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Pharmacognostical investigation of *Curcuma albiflora* Thw: A review (Sri Lanka)

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Abstract

Curcuma is an important genus used in traditional medicine. Due to similar morphological characters of *Curcuma* rhizomes, adulteration or substitution occurs. But endemic species of *C. albiflora* has not been studied extensively. Present review was conducted to establish morphological, microscopic, physicochemical, preliminary phytochemical properties. Moreover, TLC fingerprinting, GC-MS, DNA barcoding and pharmacological analysis of *C. albiflora* was performed. Morphological characters of *C. albiflora*; 35-50 cm tall, bright green lamina, glabrous both sides, coma bract absent, lower fertile bracts spreading. Microscopically, shapes, sizes, amounts of starch grains per cell, type of stomata, size, shapes and amount of crystals per cell, type of vascular bundles in petiole and midrib anatomy all together is needed to analyze to detect each species. Physicochemical, preliminary phytochemical, TLC finger printing, GC-MS analysis, and pharmacological actions showed differences and similarities which were correlated with the particular chemical compounds of each species. TLC finger printing must be standardized as done in *C. longa* using curcuminoids. DNA barcoding, *matK* gene of *C. albiflora* shows as a distinct group from *C. zedoaria*. Antioxidant activity of *Curcuma* species reported were arranged in the decreasing order; *C. longa* > *C. zedoaria* > *C. aromatica* > *C. albiflora*. Since *C. albiflora* is a threaten species, plant regeneration of *C. albiflora* is necessary to save this plant for the next generation.

Keywords: *curcuma albiflora*, GC-MS, microscopy

1. Introduction

New drugs with better action and lesser side effects are needed as an alternative for non-steroidal anti-inflammatory drugs (NSAIDs). Since NSAIDs are with lots of disadvantages, many researches are undertaken to discover new herbal medicine along with pharmacognostical evaluation. Among plant genus, *Curcuma* claimed to have clinically valuable medicinal plants in indigenous and traditional medicine in the world. Genus *Curcuma* (family *Zingiberaceae*) comprises about 100 species all over the world, only 5 species are reported in Sri Lanka (*C. albiflora*, *C. aromatica*, *C. longa*, *C. oligantha*, and *C. zedoaria*)^[1]. *Curcuma* is claimed as a potential source of raw material in herbal medicine to combat a variety of ailments such as arthritis, cancer, diabetes, cough, skin disorders, and oxidative stress-related pathogenesis etc. *Curcuma* species are similar looking species, which leads to adulteration. As an example, under same vernacular name there are two *Curcuma* species such as *C. albiflora* and *C. zedoaria*. But *C. albiflora* is endemic, poorly-explored, and threaten species^[2]. The present review evaluates pharmacognostical study on *C. albiflora* in terms of morphology, microscopy, TLC fingerprint, GC-MS profile, and pharmacological properties.

2. Taxonomical classification of *C. albiflora*

Kingdom: Plantae, Phylum: Tracheophyta, Class: Equisetopsia, Subclass: Magnoliidae, Order: Zingiberales, Family: Zingiberaceae, Genus: *Curcuma*, Species: *C. albiflora* Thw

3. Distribution

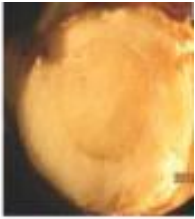
Curcuma grows well in marshy, watery, and sandy soil in shaded areas. *C. albiflora* is endemic, which distributes in Kegalle and Ratnapura districts only.

4. Morphological characters of *C. albiflora*

Plant height was 35 ± 15 cm. Morphological characters are reported in Table 1 and Plate 1^[1, 3].

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Table 1: Comparison of plant size and rhizome of five *Curcuma* species

Part	Description
<div>Rhizome</div> <div></div>	Oblong, small, off-white inside, Rootstock of <i>C. albiflora</i> is bearing a few small tubers at the ends of the root fibers, and off-white colour was observed rhizome inside.
Leaf	Petiole 9 cm, Lamina $15 \pm 3 \times 7 \pm 1$ cm, 5-6 leaves, bright green lamina, oblong, rounded at the base, apex acute and non-ciliate, petiole shorter than lamina, both surfaces glabrous
Bract	Peduncle 12 cm, 7×6 cm, coma absent, fertile bracts (5×1.5 cm) lower part of spike, shorter towards apex, lanceolate to oblong-lanceolate, rounded at the apex, upper much shorter, glabrous, joined at bases, bracteoles (2×0.8 cm), lanceolate, rounded at the apex, few flowered
Flower	Calyx is 1.3-1.8 cm, about half the lengths of the corolla tube, 3 lobed, and unilaterally split. Corolla is white tube up to 3 cm, and narrowly funnel shaped. Lobes are oblong, and obtuse. Dorsal lobe is broader than laterals, and staminodes are broad, and obtuse. Labellum is tinged yellow, suborbicular, emarginated, reflexed, and sides are incurved. Unilaterally split, corolla tube white, lobes oblong, obtuse, dorsal lobe broader than laterals, labellum suborbicular, emarginated.

