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Morphological & microscopic identification of *Curcum albiflora* Thw

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ABSTRACT

Curcuma albiflora Thw.(Harankaha) is an endemic medicinal plant used in Sri Lankan Traditional Medicine. However, it has not been studied systematically in terms of its identity. Therefore, several other plants have been used as adulterants or substitutes. In order to establish its correct botanical identity, morphological and microscopic studies were carried out on various its plant parts of *C. albiflora*. Plants were collected from Ratnapura/Kegallearea in flowering season and procedures were performed according to WHO guidelines and other published data. Morphologically, heights of the plant up to 35 ± 5 cm and 5-7 leaves, both surfaces were glabrous. Inflorescence up to 10 x 8 ± 2 cm, but coma bracts were absent. Fertile bract tip rounded and curved (light green). Flower was white with centre yellow tinged on labellum. Rhizome has many primary fingers, and remote tubers were absent, not as *C. zedoaria*. Microscopically double layer palisade, subsidiaries dicyclic stomata, palisade ratio 1:5-7, and two sizes of prismatic calcium oxalate crystals (5 ± 2 µm, and 10 ± 2 µm) were found in leaf. Stomatal index was 13% in ventral side and 5% in dorsal side of the leaf. Three sizes of starch grains (small: 5-10 µm, medium: 15-25 µm, and large: 30 µm) in rhizome, and collateral vascular bundles were found. External morphologically, the bracts were angularly attached to the inflorescence and lower bracts are spreading which is a differentiation character from the other *Curcuma* species. Microscopically, absence of crystals in cortical region of rhizome, cup shaped starch grains and double layered palisade cells under the upper epidermis of the leaf were found to be significant features of the identify of *C. albiflora*.

Keywords: *Curcuma albiflora*, Starch grains, Microscopy.

INTRODUCTION

Curcuma albiflora Thw. (*Harankaha*) is an endemic medicinal plant used in Sri Lankan Traditional Medicine. It is used as an anti-inflammatory drug, but it has not been studied systematically in terms its identity is concerned. Therefore, under the name of Harankaha, several other plants (e.g. *Curcuma zedoaria* and *Zingiber zerumbet*) reported and this leads to adulteration or substitution [1, 2]. To establish its correct identity morphological and microscopic identity has been established by the present study.

MATERIALS AND METHODS

Whole plants of *C. albiflora* were collected in the months (from June to October) of, 2015 from Kitulgala (Kegalle district), Erathna, and Bopathella (Ratnapura district) area, where naturally grown. Voucher specimens 02, 03, 04, and 05 of the plants (herbariums) were authenticated & deposited in the National Herbarium, Peradeniya, Sri Lanka and Herbal Technology Section, ITI for future reference. Preparation, preservation and storage of plants and experiments were done according to WHO guidelines [1, 3]. The organoleptic characters and historical characters of the plants were noted down. The outer appearance of thirty samples each of leaf, flower, and rhizome were observed through Leica MS 5 microscope and thin hand-cut specimens were observed under Labomed (Sigma, Labo America, Inc. U.S.A.) microscope with 100x and 400x magnification [4]. Sections were drawn by using camera Lucida [3, 5].

RESULTS

Morphology of *C. albiflora*

Heights of the plant up to 35 ± 5 cm and 5-7 leaves were observed (See plate1 (1, 2)).

Morphological characters of leaf; Composition of the *C. albiflora* leaf was simple and pinnate. Leaf was

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non-ciliate, apex acuminate, margin entire, and venation pinnate. Base was symmetrical and decurrent. Shape was oblong and13 x 3 ± 2cm in size. Thickness was 300 ± 20 µm. Both surfaces were glabrous. Duration was deciduous, petiolate, and petiole up to 15 cm ± 5 cm. Sheathing leaf was terminal, sheath leaf overlap and gave rise to aerial shoot. Phylotaxis distichous was in foliage leaves. Foliage leaves emerged from buds on the axile of the nodes of central main rhizome (See plate 1(3, 4)).

