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Morpho-anatomical characterisation of the rhizomes of ten species of *Curcuma* L. (Zingiberaceae) from South India

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Abstract

The morphological and anatomical characterisation of ten medicinally and economically important species of *Curcuma* L. from South India namely, *C. aeruginosa* (neela-kua), *C. amada* (manga-inchi), *C. aromatica* (kasturi-manjal), *C. aurantiaca*, *C. caesia* (kari-manjal), *C. haritha* (karpura-kua), *C. longa* (manjal), *C. montana*, *C. zanthorrhiza* (manja-kua) and *C. zedoaria* (chenthandan-kua) were studied and compared. Eventhough, all the species show similarity in their characters, striking differences were noticed with respect to morphological characters such as shape and size of mother rhizome and lateral branches, colour of the cut surface, aroma and taste of rhizomes etc. Differences were also observed in some anatomical characters such as nature of endodermoid layer, size and shape of starch grains, oil cells and curcumin cells, etc. Based on the distinct morpho-anatomical features, an artificial dichotomous key was proposed for taxonomic delimitation of the species with their rhizome.

Keywords: anatomy, *Curcuma*, curcumin cells, morphology, oil cells, starch grains

Introduction

The genus *Curcuma* L. of the family Zingiberaceae is economically and medicinally important and used as spices, medicines, food, dyes, cosmetics and ornamentals. It is widely distributed in South East Asia with about 104 species (Mabberely 2017), around 40 species in India (Sasikumar 2005) and about 20 species in South India (Sabu 2006). Most members of the genus are widely used for medicinal purposes and as a colouring agent. Moreover, rhizomatous species are excellent sources of starch. Several species have the potential to maintain the biodiversity of hilly regions (Bhutia & Sharangi 2017).

According to Skornickova *et al*. (2008) the genus *Curcuma* L. is a taxonomically difficult one in identification due to the close resemblances with vegetative parts and short flowering period. Likewise, they exhibit significant morphological variations at both intra-specific and inter-specific levels, but the similarities of some species create problems during their

identification. The taxonomic identification of the species is important to search and confirm their different potential uses. In the case of genus *Curcuma*, taxonomic delimitation in sterile phase when the plants are devoid of aerial portions is not feasible. Since the rhizomes are the useful part, determination of species using the rhizome alone is often challenging. Out of the 20 species available in southern India, hardly 10 species are with prominent rhizomes and hence those 10 species are included in the present study.

Anatomy is the scientific study of the structure of body, while histochemistry and the powder microscopy deals with the minute structure and composition, which are often species specific and played a role to resolve taxonomic identification problems. Anatomical and micro-morphological characters have an important role in raw drug standardization as well as in the identification of genuine medicinal plants (Dan 2011). In recent years, anatomical and histochemical characters are being used by several researchers to deduce the taxonomic conclusions (Edeoga & Okoli 1995; Remashree & Balachandran 2006; Eminagaoglu *et al*. 2012; Ozcan & Eminagaoglu 2014). Characterisation of the root powder through powder microscopy was also species-specific and significant for species delimitation (Navas *et al*. 2013).

Tomlinson (1956) used the morphology of the rhizomes of each species with its respective anatomical features to identify Zingiberaceae species. Though the anatomical characterisation has been worked out among certain species in the genus *Curcuma,* there is a big lacuna in the comparative characterisation of morpho-anatomical features of all the rhizomatous *Curcuma* species from south India. Relevant morpho-anatomical, histochemical and powder microscopic features of rhizomes were used to establish an artificial key to identify the candidate *Curcuma* species in the present study.

