Plant Archives Volume 20 No. 1, 2020 pp. 35-41 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

TAXONOMIC SIGNIFICANCE ON COMPARATIVE PETIOLE ANATOMY OF TWELVE SPECIES OF *CURCUMA* L. (ZINGIBERACEAE) FROM SOUTH INDIA

Anu S.[1, 2 *] and Mathew Dan[1]

[1*]Plant Genetic Resource Division, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram, Pin-695562 (Kerala) India.
[2*]University of Kerala, Thiruvananthapuram, (Kerala), India.

Abstract

The anatomy of vegetative organs can provide valuable evidence in taxonomy. Petiole anatomy of 12 species of *Curcuma* L. of the family Zingiberaceae distributed in south India was investigated with a view to exploit its systematic and taxonomic significance. Specimens of the selected species were obtained from south India and subjected to standard procedures for microscopic observation and micrographic documentation. The results showed that, though there were more similarities in the anatomical structure of petiole, certain features can be used for species delimitation. Based on these, a taxonomic key for identifying the twelve candidate *Curcuma* species was prepared. Distinguishing characters of taxonomic value include, outline shape of the petiole, variation in the number and arrangement of vascular bundles, nature and shape of epidermis, cortex and pith. The presence of trichomes in *C. aurantiaca* and the absence of a wavy chlorenchymatous layer in *C. zedoaria*, are exceptional characters.

Key words: Anatomy, *Curcuma*, Petiole, Zingiberaceae

Introduction

The genus *Curcuma* L. belongs to tribe Zingibereae, sub-family Zingiberoideae of family Zingiberaceae, consisting of c. 120 species (Sabu, 2006; Leong-Skornickova *et al*., 2008) through out South and South East Asia with a few species extending to China, Australia and the South Pacific. The highest diversity is in India with c. 45 species (Leong-Skornickova *et al*., 2010), most of them are distributed in south India.

The petiole is a leaf organ which connects the lamina with the stem, endowed with anatomical characters of significant taxonomic value. In genus *Curcuma*, the leaves are directly arising from the underground rhizome and the leafsheaths often appressed to form an aerial pseudostem. Morphological attributes of vegetative organs have often constituted the mainstay of taxonomic studies in plants (Polhill, 1968; Pilbeam and Bell, 1979; Agbagwa and Ndukwu, 2004; Adedeji, 2005; Kharazian, 2007; Eminagaoglu *et al*., 2012; Ozcan *et al*., 2014). The anatomical characters are more fixed than the others and it is less plastic that the systematic anatomist rely on because they are not affected by environmental conditions (Barthlott, 1981). Howard, (1979), Adedeji, (2004), Adedeji and llloh, (2004) and Stuessy, (2009) emphasized the taxonomic importance of anatomical features along with other characters for the identification and classification of plants as well as to ascertain their taxonomic relationship.

Anatomical data has not only been useful at the higher levels but in certain instances, successfully employed even at the specific level. Howard, (1963) and Metcalfe and Chalk, (1979) also suggested the taxonomic significance of petiole anatomy. Later several studies on petiole anatomy were proved to be taxonomically and diagnostically valuable in various groups at different taxonomic levels (Novikoff and Mitka, 2015; Talip *et al*., 2017). *Curcuma* species differ in fine anatomical features as reported by Das *et al*., (2004) and Jayasree, (2007). Das *et al*., (2004) investigated the transverse section of the sheathing petiole of three *Curcuma* species such as *C. amada, C. caesia and C. longa*. Various characters have been used in describing and delimiting the species

***Author for correspondence :** E-mail: anumanoj2020@gmail.com

in family Zingiberaceae but not much attention has been drawn to petiole anatomy. It is obvious that precise and detailed analysis of various features of petiole will support accurate characterization of the taxa. Therefore, the present study assessed the importance of petiole anatomy in establishing its taxonomic role among twelve species of *Curcuma* distributed in south India.