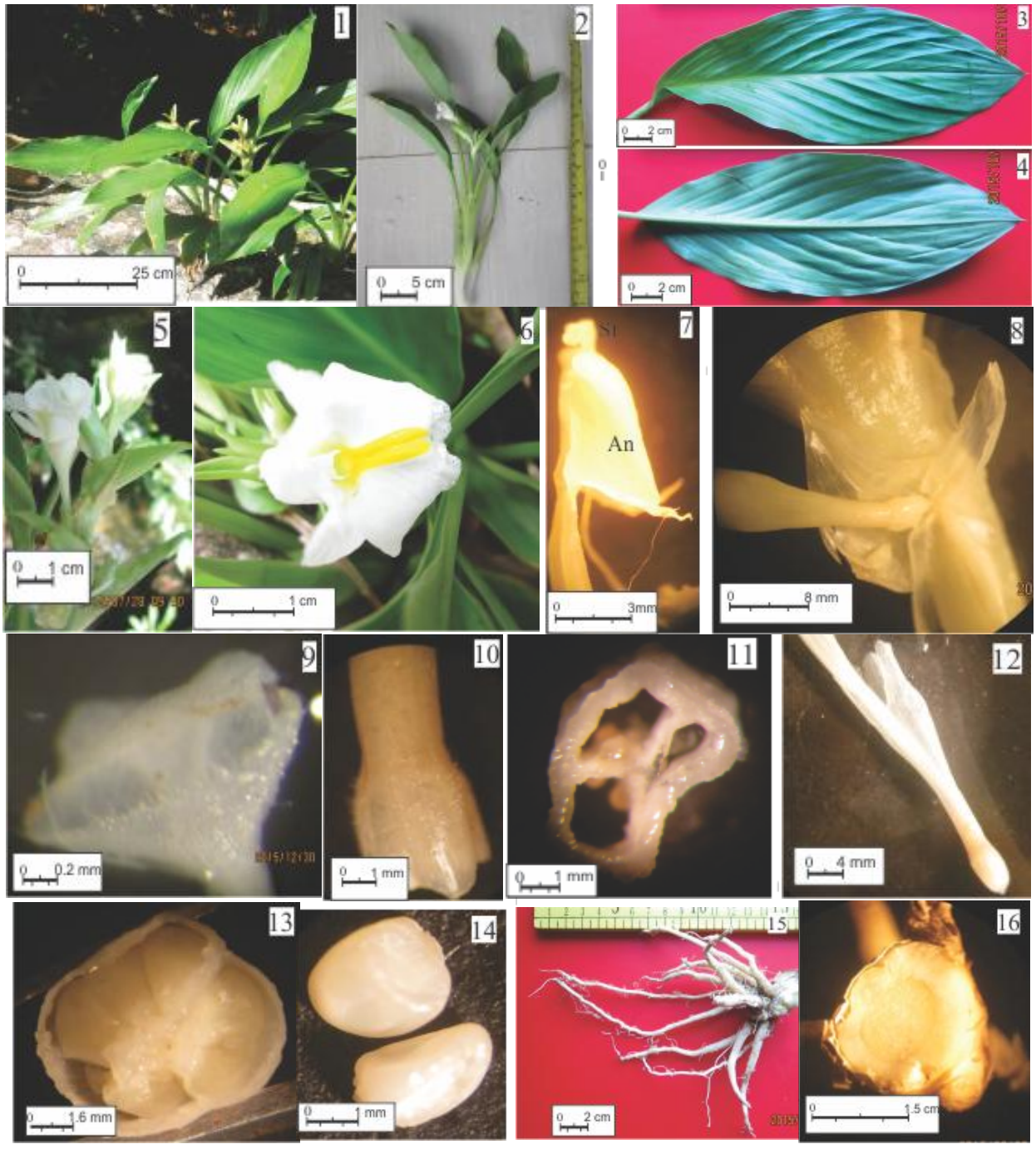


Plate 1: Morphology of *C. albiflora* Thw.; 1,2 – Whole plant. 3- Leaf dorsal side. 4- Leaf ventral side. 5, 6- flower. 7- Stigma and anther. 8- Bract base inside. 9- Stigma. 10- Calyx. 11- Ovary. 12- Flower base. 13- Fruit. 14- Seeds. 15- Rhizome and roots. 16- Rhizome.