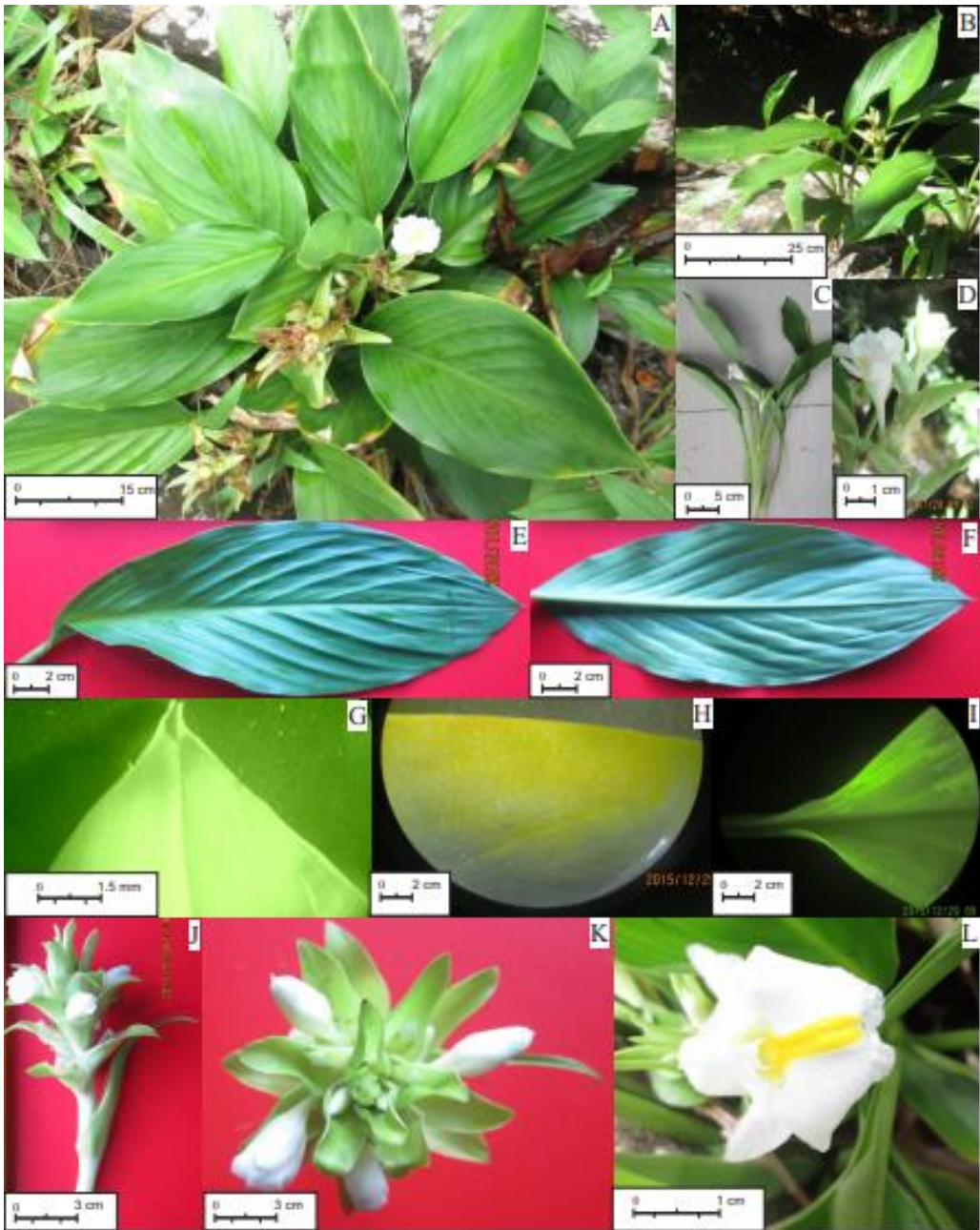


Plate 1: A, B, C, D: Plant leaf morphology of *C. albiflora*. E: Upper surface of leaf. F: Lower surface of leaf. G: Apex of leaf, 1x. H: Margin, 0.63x. I: Base of leaf, 0.63x. J: Inflorescence. K: Inflorescencetop view. L: Flower top view.

5. Microscopic characters of *C. albiflora*

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 are found to be significant features of the identify of *C. albiflora*^[4]. The main vascular bundles are furnished with a massive fibrous or sclerenchymatous sheath above the xylem and below the phloem, extruded proto-xylem, small mass of meta-xylems, and phloem tissue. Abaxial bundles are enveloped within almost a complete fibrous sheath. The abaxial type I main bundles alternate with large air canals. Air canals are traversed internally. *C. albiflora* have 2 types of VBs (main type I, and adaxial type III) and air canals are in the same level of VBs (Plate 2). Air canals are single arc pectinating with main veins and embedded in a distinct abaxial band of collenchyma. Collenchyma of midrib is not continued with lamina. Fibrous sclerenchymatous cells are observed above and below large veins and below smaller veins. Vascular bundles are collateral of the leaf. Thin walled ground parenchyma is observed. The ground tissue is differentiated into two regions, the outer cortex and the inner cortex by a distinct endodermis. Modified epidermal cells are absent. Double layer of palisade are observed in the leaf of *C. albiflora* lamina TS. Stomata in adaxial surface are infrequent and frequent in abaxial surface. Pair of lateral subsidiary and terminal cells is present. Subsidiary cells are shallow and guard cells are not sunken.

