

Macro-micro-morphological diagnosis of leaves of two species of *Cinnamomum* (*C. sulphuratum* and *C. verum*) used as resource of bay leaf

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Abstract

Background: *Cinnamomum verum* (true cinnamon or *Tejpatra*) is a well-known spice with immense medicinal properties. Its adulteration with leaf and bark of other species belonging to genus *Cinnamomum* is found to be a common practice in India. **Aims:** *Cinnamomum sulphuratum* is used as a substitute of *C. verum* owing to its apparent macroscopic similarities. **Materials and Methods:** Fresh leaves of *C. verum* and *C. sulphuratum* growing in South India were collected and studied to establish their macro-microscopic identity with pharmacognostical perspective. **Results:** Detailed microscopic evaluation by transverse section, maceration, and powder microscopy was conducted to delineate the two species. Detailed macroscopic identification served the purpose of identification of the entire drug on the spot, and microscopy has helped in the identification of fragmented and powdered form of the drugs. **Conclusion:** Further chemical and biological studies may be necessary to confirm whether these leaves can be used as a substitute or adulterant for other species *Cinnamon*.

Keywords: *Cinnamomum sulphuratum*, maceration, micrometry, powder microscopy, quantitative microscopy, *Tejpatra*

Introduction

In India, significant quantities of medicinal plant resources are consumed by traditional healers and practitioners of Indian Systems of Medicine, which require continuous supply of medicinal plants for the manufacturing of medical formulations. *Cinnamomum verum* J. Presl (synonym *Cinnamomum zeylanicum* Blume) commonly known as the true cinnamon is one of the earliest known spices and medicinal plants.^[1] It is traditionally used for bloating, nausea, flatulence, colic, and gastrointestinal tract spastic conditions and finds application in modern medicines too.^[2] Almost every part of the cinnamon tree such as bark, leaves, flowers, fruits, and roots has some medicinal or culinary uses. Among the various species occurring in India, *C. verum*, *Cinnamomum malabratum*, *Cinnamomum sulphuratum*, and *Cinnamomum bejolghota* are morphologically similar to one another. *C. sulphuratum* locally known as “*Jangali dalchini*” is one of the most traded medicinal plants sourced from tropical forests.^[3] It is medium-to-large aromatic evergreen tree, distributed mainly in south^[4] and northeastern parts^[5] of India. On account of

easy availability and similarity in flavor, different parts of *C. sulphuratum* are in use as substitutes for commercial cinnamon-derived spices.^[6] In South India, it is distributed in Western Ghats regions of Tamil Nadu; Thiruvananthapuram and Wynad in Kerala; and Coorg, South Kannada, Hassan, Mysore, Shimoga, and North Kannada districts in Karnataka.^[7] The leaves and bark of the tree are aromatic; leaves are known by vernacular name *Tejpatra* by the Northeast Indian people and are used as a spice.^[8] Medicinal uses of *C. sulphuratum* are similar to *C. verum* which include treating wounds, fever, intestinal worms, headache, and menstrual problems.^[9] Leaves

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of the same genus are found to exhibit the number of similar common macro-microscopic characters and hence most likely to be used as an adulterant for official drug. In this article, a detailed macro-microscopic examination was carried out to differentiate these two species.

Materials and Methods

Plant materials

The authentic leaf samples of *C. verum* were collected from botanical garden of Alva's Ayurveda Medical College, Moodbidri, Karnataka, and *C. sulphuratum* from wild population in Tala Kaveri of Kodagu (Coorg) district, Karnataka. Leaves were dried in the shade; a few preserved in formalin-ethyl alcohol-acetic acid solution, were used for histological studies. Powder of the dried leaves sieved through mesh 60 was stored in glass vials and used for microscopic evaluation.

Instrumentation and techniques

Detailed macroscopy of the leaves of *C. verum* and *C. sulphuratum* was studied with respect to the description provided in floras.^[10,11] Leaf petiole and lamina were transversely cut and slides were prepared^[12] as per the standard procedures for histological examinations.^[12] Photomicrographs were taken using Leica microscope attached with Canon digital camera. Isolation of the tissues was carried out by Schultz's maceration process or by boiling with 5% KOH.^[13] Measurements of the various elements^[14] were taken with the help of 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.^[15] Microscopic characters were drawn with Prism Type Camera Lucida.

