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Macro-microscopic examination of leaves of *Cinnamomum malabatrum* (Burm. f.) Blume sold as *Tamalapatra*

K. N. Sunil Kumar

Senior Research Officer, Department of Pharmacognosy, SDM Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi, Karnataka, India

Abstract

Leaves of *Cinnamomum tamala* Nees & Eberm. (Lauraceae) commonly known as '*Tamalapatra*' is a highly reputed commodity in drug and spice trade. Its adulteration with other leaf species belonging to genus *Cinnamomum* is found to be a common practice in India and other parts of the world. Thorough macroscopic and microscopic investigations are essential to differentiate them. Survey of South Indian crude drug markets revealed that in place of *C. tamala* some other leaves of *Cinnamomum* species are sold. Fresh leaves of various *Cinnamomum* species, including *C. tamala*, growing in south India were collected and studied to establish their correct identity. Leaves sold in markets of S. India under the name of *Tamalapatra* were subjected for detailed macro-microscopic evaluation including maceration and powder microscopy. Leaves of *Cinnamomum malabatrum* showed many distinguishing macro-microscopic characters, which will serve as markers to differentiate them from *C. tamala* the official source of *Tamalapatra*. Though macroscopy will serve the purpose of identification of the entire drug, microscopy had revealed the identity of the commercial substitute even in fragmented and powdered form. Macro-microscopic identity of *C. malabatrum* is established in comparison with the official drug, further chemical and biological studies may be confirmative in deciding the leaves as a substitute or adulterant.

Key words: *Cinnamomum tamala*, *Cinnamomum malabatrum*, maceration, micrometry, powder microscopy, quantitative microscopy

Introduction

Adulteration is a major problem met during the assessment of identity and quality of many herbal drugs.[1] Difficulty arises when they are in the dry state and especially belonging to leaf parts of the plants, where they are usually found to be broken or powdered. For establishing their correct identity they are needed to be examined thoroughly under the microscope. Leaves of the same genus are found to exhibit number of similar common macro-microscopic characters and hence most likely to be used as an adulterant for official drug. *Cinnamomum* is a genus of the family Lauraceae having many number of examples related to the issue of adulteration with closely knit species.[2] Survey of the local crude drug market of south India revealed that leaves of *Cinnamomum malabatrum* are sold commonly in place of the official drug *C. tamala*. As *Tamalapatra* is an important commodity in Ayurvedic medicine,[3] it was thought worth to undertake the present investigation.

Address for correspondence: Dr. K. N. Sunil Kumar, Senior Research Officer, Department of Pharmacognosy, SDM Centre for Research in Ayurveda and Allied Sciences, Laxminarayana Nagar, Kuthpady, Udupi - 574 118, Karnataka, India.
E-mail: sunilkumarnarayanan@gmail.com

Materials and Methods

Plant materials

Dried leaves sold as *Tamalapatra* were collected from markets of various places of India. The authentic samples of leaves of *C. malabatrum* were collected from Tropical Botanical Garden and Research Institute, Palode - Trivandrum in Kerala. Leaves were dried in the shade and few leaves preserved in Formalin-Alcohol-Acetic acid solution were used for histological studies. Powder of the dried leaves sift through mesh 40 was stored in glass vials and used for microscopic evaluation.

Instrumentation and techniques

Detailed macroscopy of leaves of *C. malabatrum* was studied and authenticated with the help of floras.[4,5] Leaves were processed as per the standard procedures for histological examinations, and microscopic characters were drawn with prism type of camera lucida. Isolation of various elements

was carried out by Schultz's maceration process or by boiling with 5% KOH. Measurements of the various elements were taken by using stage and ocular micrometers. The microscopic diagnostic characters of the powder were studied by clearing the powder with chloral hydrate. Cell contents were tested with usual reagents.[6,7] Photomicrographs were taken using leica microscope attached with canon digital camera.

Results

Photographs detailing the macroscopic features of both the leaves are shown in Figure 1. Morphological descriptions outlined in floras[8,9] are used as a guideline to propose diagnostic differentiating macro-microscopic characters of the official source of *Tamalapatra*. Macroscopic and sensory characters of the leaf were compared with *C. tamala* leaf [Table 1]. The results indicated the variation in size of leaf blade and petiole (almost double than that of *C. tamala*), surface (glabrous, unlike *C. tamala*), venation (veins reaching up to the tip unlike that of *C. tamala*) and taste (more mucilaginous in contrast to that of *C. tamala*).