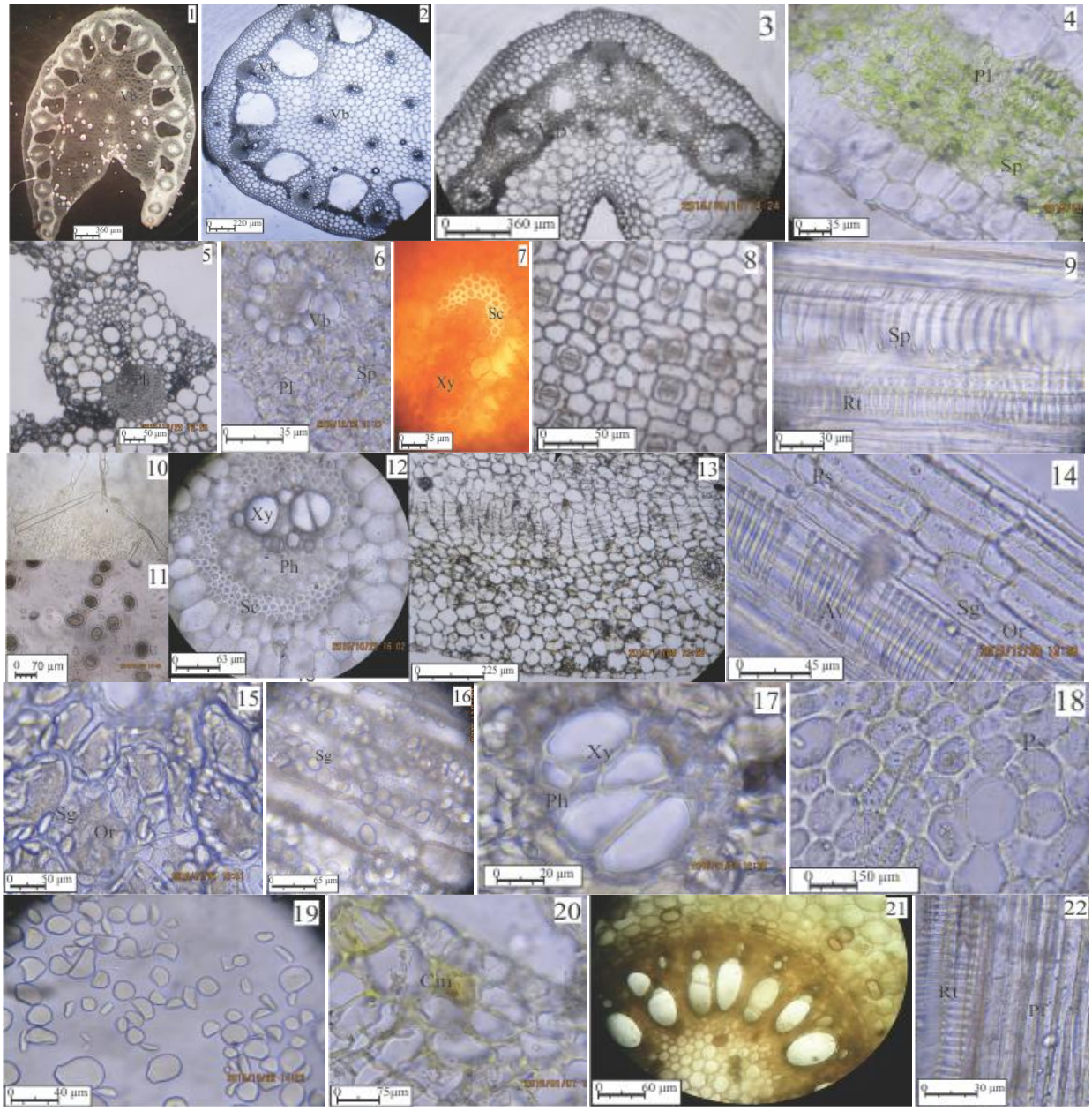
Morphological characters of inflorescence, flower, fruit, and seed; Peduncle was up to 15 ± 3 cm, and inflorescence up to 10 x 8 ± 2 cm. One or two sheaths were observed. Coma bracts were absent. Fertile bract tip was rounded and curved, narrower at apex than base. Shape of bract was lanceolate or oblong-lanceolate, glabrous, and joined at bases only. Bracts were angularly attached. Bracteoles were transparent white and 1.8 x 0.7 ± 0.3 cm in size. They were glabrous, lanceolate, and rounded at the apex. Cincinni few flowered. Flowers were blooming one at a time. Calyxwas 2.3 ± 0.3 cm high, whitish green, and three lobed. But base was pubescent. Flower was white with centre yellow tinged on labellum. Labellum was suborbicular. Corolla was polypetalous, and three lobed. Lobes were oblong and obtuse. Dorsal lobes were wider than lateral lobes. Corolla tube was funnel shape, and 3 ± 0.5 cm. Further, flower was hermaphrodite, zygomorphic, epigenous and receptacle absent. Unbranched rachis, spike flower, papilose surface stigma were observed. Anther wall was lightly papilose and thecae. It was prevented self-pollination by, stigma far away from anther. Stigma mouth was pubescent, and anther glabrous (See plate 1(7-12)). The ovary was 5 x 6 ± 1 mm, more or less circular. Ovules were with many seeds, anatropous, and axile. Fruit were simple, dry, and schizocarp. Seeds were oblong shape(See plate 1(13,14)).

