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Research

Integrative Cladistic, Morphological Traits, and Anatomical Profiling of *Averrhoa bilimbi* Linn. Leaves: A Comprehensive Study



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	Abstract
Published on: 07 Dec 2024	<p>This study investigates the pharmacognostic characteristics of <i>Averrhoa bilimbi</i> leaves, encompassing macro and microscopic examinations, quantitative microscopy, and physicochemical analyses. Fresh and dried leaves were subjected to macroscopic evaluation to document their morphological features. Microscopic analysis revealed the presence of various cellular structures, including stomata, trichomes, and vascular bundles, which were examined in detail. Quantitative microscopy provided stomatal density, and other pertinent parameters, contributing to a comprehensive understanding of the leaf's anatomical traits. Additionally, physicochemical studies assessed parameters such as moisture content, ash value, extractive value, and flow properties of leaf powder ensuring a thorough characterization of the leaf material. The findings underscore the significance of <i>Averrhoa bilimbi</i> leaves in traditional medicine and their potential as a source of bioactive compounds, paving the way for further pharmacological research.</p>
Published by: DrSriram Publications	<p>Keywords: <i>Averrhoa bilimbi</i>, Taxonomy, Phylogenetical revision, Alternipinnate, Transverse section.</p>
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INTRODUCTION

Averrhoa bilimbi is a traditional medicinal plant that thrives in tropical and subtropical regions [1]. *Averrhoa bilimbi* is found in Indonesia at elevations of up to 500 meters above sea level (MASL) and can bear fruit year-round [2]. *Averrhoa bilimbi*, a member of the *Oxalidaceae* family, grows to a height of 3-10 meters and features multiple branching stems. *Averrhoa bilimbi* is medicinally used as a folk remedy for many symptoms. It is used for the treatment of fever, mumps, pimples, inflammation of the rectum and diabetes, itches, boils, rheumatism, syphilis, bilious colic, whooping cough, hypertension, stomach ache, ulcer and as a cooling drink [3]. The genus under analysis possesses remarkable medicinal potential, warranting more extensive investigation for its application in treating various diseases. Due to the presence of bioactive compounds, this plant is considered

a promising source of these compounds, necessitating further research. Therefore, the objective of this study is to provide a comprehensive description of the species' macro, micro, and physicochemical properties, contributing to the development of a detailed monograph. Additionally, the identification process will offer new insights into species that have not been previously studied, advancing our understanding of their biological activities and their significance in drug discovery [4].

MATERIALS AND METHODS

Plant collection: The leaves of *Averrhoa bilimbi* belongs to *Oxalidaceae* family were collected from a location of Ezhudesam village, Kanyakumari district in Tamil Nadu, India during the month of February 2024.

Authentication: The leaves of *Averrhoa bilimbi* was authenticated by **Taxonomist, Numerical taxonomy & DNA Barcoding.**

The macroscopic study is the morphological description of the plant parts which are seen by naked eye or magnifying lens. Free hand transverse sections of petiole and leaf were taken and stained with 0.8% Safranin and 0.5% Astra blue and observed for their peculiar characters. The powder microscopy characters of the *Averrhoa bilimbi* leaves were observed. Physiochemical character such as foreign matters, extractive values, loss on drying, Ash values, viz. total ash, acid soluble ash, sulphated ash and water-soluble ash were determined as per standard procedure [5,6].

RESULTS

AUTHENTICATION

Taxonomist: The plant sample was authenticated by a Botanist **Dr. D. Stephen, Professor, Department of Botany, The American College, Madurai.**

Numerical taxonomy: The two most well-known species are *Averrhoa bilimbi* (bilimbi) and *Averrhoa carambola* (starfruit), which are cultivated worldwide for their edible fruits. Other lesser-known species include *Averrhoa leucopetala* and *Averrhoa dolichocarpa*, which are mainly found in tropical rainforests of Southeast Asia and the Pacific islands. The following are the four species of *Averrhoa* was selected for numerical taxonomy

Averrhoa bilimbi

Averrhoa carambola

Averrhoa leucopetala

Averrhoa dolichocarpa

The organoleptic and morphological typoscripts of aerial parts of various species in genus *Averrhoa*.

Table 1: Chart of similarity for four species of *Averrhoa*

SPECIES Characters	<i>Averrhoa bilimbi</i>	<i>Averrhoa carambola</i>	<i>Averrhoa leucopetala</i>	<i>Averrhoa dolichocarpa</i>
Leaf arrangement	1	1	1	1
Leaf length	1	2	2	2
Shape	1	2	1	1
Apex	1	1	2	2
Base	1	1	2	2
Number of leaflets	1	2	2	2
Petiole length	1	2	2	2
Leaflets surface	1	2	2	2
Leaf Nerves	1	2	2	2

Flowers inflorescence	1	2	2	2
Position	1	2	1	1
flower size	1	2	2	2
Sepal surface	1	2	1	1
Filament	1	2	2	2
Style	1	2	2	2
Calyx	1	2	1	1
Fruit shape	1	2	2	2
Size	1	2	2	2
Colour	1	2	2	2
Taste	1	2	1	1

Leaf arrangement: imparipinnate – 1, Length: 43.6 to 45.6cm – 1, other length - 2
 Shape: oblong to ovate -1, not oblong to ovate -2, Apex: Attenuate-1, not attenuate -2
 Base: asymmetrical -1, not asymmetrical -2, Number of leaflets: 14 to 19 pairs -1, others -2
 Petiole: length:5 to 6 cm -1, others -2, Leaflets surface: densely hairs -1, others -2
 Leaf nerves: 7pairs -1, not 7pairs -2, Flower Position: Cauliflorous -1, not cauliflorous -2,
 Flowers inflorescence:Panicle fasciculate -1,not panicle -2
 Flower size: 1.3 to 1.6 cm – 1, not 1.3 to 1.6 cm -2, Calyx: absent -1, not absent -2
 Sepal surface: glabrous inside & hairy outside -1, glabrous on both sides - 2
 No of Filament: 5 – 1, not 5- 2, Style: 3 mm long -1, not 3mm long -2
 Fruit shape: oblong- 1, not oblong -2, Size: 10 X 5cm -1, not 10 X 5 cm -2
 Colour: green -1, not green -2, Taste: sour-1, not sour -2

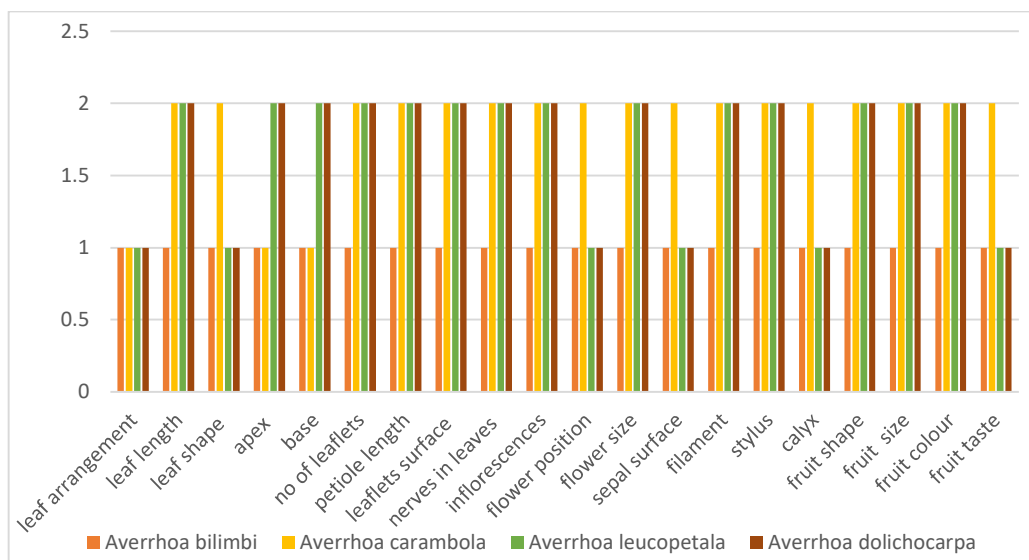


Fig 1: Chart of similarity for four species of *Averrhoa*

Table 2: Chart of Dissimilarity for four species of *Averrhoa*

SPECIES	<i>Averrhoa bilimbi</i>	<i>Averrhoa carambola</i>	<i>Averrhoa leucopetala</i>	<i>Averrhoa dolichocarpa</i>
<i>Averrhoa bilimbi</i>	1	0.2	0.3	0.35
<i>Averrhoa carambola</i>	0.2	1	0.1	0.2
<i>Averrhoa leucopetala</i>	0.3	0.1	1	0.4
<i>Averrhoa dolichocarpa</i>	0.35	0.2	0.4	1