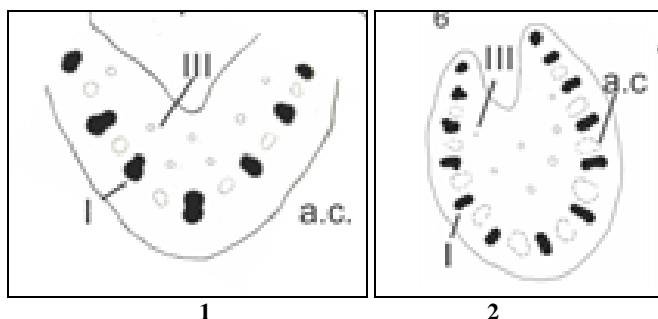


Plate 2^[4]: Diagrammatic of anatomy of midrib and petiole of *C. albiflora*; 1: midrib; 2: petiole, 4×. I: type I main Vb, III: Type III Vb.

5.1 Microscopic characters of the leaf- *C. albiflora*

Epidermis cells are large, thin walled, uniseriate, uniform, hexagonal or polygonal, and both side of the leaf^[5-7]. Modified epidermal cells are absent. Double layer palisade cells are observed in upper surface continuously in leaf lamina. Single layer of hypodermis is observed on both sides of the leaf. Main vascular bundles are attached to both side of the lamina TS. Smaller vascular bundles between both sides of the lamina are observed at the centre. Bundle sheath is absent and larger single layer parenchymatous cells interrupted below and above veins in VBs. These cells are loaded with prismatic calcium oxalate crystals. Phloem strands undivided. Vascular bundles of main and subsidiary arcs are not found at the same level. Stomata aperture is elliptical and incomplete rim around. More number of stomata (13%) is observed in lower surface, but 3% is observed on the dorsal side of the leaf. 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 are found in leaf petioles (Table 2). Colouring matter in heptagonal parenchyma cell is found in leaf midrib.

5.2 Microscopic characters of the rhizome – *C. albiflora*

Primary fingers transverse sections showed a zone of narrow cells separating inner and outer ground tissue. Surface layer is cutinized. Periderm is 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 are loaded with oleoresin. Boarded centre pitted fibers, and pitted sclerenchyma is found in cortical region and pitted fibers and parenchyma cells are observed. Collateral poly arc vascular bundles and three types of annular, spiral, and reticulate xylem vessels are found. Whereas more number of prismatic and rosette crystals are found in *C. zedoaria* rhizome, prismatic crystals are found only in *C. albiflora* rhizome in pitted parenchyma cells, but absent in cortical region. Cuboidal shaped crystals are found in rhizome of *C. albiflora*. In terms of starch grains; hilum of *C. albiflora* is visible at the corner, and centre. Striations and hilum of few starch grains were found. Hilum is 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 are significant to *C. albiflora* comparing *C. zedoaria*^[8]. Three sizes of starch grains are found (small: 5-10 µm, medium: 15-25 µm, and large: 30 µm). Circular shaped small starch grains hilum is at the centre and striations are not visible. Striations of large globular starch grains are visible and at the corner. Colouring matter loaded cells are found (Plate 3).

5.3 Microscopic characters of the root – *C. albiflora*

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 are arranged in a circle, just below endodermis. Proto-xylem towards endodermis and meta-xylem towards pith are 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 are observed in root of *C. albiflora* (Plate 3).

5.4 Powder microscopy– *C. albiflora*

Off-white colour, aromatic, powder of *C. albiflora*; oleoresin and starch grain loaded parenchyma cells, annular, boarded and centre pitted vessels, parenchyma studded with calcium oxalate crystals, simple starch grains and pollens (68 ± 7 µm in diameter) which are inaperturate, thin exine and thick intine were observed in powder microscopical studies. Two types of pollen shapes were found; spheroidal or ovoid shape (Plate 4).