Microscopy

The transverse section passing through the midrib of the leaf

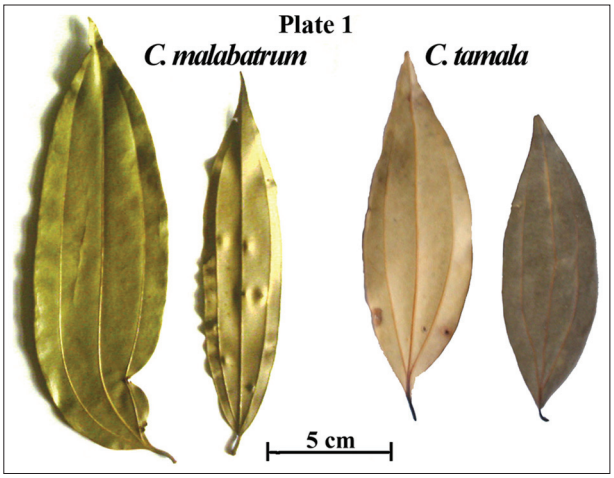

Figure 1: Macroscopic characters of leaves of *Cinnamomum malabatrum* and *Cinnamomum tamala*

Table 1: Comparative macroscopic and organoleptic characters of leaves of *C. malabatrum* and *C. tamala*

Characters	*C. malabatrum*	*C. tamala*
Size whole leaf (cm)	15-30 × 5-10	8-18 × 2.3-4.5
petiole (mm) shape	5-20	7.5-13
	Elliptic to oblong or elliptic lanceolate	Elliptic lanceolate to ovate lanceolate
Base	Acute	Acute
Tip	Acuminate	Acute-acuminate
Texture	Gabrous above, smooth and shining below	Glabrous smooth and shining above, slightly rough and pubescent below
Venation	Lateral nerves reaching to the tip, outer pair arising from the base or slightly above the base; sometimes obliquely placed	Lateral nerves not reaching the tip, insertion basal to suprabasal, secondary veins distinct sub-parallel
Petiole	Highly corrugated, glabrous, upper surface centrally grooved	Slightly corrugated, moderately hairy, upper surface flat
Color	Yellowish-green above, pale below	Brownish-green above, pale below
Taste	Mucilaginous, slightly astringent	Astringent, slightly pungent
Odor	Fragrant	Aromatic

C. malabatrum: Cinnamomum malabatrum, C. tamala: Cinnamomum tamala

Table 2: Comparative microscopic characters of the transverse section of petiole of *C. malabatrum* and *C. tamala*

Characters	*C. malabatrum*	*C. tamala*
Outline	Broadly ovate, with a number of winged projections and a depression on the upper side	Semi-lunar slightly elevated at places
Hair	Rare, simple uni to bi-cellular, thick walled, pearl gland absent	Frequent, glandular and non-glandular, bent, short, thick or thin-walled, uni to tri-cellular, pearl gland present
Epidermis	Thick walled, lignified, without any papilla	Thick walled, lignified, papillose and hairy
Pericycle	Surrounded by stone cells and discontinuous groups of sclerenchyma	Non-lignified group of sclerenchyma on the lower side of vascular bundle
Phloem	Three patches of phloem in the lower side shows few lignified fibers and sieve tubes	Completely surrounds vascular bundle, lower continuous, upper in 2-3 patches. Stone cells present sieve tubes with granular content
Xylem	50-60 radial rows of vessels, each row with 2-14 vessels	45-50 radial rows of vessels, each row with 3-6 vessels

C. malabatrum: Cinnamomum malabatrum, C. tamala: Cinnamomum tamala

and petiole of both the leaves are shown in Figures 2 and 3 and their comparative histological characters are detailed in Tables 2 and 3. The microscopical characters of *C. malabatrum* are as follows:

Transverse section of petiole

Circular to somewhat reniform in outline, with elevated and grooved margin (plano-convex in *C. tamala*), covered with thick cuticle and bear few simple unicellular and bi-cellular short trichomes, an arc of meristele with broad xylem rays (narrow longer and sinuously running in *C. tamala*) located in the center is encircled by discontinuously running band of stone cells and fibers, which are bigger in size, highly lignified and more crowded at the basal region of the meristele (uniformly distributed in *C. tamala* and smaller in size), the ground tissue is collenchymatous and embedded with dark brown coloring matter and at places occasional lignified fibers (absent in *C. tamala*), rows of volatile oil cells are located underneath the epidermis and mucilage, tannin and oleoresin are traversed throughout the ground tissue; prismatic, acicular, raphides and rosette crystals of calcium oxalate are embedded in the collenchymatous tissue [Figures 2a and 3a].