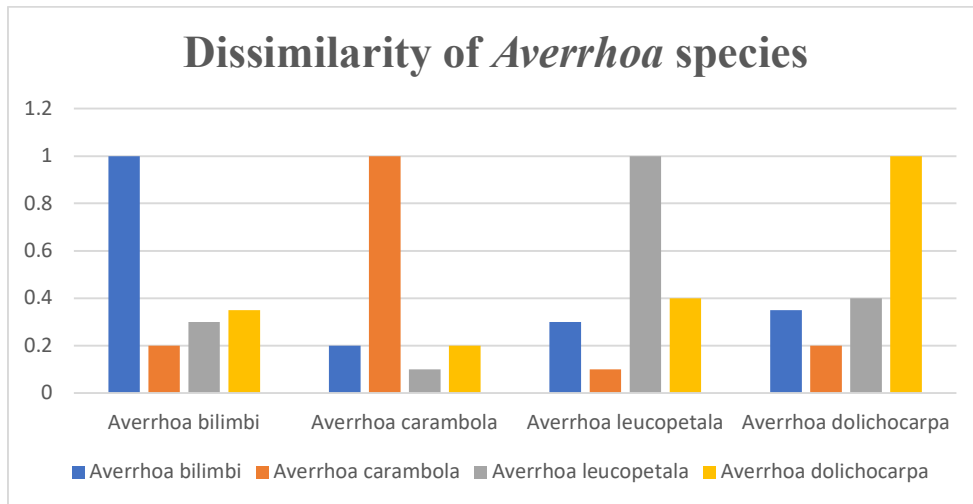


Fig 2: Chart of dissimilarity for four species of *Aerrhoa*

$$\begin{aligned} \text{Sample Matching Coefficient SSM} &= \text{NS} / \text{NS} + \text{ND} \times 100 \\ &= 35 / 35 + 45 \times 100 \\ \text{SSM} &= 43.75\% \end{aligned}$$

NS – Number of similar characters,
 ND- Number of dissimilar characters.

The matching co-efficient of *Aerrhoa bilimbi* with respect to other species was found to be 43.75%

DNA Barcoding

Table 3: Quality check and quantification of DNA

Sample	Concentration in ng/μl	A _{260/280}	A _{230/260}
<i>Aerrhoa bilimbi</i>	992.8	2.09	1.85

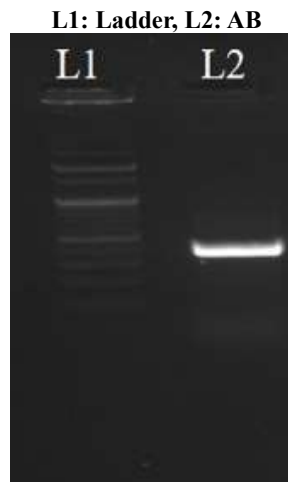


Fig 3: Gel image of PCR amplified product

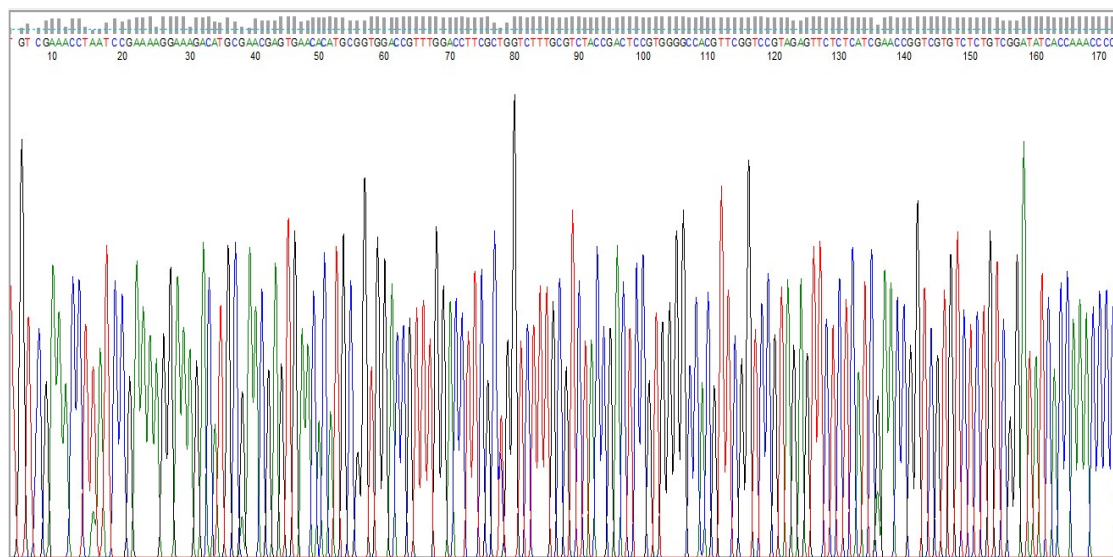


Fig 4: Chromatograms of Averrhoa bilimbi

Table 4: Sequence obtained

Sample code	Sequence with Barcode and BLAST Hit
> SCRIPCOGAB29	<p>Tcccgggaaggatcattgtcagaaacctcatagcagaaccaccgcgaacgtgctgtgacaacaactgtggccgaggatcgggcccaccgccagcggcgacgtccgtgccctccggggcccacatcgaacccccggcgcaagccgcgccaaggaactgtaatcgggagcgtgcgctccccgagctcggaaacgggtctagcggggggcgactgtctcgtgatcttgcgaataatgactctcggaacggatctcggctctcgatcgatgaagaacgtagcgaatcgatactgggtgtaattgcagaatcccgtgaacatcgaatcttgaacgcaaa</p>

Table 5: Gene bank Submission Details

Voucher No	Specimen Name	Submission Id	Accession No	Gene Bank Link
SCRIPCOGAB29	<i>Averrhoa bilimbi</i>	SUB14406360	PP732169	https://www.ncbi.nlm.nih.gov/