Materials and methods

The rhizomes of the ten *Curcuma* species namely *C. aeruginosa* Roxb. (TBGT 83451), *C. amada* Roxb. (TBGT 93668), *C. aromatica* Salisb. (TBGT 93669), *C. aurantiaca* Zijp (TBGT 83461), *C. caesia* Roxb. (TBGT 93659), *C. haritha* Mangaly & M Sabu (TBGT 93670), *C. longa* L. (TBGT 93673), *C. montana* Roxb. (TBGT 91041), *C. zanthorrhiza* Roxb. (TBGT 83452) and *C. zedoaria* (Christm.) Roscoe. (TBGT 93672) were collected from Medicinal Garden of Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Thiruvananthapuram during October-November at its maturity. The voucher specimens of all the species studied were deposited in the Herbarium of JNTBGRI (TBGT). Morphology of the fresh mature rhizomes was examined by naked eye and described based on remarkable visual characters. The materials for anatomical study were fixed in FAA and freehand sections were taken from the central portion of the primary finger of each species, except in *C. aurantiaca,* where mother rhizome itself was studied since lateral branches/finger rhizomes are absent. Good sections were stained with safranine (0.3%), mounted in glycerine and observed under microscope. For histochemical studies, the sections were stained with Lugol's iodine solution for 2–3 minutes and mounted on a glass slide to localize starch granules.

For powder microscopy, fresh mature rhizomes were washed thoroughly with water, chopped into pieces, shade dried and powdered coarsely. Powder was taken randomly and placed over slides, mounted in glycerine after staining to study different cell components and recorded the details as per standard procedure (Wallis 1997; Jackson & Snowdon 1990). All the microscopic observations were carried out using Leica DM2500 stereomicroscope attached with Leica DFC450 camera. Leica Application Suite Software was used for the observation and measuring or analyzing the microscopic images.

For morphological characters 10 consecutive readings and for microscopic characters 5 readings were taken in quantitative characters and their standard deviation was calculated.

Results and discussion

Morphological features of rhizomes

In general, all the ten *Curcuma* species showed morphological similarity (Fig. 1: A-J). On detailed study, distinct variations were observed in the morphological characters *viz*., shape,

size and internodal length of mother rhizome and lateral branches along with the colour of the cut surface (Fig. 1: A1-J1) as well as aroma, taste and fresh weight (Table 1). In most of the species, the rhizome was branched except in *C. aurantiaca*, with cylindrical condensed mother rhizome (Fig. 1: D). The shape of the mother rhizomes vary among the selected species as oblong (*C. aromatica*, *C. haritha* and *C. motana*), ovate-oblong (*C. aeruginosa*), broadly ovoid (*C. zanthorrhiza*), conical (*C. caesia* and *C. zedoaria*), cylindrical (*C. aurantiaca*), cylindrical or ellipsoid (*C. amada*) and cylindrical or oblong (*C. longa*). In all the candidate species, mother rhizome and lateral branches were marked with annular scars demarcating nodes and internodes covered with scale leaves. The lateral branches are small or large finger-shaped. The intermodal length of mother rhizome (0.32–2.5 cm) and lateral branches (0.67–1.58 cm) vary considerably. The characters such as diameter, colour of the cut surface, aroma and taste etc. of the mother rhizome and lateral branches were also noticed. The highest average diameter for mother rhizome was observed in *C. zanthorrhiza* (5.5 cm) and lowest in *C. aurantiaca* (1.3 cm). In the case of lateral branches, highest diameter was showed in *C. zanthorrhiza* (2.05 cm) and lowest in *C. caesia* (0.9 cm). The maximum number of lateral branches were produced by *C. amada* (3–8) and minimum by *C. haritha* (2–4).

Outer colour of the rhizome was almost similar among the species as pale yellowish-brown and dark yellowish brown. The colour of the cut surface showed prominent variations among the species. Rhizomes were bluish in *C. aeruginosa* and *C. caesia*, bright orange yellow to reddish yellow in *C. longa* and *C. zanthorrhiza*, whereas in other species it was creamy white or yellowish tinge (Fig. 1: A1- J1). The present observation supports the study conducted by Remashree & Balachandran (2006) in four *Curcuma* species *viz*., *C. amada*, *C. aromatica*, *C. long*a and *C. zedoaria*. Rhizome aroma possess a variable amount of camphoraceous smell in most of the candidate species apart from *C. amada*, where it was that of raw mango. The warm bitter, bitter or slightly pungent taste of rhizome was identified in most the species except for *C. amada*, where raw mango taste was identified. Fresh weight of the whole rhizome was observed highest in *C. zanthorrhiza* (445±45 g) and lowest in *C. aurantiaca* (39±6 g). In rhizomes, some of the roots end in fleshy, succulent tubers (root tubers), which were turgid when fresh but turned completely shrivelled on drying. Root tubers do not possess the taste and aroma characters of the rhizome and their colour was whitish in most of the species except in *C. longa and C. zanthorrhiza*, where it was light yellow. Eventhough several qualitative morphological characters of the rhizome among the candidate *Curcuma* species were similar, certain variations were obtained quantitatively (Table 1).