Materials and Methods

Fresh, mature petiole of 12 *Curcuma* species was collected from the conservatory garden for Medicinal and Aromatic Plants at JNTBGRI, Thiruvananthapuram, where they are cultivated and maintained under uniform conditions, which were introduced from natural habitats in south India. The voucher specimens were deposited at TBGT, JNTBGRI. The candidate species (voucher number in paranthesis) selected for the study were *C. aeruginosa* Roxb. (83451), *C. amada* Roxb. (93668), *C. aromatica* Salisb. (93669), *C. aurantiaca* Zijp (83461), *C. caesia* Roxb. (93659), *C. haritha* Mangaly & M. Sabu (93670), *C. longa* L. (91027), *C. oligantha* Trimen. (93671), *C. pseudomontana* J. Graham (93661), *C. vamana* M. Sabu & Mangaly (83460), *C. zanthorrhiza* Roxb. (83452) and *C. zedoaria* (Christm.) Roscoe (93672). Petiole anatomical investigations were carried out on the transverse-sections from the central portion of the petiole of each species. The third leaf from the base was taken for the study. Five transverse sections from at least three different individual plants of each taxon were measured to assess the consistency of quantitative anatomical characters. Freehand sections (transverse sections) were taken, stained in safranine (1g in 100ml distilled water), mounted using 2% glycerine solution and were viewed under the microscope. Microscopic observations were carried out using Leica DM2500 light microscope attached with Leica DFC450 camera. Leica Application Suite Software was used for the observation and measuring/analysing the microscopic images.

Results and Discussion

Anatomical features of transverse sections from the petiole of 12 candidate *Curcuma* species are discussed below. The comparative characters are summarized in table 1, table 2 and illustrated in Plate 1.

General Anatomical Features

• Ground tissue: The epidermis is followed by the cortical region, where occurs a parenchymatous hypodermis made up of oval or hexagonal cells, along with the small abaxial vascular bundles (bundle arc II). There is 1-3 layered chlorenchyma followed by hypodermis just above the main vascular bundles (bundle arc I), forming a wavy pattern, which extends to

Table 1: Comparative qualitative characters in petiole anatomy of 12 *Curcuma* species.

Characters	SELECTED *CURCUMA* SPECIES											
	C. aeruginosa	*C. amada*	*C. aromatica*	*C. aurantiaca*	*C. caesia*	*C. haritha*	*C. longa*	*C. oligantha*	*C. pseudomontana*	*C. vamana*	*C. zanthorriza*	*C. zedoaria*
Shape of Epidermal cells	Rectangular or Elliptical	Rectangular or Elliptical	Rectangular	Rectangular or Elliptical	Circular	Rectangular or Circular	Rectangular or Circular	Circular	Circular	Rectangular or Irregular	Circular or Irregular	Circular or Irregular
Trichomes	Absent	Absent	Absent	Present Unicellular	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Collenchymatous layer	Wavy	Wavy	Wavy	Wavy	Wavy	Wavy	Wavy	Wavy	Wavy	Wavy	Wavy	Absent
Main Vascular Bundles	Close collateral alternating with air cavities	Close collateral alternating with air cavities	Close collateral alternating with air cavities	Close collateral alternating with air cavities	Close collateral alternating with air cavities	Close collateral alternating with air cavities	Close collateral alternating with air cavities	Close collateral alternating with air cavities	Close collateral alternating with air cavities	Close collateral alternating with air cavities	Close collateral alternating with air cavities	Close collateral alternating with air cavities
The shape of air canals	Round / Oval	Round / Oval	Round / Oval	Oval / Irregularly oval	Round / Oval	Rectangular / Oval	Round / Oval	Round / Oval	Round/ Oval	Rectangular/ Squarish	Round/ Oval	Rectangular/ Squarish
Outline shape of T. S.	Open 'U' shaped	Open 'U' shaped	Open 'V' shaped	'V' shaped	'U' shaped	'U' shaped	Horseshoe shaped	'V' shaped	Open 'U' shaped	Open 'V' Shaped	Open 'U' shaped	Open 'V' shaped

Table 2: Comparative quantitative characters in petiole anatomy of 12 *Curcuma* species.