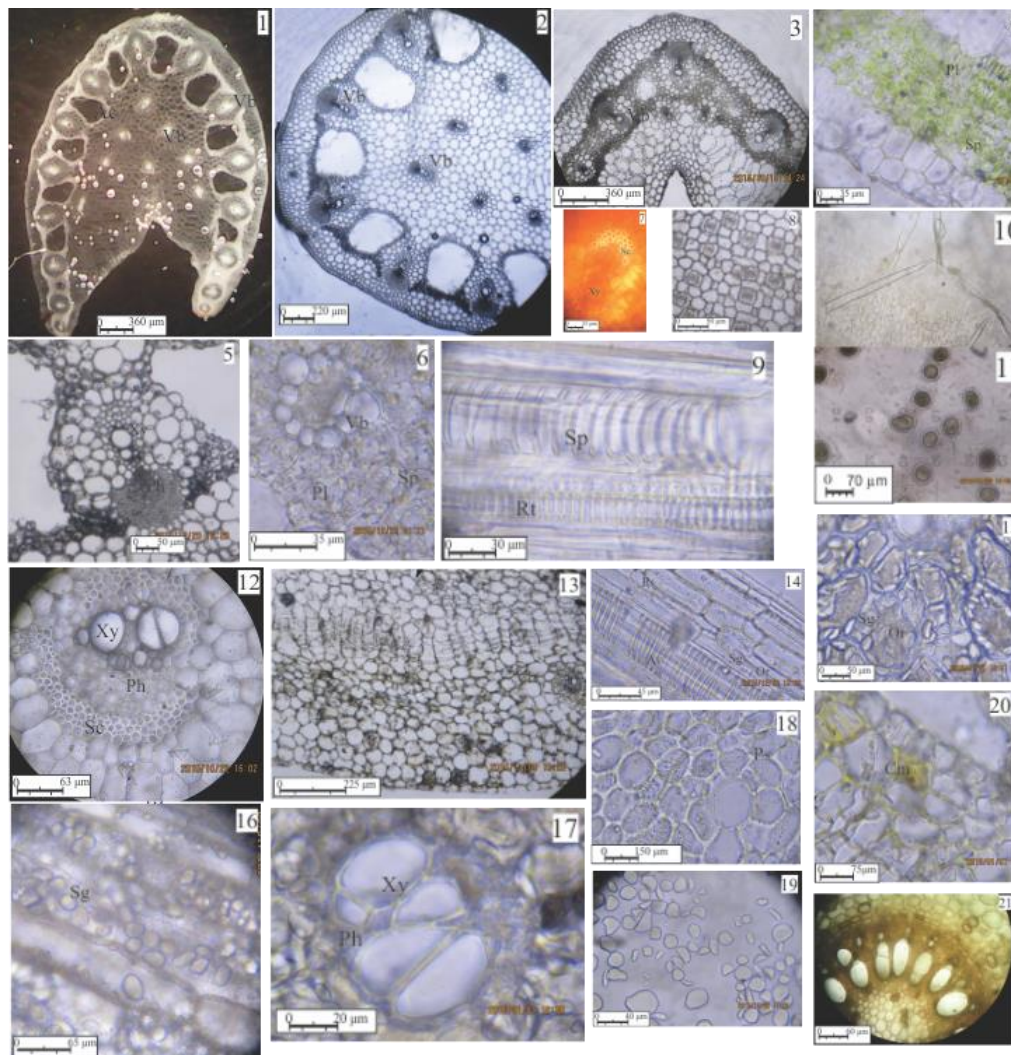


Plate 3: Microscopic characters of *C. albiflora*; 1, 2- Transverse section of petiole. 3- Transverse section of midrib. 4- Transverse section of lamina. 5, 6- Collateral vascular bundles. 7- Vascular bundle after phloroglucinol test. 8- Subsidiaries dicyclic stomata. 9- Annular and reticulate vessels. 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.

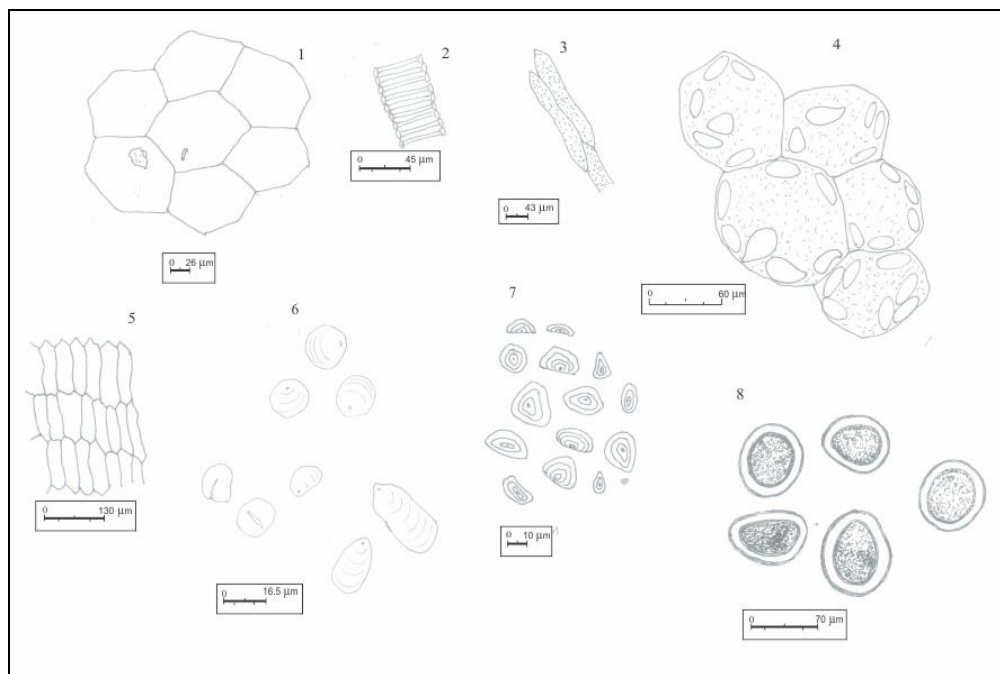


Plate 4: Powder microscopy of *C. albiflora*; 1- oleoresin and starch grain loaded cells. 2- Annular vessels. 3- Boarded and centre pitted vessels. 4- Boarded and centre pitted cells. 5- Parenchyma studded with crystals. 6- Cork cells in surface view. 7, 8- Starch grains. 9 – Pollens.