Anatomical features of rhizomes

Transverse section (T.S.) of fresh mature rhizomes was microscopically observed (Fig. 2: A-J) and compared the anatomical features of the ten selected *Curcuma* species (Table 2). T. S. of the rhizomes of all the species was more or less circular in outline, bounded by epidermis composed of small, thin-walled, rectangular tangentially elongated cells as observed by Srivastava *et al.* (2006), in *C. aeruginosa*. The epidermal layer was uniseriate in most of the species, but it was multiseriate in *C. aurantiaca* (Fig. 2: L). Unicellular trichomes were present in eight *Curcuma* species studied, except in *C. aurantiaca* and *C. caesia*, where trichomes were absent (Table 2). The epidermis was followed by periderm layer, which varied in number (4–9) in different species. According to Tomlinson (1969), development of periderm is associated with habitat conditions since they are not found in all individuals of the same species and hence they have no taxonomic significance. However, in the present study, all the ten species were grown under same habitat conditions and thus the variations observed in number of periderm layers is important.

The rhizomes were internally differentiated into an outer cortex and a central cylinder of stele with collaterally closed vascular bundles distributed irregularly. The inner limit of the cortex separated from stele was demarked with endodermoid layer, which was seen continuous

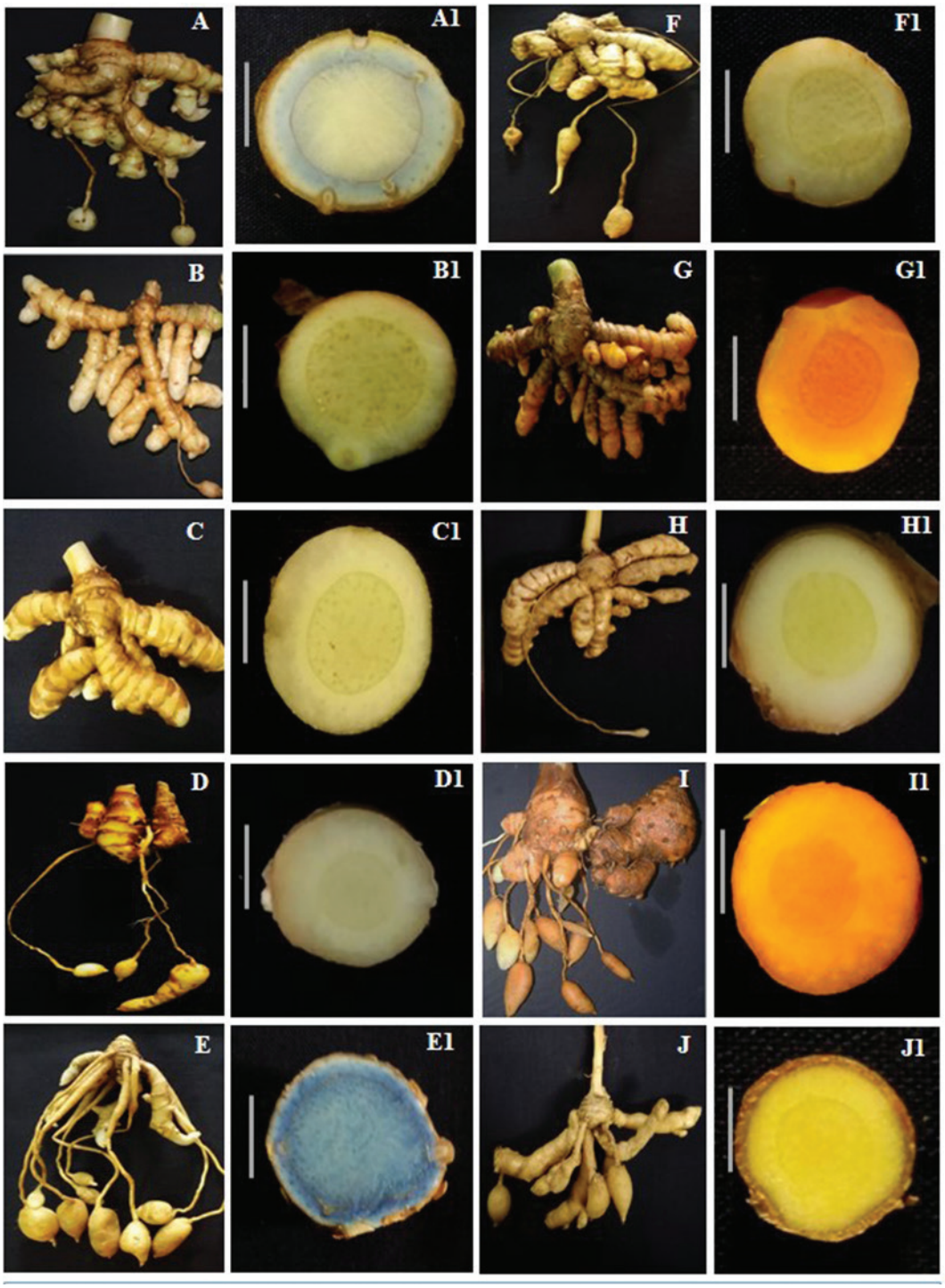

Fig. 1. Whole rhizome and C. S. of finger rhizome of *Curcuma* species (Scale 0.5 cm)

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. montana*
I, I1 – *C. zanthorrhiza*
J, J1 – *C. zedoaria*

Table 1. Comparative rhizome morphology and organoleptic characters of *Curcuma* species

Character	*Curcuma* Species	*C. aeruginosa*	*C. amada*	*C. aromatica*	*C. aurantiaca*	*C. caesia*	*C. haritha*	*C. longa*	*C. montana*	*C. zanthorrhiza*	*C. zedoaria*