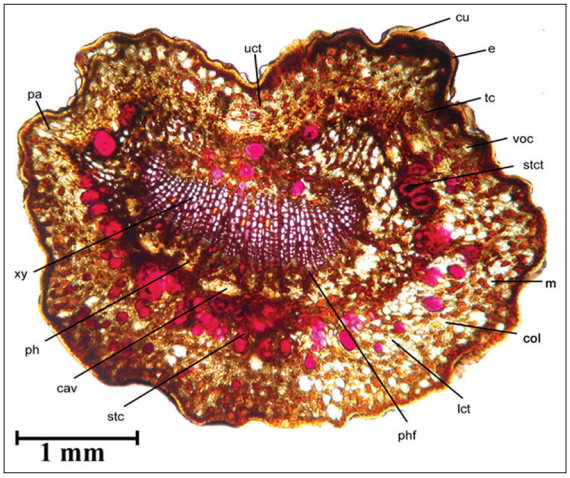

Figure 2a: Microscopic characters of leaf of *Cinnamomum malabatrum*. Detailed transverse section of petiole cav: Cavity, col: Collenchyma, cu: Cuticle, e: Epidermis, lct: Lower cortex, m: Mucilage, pa: Parenchyma, ph: Phloem, phf: Phloem fiber, stc: Stone cell, stct: Stone cell with tannin, tc: Tannin cell, uct: Upper cortex, voc: Volatile oil cell, xy: Xylem. Phloroglucinol and HCl staining

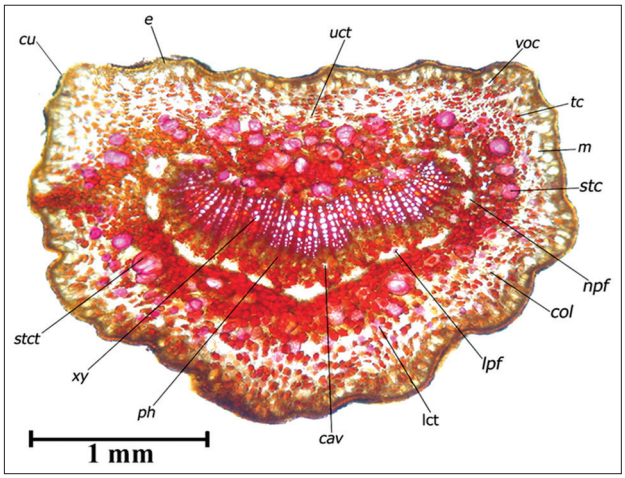

Figure 3a: Microscopic characters of leaf of *Cinnamomum tamala*. Detailed transverse section of petiole. cav: Cavity, col: Collenchyma, cu: Cuticle, e: Epidermis, lct: Lower cortex, lpf: Lignified pericyclic fibers, m: Mucilage, npf: Nonlignified pericyclic fibers, ph: Phloem, stc: Stone cell, stct: Stone cell with tannin, tc: Tannin cell, uct: Upper cortex, voc: Volatile oil cell, xy: Xylem. Phloroglucinol and HCl staining

Transverse section of leaf passing through midrib

Broadly convex dorsiventrally (shows number of winged projecting elevations at the lower side in *C. tamala*) shows

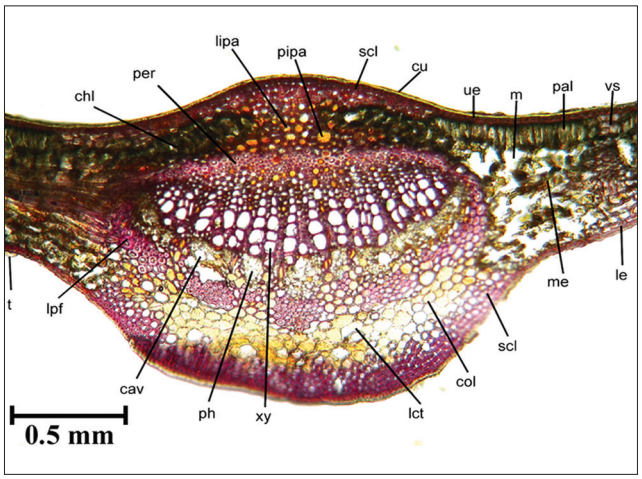

Figure 2b: Microscopic characters of leaf of *Cinnamomum malabatrum*. Detailed transverse section of leaf passing through midrib. cav: Cavity, chl: Chlorenchyma, col: Collenchyma, cu: Cuticle, e: Epidermis, le: Lower epidermis, lct: Lower cortex, lipa: Lignified parenchyma, lpf: Lignified pericyclic fiber, m: Mucilage, me: Mesophyll, pal: Palisade, per: Pericycle, ph: Phloem, pipa: Pitted parenchyma, scl: Sclerenchyma, t: Trichome, ue: Upper epidermis, vs: Vascular strands, xy: Xylem. Phloroglucinol and HCl staining