Morphological characters of rhizome; Rhizomes of *C. albiflora* were short, fleshy, and bearing more number of primary fingers (See plate 1(15)). But it was not bear localized tubers as *C. zedoaria* [6]. Short central cylindrical bulb rhizome, primary branches bearing roots and rootlets were observed. If roots present, root emerged from primary fingers or from short rhizome. Rhizome terminal had changed to leafy shoot. Cross section of rhizome showed an inner zone and an outer zone, were separated by intermediate layers by using Leica MS 5 microscope with magnification of 1.6 x (See plate 1(16)). Rhizome was short, fleshy, and sympodial. Inside was off white and surface light-brownish. However, white or off white cylindrical tubers shorter towards end. Matured rhizome is with 1.5 ± 0.5 cm in diameter and 3.5 ± 0.5 cm in length. The aerial stems were invariably short, usually leafless. But leaf scars present. Inter-scar length was 1 ± 0.3 cm. Each rhizome terminates with tuberous roots with 0.6 ± 0.2 cm in diameter and 12 ± 0.3 cm in length. Primary fingers are pale white in colour and either with roots, rootlets or sometimes without roots. All of the plants were disturbed to grow, because plants were grown in rocky places surrounded by natural water bodies. Rhizomes were fibrous, camperous smell and bitter taste.

Morphological characters of roots; Root tips were pointed.

Microscopy of *C. albiflora*

Microscopic characters of leaf; Transverse sections of *C. albiflora* petiole were shown in plate 2(1-2) and midrib in plate 2(3). Surface layer above epidermal cells was uneven and cuticle. Epidermis cells were large, thin walled, uniseriate, uniform, hexagonal or polygonal, and both side of the leaf.



Av- annular vessels; **Cm**- colouring matter; **Ep**: epidermis; **Hp**: hyperdermis; **Or**: oleo resin; **Pe**: pericycle; **Pf**: pitted fibre; **Ph**: phloem; **Pl**: palisade; **Pr**: prismatic crystals; **Ps**: pitted sclerenchyma; Rt: reticulate vessels; Sc: Sclerenchyma; Sg: starch grains; **Sp**: spiral vessels; **Sp**: spongy parenchyma; **Vb**: vascular bundles; **Xy**: Xylem.

Plate 2: Microscopic characters of *C. albiflora*; 1, 2- Transverse section of petiole. 3- Transverse section of midrib. 4- Transverse section of lamina. 5, 6- Collateral vascular bundles. 7- Vascular bundle after phloroglucinol test. 8- Subsidiaries dicyclic stomata. 9- Annular and reticulate vessels. 10- Hair on stigma. 11- Pollens. 12- Vascular bundle in peduncle. 13- Transverse section of rhizome. 14- Annular and pitted vessels. 15, 16- Transverse section and longitudinal section oleoresin and starch loaded parenchyma. 17- Vascular bundle of rhizome. 18- Prismatic crystals in vascular region. 19- Starch grains. 20- Colouring matter. 21- Root. 22- Reticulate and fibers of root.