Results

Macroscopy

Morphological descriptions outlined in floras were used as a guideline to propose diagnostic differentiating

macrocharacters of the two drugs. The macroscopic and sensory characters of the two species were compared, and the differences were observed in size of leaf blade and petiole (*C. verum* is bigger than *C. sulphuratum*). Petiole is not hairy in *C. verum* when compared to *C. sulphuratum* and is lamina glabrous and smooth in *C. verum* while coriaceous and tomentose in *C. sulphuratum* which also shows algal growth on the surface. The lower surface of the leaves of *C. verum* is paler compared to *C. sulphuratum*. The secondary veins were distinct in *C. verum* while faint in *C. sulphuratum*. Leaves of *C. verum* are sweet, pungent, and aromatic while of *C. sulphuratum* are astringent and fragrant [Table 1 and Figure 1a, b].

Microscopy

Transverse section of the petiole

C. verum is cordate with a small projection within the depressed upper surface (circular to almost elliptical without any projections in *C. sulphuratum*); below the epidermis, cavities containing volatile oils are present in *C. verum* (absent in *C. sulphuratum*); rosette crystals are present in *C. sulphuratum* (absent in *C. verum*); stomata anomocytic cells are present in *C. verum* (paracytic in *C. sulphuratum*); and isolated sclerenchyma cells are seen on both sides of the vascular bundles in *C. verum* (in *C. sulphuratum*, it is present on the outer side) [Table 2 and Figure 1c, d].

Transverse section of the leaf passing through midrib

Both upper and lower surfaces are broad and conical in *C. verum*, while the upper region is semicircular and the lower is broad in *C. sulphuratum*; hypodermal sclerenchymatous patch is 10–12 cells wide in *C. verum* while 8–10 cells wide in *C. sulphuratum*; lower cortex is made up of collenchymatous cells of 2–3 layers in *C. verum* while 6–8 layers in *C. sulphuratum*. Xylem in *C. verum* is elongated, roughly ellipsoidal with 28–30 radial rows of vessels while in *C. sulphuratum* is concavo convex in outline with 20 radial rows of xylem vessels.

Table 1: Comparative macroscopic and organoleptic characters of leaves of *Cinnamomum* spp.

Characters	<i>Cinnamomum verum</i>	<i>Cinnamomum sulphuratum</i>
Size of whole leaf (cm)	7-25×3.8	9.5-18×2.6-4.8
Petiole (mm)	13-25	8-11
Shape	Oval or elliptic to lanceolate or narrowly elliptic	Elliptic to lanceolate to narrowly ovate lanceolate
Base	Acutish or cuneate, usually rounded	Acute to cuneately acute
Tip	Shortly or broadly acuminate	Sharply acute to shortly acuminate
Texture	Smooth shining, glabrous; highly reticulate below, thinly to stiffly coriaceous	Coriaceous, young leaves highly tomentose, mature leaves perfectly glabrous
Venation	Triplinerved or with very slender additional basal nerves (5 nerved), three main nerves prominent on both surfaces, the basal or subbasal ones running out near the base of acumen, secondary nerves faint, bent in the middle more or less parallel	Triplinerved; lateral nerves not reaching the tip of apex, basal to suprabasal, perfect-imperfect. Midrib modulate, secondary veins distinct, subparallel, bent at middle
Petiole	Rather stout, slightly concave above	Concave above, usually short and stout, highly hairy
Color	Upper surface dark green, lower surface paler, and dull	Dark green above, pale beneath
Taste	Mucilaginous, sweet, and pungent.	More mucilaginous and astringent
Odor	Aromatic	Fragrant