Mother Rhizome	Shape	Ovate-Oblong	Cylindrical or Ellipsoid	Oblong	Cylindrical	Conical	Oblong	Cylindrical or Oblong	Oblong	Broadly ovoid	Conical
	Internodal length (cm)	0.76±0.19	1.1±0.16	1±0.20	2.5±0.37	0.6±0.12	1.3±0.50	0.36±0.50	0.68±0.23	1.0±0.38	0.32±0.53
	Diameter (cm)	3.8±0.90	4.4±1.30	3.9±1.00	1.3±0.56	1.7±0.32	3.5±1.00	3.5±1.00	3.6±0.80	5.5±1.14	3.7±0.33
Lateral branches/ finger rhizomes	Shape	Finger like	Finger like	Finger like	Nil	Small finger like	Finger like	Finger like	Finger like	Large finger like	Finger like
	Number of fingers	2-6	3-8	2-5	Nil	2-6	2-4	3-5	2-6	1-5	2-5
	Internodal length (cm)	1.3±0.70	1.2±0.57	0.9±0.27	Nil	1±0.41	1.5±0.12	0.67±0.30	1.58±0.23	1.06±0.43	1.4±0.36
	Diameter (cm)	1.75±0.90	1.55±0.55	1.55±0.95	Nil	0.9±0.50	1.73±0.52	1.3±0.50	1.56±0.44	2.05±0.20	1.55±0.80
Colour	Outer	Pale yellowish brown	Pale yellowish brown	Pale yellowish brown	Pale yellowish brown, whitish at tip	Dark yellowish brown	Pale yellowish brown	Pale yellowish brown or Pale orange-yellow	Pale yellowish brown	Pale yellowish brown or Pale orange-yellow	Pale yellowish brown
	Inner	Bluish-green, inwardly pale pearly	Light yellow/ Creamy white	Greyish yellow/ Creamy white	Creamy	Dark blue or Blackish	Greyish yellow/ Creamy white	Orange yellow to reddish yellow	Light yellow/ Creamy white	Yellow to deep orange yellow	Light yellow
Aroma/ Odour		Strong Camphoraceous	Raw Mango	Strong Camphoraceous	Sweet Campharaceous	Strong Camphoraceous	Mild Camphoraceous	Mild Camphoraceous	Mild Camphoraceous	Mild Camphoraceous	Mild Camphoraceous
Taste		Bitter	Raw mangoish	Bitter	Slightly pungent	Bitter	Slightly pungent	Warm bitter	Bitter	Warm bitter	Slightly pungent
Root tubers		Whitish	Whitish	Whitish	Whitish	Whitish	Whitish	Light yellow	Whitish	Light yellow	Whitish
Fresh weight of rhizome (~) g/plant		190±34	319±50	210± 33	39±6	95±15	186±33	121±10	285±40	445±45	149±17