Modified epidermal cells were absent. Thin walled, large, colourless, irregular polygonal cells of single layer hypodermis were observed below both epidermal layers. Double layer palisade cells were observed in upper surface continuously in leaf lamina, but observed in ventral side where main vascular bundle, towards ventral side. Palisade cells were compact, anticlinal extended, and longer than abaxial mesophyll which was lobed and loose tissue (See plate 2(4)). Palisade ratio was 1: 5-7. Vascular bundles formed a single conspicuous abaxial arc, alternating with air canals, and embedded in collenchyma observed in *C. albiflora* leaf midrib (See plate 2(5)). Two types of vascular bundles were observed, type-I of main and type-III of centre vascular bundles were observed in midrib region [7]. Main vascular bundles were attached to both side of the lamina. Smaller vascular bundles were between both sides of the lamina (i.e. centre). Both type-I towards abaxial side and type-III towards adaxial side were present in midrib and petiole area. Bundle sheath was absent and larger single layer parenchymatous cells interrupted below and above veins. These cells were loaded with prismatic calcium oxalate crystals. Fibrous sclerenchymatous cells were observed above and below large veins and below smaller veins. Tracheal elements or silica were not found. Phloem strands undivided. Air canals were single arc pectinating with main veins and embedded in a distinct abaxial band of collenchyma. Collenchyma of midrib was not continued with lamina. Vascular bundles of main and subsidiary arcs were not found at the same level. Massive fibrous sheath was presented in the above xylem and below phloem; vascular bundles were collateral of the leaf. Thin walled ground parenchyma was observed. Red colour of sclerenchyma and xylem tissues indicated, presence of lignin for phloroglucinol test (See plate 2(7)). Single type of stomata (Subsidiaries dicyclic) was observed longitudinal section of leaf surface. Stomata aperture was elliptical and incomplete rim around. Stomata in adaxial surface were infrequent and frequent in abaxial surface. While stomata were present in both sides of the leaf, more number of them (13%) in lower surface. Further only 3 % stomatal index of dorsal side of the leaf. Pair of lateral subsidiary and terminal cells was present. Subsidiary cells were shallow and guard cells were not sunken (See plate 2(8)). Two sizes of prismatic crystals were found; 5 ± 2µmand 10 ± 2 µm (See plate 2(5) and plate 3(5)). Colouring matter filled quadrilateral parenchyma cells, parenchyma with hexagonal prismatic crystal, prismatic crystal with hexagonal parenchyma cells, and colouring matter filled hexagonal parenchyma cells, large starch grain in hexagonal parenchyma cell, and octagonal parenchyma cells were found in leaf petioles. Colouring matter in heptagonal parenchyma cell was found in leaf midrib. Annular, reticulate, spiral vessels and pitted sclerenchyma were found (See plate 2(9) and plate 3(1-4)).