Table 2: Microscopic characters of *C. albiflora*

Description	Characteristics
Number of layers of palisade	Double layer
Surface layer	epidermal cells is uneven and cuticle
Epidermis of leaf	Single layer on both surfaces
Hypodermis	Thin walled, large, colourless, irregular polygonal cells of single layer on both-side
Periderm	15-16 layers
Outer zone	Small outer zone, primary vascular bundles, starch, oil cells
Primary vascular bundles	15-20 vascular bundles in inner core
Endodermoidal layer	Discontinuous
Cambium	3-4 layers
Inner zone	Large inner zone, secondary vascular bundles are more, just below the endodermoidal layer than inner-core
Secondary vascular bundles	In groups below the endodermoidal layers and few in the inner core
Xylem tracheides	Annular, reticulate, spiral vessels and pitted sclerenchyma
Fibers	Present
Phloem	Sieve tube, 1-2 companion cells and phloem parenchyma
Bundle sheath	Absent
Oil cells	
Curcumin cells	Absent
Starch grains	Eccentric and concentric, Striations and hilum of few starch grains, Globular, circular, elongated, oval and semicircular shape, 5-20/cell small: 5-10 μm , medium: 15-25 μm , and large: 30 μm
Crystals	Two sizes of prismatic crystals; $5 \pm 2 \mu\text{m}$ and $10 \pm 2 \mu\text{m}$
Fibers	Boarded and centred pitted
Palisade ratio	1:5-7
Stomatal index	Up
	Lo
	3%
	13%
Stomata type	Subsidiaries dicyclic/ paracytic

5.5 Physicochemical parameters

Physicochemical parameters were reported in Table 3 [4].

Table 3: Physicochemical parameters

Species	Moisture%	Total ash%	Acid insoluble ash%	pH
<i>C. albiflora</i>	11.60 (± 0.21)	5.07 (± 0.01)	0.55 (± 0.03)	5.82

5.6 Preliminary phytochemical analysis

Extractable percentage (w/w) of various solvents from whole plant of *C. albiflora* by Soxhlet apparatus are found; $2.86 \pm 0.05\%$, $3.21 \pm 0.04\%$, $5.65 \pm 0.02\%$, $2.59 \pm 0.05\%$, $5.93 \pm 0.23\%$, $6.64 \pm 0.03\%$ have shown with petroleum ether, dichloromethane, acetone, methanol, water and DCM/methanol (1:1) extracts respectively. The petroleum ether, DCM, acetone, and methanol all extracts of *C. albiflora* have shown the presence of alkaloids, terpenoids, sterols, protein.

Table 4: Thin layer chromatography analysis of the whole plant of *C. albiflora*

Condition	R _f values
Petroleum ether extract (Solvent system: DCM: Ethyl Acetate: Formic acid (90:10:0.05))	0.08, 0.17, 0.22, 0.27, 0.31, 0.39, 0.45, 0.55, 0.64, 0.69, 0.73, 0.76, 0.81, 0.87
DCM extract (Solvent system: Hexane: Methanol: Formic acid (98:2:0.05) v/v/v)	0.04, 0.10, 0.22, 0.33, 0.40, 0.49, 0.70, 0.76, 0.88
Methanol extract (Solvent system: Dichloromethane: Ethyl Acetate: Formic acid (95:5:0.05) v/v/v).	0.21, 0.34, 0.50, 0.58, 0.65, 0.77, 0.79, 0.81, 0.84, 0.90, 0.95

5.8. Phytochemistry and GC-MS analysis of *C. albiflora*

GC-MS study performed in RTX-WAX column. Since retention index values related RTX-WAX is rare, DB-WAX and PE values were taken for comparison (RI ref) [22] A total of 64 constituents were found by GC-MS, but 20 constituents

Dichloromethane, acetone, methanol, and water extracts show the presence of cardiac glycosides, flavonoids, and tannins. Essential oil of whole plant of *C. albiflora* is yellowish and oil content is about 0.2% v/w.

5.7 TLC fingerprint

In terms of TLC analysis, the petroleum ether extract of *C. albiflora* showed 14 spots when viewed after derivatization by DCM: Ethyl Acetate: Formic acid (90:10:0.05) solvent system. The dichloromethane extract showed 11 spots when viewed after derivatization by Hexane: Methanol: Formic acid (98:2:0.05) solvent system. The methanol extract showed 11 spots when viewed after derivatization by DCM: Ethyl Acetate: Formic acid (95:5:0.05) solvent system, and R_f values are reported in the Table 4 [9].

of essential oil were identified; α -pinene (10.87%), caryophyllene oxide (8.85%), and isoborneol (3.4%) as major compounds, which were more than 3%. Identified chemical constituents in essential oil of *C. albiflora* were listed in Table 5.