MACROSCOPY OF *Averrhoa bilimbi* Linn LEAVES

Plate 1: *Averrhoa bilimbi* Leaflet



Dorsal view of leaf

Ventral view of leaves



Plate 2 :Dorsal & Ventral view of various sized leaves



Large leaf



medium size leaf



small leaf

DORSAL VIEW OF BASE

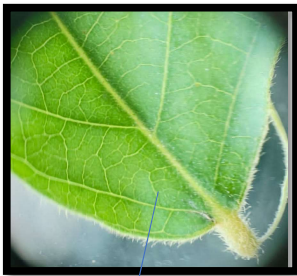

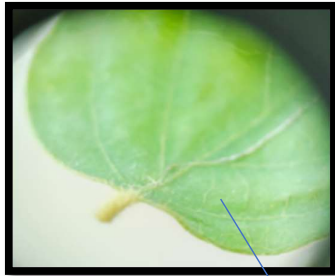


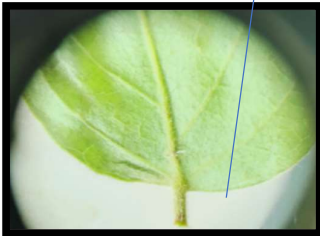
Large leaf	Medium leaf	Small leaf
		
Asymmetrical	Rounded and Symmetrical	Asymmetrically Obtuse
VENTRAL VIEW OF BASE		
		
Large leaf	Medium leaf	Small leaf

Plate 3: Dorsal & Ventral view of Base

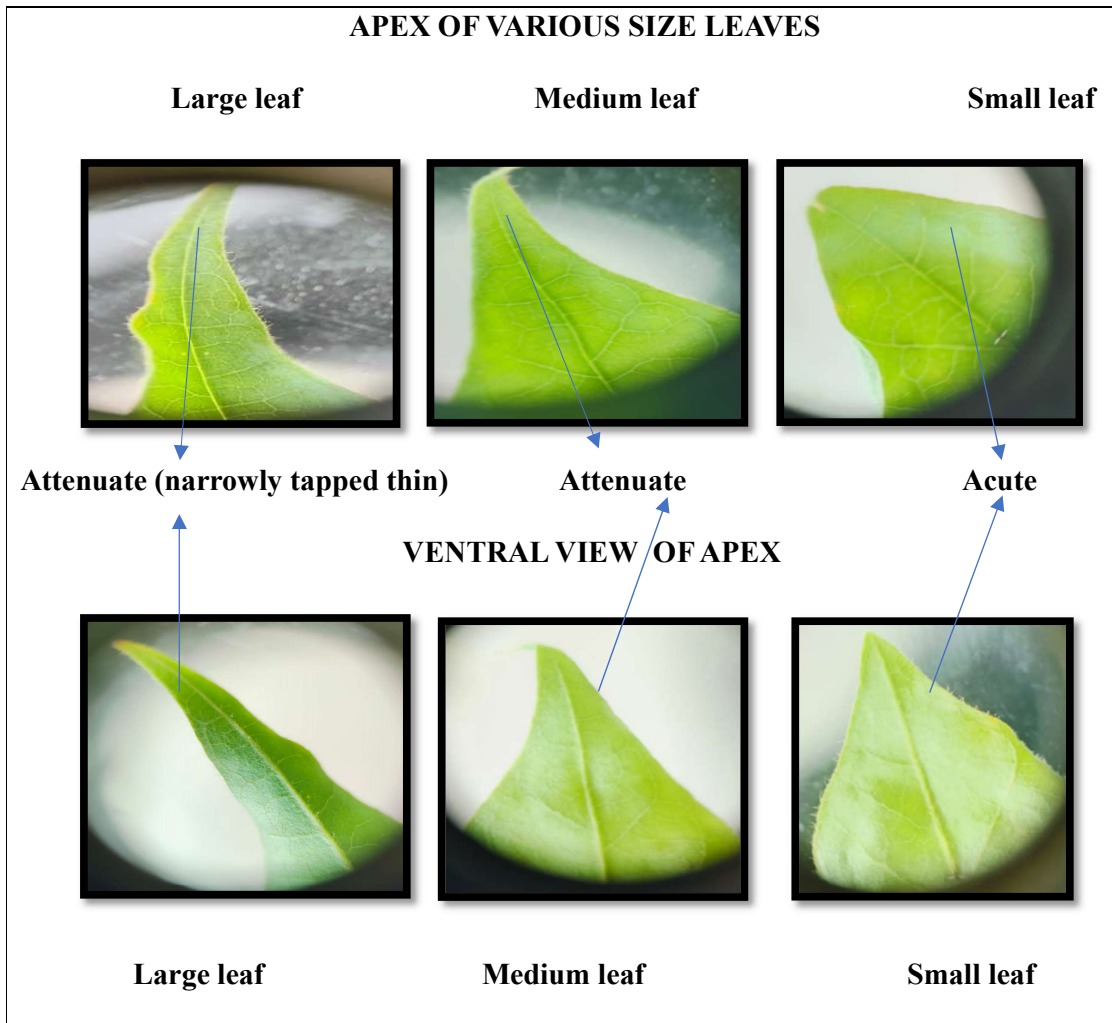


Plate 4: Dorsal & Ventral view of Apex

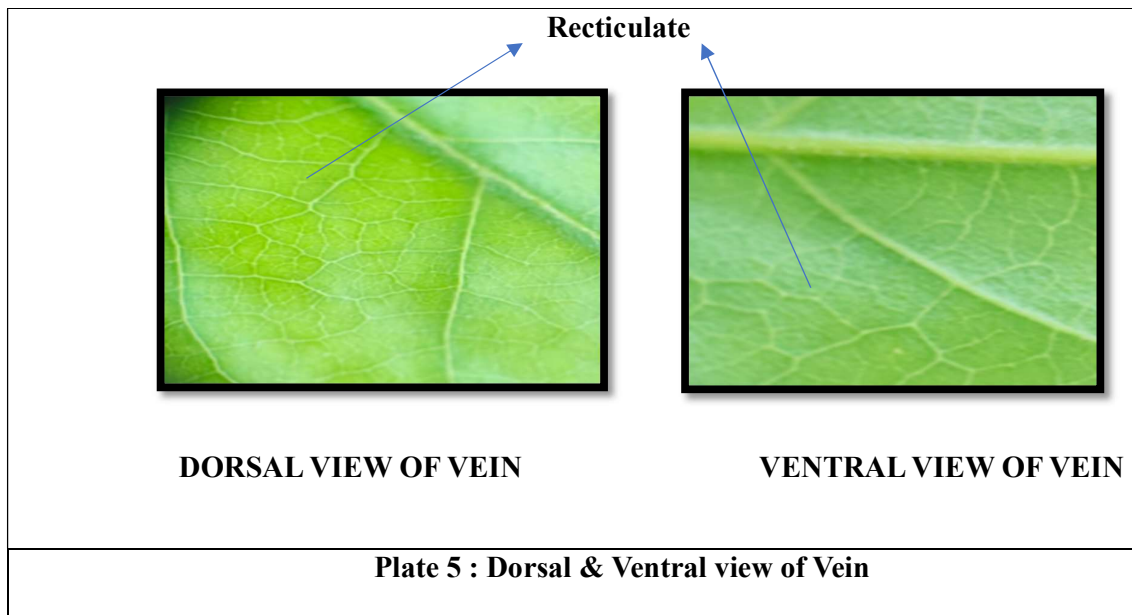


Plate 6: Dorsal & Ventral view of Margin

Entire with ciliated Margin



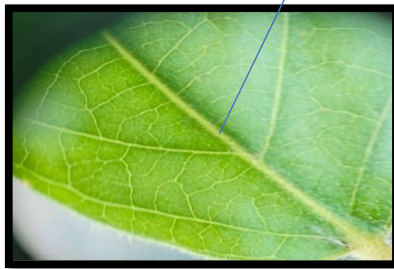
DORSAL VIEW OF MARGIN



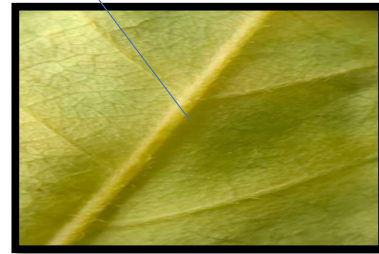
VENTRAL VIEW OF MARGIN

Plate 7: Dorsal & Ventral view of Midrib

Minute glands with pubescent midrib



DORSAL VIEW OF MIDRIB



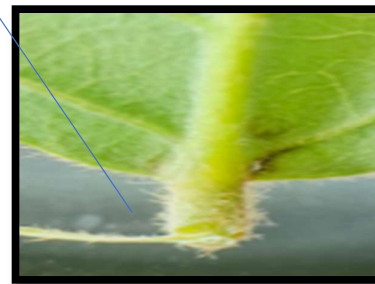
VENTRAL VIEW OF MIDRIB

Plate 8: Dorsal & Ventral view of Petiole

Bulbous Densely pubescent Petiole



DORSAL VIEW OF PETIOLE



VENTRAL VIEW OF PETIOLE

Table 6: Organoleptic & Macroscopical features of leaves *Averrhoa bilimbi*

S.no	Morphological features	<i>Averrhoa bilimbi</i> linn leaf
1	Condition	Fresh
2	Colour	Adaxial surface -Medium Green Abaxial surface - Pale Green Young leaf- Brown Green
3	Odour	Characteristic odour
4	Taste	Characteristic
5	Type of leaf	Compound
6	Leaf arrangement	Alternate, imparipinnate
7	Shape	Large leaf-Ovate to Oblong Medium leaf-Lanceolate Small leaf – Deltoid
8	Size	Large leaf -10 cm long and 2 cm broad Medium leaf -5.5cm long and 2 cm broad Small leaf -2.9cm long and 2 cm broad
9	Surface	Finely pubescent
10	Apex	Large leaf-Attenuate (narrowly tapered thin) Medium leaf-Attenuate Small leaf-Acute
11	Leaf blades	Non-sheathing, Unifoliate or ternate
12	Base	Large leaf- asymmetrical Medium leaf- rounded and symmetrical Small leaf-asymmetrical
13	Number of leaflets	26-31 leaves
14	Venation	Reticulate
15	Stipules	Exstipulate
16	Margin	Entire with ciliated
17	Pinnate	Alternipinnate
18	Rachis	Present
19	Petiole	Swollen
20	Leaf area	1444.6 mm ²

Transverse Section of Petiole of *Averrhoa bilimbi* Leaves

The transverse section (T.S.) of the petiole in *Averrhoa bilimbi* shows an irregularly circular shape with a wavy outline. The adaxial and abaxial epidermis, both covered by a thick cuticle, contain unicellular trichomes, more abundant on the adaxial surface. Beneath the epidermis, 5 layers of collenchymatous cells and a broad parenchymatous ground tissue with pigment cells are observed. The centrally located, closed vascular bundle is surrounded by non-lignified pericyclic fibers, with radially arranged xylem and polygonal phloem cells. Prismatic crystals and brownish-red secretory cavities, particularly on the adaxial side, are present within the ground tissue.