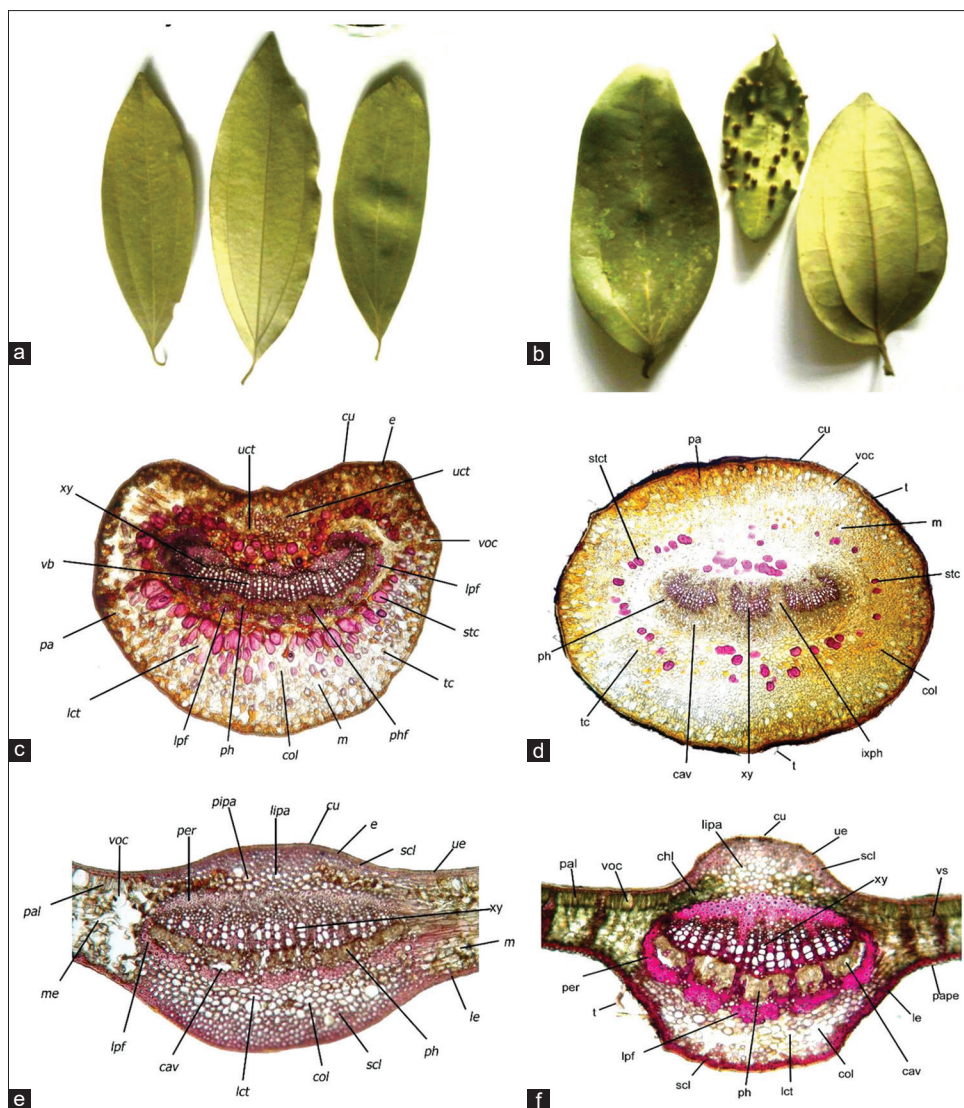


Figure 1: (a) Macroscopic characters of the leaves of *Cinnamomum verum*, (b) macroscopic characters of the leaves of *Cinnamomum sulphuratum*, (c) Detailed transverse section of petiole of *Cinnamomum verum*, (d) detailed transverse section of petiole of *Cinnamomum sulphuratum*, (e) detailed transverse section of the leaf passing through midrib of *Cinnamomum verum*, (f) detailed transverse section of the leaf passing through midrib of *Cinnamomum sulphuratum*

Table 2: Comparative microscopic characters of the transverse section of petiole of *Cinnamomum* spp.