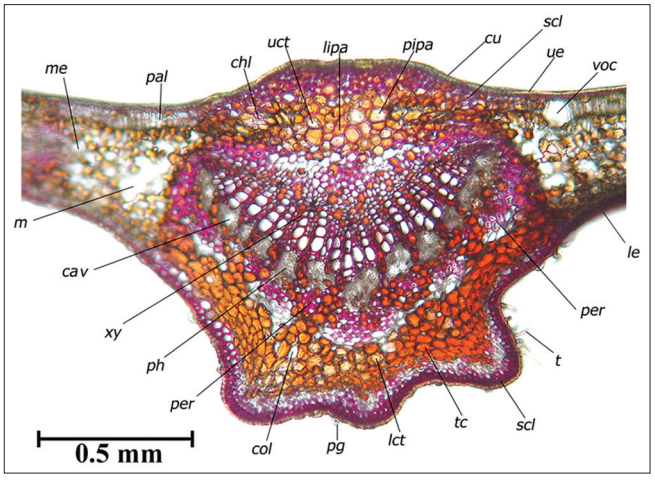

Figure 3b: Microscopic characters of leaf of *Cinnamomum tamala*. Detailed transverse section of leaf passing through midrib. cav: Cavity, chl: Chlorenchyma, col: Collenchyma, cu: Cuticle, e: Epidermis, le: Lower epidermis, lct: Lower cortex, lipa: Lignified parenchyma, m: Mucilage, me: Mesophyll, pal: Palisade, per: Pericycle, pg: Pearl gland, ph: Phloem, pipa: Pitted parenchyma, scl: Sclerenchyma, t: Trichome, tc: Tannin cell, uct: Upper cortex, ue: Upper epidermis, voc: Volatile oil cell, xy: Xylem. Phloroglucinol and HCl staining

Table 3: Comparative microscopic characters of the transverse section of midrib of *C. malabatrum* and *C. tamala*

Characters	*C. malabatrum*	*C. tamala*
Upper elevation	Small, conical shaped	Small, convex shaped
Lower elevation	Broad, not winged	Broad, with 3 winged projections
Upper epidermis	Thick walled cells covered by thick cuticle, hairs seldom	Radially elongated cells with multilayered cuticle, hairs plenty
Lower epidermis	Thick walled, lignified cells with scanty bent unicellular and thick walled trichomes, epidermal cells not papillose	Thick walled, with straight and bent hairs with 1-3 cells, globular papillae and pearl gland present
Palisade	Single layered, oval or spherical cells containing mucilage found embedded between the cells, volatile oil cells less often	Some places a second layer with short cells are found, oval shaped cavities with volatile oil is found embedded between the cells, mucilage cells less often
Spongy	5-6 layers of loosely arranged chlorenchyma	10-12 layers of loosely arranged chlorenchyma
Sclerenchyma traversing in mesophyll	About 2-4 cells wide, 20 cells high, 15-25 palisade cells in between such traversions	About 3 cells wide, 12-15 cells high, 12-22 palisade cells between such traversions
Stomata	Anomocytic	Paracytic

C. malabatrum: Cinnamomum malabatrum, C. tamala: Cinnamomum tamala

Table 4: Micrometry of leaves of *C. malabatrum* and *C. tamala*

Character	*C. malabatrum*	*C. tamala*
Upper epidermal cells	24 to 25×24 to 25	25 to 27×13 to 14
Parenchyma with crystals	40 to 115×50 to 60	58 to 70×40 to 50
Parenchyma with tannin	35 to 60×14 to 40	49 to 58×18 to 46
Mucilage (swollen)	30 to 46×30 to 46	58 to 70×35 to 58
Volatile oil cell	46 to 50×30 to 40	46 to 58×35 to 50
Crystals	2 to 23×2 to 30	2 to 13×2 to 18
Stone cells		
Smaller	28 to 56×18 to 54	52 to 87×35 to 105
Larger	77 to 245×42 to 140	-
Sclereids from lamina	260 to 300×25 to 32	290 to 350×38 to 40
Fibers		
Simple	150 to 350×10 to 25	172 to 276×12 to –23
Broad lumened	-	586 to 828×207 to 253
Vessels		
Annular	-	396 to 432×36 to –42
Reticulate	410 to 500×40 to 45	-
Spiral	280 to 340×22 to 28	144 to 304×12 to 25
Tracheids	30 to 100×15 to 30	46 to 92×9 to 28
Pitted parenchyma	-	88 to 120×28 to 46
Covering trichome	90 to 120×70 to 10	53 to 175×7 to 14