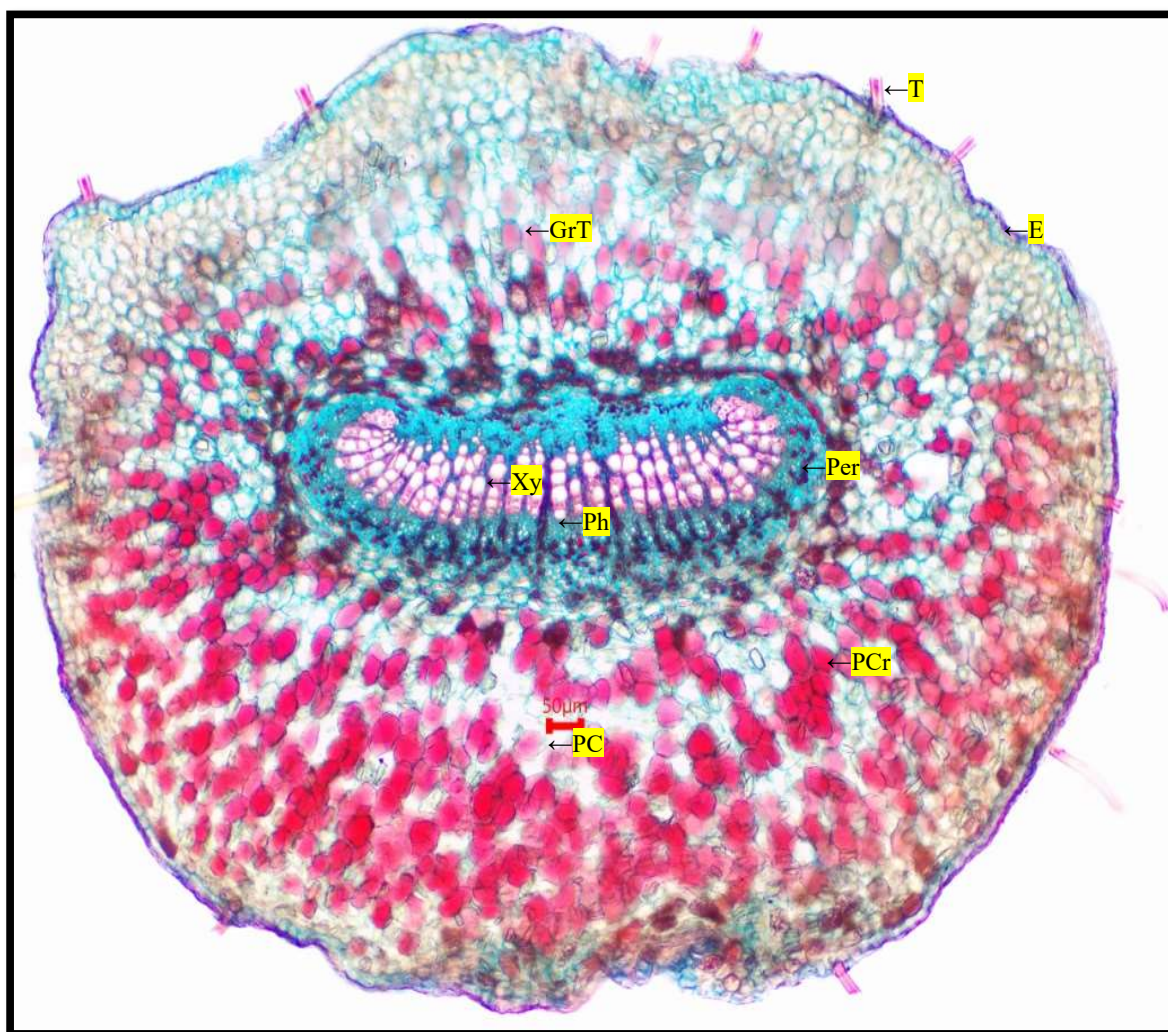


Plate 9:TS of *Averrhoa bilimbi* petiole

Ct - cortex; **E** - epidermis; **GrT** - ground tissue; **Pa** - parenchyma; **PC** - pigment cell; **PCr** - prismatic crystals; **Per** - pericycle; **Ph** - phloem; **T** - trichome; **V** - vessel; **Xy** - xylem

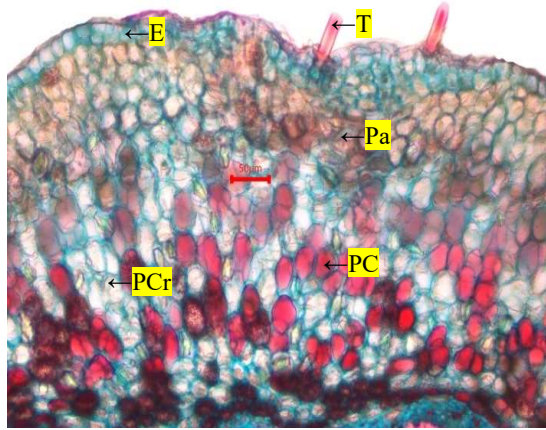


Plate 10: Upper portion enlarged

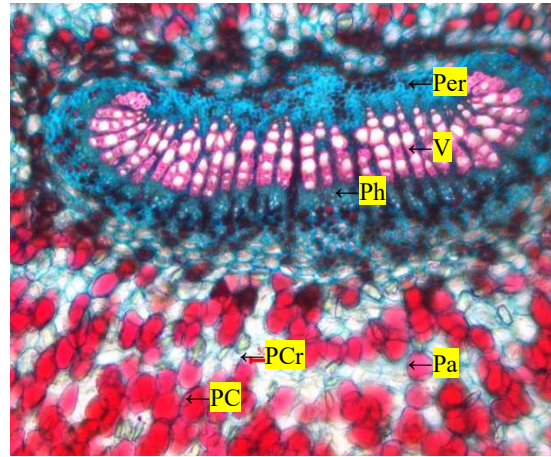


Plate 11: Middle portion enlarged

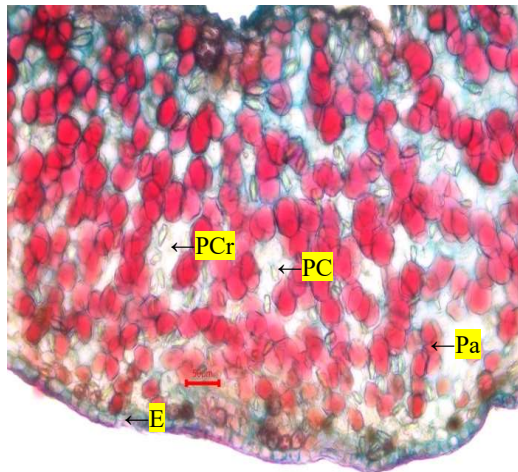


Plate 12: Lower portion enlarged

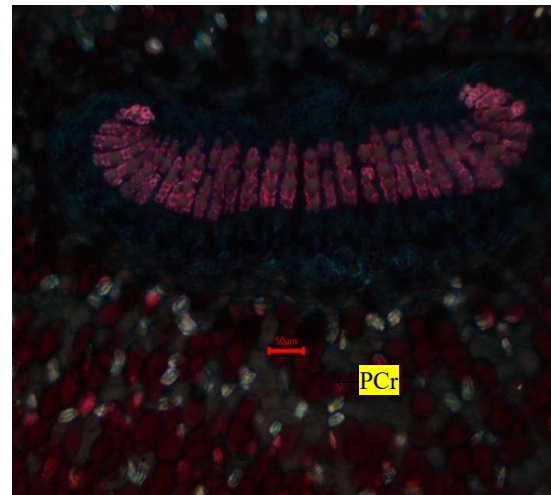


Plate 13: TS of petiole under polarized field

Ct - cortex; E - epidermis; GrT - ground tissue; Pa - parenchyma; PC - pigment cell; PCr - prismatic crystals; Per - pericycle; Ph - phloem; T - trichome; V - vessel; Xy - xylem

Transverse Section of Leaflet -Midrib of *Averrhoa bilimbi linn*

The adaxial and abaxial epidermis in *Averrhoa bilimbi* are single-cell layers with thick cuticles and unicellular trichomes. Beneath the adaxial epidermis are 3-4 layers of circular collenchyma cells, while a single collenchymatous hypodermis is present above the abaxial epidermis. The parenchymatous ground tissue has a large, crescent-shaped vascular bundle, covered by pericyclic fibers on both sides. The xylem, oriented towards the adaxial side, has smaller elements than the abaxial side, and the phloem, facing the abaxial side, contains pigment cells. Prismatic crystals, pigment cells, and starch grains (3-6 per cell) are scattered throughout the ground tissue and within the parenchyma and collenchyma.