CHARACTERS	SELECTED *CURCUMA* SPECIES											
	C. aeruginosa	*C. amada*	*C. aromatica*	*C. aurantiaca*	*C. caesia*	*C. haritha*	*C. longa*	*C. oligantha*	*C. pseudomontana*	*C. vamana*	*C. zanthorriza*	*C. zedoaria*
Epidermal cells length (µm) width (µm)	43±7.70 19±4.20	25±5.56 13±2.98	23±8.83 15±4.18	24±5.05 13±7.65	17±2.90 11±1.8	26±8.48 15±4.03	22±4.24 10±7.24	37±9.79 18±5.88	31±8.48 19±4.44	17±4.25 16±3.81	18±1.99 12±1.66	17±5.15 15±2.31
Trichome length (µm) width (µm)	Absent	Absent	Absent	36±4.69 5±0.69	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Hypodermal layer number thickness (µm)	2-5 133±55.16	2-6 125±75.26	2-4 94±30.07	2-6 96±4.69	1-4 85±30.57	1-5 102±71.74	2-5 92±12.36	1-4 82±25.11	2-6 240±62.96	1-4 92±14.63	2-5 90±10.26	2-5 93±15.06
Chlorenchyma Layer number thickness (µm)	1-3 64±30.59	1-3 49±19.10	1-2 42±25.86	1-3 45±19.66	1-3 49±16.34	2-3 60±23.66	1-2 51±6.66	1-2 43±21.36	1-3 106±56	1-3 93±21.80	1-3 104±25	Absent
Main Vascular Bundles number xylem length (µm) phloem length(µm)	26-30 212±34.87 100±12.72	24-26 242±13.59 107±15.59	20-22 157±16.81 63±11.20	14-16 146±14.57 53±10.97	16-18 157±42.45 63±11.20	24-26 231±24.55 83±8.66	18-20 150±14.97 56±15.50	14-16 144±18.16 52±11.17	24-26 313±27.19 134±39.44	18-22 238±17.67 126±14.33	21-25 163±15.16 103±15.54	18-24 156±18.19 64±13.79
Pith cell length(µm) width(µm)	70±45.67 44±23.49	116±42.33 96±37.84	99±16.98 83±19.81	60±11.31 46±7.65	88±29.13 79±34.74	125±45.93 86±28.54	94±19.54 61±16.76	55±18.16 27±10.93	181±90 116±39	59±7.67 32±14.33	57±8.89 43±14.18	65±10.65 42±8.97
Middle (adaxial to abaxial) width (µm)	3559±89	4084±168	1987±71	1835±48	2008±66	2495±43	1760±50	1671±55.91	4834±110	1538±35	1984±78	2345±32
Sheath width (µm)	175±58	584±30	137±23	185±26	114±16	146±35	137±18	126±15	175±24	156±29	148±34	134±28