Minimum length to maximum length x minimum breadth to maximum breadth. All figures in µm; measurements are expressed from an average of 3 readings, *C. malabatrum: Cinnamomum malabatrum, C. tamala: Cinnamomum tamala*

an arc of well-developed conjoint collateral oval shaped meristele (triangular in *C. tamala*) in the center of the midrib and dorsiventral laminar extensions on its lateral sides. Pericyclic band encircling the meristele shows continuously running narrow arc of fibers on the upper side and discontinuously running groups of fibers on its lower side (in *C. tamala* it is discontinuous throughout except at two lateral sides). Both the upper and lower epidermal cells are thick-walled lignified on their inner walls, embedded with stomata on the lower side only, covered with thick cuticle and bear few simple and glandular trichomes like that of petiole (they being plenty on the lower side of the midrib region in *C. tamala*). 5 and 6 rows of lignified thick-walled parenchymatous cells are located underneath the upper and lower epidermis of the midrib it being 10-12 rows and extending up to the pericyclic band in the middle region underneath the upper epidermis. 5 and 6 rows of parenchyma cells embedded with tannins are located on both, dorsal and ventral sides of the meristele.

Lamina shows a row of narrow and compactly arranged palisade cells embedded with oval to spherical oil cells followed by few rows of spongy parenchyma embedded with mucilage cells, small parenchyma embedded with mucilage cells and small vascular bundles sheathed dorsiventrally with sclerenchymatous

band reaching up to both epidermis of the lamina [Figures 2b and 3b].

Microscopy of macerated leaf

Leaf on maceration yielded information on different tissue system from the petiole and lamina, the inference is found to be more informative in differentiating the two species. The characters observed are illustrated in Figure 4.

Micrometry

Micrometric measurements were taken for various tissues in the macerated leaf. The data is presented in Table 4.

Quantitative microscopy

Values for different leaf constants are presented in Table 5.

Powder

Presence of anomocytic stomata embedded in sinuously walled lower epidermal fragments (often less sinuously walled, papillate epidermis bearing paracytic stomata in *C. tamala*); rare occurrence of unicellular, rarely septate covering trichomes (up to three-celled covering trichomes and pearl-glands abundant in *C. tamala*); long sclereidal fibers with wide lumen and pitted wall and large stone cells (absent in *C. tamala*); fragments of annular vessels (reticulate in *C. tamala*) and spiral vessels; comparatively abundance of mucilage cells (volatile oil cell abundant in *C. tamala*) and plenty of square-like and hexagonal prismatic crystals of calcium oxalate (predominantly rod shaped and few acicular in *C. tamala*) are of diagnostic value in identifying the leaf powder under microscope. Characters of the powdered leaves are illustrated in Figure 5.

Table 5: Quantitative microscopy of leaves of *C. malabatrum* and *C. tamala*

Parameters	*C. malabatrum*	*C. tamala*
Vein islet number	24-26	36-38
Upper epidermal cells	1550-1700	2550-2700
Stomata in lower epidermis	320-360	475-540

All figures/sq.mm; measurements are expressed from an average of three readings, *C. malabatrum: Cinnamomum malabatrum, C. tamala: Cinnamomum tamala*