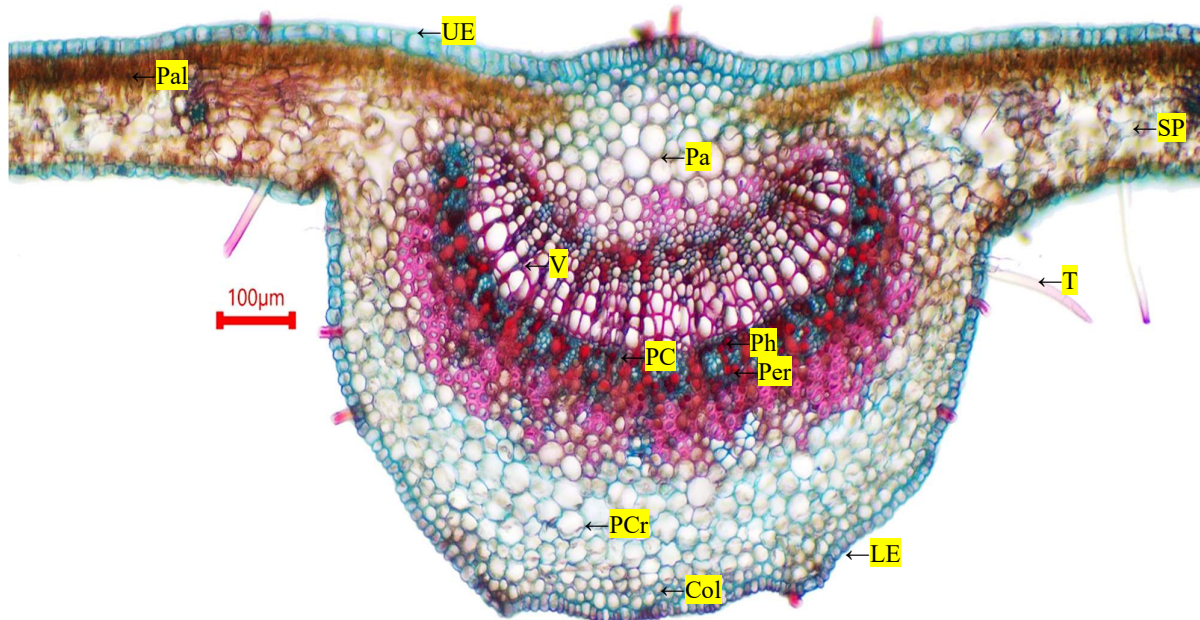


Plate 14: TS of *Averrhoa bilimbi* leaf passing through midrib

Col - collenchyma; **Cu** - cuticle; **LE** - lower epidermis; **Pa** - parenchyma; **PC** - pigment cell; **PCr** - prismatic crystals; **Pal** - palisade parenchyma; **Per** - pericycle; **Ph** - phloem; **SP** - spongy parenchyma; **SG** - starch grains; **T** - trichome; **UE** - upper epidermis; **V** - vessel; **Ve** - vein

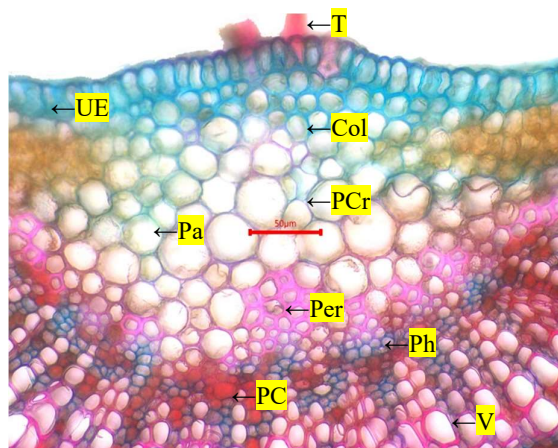


Plate 15: Midrib upper enlarged view

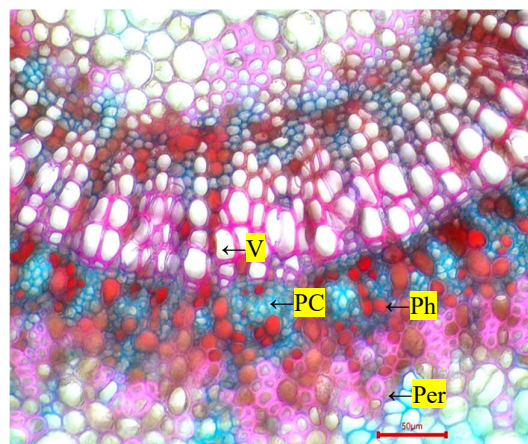


Plate 16: Enlarged view of vascular bundle

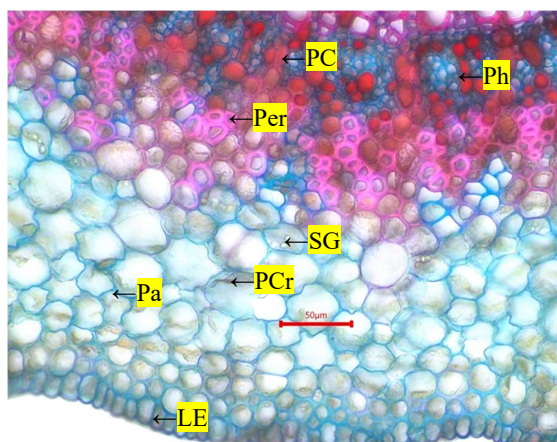


Plate 17: Midrib lower portion enlarged

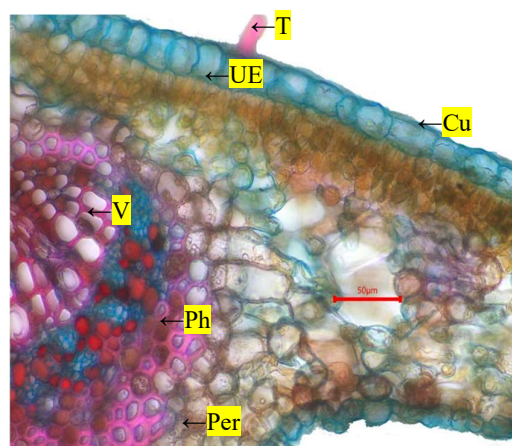


Plate 18: Laminar extension from midrib

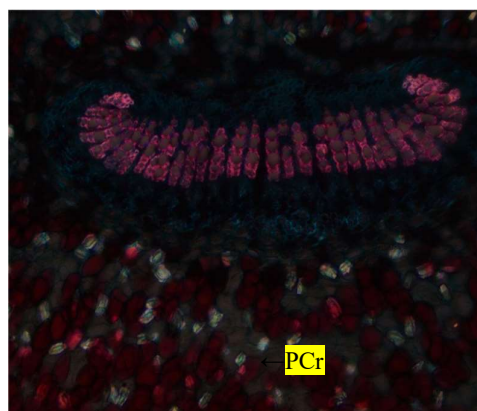
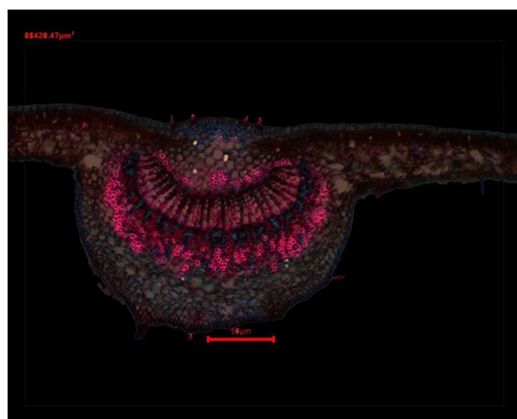