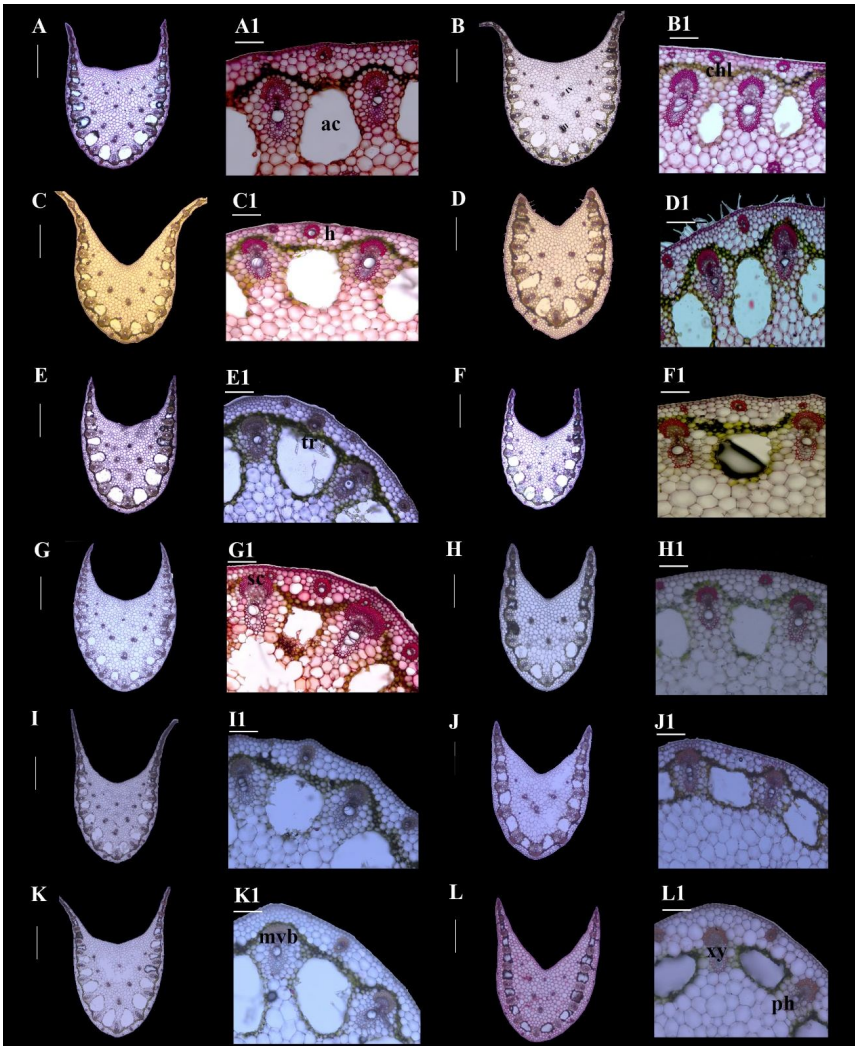
demarcate the wall of air canals. The rest of the ground tissue is composed of thin-walled parenchyma with two inner arcs of vascular bundles (bundle arc III and IV) of different sizes (Plate 1: A-L). Subepidermal parenchyma are smaller with a gradual transition towards the centre with larger cells, forming the pith. The same pattern of distribution of ground tissue was observed by Jayasree (2007) in the petiole of some *Curcuma* species studied.

• Vascular bundles

(a) Arrangement: The vascular bundles in the petiole are arranged in three or four main arcs (Plate 1: A-L), which are numbered in the present study as I to IV. The large main bundle arc (bundle arc I) is close to the abaxial surface and the bundles are of the same size in the median region, gradually becoming smaller towards the margin. Each main vascular bundle is between two air canals (Plate 1, A1-L1) and the air canals are internally traversed by trabeculae or diaphragm. Bundle arc II, consists of bundles of smaller size and are arranged alternately with the arc I bundles. Arc III is adaxial to the bundle arc I which is with medium-sized bundles. In addition to these, some small vascular bundles are seen close to the adaxial surface, which are scattered or sometimes forming one more arcs (bundle arc IV with 1-4 vascular bundles). The degree of the fusion of bundle arc varies with the level of maturity of section and the species. The termination of bundle arc IV is marked a gradual fusion with bundle arc III. The cortical bundles (bundle arc III) showed oblique /inverse orientation of vascular tissues. The vascular arrangement of petiole showed various types of vascular structure as illustrated by Metcalfe and Chalk, (1979). Similar vascular arrangement was reported by Tomlinson, (1956), that the Zingiberaceae petiole has a maximum of 4 bundle arcs, with the main bundle arc (I) close to the abaxial surface and subsidiary arcs, two above and one below the main arc, if all present. Hare, (1944) described various arrangements of vascular bundles in petiole and stated that the petiole anatomical characters are diagnostic and possibly of value for classification.

(b) Structure: Vascular bundles are conjoint, collateral, open and the main vascular bundles (bundle arc I) have incomplete fibrous or sclerenchymatous bundle sheath, which is seen above the phloem and below the xylem. The smaller vascular bundles are circular while larger bundles are elliptic with the length twice as much as width. Solerender and Meyer, (1930) commented that in Zingiberaceae vascular bundles in the main arc (bundle arc I) are as Musa-type. The bundles are almost pear-shaped as they are wider towards the abaxial surface. In all the main vascular bundles xylem remain towards the

centre and the phloem towards the periphery. Xylem consists of one or two large metaxylem elements, adaxial to which one to three protoxylem elements are arranged. Vascular bundles of accessory arcs (bundle arc II, III and IV) have reduced vascular tissues and contracted protoxylem. A layer of angular xylem parenchyma separates the xylem from the phloem; which is arranged towards the abaxial surface. Phloem is composed of sieve-tubes, companion cells, and phloem parenchyma. Bundle arc II consists of bundles mostly with a metaxylem and a small group of phloem. Xylem parenchyma may or may not be present. These bundles are enveloped with a well-developed complete fibrous sheath. Bundles in arc III may be completely or incompletely surrounds the sclerenchymatous or fibrous sheath. The bundle arc IV may be very much reduced, seen near to the adaxial surface and are rich in mechanical tissues. Some of these bundles are devoid of any vascular orientation of the xylem and phloem.