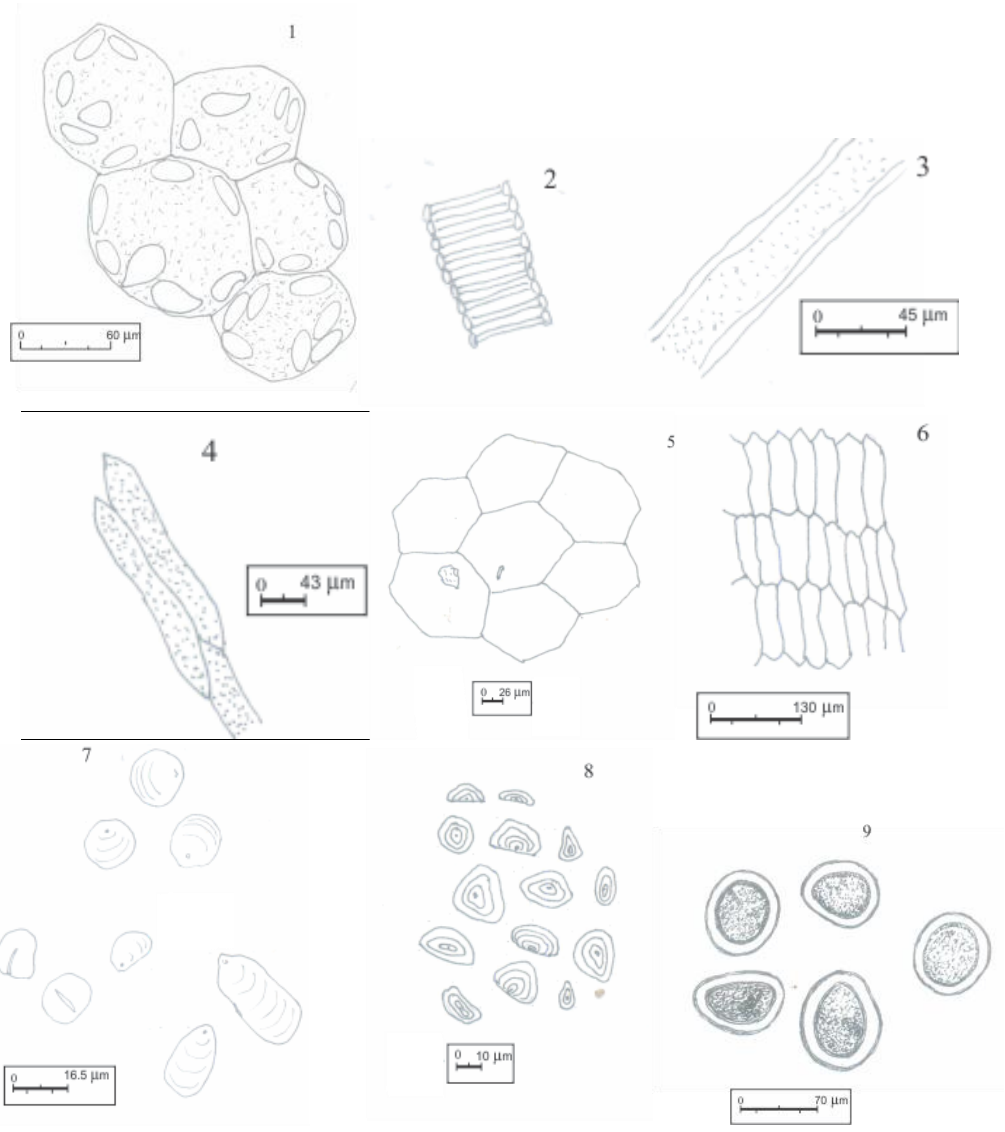


Plate 3: Powder microscopy of *C. albiflora* Thw.; 1- oleoresin and starch grain loaded cells. 2- Annular vessels. 3- Boarded and centre pitted vessels. 4- Boarded and centre pitted cells. 5- Parenchyma studded with crystals. 6- Cork cells in surface view. 7, 8- Starch grains. 9 – Pollens.

Microscopic characters of inflorescence, flower, fruit, and seed; Single layer epidermis, scattered vascular bundles, spiral, reticulate and annular vessels were found in peduncle (See plate 2(12)). Single cell non glandular sunken hairs were observed on stigma mouth (See plate 2(10)). Pollen grains were 68 ± 7 µm in diameter, inaperturate, thin exine and thick intine. Two types of pollen shapes were found; spheroidal or ovoid shape (See plate 2(11) and plate 3(9)). Transvers section of ovule before fertilization was anatropous.