Characters	<i>Cinnamomum verum</i>	<i>Cinnamomum sulphuratum</i>
Outline	Almost cordate with a small projection within the depressed upper surface	Almost elliptical without any projections
Hair	Unicellular thick-walled, few are of pearl type	Highly ranging, very long, with blunt end, sharp end, short with some black content, hair with mucilaginous wall only unicellular or pearl gland
Epidermis	Thick-walled, lignified, rectangular shaped cells without any hairs	Highly thickened inner wall with plenty of hairs, slightly papillose
Pericycle	Lignified, isolated, or grouped sclerenchyma fibers surrounding the whole vascular bundle from both the sides. Between stone cell sheath and pericycle fibers, there is a single layer of parenchyma with tannin surrounding to the vascular bundle	Formed by isolated stone cells. there are few isolated sclerenchyma fibers inner to stone cells
Phloem	Only on lower side, lignified sclerenchyma cells are found distributed throughout phloem. Small cavities are also evident	Much broader with small cavities, few sclerenchyma cells are also found in the phloem
Xylem	Boat shaped continuous, 55-60 radial and 6-7 vessels in each row	In three groups, each C/U-shaped, lateral two are wider, about 20 radial row in each group, 10-12 vessels in each

Table 3: Comparative microscopic characters of the transverse section of lamina passing through the midrib of *Cinnamomum* spp.

Characters	<i>Cinnamomum verum</i>	<i>Cinnamomum sulphuratum</i>
Upper elevation	Upper and lower epidermis with broad conical elevation	Semicircular
Lower elevation	almost similar in shape	Broad
Upper epidermis	Thick walled, slightly papillose cells with lignifications on their lower wall	Thick walled, lignified, slightly papillose lignified cells forming wavy epidermis
Lower epidermis	Cells very small compared to upper, some cells with brownish tannin content, lignified and rarely with simple unicellular thick walled glandular hairs (pearl gland)	Moderately papillose, thick-walled, lignified cells with good number of simple unicellular covering trichomes
Palisade	Single-layered, embedded with almost circular cells with volatile oil, sometimes second layer of short cells are found	Single layered, rarely a short second layer is found, idioblast with volatile oil is found in between the cells
Spongy	4-5 layers of loosely arranged chlorenchyma with mucilage and volatile oil cell	7-8 layers of loosely arranged chlorenchyma with mucilage cells, it intrudes into the midrib region
Sclerenchyma traversing in mesophyll	Small, thick-walled cells, 3-4 cells wide, 18-24 cells high, very closely arranged	Small, thick-walled, narrow-lumened cell, 2-3 cells wide, 30-35 palisade cells in between such traversions. 18-20 cells high, 4-8 cells wide
Stomata	Anomocytic	Paracytic

Lamina of both species have thick cuticle covering the upper epidermis and thin cuticle covering the lower cells, being nonlignified, nonpapillose in *C. verum* while being lignified and highly papillose in *C. sulphuratum*. Single-layered palisade cells are present in both; the number of layers of spongy mesophyll varies from 4 to 5 layers in *C. verum* while 7 to 8 layers in *C. sulphuratum* [Table 3 and Figure 1e, f].

Microscopy of the macerated leaf

Leaf on maceration with KOH yielded information on different tissue systems such as type of hairs, xylem vessels, tracheids, stone cell fibers, crystals of calcium oxalate from the petiole and lamina. Brief differentiations are depicted Figures 2A, B, and 3A, B. The inference is found to be more informative in differentiating the two species.

Micrometry

Micrometric measurements were taken for various tissues in the macerated leaf [Table 4 and Figure 4a, b].

Quantitative microscopy

Values for different leaf constants such as upper epidermal cells, vein islet number, and stomatal number are documented in Table 5.

Powder

Pearl glands were observed in *C. verum* (absent in *C. sulphuratum*); fragments of pitted and reticulate vessels present in *C. verum* (absent in *C. sulphuratum*); tracheidal elements in *C. sulphuratum* possessed wide lumen (narrow lumen in *C. verum*); stone cells with wide lumen are present in *C. verum* (found rarely in *C. sulphuratum*); oval-shaped stone cells are extant in both the species, but the boat-shaped one is present exclusively in *C. sulphuratum*; stone cells with three-sided thickening was seen in *C. verum* (absent in *C. sulphuratum*); prismatic crystals of calcium oxalate predominantly were present in *C. verum* (absent in *C. sulphuratum*) [Figure 5a and b].

Table 4: Micrometry of leaves of *Cinnamomum* spp.