Petiole outline and T. S. portion enlarged (ac-air canal, chl-chlorenchymatous layer, h-hypodermis, ph-phloem, sc- sclerenchymatous sheath, tr-trabeculae, xy-xylem, mvb-main vacular bundle, I, II, III & IV-bundle arc I, II, III & IV): A, A1 : *C. aeruginosa*, **B, B1 :** *C. amada*, **C, C1 :** *C. aromatica*, **D, D1:** *C. aurantiaca* **E, E1 :** *C. caesia*, **F, F1:** *C. haritha*, **G, G1:** *C. longa*, **H, H1 :** *C. oligantha*,**I, I1 :** *C. pseudomontana*, **J, J1:***C. vamana*,**K, K1 :** *C. zanthorrhiza*, **L, L1:** *C. zedoaria* **(Scale bar : A-L =1mm,A1-L1=200µm).**

Plate 1

Comparative Anatomy

• Outline Shape: The adaxial surface is with a concave groove and the abaxial surface is convex, forming different outline shapes. The outline was 'U' shaped in *C. caesia* and *C. haritha* (Plate 1: E & F); open 'U' shaped in *C. aeruginosa*, *C. amada*, *C. pseudomontana* and *C. zanthorrhiza* (Plate 1: A, B, I & K); 'V' shaped in *C. aurantiaca* and *C. oligantha* (Plate 1: D & H); open 'V' shaped in *C. aromatica*, *C. vamana* and *C. zedoaria* (Plate 1: C, J & L); whereas it is horseshoe-shaped in *C. longa* (Plate 1: G). The investigation carried out by Das *et al*., (2004) supports the horseshoe-shaped outline of petiole in *C. longa* but his observation in the case of *C. caesia* and *C. amada* is not matching where the outline is 'U' and open ' U' shaped respectively. Though the petiolar anatomical characters in the species studied varied, it is difficult to identify and delimit the taxa solely based on petiole outline shape. This result is consistent with that of Haddad and Barnett, (1989), who suggested that variations in petiole outline are not taxonomically much significant. But Noraini and Cutler, (2009) stated that the variability in vascular structure and the combination of petiole outline could be very useful in the classification of genus *Parashorea* of Dipterocarpaceae.

• Epidermis: Epidermis in all species are uniseriate with similar-sized cells in both adaxial and abaxial surface. Epidermis is with circular cells in *C. caesia*, *C. oligantha* and *C. pseudomontana* (Plate 1: E1, H1 & I1) where as in other species, cell shapes differ as elliptical, rectangular or irregular, with a cuticularised outer walls (Table 1). Transverse sections are with unicellular, non-glandular trichomes on both adaxial and abaxial surfaces of *C. aurantiaca*, at a high density (Plate 1: D1). All the other candidate species have no trichomes. The presence or absence of trichomes on the epidermis of the petiole is taxonomically significant. Sometimes trichome types,

distribution, and density provide identification at the generic and even specific levels. Trichome characters are reported to be taxonomically useful at various levels in Leguminosae (Shaheen, 2007) Lamiaceae (Atalay *et al*., 2016) and Rosaceae (Faghir *et al*., 2011 & 2016). Mabel, *et al*., (2013) also reported that the presence or absence of trichome in the epidermis of the petioles is taxonomically valuable.