Table 5: Percentage composition of essential oil of *C. albiflora*

Compound	RI cal	RI ref ^[22]	Area%	MW
α -pinene	1046	1035	10.87	136
α -myrcene	1075	1158	0.03	136
1,8 cineol	1141	1222	2.15	154
o-cymene	1205	1274	0.21	134
α -linalool	1475	1544	0.69	154
Pinocarpone	1502	1575	0.48	150
(1R)-(+)-nopinon	1514	1573	0.2	138
Caryophyllene	1529	1588	2.49	204
4-terpinenol	1533	1616	0.25	154
Isoborneol	1600	1659	3.4	154
cis-verbenol	1609	1659	0.31	152
α -terpineol	1628	1694	0.64	154
endo-borneol	1633	1699	1.44	154
Cedrene	1648	1582	0.07	204
(-)-carvone	1670	1765	0.08	150
(-)-myrtenol	1726	1790	0.84	152
2-tridecanone	1742	1808	0.15	198
Caryophyllene oxide	1923	1986	8.85	220
Nerolidol	1975	2036	0.17	222
7-epi-cis-sesquibabinene	1997	2082	0.3	222

Note: MW- molecular weight, RI: retention index, RI cal: RI calculated, RI ref: RI references

5.9 DNA barcoding

Using standard modified CTAB method for the extraction and purification, by matK and rbcL genes in chloroplast, which are analyzed and used for DNA barcoding. The matK sequence is selected for identification of *C. albiflora* (Accession No KF 521885) from *C. zedoaria* by the Neighbor-Joining method^[10].

5.10 Antioxidant properties of *C. albiflora*

All *in vitro* antioxidant experiments were performed on dichloromethane/ methanol (1:1) extract of *C. albiflora*. Polar and non-polar dichloromethane/ methanol solvent system was used to extract polar and non-polar compounds present in *C. albiflora*. The standard and the plant extracts were shown their maximum percent inhibitory activity against concentration; standard was shown 70.56 ± 0.30 at 12.50 ppm, and *C. albiflora* DCM/methanol (1:1) extract was shown $65.76 \pm 0.72\%$ at 1250 ppm assay concentration on DPPH bioassay. Moreover, *C. albiflora* DCM/methanol (1:1) extract was shown $99.19 \pm 0.42\%$ at 500 ppm assay concentration on ABTS⁺ bio assay. The correlations of TFC against the antioxidant activity based on the DPPH, ABTS⁺, FRAP, and ORAC assay were significant. Further, the negative correlation between TPC and antioxidant activity were suggested that it could be related to other antioxidant compounds contained in the plants^[11]. Although TPC, and TFC values of *C. albiflora* were reported as 31.25 ± 1.48 (mg GAE/g of extract), and 11.22 ± 0.13 (mg QE/g of extract), *C. longa* were reported as 260 ± 0.25 (mg GAE/ g of extract) and 79.36 ± 0.01 (mg QE/g of extract) respectively^[12]. Whereas the concentration of 1250 $\mu\text{g/mL}$ of *C. albiflora* DCM/methanol extract was shown that $65.76 \pm 0.72\%$, the concentration of 100 $\mu\text{g/mL}$ of water extract of *C. zedoaria*

reported to have 98.95% inhibition^[13]. Although, IC₅₀ values of *C. albiflora* DCM/methanol extract on DPPH and ABTS⁺ assays were 827.78 ± 6.06 ppm, and 188.84 ± 2.99 ppm respectively, water extract of *C. aromatica* was reported to have 427.75 ± 1.43 ppm and 11.674 ± 1.98 ppm respectively^[14]. Low IC₅₀ values were shown higher antioxidant activity. Although, ORAC value of *C. albiflora* was reported as 128.10 ± 3.29 mg TE/g of extract in *C. longa*. It was reported $1592.77 \mu\text{M TE/g}$ of extract^[15]. According to the results obtained from the current study, antioxidant activity of *Curcuma* species reported were arranged in the decreasing order; *C. longa* > *C. zedoaria* > *C. aromatica* > *C. albiflora*.

5.11 Anti-inflammatory activity

The 200 mg/kg was significantly impaired the paw oedema, at 1h (by 61%) by carrageenan induced paw-oedema in Wistar rats. In contrast, the 400 and 600 mg/kg tested were significantly inhibited the paw oedema measured; 1h (by 45-58%), 2h (by 24-46%), 3h (by 21-27%). Indomethacin induced significantly impairment of oedema at all-time points measured (58-89%). Initial phase lasting primarily mediated via production of cox-1, histamine, serotonin, bradykinins etc^[16]. In contrast, *C. zedoaria* shows 54- 56% inhibition in the initial phase and 56-59% in late phase by the concentration 200 mg/kg of petroleum ether on carrageenan induced paw oedema test. However it was shown that the 58% inhibition at 2h by the concentration of 200 mg/kg of chloroform^[17]. Therefore, it was shown that the anti-inflammatory activity of *C. zedoaria* on both the initial and late phases.