Character	<i>Cinnamomum verum</i>	<i>Cinnamomum sulphuratum</i>
Upper epidermal cells	18-36×7-16	7-14×6-7
Parenchyma with crystals	46-120×46-70	30-115×30-46
Parenchyma with tannin	58-105×35-46	30-70×20-30
Mucilage (swollen)	58-80×42-50	30-40×20-30
Volatile oil cell	80-88×46-70	60-105×36-40
Crystals	46-120×46-70	2.5-23×2-28
Stone cells	88-193×35-88	63-245×35-158
Sclereids from lamina	With crystals, 184-218×30-70	550×30
Fibers	Laminal, 69-184×9.3-23	
Simple	190-320×11-22	-
Broad lumened	Without pits 620×26, fiber with crystals 184×23, septate with acicle 300×20	156-600×12-24
Vessels		
Annular	Pitted 288×25	Pitted 460×34
Reticulate	384×64	-
Spiral	272×26	-
Tracheids	57-92×11-16	60-90×12-18
Pitted parenchyma	263×14-32	105-140×28-35
Covering trichome	-	105-228×14-25

Minimum length to maximum length × minimum breadth to maximum breadth. All figures in µm; measurements are expressed from an average of three readings, *C. verum*: *Cinnamomum verum*, *C. sulphuratum*: *Cinnamomum sulphuratum*

Discussion

The morphological similarities, wider distribution, and resemblance in fragrance of the leaves of various species of *Cinnamomum* have created a great difficulty in differentiating