'±' indicates standard deviation

in seven species, whereas discontinuous in *C. haritha, C. montana* and *C. zanthorrhiza* (Fig. 2: P). The endodermis was composed of a row of thin-walled tangentially elongated cells with their radial walls slightly thickened. Just inner to the endodermis, smaller narrow thin-walled cells formed the pericycle. The characters such as the number of primary and secondary vascular bundles and presence or absence of cambium and bundle sheath were notable. In all the candidate species, vascular bundles were found to be more closely distributed near the endodermis and pericycle at both sides. Similar type of vascular bundle distribution was observed by Aiyer & Kolammal (1964) and Kolammal (1979) in *C. zedoaria* and *C. longa* respectively.

The number of primary vascular bundles were found to be more in *C. montana* (131–199) followed by *C. haritha* (60–176), *C. aeruginosa* (94–139) and *C. aurantiaca* (88–136) and the minimum number was observed in *C. zedoaria* (43–49). The highest number of secondary vascular bundles were showed by *C. longa* (56–74) and lowest by *C. zedoaria* (32–37). The number of primary and secondary vascular bundles varied in all the candidate species and the number of primary vascular bundle was more than that of secondary vascular bundles and the vascular bundles within the endodermis were smaller. Vascular bundles were collateral in both the cortical and stelar region. Xylem was composed of vessels, fibres, and xylem parenchyma. There were more number of xylem vessels in the vascular bundles outwards the endodermis whereas only 2–5 inwards. The phloem was a small patch associated with the xylem and composed of very small, thin-walled polygonal cells. Each vascular bundle has got a sheath of small-sized cells, which completely encircled it and few slightly thickened cells were also found associated that form the bundle sheath in some vascular bundles, as reported in *C. zedoaria* by Aiyer & Kolammal (1964). Bundle sheath was absent in most of the candidate *Curcuma* species, but present in *C. aromatica* and *C. zedoaria*. Mechanical elements of any sort were not observed either associated with the vascular bundle or elsewhere. Length of both the xylem and phloem regions were observed maximum in *C. aromatica* (362±17 μm and 239±11.36 μm respectively) whereas minimum xylem region was found in *C. haritha* (121±9.20 μm) and minimum phloem region in *C. zanthorrhiza* (97±5.54 μm). The cortical cells were polygonal to round. Meristematic cells were noticed as a 2–3 layered cambium in the endodermal region in four species *viz*., *C. aurantiaca*, *C. longa*, *C. montana* and C. *zedoaria* and the other six species were without cambial layer. In *C. longa,* presence of true cambium as an adaptation for rhizome growth and development was reported by Sherlija *et al*. (1998). Tomlinson (1969) pointed out the phylogenetic possibility of lack of cambium in monocotyledons which may provide evidence for cambial activity lost during the evolutionary period. Generally, parenchyma cells of the ground tissue contain abundant starch grains, a variable quantity of oil cells and curcumin cells.