The drug group (400 mg/kg and 600 mg/kg) significantly ($P < 0.05$) reduced the paw oedema from the day 5 to 7 by 400 mg/kg when compared with the control by formaldehyde induced paw-oedema in Wistar rats. Since, *C. albiflora* was shown low (as 19.5% on 400 mg/kg) anti-inflammatory activity on cotton pellet granuloma test, it can be concluded that anti-inflammatory activity of *C. albiflora* is not linked with prostaglandin synthesis.

5.12 Survey on *C. albiflora* ointment

After screening past diagnosis and current health status of patients were assessed by Ayurvedic doctors ($n=2$), patients with arthritis and joint disorders only have received the *C. albiflora* (CA) ointment. Patients were advised to use ointment only for two weeks without using any other anti-inflammatory drug. Out of these 175 people, 98 (56%) were eligible for receiving CA ointment. After use of CA ointment, nobody has experienced any side-effect. According to odd ratio, CA ointment treatment group satisfaction was about 31 times comparing the placebo group. The binary logistic models indicated that above 60 years of age, female, previous TM users, duration of current anti-inflammatory condition (≥ 1 y) were more likely to effectively response for CA ointment^[19]. Morphologically, by cluster varibale analysis of morphological characters of five *Curcuma* species grown in Sri Lanka, *C. longa* and *C. zedoaria* showed similar morphological characters (Plate 5)^[20].

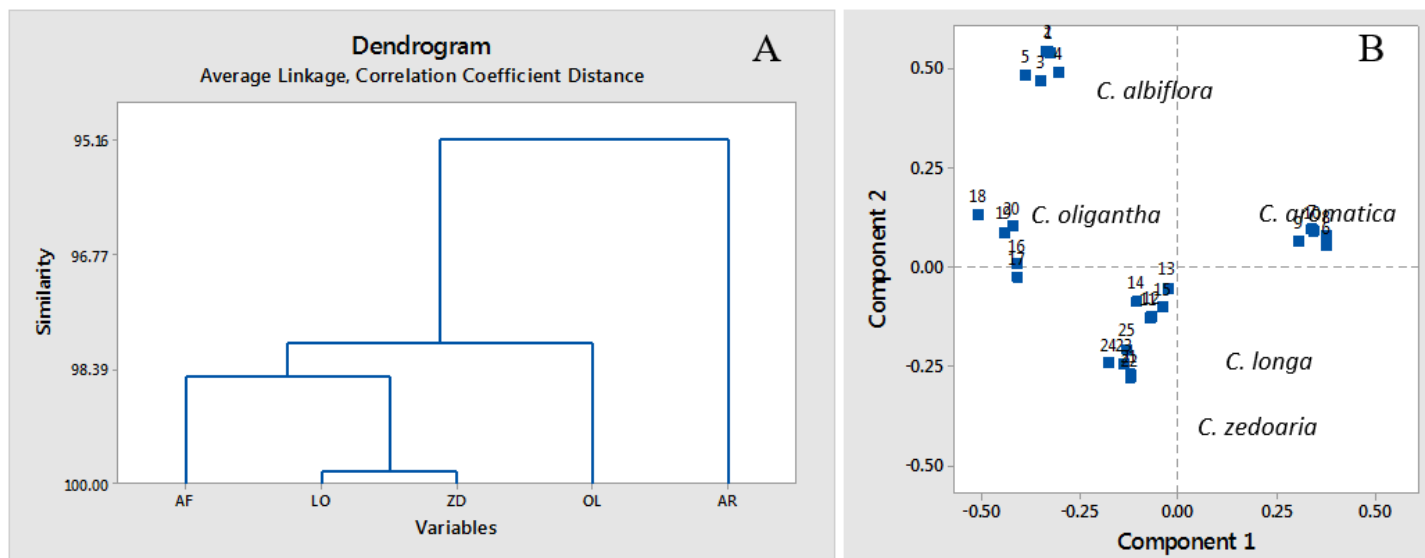


Plate 5: Dendrogram by cluster variable analysis (A) and Column plot (B) obtained from morphological characters; AF: *C. albiflora*, LO: *C. longa*, ZD: *C. zedoaria*, OL: *C. oligantha*, AR: *C. aromatica*

Microscopically shapes, sizes of starch grains, shapes of calcium oxalate crystals, microscopic features of leaf, and rhizome also helpful in identification of *C. albiflora*. However, microscopic identification is one of the oldest, simplest and cheapest methods for plant characterization, using statistics and microscopical features in multivariate test will be useful [18]. Using microscopic data reported on five

Curcuma species grown in Sri Lanka, multivariate test was performed to identify similarities and dissimilarities; As per the cluster variable analysis, group 1 (*C. albiflora* and *C. zedoaria*) and group 2 (*C. aromatica* and *C. longa*) were identified. This was further evidenced by column plot, *C. aromatica*, and *C. longa* showed similar microscopic characters (Plate 6) [20].