Plate 19: TS of leaf under polarized field

Col - collenchyma; Cu - cuticle; LE - lower epidermis; Pa - parenchyma; PC - pigment cell; PCr - prismatic crystals; Pal - palisade parenchyma; Per - pericycle; Ph - phloem; SP - spongy parenchyma; SG - starch grains; T - trichome; UE - upper epidermis; V - vessel; Ve – vein

Transverse Section of Lamina of *Averrhoa bilimbi* linn

The adaxial and abaxial epidermis consist of a single layer of cells covered by a cuticle, with a few simple, unicellular covering trichomes. The mesophyll tissue is differentiated into palisade cells and spongy parenchyma cells. Beneath the adaxial epidermis, there is a single layer of narrow, polygonal, thick-walled palisade cells. These palisade cells contain secretory cavities, which appear brownish in color. In the abaxial region, 3 to 4 layers of spongy parenchyma cells are present, with noticeable intercellular spaces. Veins traverse through the mesophyll tissue, and starch grains are also present within the cells.

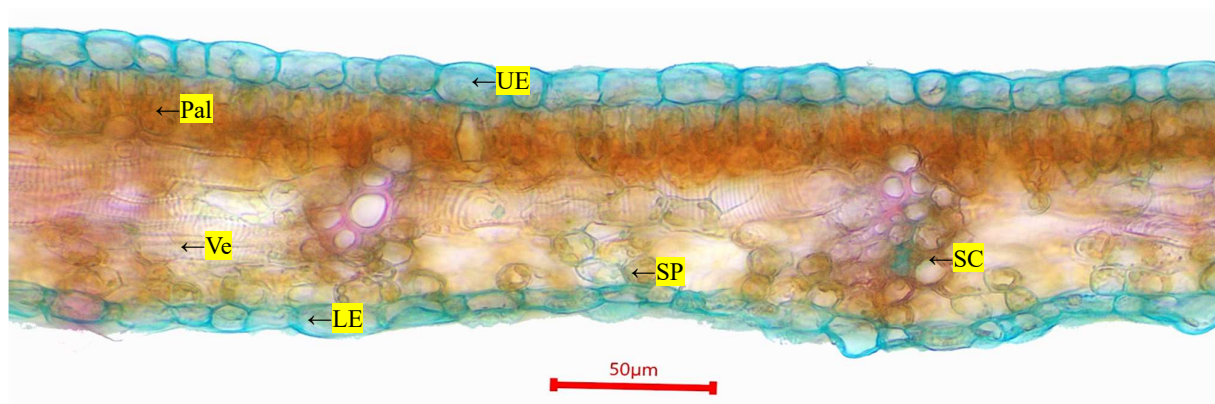


Plate 20: TS of lamina

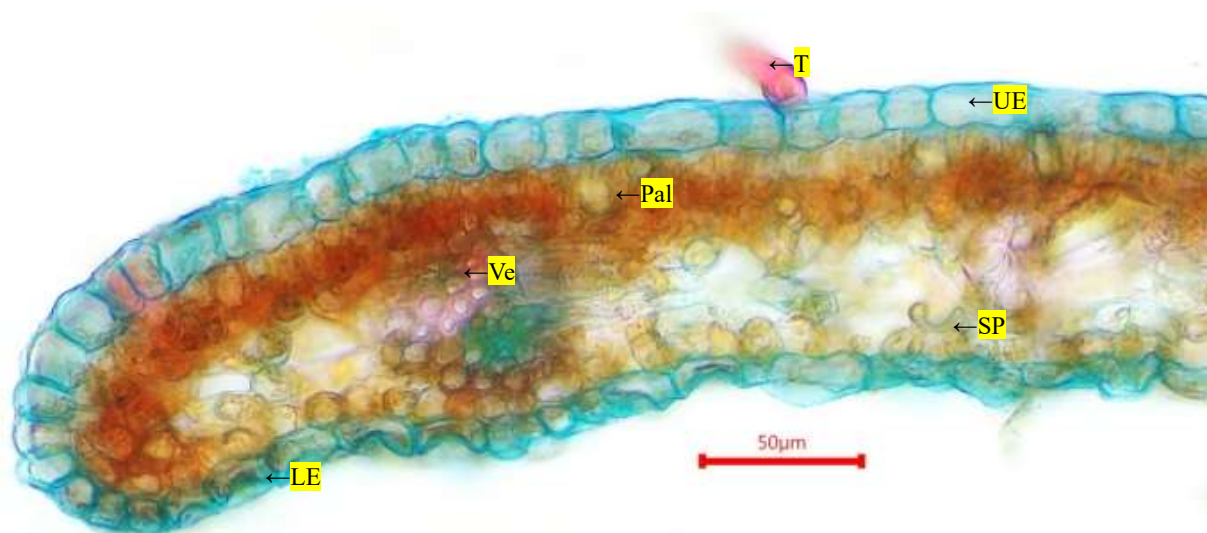


Plate 21: TS of leaf margin

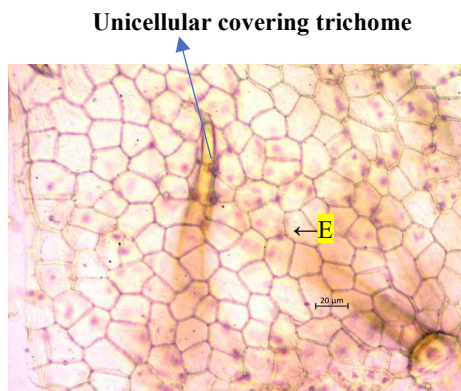
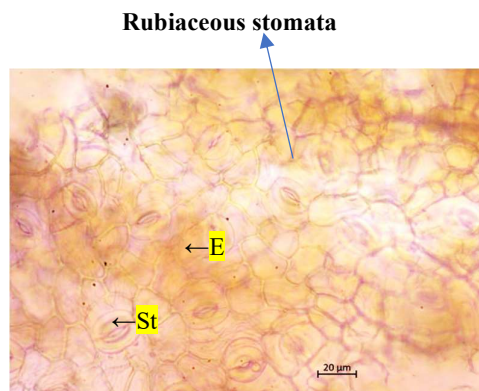
Col - collenchyma; **Cu** - cuticle; **LE** - lower epidermis; **Pa** - parenchyma; **PC** - pigment cell; **PCr** - prismatic crystals; **Pal** - palisade parenchyma; **Per** - pericycle; **Ph** - phloem; **SP** - spongy parenchyma; **SG** - starch grains; **T** - trichome; **UE** - upper epidermis; **V** - vessel; **Ve** - vein

Table 7: Microscopic characters of leaves of *Averrhoa bilimbi* linn

S.no	Transverse section	Microscopical characters
1	Petiole	<p>Epidermis formed of round cells with numerous simple unicellular covering trichomes.</p> <p>Epidermis followed by 5 layers of collenchyma cells succeeded by 7-8 layers of parenchyma cells</p> <p>Pigments cells are present in parenchyma, phloem and ground tissues.</p> <p>Closed vascular bundles consists of xylem and phloem, covered by pericyclic fibers.</p> <p>Prismatic crystals are found in ground tissues.</p> <p>Brownish or reddish secretory cavities are present in both adaxial and abaxial surfaces.</p>
2	Midrib	<p>Trichomes-simple covering trichomes are present in adaxial and abaxial surface.</p> <p>Adaxial epidermis cells are rectangular in shape, whereas abaxial epidermis is formed of synovate thick-walled cells.</p> <p>Epidermis followed by 3-4 layers of collenchymatous cells succeeded by parenchymatous cells.</p> <p>Vascular bundles consist of xylem and phloem, covered by pericyclic fibers.</p> <p>Pigments cells are scattered in ground tissues.</p> <p>Prismatic crystals are present in parenchyma cells and also scattered in ground tissues.</p> <p>Starch grains are present in collenchyma, parenchyma cells.</p>
3	Lamina	<p>Lamina shows adaxial and abaxial epidermis covered by thick cuticle.</p> <p>Epidermis is followed by well differentiated mesophyll tissue consisting of upper palisade parenchyma and lower spongy parenchyma with intracellular spaces.</p> <p>Papillose are seen in lower epidermis.</p> <p>Veins are transversing through the mesophyll tissue.</p> <p>Starch grains are also present in lamina.</p>