• Assimilatory tissue and air canals: There is a band of wavy chlorenchymatous layer (assimilatory tissue) abaxial to the main arc bundles (bundle arc I) (Plate 1: A1-L1). Within the band, and alternating with the main arc bundles, is a system of air canals, which is lined by 1 or 2 chlorenchymatous layer. The chlorenchyma band and air canals show a maximum development in the median region, on the other hand towards the margin, there is progressive decrease, in air canals and chlorenchyma layer and are become narrow. The air canals contain, elongated, loosely arranged, chlorophyllous tissue forming partitions called diaphragm or trabeculae (Plate 1: A-L), which are present in all the selected *Curcuma* species. The shape of air canals are round or oval or irregularly oval in most of the species. But it is rectangular or squarish in *C. vamana* and *C. zedoaria* and rectangular or oval in *C. haritha* (Table 1). A continuous wavy chlorenchymatous layer is present in all the selected species except *C. zedoaria*, where it is seen only as surrounding the air canals (Plate1: L, L1).

Data on qualitative and quantitative anatomical features of the petiole (Table 1 & 2), provided significant characters to be considered in taxonomic studies of the genus *Curcuma*. A combination of petiolar anatomical vascular and non-vascular features may be helpful in the identification of genus and species. Though the pattern of distribution and composition of tissues are almost uniform in the *Curcuma* species studied, there are distinguishing qualitative characters such as outline shape of petiole (T.S.) and air canals, presence or absence of chlorenchymatous layer and trichomes and nature and shape of epidermal cells (Table 1). Significant quantitative characters include length and width of epidermal cells, thickness of chlorenchymatous and hypodermal layers, number of main vascular bundles and length and width of middle (adaxial to abaxial) portion and sheaths (Table 2). Anatomical observations on the trans-sections of the petiole of twelve candidate *Curcuma* species revealed interspecific variations that are important in the classification and delimitation of the species. The vascular pattern, outline shape etc. of the petiole of *Alpinia* species studied by Hussin *et al*., (2000) also showed variations, which can be used for species identification. Aiswarya, (2019) clearly mentioned about the taxonomic significance of foliar anatomy based on a study on Indian species of *Kaempferia* of Zingiberaceae. Petiole anatomy of 64 species of *Baphia* of Leguminosae (Soladoye, 1982) and some species of *Phlomis* and *Eremostachys* of Labiatae (Azizian and Cutler, 1982) provide clear support of its use in the taxonomy of these genera. According to Howard, (1963), families, genera and even species may be identified using petiole characters and he emphasised its importance to delimit generic level.

Some qualitative and quantitative petiole anatomical characters were found to provide useful information for taxonomic studies within the species of *Curcuma*. The data recorded are useful to distinguish the taxa because of some identifying characters. Based on these, an artificial dichotomous key for identification at the species level is provided.

Identification key to the species

1. Trichomes present - *C. aurantiaca*
1. Trichomes absent - 2
2. Wavy chlorenchymatous layer present - 3
2. Wavy chlorenchymatous layer absent - *C. zedoaria*
3. T. S. horseshoe-shaped in outline - *C. longa*
3. T. S. not in horseshoe-shaped outline - 4
4. T. S. 'V' shaped in outline - *C. oligantha*
4. T. S. not in 'V' shaped in outline - 5
5. Epidermal cells circular - 6
5. Epidermal cells not circular - 7
6. Middle (abaxial to adaxial) width (mean) is > 4000µm - *C. pseudomontana*
6. Middle (abaxial to adaxial) width (mean) is < 4000µm - *C. caesia*
7. The average number of main vascular bundles > 25 - *C. aeruginosa*
7. The average number of main vascular bundles < 25 - 8
8. Sheath width (mean) > 500µm - *C. amada*
8. Sheath width (mean) < 500µm - 9
9. Xylem length (mean) > 200µm - 10
9. Xylem length (mean) < 200µm - 11
10. Hypodermal layer thickness (mean) > 100µm - *C. haritha*
10. Hypodermal layer thickness (mean) < 100µm - *C. vamana*
11. Chlorenchymatous layer thickness (mean) > 100µm

- C. zanthorrhiza

11. Chlorenchymatous layer thickness (mean) < 100µm *- C. aromatica*

Conclusion

The petiole anatomical characters on the twelve species of the genus *Curcuma* showed almost similar anatomical characteristics, but some important distinct features are observed. The resulted anatomical data provides reliable characters useful for the delimitation of species. It is obvious that by a comparative analysis of the various petiolar features, it will be possible to accurately characterize the taxa. The presence of trichomes, outline shapes of the petiole (T.S.) and air canals, number of main vascular bundles, the thickness of chlorenchymatous and hypodermal layers and the width of middle portion and sheaths, etc. are indeed important for taxonomic evaluation. Our observations suggest that a combination of qualitative and quantitative petiole anatomical characters will be helpful in the taxonomic treatment of these species even in its vegetative phase.