Rhizomes of *Curcuma* were found rich in secretory and storage elements based on histochemical studies. It revealed the distribution, shape and size of curcumin cells, starch grains and oil cells (Fig. 2: Q, R & S). Almost all the parenchyma cells of ground tissue, except those towards periphery, were densely packed with starch grains. The starch grains were simple, comparatively big and flattened, with different shapes such as oval, fusiform, phaseoliform to versiform, elliptical, cylindrical and spherical. The size and shape of the starch grains varied remarkably and possess a slight projection at one end. The striations on the starch grains were numerous and transversely faint or indistinct with the hilum at the narrow end. Larger starch grains were found in *C. aromatica* (33.07 × 14.51 μm) and smaller in *C. aurantiaca* (17.78 × 11.73 μm). Navas *et al*. (2013) reported that the variation in the starch grains is a supporting character for taxonomic delimitation of the species. Several parenchymatous cells of yellow or orange contents that fill the cells were noticed in all the candidate species as curcumin cells and oil cells. The shape of oil cells and curcumin cells was round or oval in general and rarely irregular. The size of oil cells and curcumin cells ranged from 37–173 μm and from 85–234 μm, respectively. Largest oil cells were

observed in *C. zanthorrhiza* (173±11.69 µm) and the smallest in *C. aromatica* (37±13.64 µm). Oils and fats are important reserve food materials which possess great taxonomic significance (Metcalfe & Chalk 1983; Baas & Gregory 1985; Fahn 1990; Remashree 2003). Maximum number of curcumin cells were found in *C. longa* and C. *zanthorrhiza* justifying the bright yellow colour of the rhizomes.

Powder microscopy showed variation in colour and type of vascular elements present in the species. The study showed the presence of scalariform vessels and long, narrow fibres in all the candidate species (Fig. 2: T&U). Navas *et al*. (2013) observed that stone cells are a diagnostic feature in the identification of powder drugs. In the present study, thin-walled stone cells were observed in *C. aurantiaca* (Fig 2: V), while stone cells were absent in other species. From the notable morpho-anatomical features in rhizome of the ten selected species of *Curcuma,* a key was prepared indicating taxonomic delimitation, as follows.

Identification key to the species based on rhizome characters

1	Rhizome branched, epidermis uniseriate, stone cells absent	2
1	Rhizome not branched, epidermis multiseriate, stone cells present	*C. aurantiaca*
2	Trichome unicellular, cut surface of rhizome not dark blue or blackish	3
2	Trichome absent, cut surface of rhizome dark blue or blackish	*C. caesia*
3	Endodermoid layer continuous	4
3	Endodermoid layer discontinuous	8
4	Mother rhizome conical, average number of primary vascular bundle < 50	*C. zedoaria*
4	Mother rhizome not conical, average number of primary vascular bundle > 50	5
5	Rhizome yellow, Internodal length of mother rhizome < 0.5 cm	*C. longa*
5	Rhizome not yellow, Internodal length of mother rhizome > 0.5 cm	6
6	Rhizome bitter, camphoraceous aroma	7
6	Rhizome not bitter, raw mango aroma	*C. amada*
7	Bundle sheath absent, rhizome cut surface bluish-green	*C. aeruginosa*
7	Bundle sheath present, rhizome cut surface cream coloured	*C. aromatica*
8	Cambium present, internodal length < 1 cm	*C. montana*
8	Cambium absent, internodal length >1 cm	9
9	Root tubers yellowish, rhizome yellowish, taste warm bitter	*C. zanthorrhiza*
9	Root tubers whitish, rhizome creamy, taste slightly pungent	*C. haritha*

In general, all the candidate species have similarity in morpho-anatomical pattern. Variations were noticed in the morphological characters such as shape and size of mother rhizome and lateral branches, colour of the cut surface, aroma and taste of rhizomes and in the anatomical characters such as number of primary and secondary vascular bundles, nature of endodermoid layer, and also the number and shape of oil cells, starch grains and curcumin cells. These stable and striking differences in macroscopy and microscopy are useful for the taxonomic identity of the species, even with its rhizome alone.