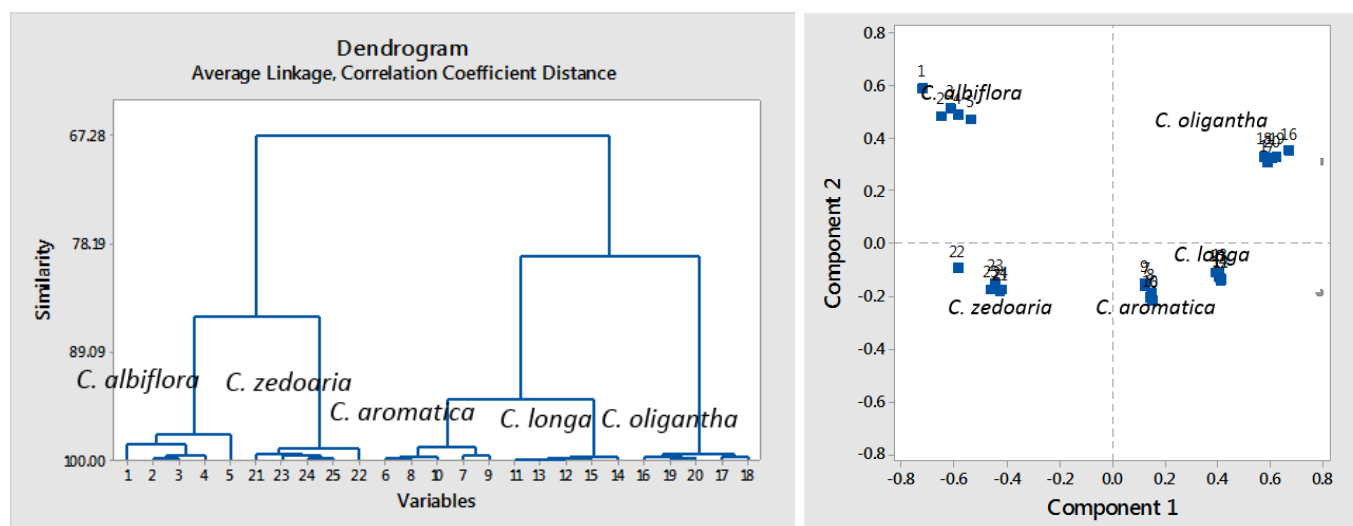


Plate 6: Dendrogram by cluster variable analysis and column plot obtained from microscopic characters of five *Curcuma* species.

Physicochemical properties vary in same species, which depends on way of drying, environmental condition, way of storing, maturity level and season etc. In order to solve this problem, DNA barcoding, which is related to macro-level molecules perform a strong action in identification of *Curcuma* species; *matk* gene of leaf is shown as a distinct group. Phytochemical analysis is provided with GC-MS

analysis. By cluster variable analysis of phytochemicals present in five *Curcuma* species grown in Sri Lanka, mainly two subgroups were clustered; *C. albiflora* and *C. oligantha* into one and *C. aromatica* and *C. zedoaria* into another. These two subgroups are separated from *C. longa* (Figure 1) [21].

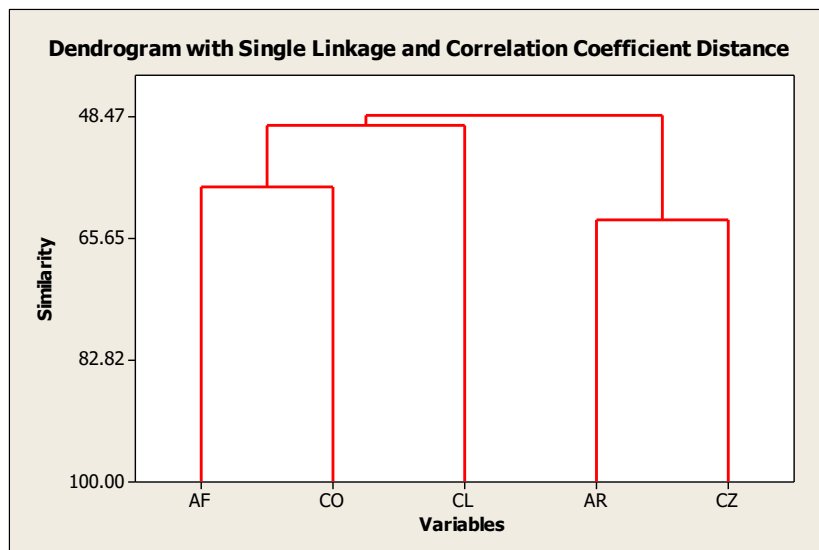


Fig 1: Cluster variable analysis of dendrogram obtained from phytochemicals present in five *Curcuma* species in Sri Lanka; AF: *C. albiflora*, AR: *C. aromatica*, CL: *C. longa*, CO: *C. oligantha*, CZ: *C. zedoaria*

Since *C. albiflora* Thw is a significant species in plant kingdom and a threatened species, plant regeneration of *C. albiflora* is necessary to save this plant for the next generation.

6. Acknowledgments

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