Acknowledgments

Authors wish to express our sincere acknowledgment to the Director, Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI) for providing the facilities; the first author is thankful to the University of Kerala for Providing the Research Fellowship.

References

Adedeji, O. (2004). Leaf epidermal studies of the species of *Emilia* Cass. (Senecioneae, Asteraceae) in Nigeria. *Bot. Lith.,* **10(2):** 121-133.

Adedeji, O. (2005). Pollen morphology of the three species of the genus *Emilia* Cass. (Asteraceae) from Nigeria. *Thaiszia- J. Bot.*, **15:** 1-9.

Adedeji, O. and H.C. Illoh (2004). Comparative foliar anatomy of ten species in the genus *Hibiscus* Linn. in Nigeria. *New Bot.,* **31:** 147-180.

Agbagwa, I.O. and B.C. Ndukwu (2004). The value of morpho-anatomical features in the systematics of *Cucurbita* L. (Cucurbitaceae) species in Nigeria. *Afr. J. Biotechnol.*, **3(10):** 541-546.

Aiswarya, K. (2019). Foliar anatomy of Indian *Kaempferia* (Zingiberaceae) - a taxonomic approach. In: Asian Zingiberales: Resume & Prospects, M/s Bishen Singh Mahendra Pal Singh, Dehra Dun, India.1-7.

Atalay, Z., F. Celep, F. Bara and M. Dogan (2016). Systematic significance of anatomy and trichome morphology in *Lamium* (Lamioideae; Lamiaceae). *Flora- Morphology, Distribution, Functional Ecology of Plants.,* **225:** 60-75.

Azizian, D. and D.F. Cutler (1982). Anatomical, cytological and phytochemical studies on *Phlomis* L. and *Eremostachys* Bunge (Labiatae). *Bot. J. Linn. Soc.,* **85(4):** 249-281.

Barthlott, W. (1981). Epidermal and seed surface characters of plants: systematic applicability and some evolutionary aspects. *Nord. J. Bot.,* **1(3):** 345-355.

Das, D., A. Bhattacharjee, I. Biswas and A. Mukherjee (2004). Foliar characteristics of some medicinal plants of Zingiberaceae. *Phytomorphology.,* **54(3-4):** 291-302.

Eminagaoglu, O., M. Ozcan and S. Kultur (2012). Contributions to the leaf and stem anatomy of *Tradescantia fluminensis*: an alien species new to the flora of Turkey. ACU. *J. For. Fac*., **13(2):** 270-277.

Faghir, M.B., F. Attar and B. Ertter (2011). Foliar anatomy of the genus *Potentilla* L. (Rosaceae) in Iran and its taxonomic implication. *Iran. J. Sci. Technol.,* **35(3):** 243-246.

Faghir, M.B., A. Mehrmanesh and F. Attar (2016). Leaf and petiole anatomical characters of the genus *Alchemilla* (Rosaceae) in Iran and their use in numerical analysis. *Taxon. Biosyst.,* **8:** 1-20.

Haddad, R.S. and J.R. Barnett (1989). Variation in petiole anatomy of European spiny species of *Astragalus* L. (Leguminosae-Papilionoideae-Galegeae). *Bot. J. Linn. Soci*., **101(2):** 241-247.

Hare, C.L. (1944). On the taxonomic value of the anatomical structure of vegetative organs of the dicotyledons: 5. The anatomy of the petiole and its taxonomic value. In: *Proc. Linn. Soc. London*., **155 (3):** 223-229.

Howard, R.A. (1963). The vascular structure of the petiole as a taxonomic character. *Adv. Hort. Sci.,* **3:** 7-13.

Howard, R.A. (1979). The petiole. In: Metcalfe, C. R. and Chalk, L. (eds.), Anatomy of the Dicotyledons. I. Systematic Anatomy of Leaf and Stem with a Brief History of the Subject, Clarendon press. 88-96.