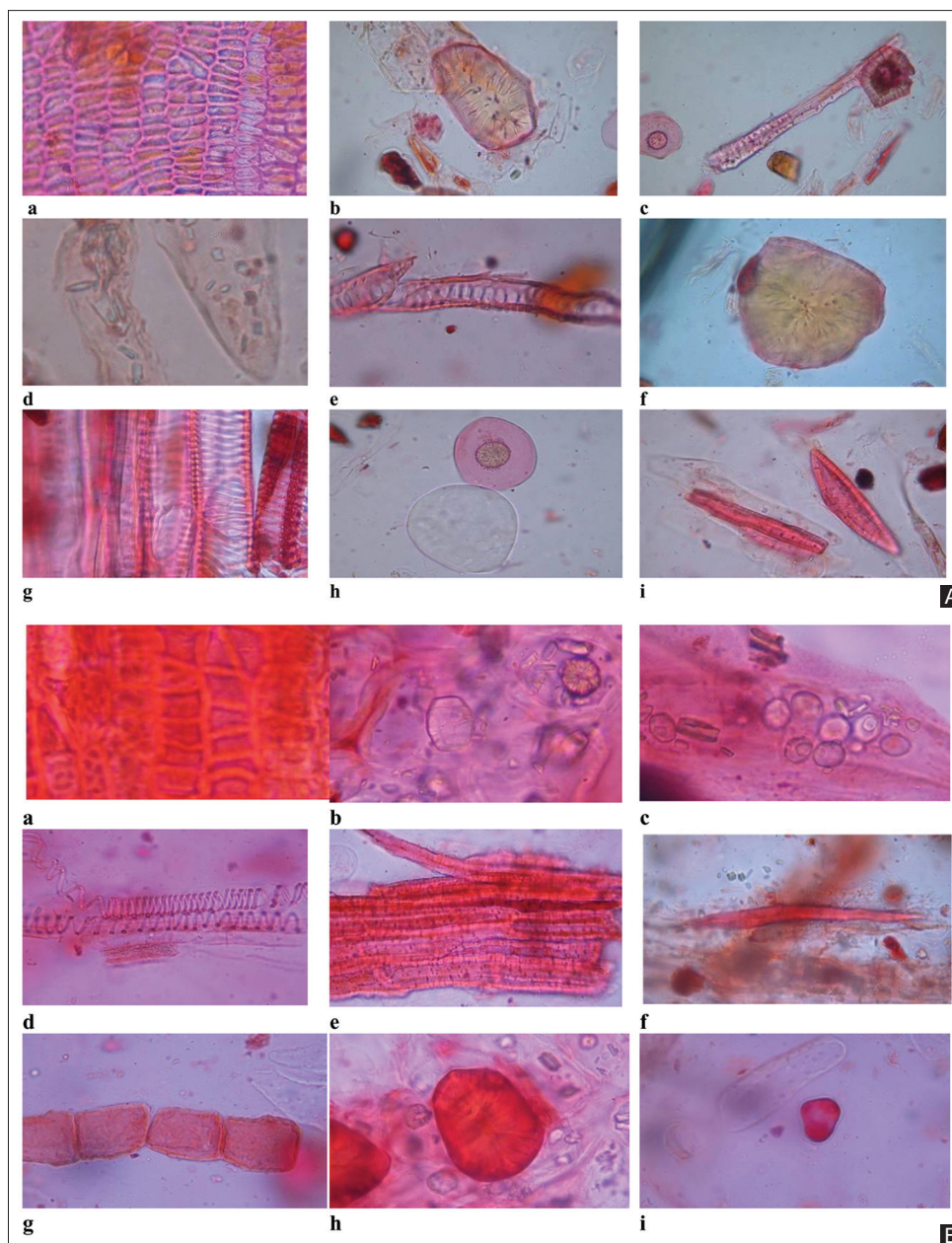


Figure 2: (A) Elements of maceration of *Cinnamomum verum* petiole (a) epidermis, (b) stone cell with wide lumen, (c) sclereisidal fiber, (d) prismatic crystals of calcium oxalate, (e) vessels with wide simple pits, (f) stone cell with narrow lumen, (g) spiral vessel, (h) mucilage and volatile oil cells, (i) sclereids. (B) Elements of maceration of *Cinnamomum sulphuratum* petiole (a) epidermis, (b) rosette crystals, (c) tablet-shaped crystals, (d) spiral vessel, (e) highly pitted vessel, (f) fiber with narrow end, (g) sclereids, (h) stone cells, (i) sclereids and oleoresin cells

and lead to the adulteration and substitution of herbal samples. *C. verum* has been used in many Ayurvedic and Siddha formulations. Due to the similarities with *C. verum*, *C. sulphuratum* is used widely as the substitute. Anatomical descriptions of leaf of *Cinnamomum* species have been provided earlier,^[16,17] but detailed anatomical studies of leaf have been published for only a restricted number of *Cinnamomum* species.^[18-26]

Even though penninerved and triplinerved leaves have been reported in *Cinnamomum*,^[27] the two species under the study possessed triplinerved leaves. *C. verum* and *C. sulphuratum*

had thick epidermis with lignin deposition in contrast to the suberin deposition in *Cinnamomum tamala*.^[26] The stomata observed in *C. verum* were anomocytic while it was paracytic in *C. sulphuratum*. Earlier anomocytic stomata have been reported in *C. malabratrum* and paracytic in *C. tamala*^[25] and *Cinnamomum camphora*.^[27] In *C. verum* and *C. sulphuratum*, volatile oil cells were present in mesophyll cells but completely absent in *Cinnamomum magnifolium*.^[23] In *Cinnamomum amoenum*, three-layered palisade parenchyma was observed,^[23] while in *C. verum* and *C. sulphuratum*, it is single layered. The variation