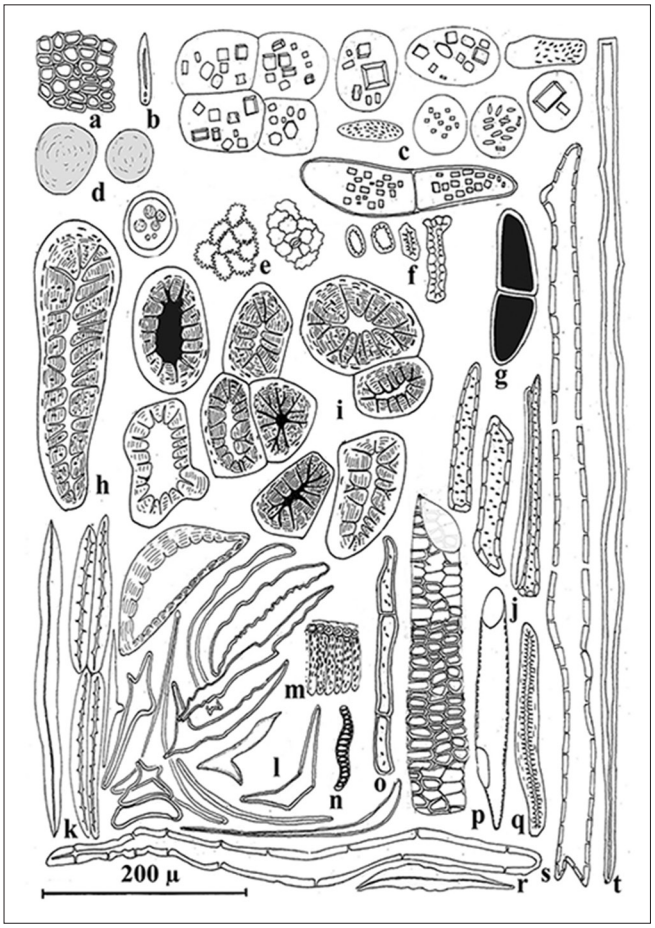

Figure 4a: Microscopy of macerated leaf of *Cinnamomum malabatrum*. (a) Epidermis of petiole; (b) Trichome; (c) Parenchyma with calcium oxalate crystals; (d) Mucilage cells; (e) Upper and lower epidermis; (f) Small stone cells; (g) Tannin cell; (h) Large sclereid; (i) Stone cells; (j) Tracheidal fibers; (k) Pitted and non-pitted fibers; (l) Thin-walled sclereids of different types; (m) Transversely cut lamina; (n) Annular tracheid; (o) Septate fiber; (p) Reticulate and pitted vessels; (q) Porous thick-walled fiber; (r) Thick-walled short fiber; (s) Sclereidal fibers; (t) Long fibre

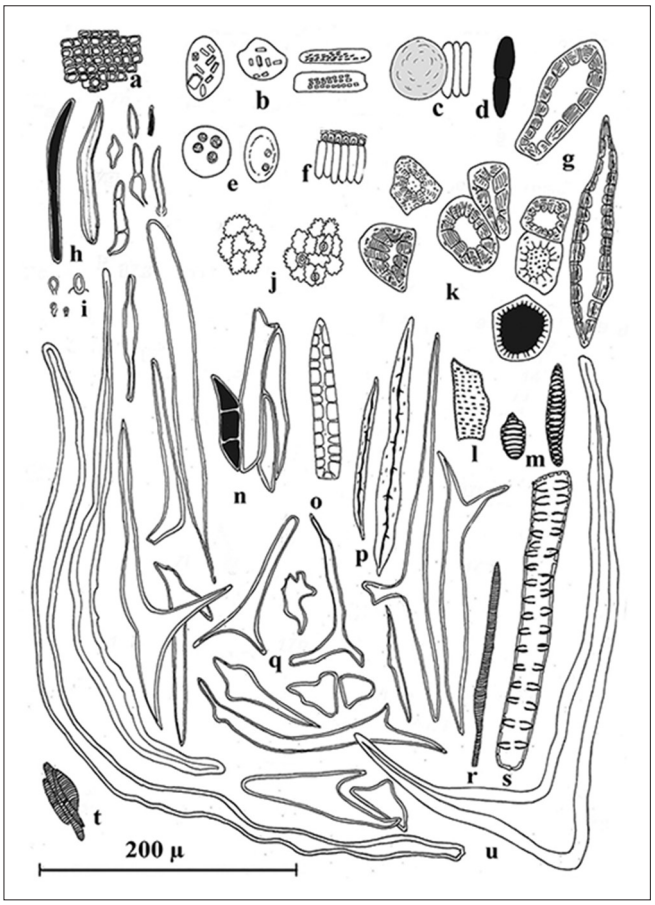

Figure 4b: Microscopy of macerated leaf of *Cinnamomum tamala*. (a) Epidermis of petiole; (b) Parenchyma with crystals; (c) Palisade and mucilage cell; (d) Tannin cell; (e) Volatile oil cell; (f) Transversely cut lamina; (g) Sclereids; (h) Trichomes; (i) Pearl glands; (j) Upper and lower epidermii; (k) Stone cells; (l) Pitted vessel; (m) Annular tracheids; (n) Sclereid and tannin cell; (o) Sclereidal fibers; (p) Thick-walled fiber; (q) Thin-walled sclereid-different types; (r) Spiral vessel; (s) Annular vessel; (t) Group of annular vessels; (u) Large thick-walled sclereid