Table 2. Comparative rhizome anatomical, histochemical and powder characters of *Curcuma* species

Curcuma Species Character		*C. aeruginosa*	*C. amada*	*C. aromatica*	*C. aurantiaca*	*C. caesia*	*C. haritha*	*C. longa*	*C. montana*	*C. zanthorriza*	*C. zedoaria*
Trichome		Unicellular	Unicellular	Unicellular	Absent	Absent	Unicellular	Unicellular	Unicellular	Unicellular	Unicellular
Epidermis		Uniserate	Uniserate	Uniserate	Multi-seriate	Uniserate	Uniserate	Uniserate	Uniserate	Uniserate	Uniserate
Periderm layers		5-7	4-7	5-8	4-7	4-6	5-6	5-6	8-9	4-6	6-8
Cortical cell		Polygonal	Polygonal and rounded	Polygonal and rounded	Polygonal	Polygonal	Polygonal and rounded	Polygonal	Polygonal and rounded	Polygonal	Polygonal
Endodermoid layer		Continuous	Continuous	Continuous	Continuous	Continuous	Discontinuous	Continuous	Discontinuous	Discontinuous	Continuous
Bundle sheath		Absent	Absent	Present	Absent	Absent	Absent	Absent	Absent	Absent	Present
Cambial layers		Absent	Absent	Absent	2-layered	Absent	Absent	3-layered	3-layered	Absent	2-layered
Vascular bundles	No of 1° V. B	94-139	45-57	53-56	88-136	94-106	60-176	88-132	131-199	46-102	43-49
	No of 2° V. B	34-46	41-53	42-48	37-53	44-84	54-66	56-74	44-63	43-53	32-37
	Xylem length (µm)	218±13.98	151±13.80	362±17.00	157±10.80	144±12.95	121±9.20	141±8.50	190±9.16	201±15.09	238±16.64
	Phloem length (µm)	184±9.02	152±12.5	239±11.36	189±14.10	146±14.40	116±13.30	132±16.40	143±12.35	97±5.54	176±16.69
Starch grains	Shape	Ovoid or fusiform	Phaseoliform to versiform, oval to elliptical or cylindrical	Ellipsoid, phaseoliform	Circular or ovid	Phaseoliform to versiform	Rounded, cylindrical or ovoid	Phaseoliform to versiform, spherical or cylindrical	Phaseoliform to versiform, ellipsoid,	Oval to elliptical, rounded	Ovoid, versiform
	Size (µm)	29.40×16.37	28.74×14.78	33.07×14.51	17.78×11.73	19.98×15.19	24.94×17.79	19.51×11.84	32.16×13.98	23.95×15.23	22.75×14.66
Oil cells	Size (µm)	106±16.70	116±10.58	37±13.64	42±10.39	79±14.54	77±7.97	118±17.89	56±8.95	173±11.69	134±14.70
Curcumin cells	Size (µm)	202±17.38	95±15.06	126±14.46	142±9.75	220±15.32	142±12.25	153±15.64	111±11.74	85±2.14	234±8.14
Powder characters	Stone cells	Absent	Absent	Absent	Present	Absent	Absent	Absent	Absent	Absent	Absent