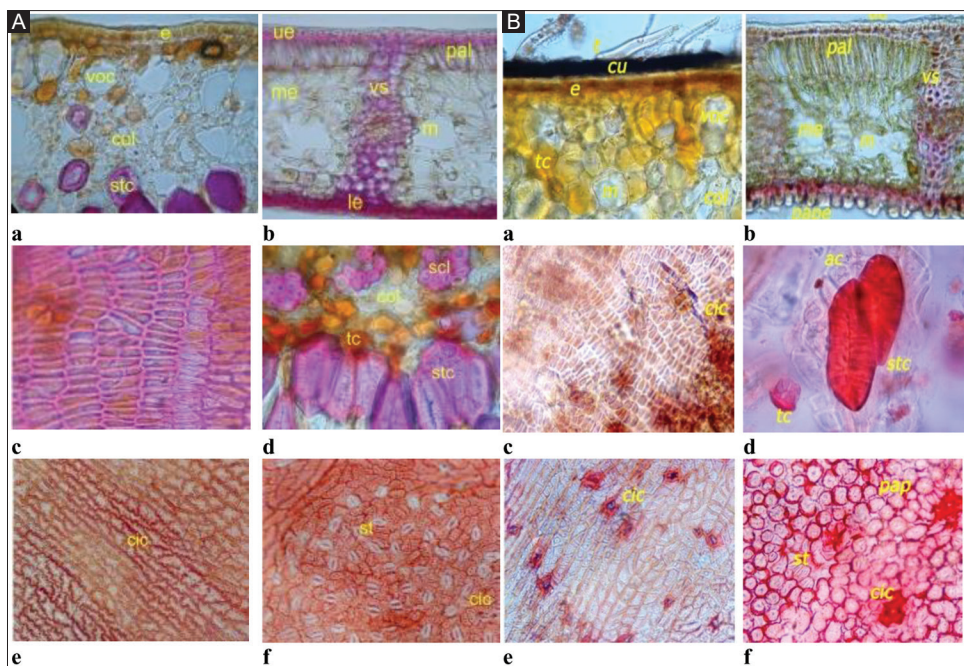


Figure 3: (A) Histology of *Cinnamomum verum* leaves: (a) transverse section of petiole, (b) transverse section of lamina showing vascular strands, (c) epidermis of petiole in surface view, (d) transverse section of petiole showing group of stone cells, (e) upper epidermis showing cicatrix, (f) lower epidermis showing stomata and cicatrix. (B) Histology of *Cinnamomum sulphuratum* leaves: (a) transverse section of petiole, (b) transverse section of lamina showing vascular strands, (c) epidermis of petiole in surface view, (d) transverse section of petiole showing group of stone cells, (e) upper epidermis showing cicatrix, (f) lower epidermis showing stomata, papillae, and cicatrix

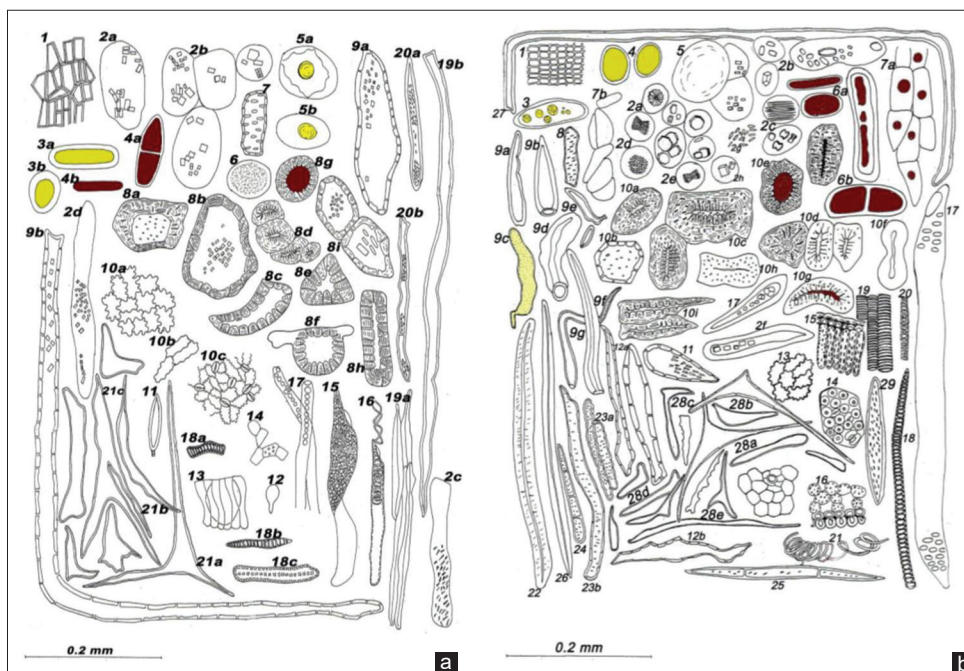


Figure 4: (a) Microscopy of macerated leaf of *Cinnamomum verum*, (b) microscopy of macerated leaf of *Cinnamomum sulphuratum*

in the number of layers of spongy parenchyma has been encountered before in *C. malabatum* (5–6) and *C. tamala* (10–12).^[20,25]

Macroscopic and organoleptic features depicting the differences in petiole, leaf, smell, and taste can help to a certain extent in delimiting the species, but the detailed and exhaustive

microscopic evaluation which displayed the differences in hypodermal sclerenchymatous layers, shape and number of xylem elements, differences in the number of phloem patches, and the presence of isolated sclerenchyma cells surrounding the vascular bundles has proved to be a reliable tool in authenticating the two species.