Table 8: Quantitative microscopy of *Averrhoa bilimbi* leaf

Parameters	Upper epidermis (/mm ²)	Lower epidermis (/mm ²)
Epidermal number	480 – 490	510 – 520
Stomatal number	-	70 – 80
Stomatal index	-	12 – 13
Palisade ratio		12-14
Vein islets number		25-30
Vein termination number		12-15

**Plate 22: Upper Epidermis****Palte 23: Lower Epidermis**

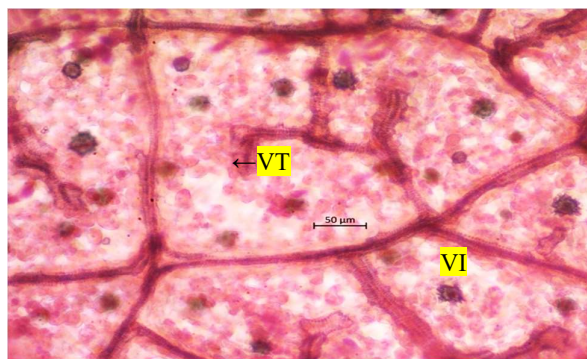


Plate 24: Vein Islets And Vein Terminations

E - Epidermis; St - Stomata; VI - Vein Islet; VT - Vein Termination

Powder Microscopy

The powder is dull green coloured with no characteristic odour and taste; shows the characters like simple covering trichomes, foliar upper epidermis devoid of stomata, lower epidermis with paracytic stomata, palisade cells in sectional view, thin-walled fibre, spiral vessels and prismatic crystals (**Plate 25**)

Plate 25: Powder microscopy of *Averrhoa bilimbi* leaf

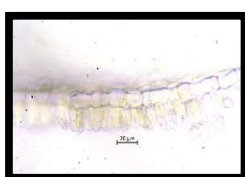
Trichomes



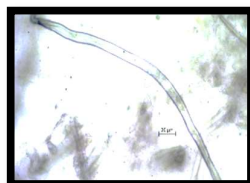
upper epidermis
devoid of stomata

lower epidermis
with stomata

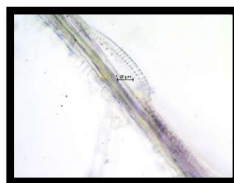
Sectional view of palisade



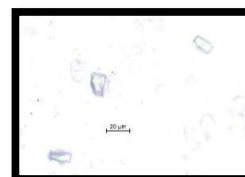
Fibre



Vessels



Crystals



Physiochemical Studies

Table 9: Organoleptic parameter of leaf powder of *Averrhoa bilimbi* Linn

S. No	Characters	Leaf powder of <i>Averrhoa bilimbi</i> Linn
1.	Color	Dark Green
2.	Odor	Characteristic odor.
3.	Taste	Sour
4.	Nature	Coarse powder

Table 10: Physiochemical constants of leaf powder of *Averrhoa bilimbi* leaf Linn

S.no	Physiochemical constants	Reports w/w%
1	Foreign Matter	Nil
2	Extractive value	
	Petroleum ether Extract	4.11±0.07
	Chloroform extract	1.96±0.09
	Toluene extract	3.13±1.0.07
	Ethyl acetate extract	0.46 ±0.07
	Ethanol extract	24.25 ± 0.19
	50% Ethanol extract	10.73 ± 0.27
	60% Ethanol extract	13.55 ± 0.19
	70% Ethanol extract	19.17 ± 0.18
	Aqueous extract	6.58 ± 1.76
3	Loss on Drying.	8.56±0.27
4	Ash value	
	Total Ash Value	9.92 ±0.06
	Water Soluble Ash Value	0.95±0.01
	Acid Insoluble Ash Value	4.62±0.21
	Sulphated ash value	3.11±0.06
5	Flow properties of the powder	
	Angle of repose	Θ = 29.43
	Carr's index	0.3024
	Hausner's ratio	1.0335

Values are expressed in means of **SEM** ±, Standard Error Mean

Qualitative analysis of inorganic elements and heavy metals in *Averrhoa bilimbi* linn Leaves

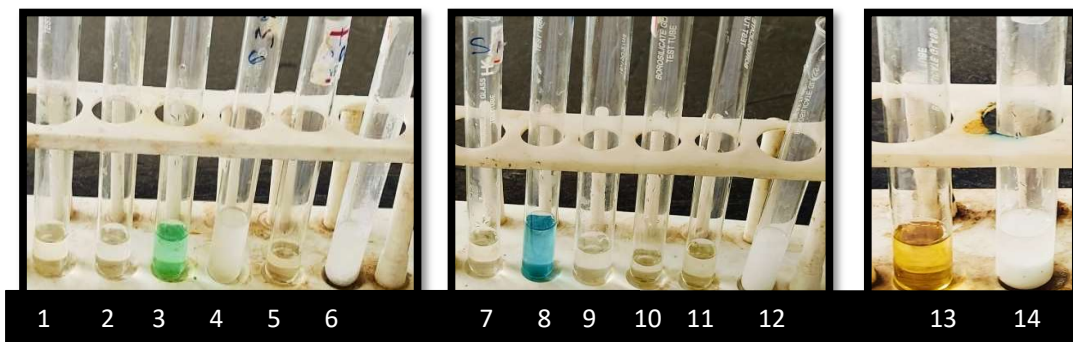


Plate 26: Qualitative analysis of inorganic elements and heavy metals in *Averrhoa bilimbi* linn Leaves

Table 11: Qualitative analysis of inorganic elements and heavy metals

S.no	Inorganic elements	Observations
1	Aluminum	-
2	Arsenic	-
3	Borate	-
4	Calcium	+
5	Carbonate	-
6	Chloride	-
7	Copper	-
8	Iron	+
9	Lead	-
10	Mercury	-
11	Phosphate	-
12	Potassium	+
13	Silver	-
14	Sulphate	+

The inorganic and heavy metal analysis reveals essential elements like calcium, iron, potassium, and sulphate beneficial for health, while the absence of harmful metals like arsenic, lead, mercury, and aluminium indicates the sample's safety and potential for consumption or formulation use.

DISCUSSIONS

Taxonomical revision

The *Oxalidaceae* family, commonly known as the wood-sorrel family, includes six to ten genera and around 900 species, predominantly distributed in tropical and subtropical regions, with some species extending into temperate zones. The genus *Averrhoa* comprising two species has been treated under the family *Oxalidaceae* of the order *Geraniales* by Engler & Prantl (1931), Takhtajan (1980), Cronquist (1981), Thorne (1981) and Dahlgren (1983). Earlier Benth (1862-1867) placed it in the tribe *Oxalidaceae* of the family *Geraniaceae*. But Hutchinson (1973) and Rouleau (1981) removed *Averrhoa* from *Oxalidaceae* and elevated it to the status of an independent family, *Averrhoaceae*, and placed it in the order Rutales and Geraniales, respectively.

The genus *Averrhoa* within the *Oxalidaceae* family, which includes small trees and shrubs like *Averrhoa bilimbi* (bilimbi) and *Averrhoa carambola* (starfruit), is notable for its edible fruits and unique traits that have piqued taxonomic interest. While some early classifications proposed placing *Averrhoa* in a separate family, *Averrhoaceae*, recent molecular and morphological studies affirm its place within *Oxalidaceae*. This includes similarities in floral and anatomical features and embryological traits, despite minor differences such as the presence of crassinucellate ovules in *Averrhoa* (Thathachar, 1942; Narayana, 1962). These ovules and the arborescent growth habit suggest that *Averrhoa* may be one of the more primitive lineages within *Oxalidaceae* (Kumari & Narayana, 1980) [7-9]. Phylogenetic research now aligns *Oxalidaceae* more closely with other members of the *Oxalidales* order, supporting the integrated approach of combining molecular data with classical morphology to deepen understanding of plant relationships, as emphasized by Bell et al. (2001) and the APG (2016). Thus, considering the various aspects, it is tentatively concluded that the separation of *Averrhoa* by Hutchinson (1973) and Rouleau (1981) from *Oxalidaceae* into an independent family, *Averrhoaceae* is not justified (D. Rama Devi et al., 1990) [10-14].