Hussin, K.H., C.T. Seng, H. Ibrahim, W.Q. Gen, L.J. Ping and L. Nian (2000). Comparative leaf anatomy of *Alpinia* Roxb. species (Zingiberaceae) from China. *Bot. J. Linn. Soc.,* **133(2):** 161-180.

Jayasree, S. (2007). Morphological and anatomical studies on South Indian Zingiberaceae, Ph. D. Thesis. Department of Botany, University of Calicut, Kerala.

Kharazian, N. (2007). The taxonomy and variation of leaf anatomical characters in the genus *Aegilops* L. (Poaceae) in Iran. *Turk. J. Bot.,* **31(1):** 1-9.

Leong- Skornickova, J., O. Sida and K. Marhold (2010). Back to types! Towards stability of names in Indian *Curcuma* L. (Zingiberaceae). *Taxon.,* **59(1):** 269-282.

Leong-Skornickova, J., O. Sida, M. Sabu and K. Marhold (2008). Taxonomic and nomenclatural puzzles in Indian *Curcuma*: the identity and nomenclatural history of *C. zedoaria* (Christm.) Roscoe and *C. zerumbet* Roxb. (Zingiberaceae). *Taxon.,* **57(3):** 949-962.

Mabel, A.F., A.A. Johnson and O.O. Temitope (2013). Petiole

anatomy of some species of Asteraceae in southwest Nigeria. *Afr. J. Plant Sci*., **7(12):** 608-612.

Metcalfe, C.R. and L. Chalk (1979). Anatomy of the Dicotyledons, Vol. I. Oxford Univ. Press. New York. 12-88.

Noraini, T. and D.F. Cutler (2009). Leaf anatomical and micromorphological characters of some Malaysian *Parashorea* (Dipterocarpaceae). *J. Trop. For. Sci*., **1:** 156-167.

Novikoff, A.V. and J. Mitka (2015). Anatomy of stem-node-leaf continuum *Aconitum* (Ranunculaceae) in the Eastern Carpathians. *Nord. J. Bot.*, **33(5):** 633-640.

Ozcan, M., M.C. Unver and O. Eminagaoglu (2014). Comparative anatomical and ecological investigations on some *Centaurea* (Asteraceae) taxa from Turkey and their taxonomic significance. *Pak. J. Bot.*, **46(4):** 1287-1301.

Pilbeam, D.J. and E.A. Bell (1979). A reappraisal of the free amino acids in seeds of *Crotalaria juncea* (Leguminosae). *Phytochem.*, **18(2):** 320-321.

Polhill, R.M (1968). Miscellaneous notes on African species of *Crotalaria* L. : ll. *Kew Bull.*, **22(2):** 169-348.

Sabu, M. (2006). Zingiberaceae and Costaceae of South India. Indian Association for Angiosperm Taxonomy, Calicut University, Kerala, India. 126-191.

Shaheen, A.S.M. (2007). Characteristics of the stem-leaf transitional zone in some species of Caesalpinioideae (Leguminosae). *Turk. J. Bot.*, **31(4):** 297-310.

Soladoye, M.O. (1982). Leaf epidermal studies in the African genus *Baphia* Lodd. and related genera (Papilionoideae-Sophoreae). *Bulletin du Jardin botanique national de Belgique/Bulletin van de Nationale Plantentuin van Belgie*, 415-437.

Solerender, H. and F.J. Meyer (1930). Zingiberaceae. *Systematische Anatomie der Monokotyledonnen*., **6:** 27-56.

Stuessy, F.T. (2009). Plant Taxonomy: The systematic evaluation of comparative data. (2nd edition), Columbia Univ. Press. 565.

Talip, N., D.F. Cutler, A.A. Paud, B.S. Ismail, A.R. Ruzi and A.A. Juhari (2017). Diagnostic and systematic significance of petiole anatomy in the identification of *Hopea* species (Dipterocarpaceae). *S. Afr. J. Bot.*, **111:** 111-125.

Tomlinson, P.B. (1956). Studies in the systematic anatomy of the Zingiberaceae. *J. proc. Linn. Soc.*, **55(361):** 547-592.