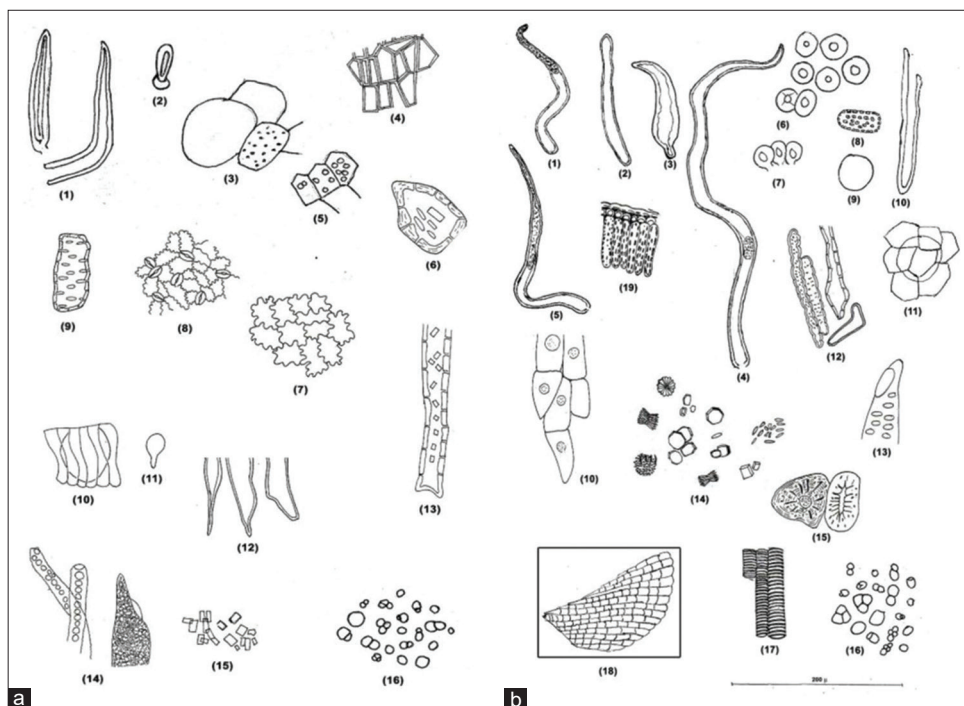


Figure 5: (a) Powder microscopy of leaf of *Cinnamomum verum*, (b) powder microscopy of leaf of *Cinnamomum sulphuratum*

Table 5: Quantitative microscopy of leaves of *Cinnamomum verum* and *Cinnamomum sulphuratum*

Parameters	<i>Cinnamomum verum</i>	<i>Cinnamomum sulphuratum</i>
Vein islet number	16-18	30-32
Upper epidermal cells	1100	1720
Stomata in the upper epidermis	180 st/sqmm	500 st/sqmm
SI	14%	22.5%

All figures/sq.mm; measurements are expressed from an average of three readings, *C. verum*: *Cinnamomum verum*: *C. sulphuratum*: *Cinnamomum sulphuratum*, SI: Stomatal index

Conclusion

The lack of botanical identity of herbal drugs may lead to adulteration and substitution. When the concerned herbal material is not present in sufficient quantities, there is a tendency to substitute it with available samples. The differences exhibited in macro-microscopy of the species may lead to the differences in their medicinal values too. Hence, the usage of allied species may not ensure the exact bioactivities for which detailed pharmacological studies may be required. The detailed macro-microscopic profiles laid down for *C. verum* and *C. sulphuratum* leaves will thus serve as diagnostic tools for their differentiation.

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Conflicts of interest

There are no conflicts of interest.

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