Microscopic characters of the rhizome; Primary fingers transverse sections showed a zone of narrow cells separating inner and outer ground tissue. Surface layer was cutinized. Periderm was with more layers. Cortical cells irregular, with several scattered vascular bundles showed joined, collateral and poly arc arrangement. Parenchyma cells of wide cortex loaded with many starch grains per cell, and they were loaded with oleoresin. Boarded centre pitted fibers, and pitted sclerenchyma was found in cortical region and pitted fibers and parenchyma cells were observed (See plate 2(13-18) and plate 3(3, 4). Collateral poly arc vascular bundles and three types of annular, spiral, and reticulate xylem vessels were found (See plate 2(14)). Whereas more number of prismatic and rosette crystals were found in *C. zedoaria* rhizome, prismatic crystals were found only in *C. albiflora* rhizome in pitted parenchyma cells (See plate 2(18)), but absent in cortical region. While many shapes of crystals including hexagonal, diamond shaped, cuboidal, cubic, and irregular and 5-6 crystals were found in single cell in cortical region of *C. zedoaria* [8], cuboidal shaped crystals were found in rhizome of *C. albiflora.* In terms of starch grains; hilum of *C. albiflora* was visible at the corner, and centre. Striations and hilum of few starch grains were found. Hilum was adjacent to straight edge of segment shape starch grains. Ground tissues or spongy parenchyma studded with lots of simple starch grains with different shapes; Globular, circular, elongated, oval and semicircular shaped starch grains. However, cup shaped starch grains were significant to *C. albiflora* comparing *C. zedoaria* [8]. Three sizes of starch grains were found (small: 5-10 µm, medium: 15-25 µm, and large: 30 µm). Circular shaped small starch grains hilum was at the centre and striations were not visible. Striations of large globular starch grains were visible and at the corner (See plate 2(19) and plate 3(7, 8)). Colouring matter loaded cells were found(See plate 2(20)).

Microscopic characters of the root; Ground tissue consists of thin-walled, circular, oval or polygonal, parenchymatous cells, filled with few simple starch grains. Stellar region demarked from cortex by single layer pericycle and endodermis.Vascular bundles closed and collateral, distributed throughout stellar region, consisting of xylem and phloem elements; vascular bundles found in the stellar region were arranged in a circle, just below endodermis. Proto-xylem towards endodermis and meta-xylem towards pith were observed in vascular tissue. The ground tissues and parenchyma cells in pith region many cells loaded with oleoresin. Single layered epidermis, large parenchymatous cells in cortex, vascular bundles; collateral and radially arranged, xylem vessels; annular, reticulate and spiral vessels and xylem parenchyma, bordered and centre pitted sclerenchyma cells, pitted fiber, parenchymatous pith were observed in root of *C. albiflora*(See plate 2(21,22)).

DISCUSSION

Harankaha is controversial medicinal plant, and three plants are reported under the same vernacular name [9]. Out of three plants under the name "Harankaha", comparative study of three plants (e. g. *Curcuma albiflora* Thw., *Curcuma zedoaria* Roscoe., and *Zingiber zerumbet* Smith.) were carried out on the rhizomes in the year of 2014 [8]. The current study of *C. albiflora* was carried out on its various plant parts in terms of its morphology and microscopy. The height of the plant is about 35 cm which is smaller than the other two species. Shape of bract was lanceolate or oblong-lanceolate, glabrous, and joined at bases only which are a differentiation character of *C. albiflora*. Bracts were angularly attached and lower bracts are spreading which is another differentiation character from the other *Curcuma* species. prismatic calcium oxalate crystals of *C. zedoaria* were found in rhizome cortical region, crystals of *C. albiflora* were absent in cortical region. Prismatic crystals of *C. albiflora* were found in leaf. But prismatic and rosette crystals are found in *C. zedoaria* rhizome [8]. Starch grains of *C. zedoaria* were different from starch grains of *C. albiflora*; cup shaped starch grains were significant to *C. albiflora* with compare to *C. zedoaria*.Parenchyma cells of *C. albiflora* loaded with starch grains and oleo resin. Whereas, single layer of palisade was observed in *C. zedoaria*, double layered palisade observed in *C. albiflora*.

CONCLUSION

External morphologically, the bracts were angularly attached to the inflorescence and lower bracts are spreading which is a differentiation character from the other *Curcuma* species. Microscopically, absence of crystals in cortical region of rhizome, cup shaped starch grains and double layered palisade cells under the upper epidermis of the leaf were found to be significant features of the identify of *C. albiflora.*

CONFLICTS OF INTEREST

No conflicts of interest.

SOURCE OF FUNDING

Nil.

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