R C, Foliar epidermal studies of *Heliotropiumsupinum* L., *Folia Geobot Phytotax*, 1984, **19**, 381–385.

3 Christenhusz M J M and Byng J W, The number of known plants species in the world and its annual increase, *Phytotaxa*, 2016, **261**(3), 201-217.

4 Zoric L, Merkulov L, Lukovic J, Boza P and Polic D, Leaf epidermal characteristics of *Trifolium* L. species from Serbia and Montenegro, *Flora*, 2009, **204**(3),198-209.

5 Wilkins P W and Sabanci C O, Genetic variation in leaf epidermal cell size and shape in *Loliumperenne*, *Euphytica*, 1990, **47**(3), 233–239.

6 Avalos S V and Salinas A D, Los tricomasfoliaresen la caracterizacion de un grupo de especies del genero *Quercus*, seccion *Lobatae* (Fagaceae), *An Inst BiolUniv Nac Auton Mex, Serie Botanica*, 2003, **74**(1), 5-15.

7 Zareh M, Faried A and Farghaly N, Micromorphological studies on the genus *Lotus* L. (Fabaceae: Loteae) from Egypt, *Turk J Bot*, 2017, **41**, 273-288.

8 Kumar A V S and and Murugan K, Taxonomic implications with special reference to stomatal variations in *Solanum* species using light and scanning electron microscope, *Int J Appl Biol Pharm*, 2015, **6**(2), 112-125.

9 Fayed A A, Zareh M, Hassan N and Faried A, A systematic revision of the genus *Teucrium* (Lamiaceae) in Egypt, *Nord J Bot*, 2015, **33**(4), 389–400.

10 Panda P, Dash P and Ghosh G, Chemometric profile, antioxidant and tyrosinase inhibitory activity of Camel's foot creeper leaves (*Bauhinia vahlii*), *Nat Prod Res*, 2017, **32**(5), 596-599.

11 Filho V C, Chemical composition and biological potential of plants from the genus *Bauhinia*, *Phytother Res*, 2009, **23**(10), 1347-1354.

12 Bonilha I F, Cuelho C H F, Brondani J C, Cossetin J F, Marangoni L D, *et al.*, Biological potential of plants from the genus *Bauhinia*, *Rev Cuba Farm*, 2015, **49**, 583-594.

13 Parotta J A, *Healing plants of peninsular India*, (CABI Publishing New York, USA), 2001.

14 Nadkarni K M and Nadkarni A K, *Dr. K. M. Nadkarni's Indian Materia medica: with ayurvedic, unani-tibbi, siddha, allopathic, homeopathic, naturopathic & home remedies, app. & indexes*. (Bombay Popular Book Depot Popular Prakashan), 1998, 968.

15 Panda P, Das D, Dash P and Ghosh G, Therapeutic potential of Bauhinia racemosa - A mini review, *Int J Pharm Sci Rev Res*, 2015, **32**(2), 169-179.

16 Kumar V, Rathore K, Jain P and Ahmed Z, Biological activity of *Bauhinia racemosa* against diabetes and interlinked disorders like obesity and hyperlipidemia, *Clin Phytoscience*, 2017, **3**(1), 1-9.

17 Dugasani S, Balijepalli M K, Tandra S and Pichika M R, Antimicrobial activity of *Bauhinia tomentosa* and *Bauhinia vahlii* roots, *Pharmacogn Mag*, 2010, **6**(23), 204-207.

18 Quriroga E N, Sampietro A R and Vattuone M A, Screening antifungal activities of selected medicinal plants, *J Ethnopharmacol*, 2010, **74**(1), 89-96.

19 Mukundan D, Mohankumar R and Vasanthakumari R, Comparative study of synthesized silver and gold nanoparticles using leaves extract of *Bauhinia tomentosa* Linn and their anticancer efficacy, *Bull Mater Sci*, 2017, **40**(2), 335-344.

20 Elbanna A H, Mahrous E A, Khaleel A E and El-Alfy T S, Morphological and anatomical features of *Bauhinia vahlii* Wright & Arnoot grown in Egypt, *J App Pharm Sci*, 2016, **6**(12),84-93.

21 Zarsky V and Cvrckova F, Plant cell morphogenesis: Methods and protocols, (Humana Press), 2014, **285**.

22 Heywood V H, Scanning Electron Microscopy (University of Reading) International Symposium Department of Botany Proceedings, (Academic Press, New York), 1971, 180-227.

23 Harbone J B, *Phytochemicals methods: A guide to modern techniques of plant analysis*, (Chapman and Hall, London and New York), 1973, 182-89.

24 Raj R S N and Radhamany P M, Pharmacognostic and physicochemical analysis on the leaves of *Brunfelsiaamericana* L, *Asian Pac J Trop Biomed*, 2012, **2**(1), S305-S307.

25 Kumar D, Kumar K, Kumar S, Kumar T, Kumar A, *et al.*, Pharmacognostic evaluation of leaf and root bark of *Holopteleaintegrifolia* Roxb, *Asian Pac J Trop Biomed*, 2012, **2**(3), 169-175.

26 Baas P, Wheeler E and Chase M, Dicotyledonous wood anatomy and the APG system of angiosperm classification, *Bot J Linn Soc*, 2000, **134**, 3–17.

27 Rashid N, Zafar M, Ahmad M, Khan M A, Malik K, *et al.*, Taxonomic significance of leaf epidermis in tribe *Trifolieae* L. (Leguminosae; Papilionoideae) in Pakistan, *Plant Biosyst - Int J Dealing Aspects Plant Biol*, 2019, **153**(3), 406-416.

28 Carpenter S B and Smith N D, Stomatal distribution and size in southern Appalachian hardwoods, *Canadian J Bot*, 1975, **53**(11), 1153–1156.

29 Tipmontiane K, Srinual A and Kesonbua W, Systematic significance of leaf anatomical characteristics in some species of Mangifera L. (Anacardiaceae) in Thailand, *Trop Nat Hist*, 2018, **18**(2), 68-83.

30 Albert S and Sharma B, Comparative foliar micromorphological studies of some *Bauhinia* (Leguminosae) species, *Turk J Bot*, 2013, **37**(2), 276-281.

31 Paul V, Sharma L, Pandey R and Meena R C, Measurements of stomatal density and stomatal index on leaf/plant surfaces, Manual of ICAR Sponsored Training Programme for Technical Staff of ICAR Institutes on "Physiological Techniques to Analyze the Impact of Climate Change on Crop Plants", 2017, **27**.

32 Dickison W C, *Integrative Plant Anatomy*, (Academic Press, San Diego), 2002.

33 Bano A, Ahmad M, Zafar M, Sultana S and Khan M A, Comparative foliar micromorphological studies of some species of *Asteraceae* from alpine zone of Deosai plateau, Western Himalayas, *J Animal Plant Sci*, 2015, **25**(2), 422–430.

34 Murugesan S K, Dhanapaul V and Rakkan P, Comparison of two Bauhinia species Based on the morphological, antimicrobial and protein markers, *J Pharmacogn Phytochem*, 2017, **6**(6), 306-309.

35 Klimko M, Nowińska R, Wilkin P and Wiland-Szymańska J, Comparative leaf micromorphology and anatomy of the dragon tree group of *Dracaena* (Asparagaceae) and their taxonomic implications, *Plant Syst Evol*, 2018, **304**(8), 1041–1055.