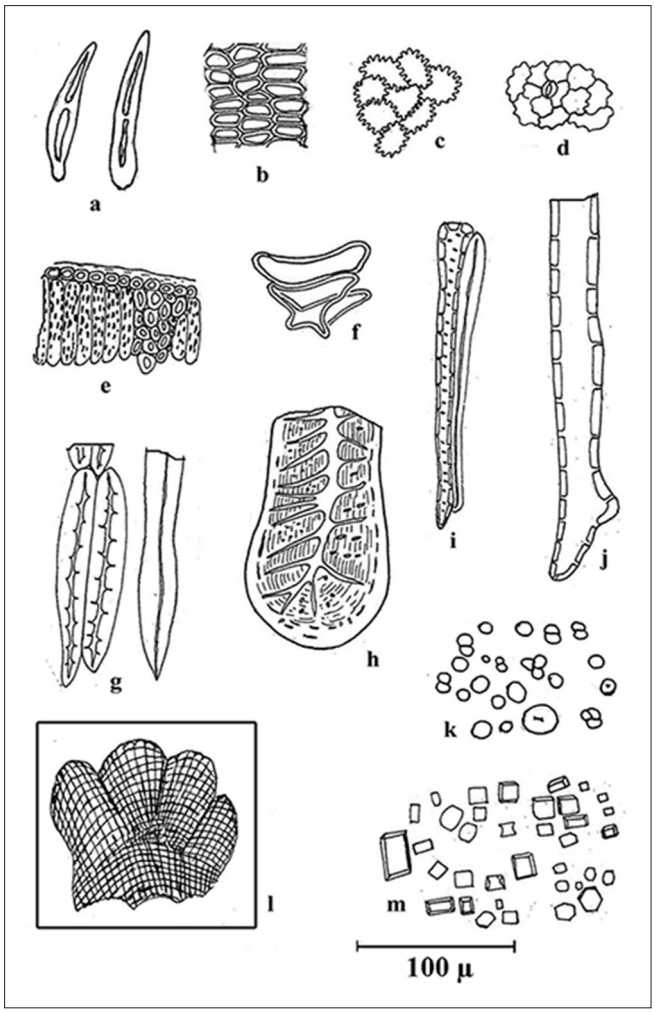

Figure 5a: Powder microscopy of leaf of *Cinnamomum malabatrum*. (a) Simple trichomes; (b) fragment of reticulate vessel; (c) Upper epidermis in surface view; (d) Lower epidermis in surface view; (e) Transversely cut lamina; (f) Thin-walled fibers; (g) Fragment of thick-walled fibers; (h) Fragment of sclereid; (i) Fragment of pitted vessel; (j). sclereidal fiber; (k) Starch grains; (l) Lichen growing on the leaf; (m) Calcium oxalate crystals