'±' indicates standard deviation

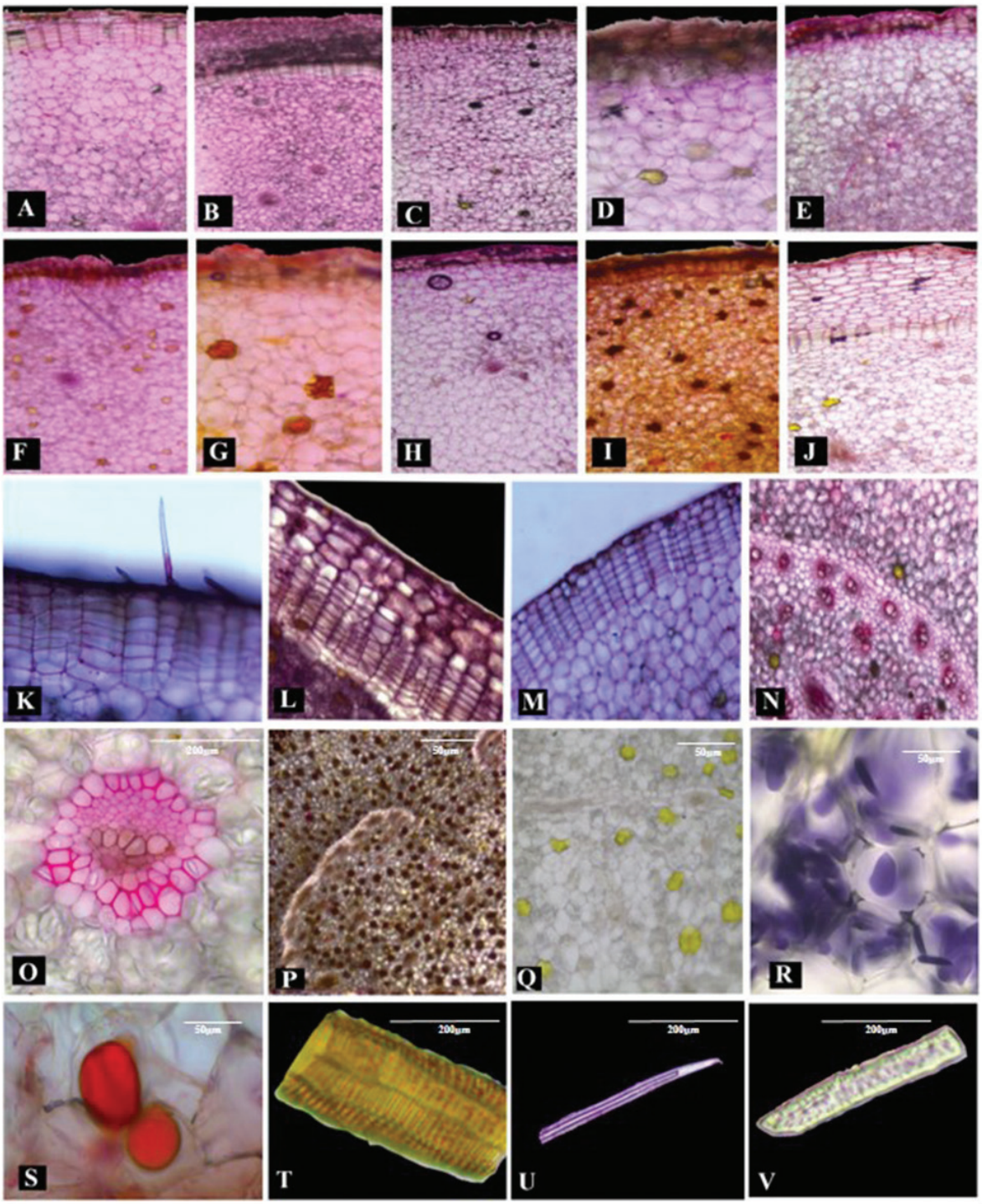

Fig. 2. Rhizome T. S. (20 ×) and diagnostic anatomical characters of *Curcuma* species: A- *C. aeruginosa*; B- *C. amada*; C- *C. aromatica*; D- *C. aurantiaca*; E- *C. caesia*; F- *C. haritha*; G- *C. longa*; H- *C. montana*; I- *C. zanthorrhiza*; J- *C. zedoaria*; K- trichomes (*C. aeruginosa*); L- multi-layered epidermis (*C. auranatiaca*); M- periderm layer (*C. aeruginosa*); N- endodermoid region (*C. amada*); O- vascular bundle (*C. caesia*); P- discontinuous endodermoid layer (*C. zanthorrhiza*); Q- curcumin cells (*C. zedoaria*); R- starch grains (*C. aeruginosa*); S- oil cells (*C. aurantiaca*); T- xylem vessel (scalariform) (*C. longa*); U- fiber (*C. zedoaria*); V- stone cell (*C. aurantiaca*)

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