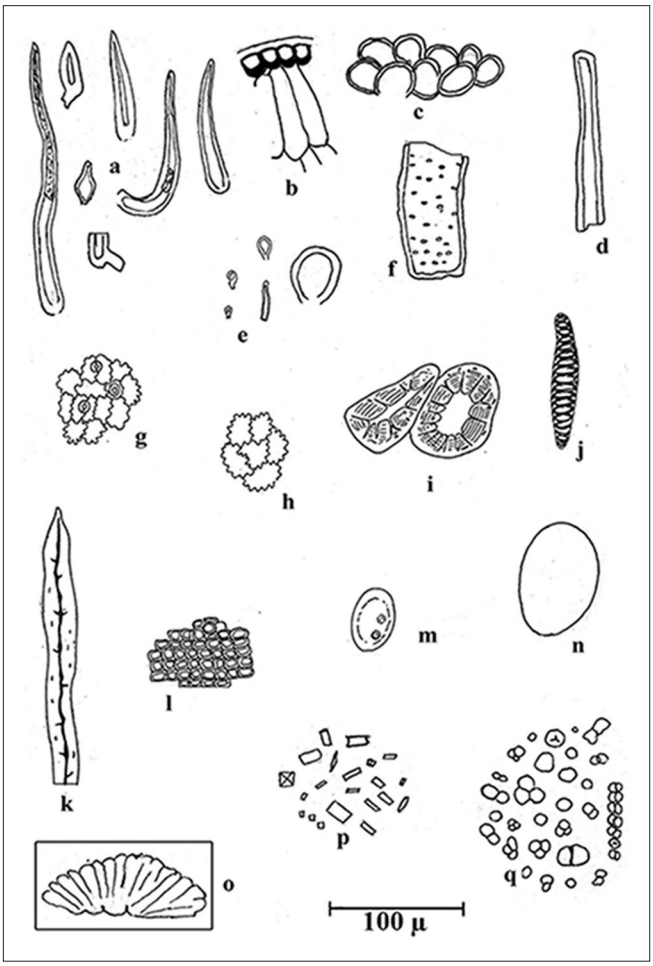

Figure 5b: Powder microscopy of leaf of *Cinnamomum tamala*. (a) Covering trichomes; (b) Transversely cut lamina; (c) Papillae of lower epidermis; (d) Fiber fragment; (e) Dwarf trichomes and pearl glands; (f) Pitted tracheid fragment; (g) Lower epidermal cells; (h) Upper epidermal cells; (i) Stone cells; (j) Annular tracheid; (k) Thick-walled fiber fragment; (l) Epidermis of petiole; (m) Volatile oil cell; (n) Mucilage cell; (o) Lichen growing on the leaf; (p) Calcium oxalate crystals; (q) Starch grains

Discussion

On account of wider distribution in Western Ghats, morphological similarity and the characteristic fragrance, leaves of *C. malabtrum* has been sold in crude drug markets for flavoring various edibles, also for use in many Ayurvedic and Siddha formulations. The market samples were analyzed to detect its identity by subjecting it to exhaustive macro-microscopic evaluation; the characters were recorded in comparison with authentic samples of *C. malabatrum*. Macrosopical and organoleptic features such as size of the leaf up to 25-30 cm, venation reaching up to the tip of the leaf, grooved and glabrous petiole, less aromatic and mucilaginous taste can easily differentiate the substitute from *C. tamala* leaf. Reniform to urn shaped outline of the TS of petiole, differences in the outline of the transverse section of leaf, rare occurrence of trichomes, and presence of anomocytic stomata in non-papillose epidermal cells in *C. malabatrum* can be diagnostic features to differentiate the two. The macro-microscopic characters of leaf petiole and lamina of samples obtained from the markets were found to be exactly in agreement with that of authentic *C. malabatrum*.

Conclusion

Detailed macro-microscopic profiles were laid down for *C. malabatrum* leaves, which will serve as diagnostic tools for its differentiation from official *C. tamala*. Exhaustive chemical and pharmacological evaluation might be conclusive to designate the species as a substitute or as an adulterant to *Tamalapatra*.

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हिन्दी सारांश

तमालपत्र के स्थान पर सिनामोमम् मलबाट्रम् (बर्म.एफ.) ब्लूम की पत्तियों का स्थूल सूक्ष्म परीक्षण

के. एन. सुनिल कुमार

सामान्यतः ङ्गतमालपत्रफके नाम पर सिनामोमम् तमाला(लाउरेसी) की पत्तियां औषध और मसाले के व्यापार में अत्यधिक प्रयोग की जाती है । जीनस सिनामोमम् से संबंधित अन्य प्रजाति के पत्रोंके साथ इसकी मिलावट भारत और दुनिया के अन्य भागों में की जाती है। उन्हें अलग करने के लिए संपूर्ण स्थूल और सूक्ष्म जांच आवश्यक हैं । दक्षिण भारतीय जडी बूटी बाजार के सर्वेक्षण से पता चला है कि सी.तमाला के स्थान पर सिनामोमम् प्रजातियों के कुछ अन्य पत्र बेचे जा रहे हैं । दक्षिण भारत में प्राप्त विभिन्न सिनामोन प्रजातियों की पत्तियों सी. तमाला को एकत्र कीयाँ और उनकी सही पहचान स्थापित करने के लिए अध्ययन किया गया । तमालपत्र के नाम पर भारत के बाजारों में बेची जानेवाली पत्तियों के द्रव संमर्दन और पाउडर माइक्रोस्कोपी सहित विस्तृत स्थूल सूक्ष्म मूल्यांकन किये गये । सी. मलबाट्रम की पत्तियां जो कई विशिष्ट स्थूल सूक्ष्म वर्ण की होती है, उन्हें तमालपत्र की आधिकारिक स्रोत – सी.तमाला से अलग करने में मार्करों जैसा काम करेंगे । मेक्रोस्कोपी पूरी पत्तियों की पहचान करेगी हालांकि, माइक्रोस्कोपी भी खंडित और पाउडर के रूप में वाणिज्यिक विकल्प की पहचान कर सकती है । सी.मलबाट्रम की स्थूल सूक्ष्म पहचान आधिकारिक औषधि के साथ तुलना करने हेतु स्थापित कि गई है, आगे रासायनिक और जैविक अध्ययन विकल्प या मिलावट के रूप में पत्तियों को अलग करने में सहायक सिद्ध हो सकता है ।