The pharmacognostical studies of *Averrhoa bilimbi* leaves, have been documented by Anitha Roy et al., 2011, Alisha Valsan et al., 2016, Gawli et al., 2021, Hari N et al., 2020. Upon comparison with our morphological observations, disparately similar with previous reports. Some unique characteristics were identified in our research reports -leaves were vary in shape, size, apex, and base. Large leaves measure 10 cm × 2 cm, medium leaves 5.5 cm × 2 cm, and small leaves 2.9 cm × 2 cm. Large leaves have an attenuate apex with a narrowed, thin tip and a symmetrical, rounded base. Medium leaves are attenuate with an asymmetrical base, while small leaves have an acute apex and an asymmetrically obtuse base. Both the dorsal and ventral surfaces of the leaves exhibit reticulate venation, consisting of primary, secondary, and tertiary veins. The primary veins contain 14 pairs, the secondary veins 5 pairs, and the tertiary veins 8 pairs. Additionally, we observed presence of minute glands at the midrib, which is covered by short, sharp and conical hairs. The species of *Averrhoa bilimbi* coincides *A.carambola*, *A.leucopetala* and *A.dolichocarpa* as per the chart of similarity. The leaf arrangement, shape of *Averrhoa bilimbi* is similar with that of *Averrhoa leucopetala* and *Averrhoa dolichocarpa*. The apex and base of the *Averrhoa bilimbi* is similar with *Averrhoa carambola*. As per the chart of dissimilarity *Averrhoa bilimbi* shows more dissimilar characters when compared with other species. Dissimilar characters of *Averrhoa bilimbi* with

other species are leaf length, petiole length, leaf surface, number of leaflets, number of veins. Taxonomical revisions of *Averrhoa* in India reveal a limited number of species i.e.) *Averrhoa bilimbi* and *Averrhoa carambola*. Macroscopic examination serves as a critical first step in plant identification and quality control in herbal medicine, ensuring the correct usage of plants for therapeutic purposes. This process is essential for maintaining the efficacy and safety of herbal treatments. When comparing the morphology of these two species, several differentiating characteristics emerge. Both *Averrhoa bilimbi* and *Averrhoa carambola* species have a similar leaf color, appearing green, and differences in shape and texture. *A. bilimbi* exhibits lanceolate-shaped leaves with a puberulous (finely hairy) texture, while *A. carambola* has ovate leaves with a smooth, glossy texture. The apex and base of the leaves in both species are similar, with an attenuate apex and asymmetrical base. The margin in *A. bilimbi* has ciliate, whereas *A. carambola* has an entire margin. Finally, the leaf area differs slightly between the *Averrhoa bilimbi* with *A. carambola* having a larger leaf area (1578.6 mm²) compared to *A. bilimbi* (1444.6 mm²). This comparative analysis highlights the distinct morphological differences between the two species, which can aid in their identification and classification in botanical studies. These morphological differentiations provide valuable insights for identifying and distinguishing between species, aiding in quality control and authenticity assurance in herbal products. Thus, cytomorphology is speculated the identification of taxonomical paradigm at species and genus level [9,10,11].

Glandular hairs with stalks of varying lengths and unicellular heads are reported to occur in *Averrhoa*. The stomata are rubiaceous, with at least one and sometimes two subsidiary cells parallel to the pore in *Averrhoa*. The vascular bundles of the veins are equipped with enlarged terminal tracheids. The fundamental vascular structure of both the stem and petiole consists of a circle of collateral bundles, often accompanied externally by a sclerenchymatous ring in the pericycle. In some species of *Oxalis*, the xylem of the stem tends to form a closed cylinder, correlating with a more woody habit. However, this character has developed even further in genera like *Averrhoa*, where the xylem and phloem of the petiole, as well as the stem, appear in transverse sections as practically continuous closed rings. As the family name suggests, oxalic acid is prevalent in the tissues, where it is believed to exist as dissolved potassium oxalate and as secreted calcium oxalate, typically in the form of small, solitary, cubical crystals (Metcalf and Chalk, 1950). The leaflets of *Averrhoa bilimbi* have epidermal cells with sinuous walls on both surfaces. SEM (Scanning Electron Microscopy) reveals that the epicuticular wax covering the epidermal cells in both species is of the squamous type, with crystalloids arranged in rosettes. Additionally, simple filiform non-glandular trichomes are present on both surfaces, though they are more abundant on the abaxial surface. Cross-sectional SEM analysis shows the midrib of *Averrhoa bilimbi* as having a plane-convex shape. Under optical microscopy, the epidermis of both species is uniseriate and covered with a thick cuticle. In *Averrhoa bilimbi*, beneath the adaxial epidermis are about two layers of collenchyma, followed by a collateral vascular bundle in the shape of an arch, surrounded by sclerenchyma. In both species, prismatic crystals can be observed in the phloem under optical and polarized microscopy. In *Averrhoa bilimbi*, the palisade parenchyma is composed of two layers of cells. Prismatic crystals are found in the mesophyll of *Averrhoa bilimbi* in both the palisade and spongy parenchyma (R.D. Sá et al., 2019). Our study identified variations in the shapes and sizes of epidermal cells in the petiole, midrib, and lamina. Special characteristics such as pigment cells, starch grains, and prismatic crystals are scattered throughout the ground tissues, and secretory canals are present in the lamina. In the lamina, the abaxial epidermis is papillose. The foliar anatomy of *Averrhoa bilimbi* leaves reveals several important microscopical features that coincide with the Statement of Metcalf and Chalk's. These findings exhibit interesting interspecific variations due to the environmental and genetic factors that emphasize to convict the area of phytotaxonomic revision of particular genus and species. The microscopic evaluation of leaf characters aids in the accurate identification of plant species, ensuring purity, authenticity, and quality control in herbal based products. It also provides insights into the leaf's functional adaptations and medicinal properties. Combining microscopic and macroscopic analysis plays a crucial role in the standardization and validation of herbal medicines, contributing to their safe and effective use.

Microscopic leaf constants including stomatal number, epidermal cell number, stomatal index, vein islet number and palisade ratio were demonstrated in above table. Determination of leaf constant number is also one of the useful tools in order to distinguish closely related species (Thimmerman et al., 1927). Stomata rubiaceous with at least 1 and sometimes 2 subsidiary cells parallel to the pore in *Averrhoa* (Metcalf and Chalk). Our study revealed that hypostomatic leaves with paracytic stomata. Finally powder microscopy was done to identify the microscopical structure for the purpose of authentication and to find the presence of adulterated substance [15-18].

The comparison of physiochemical parameters from previous studies with our current findings on *Averrhoa bilimbi* leaves provides key insights into their shelf life and quality. Important factors like moisture content, temperature, and environmental conditions greatly influence the longevity of medicinal plants. Moisture levels above 8% can lead to insect infestation, while levels exceeding 15% increase the risk of pathogenic contamination. According to the Ayurvedic pharmacopeia, total ash should not exceed 13%, water-soluble ash should be under 1%, and alcohol extractive values should be no less than 2.5%. Aforementioned studies by Alisha Valsan et al. (2016) and Akzeem et al. (2015) found the total ash content of *Averrhoa bilimbi* leaves to be 9.92%

and 10.54% w/w, respectively, with acid-insoluble ash at 5.25% and 6.44% w/w, and water-soluble ash at 0.96% w/w. The aqueous and ethanolic extractive values were 9.64% and 11.39% w/w. Our results closely align with these findings, indicating a consistency in the quality of *Averrhoa bilimbi* leaves. None of our values exceeded standard thresholds, suggesting a low risk of insect and microbial contamination, which enhances the plant's shelf life. Moreover, our findings confirm no contamination in the powdered drug, which is crucial for future studies. The flow properties of the powder are essential for product development, manufacturing, and quality control. A good flow property is indicated by an angle of repose between 25° and 30°, a Carr's index below 10, and a Hausner's ratio between 1.0 and 1.11 (Sharma et al., 2021). Our results fall within these ranges, indicating that *Averrhoa bilimbi* leaf powder has excellent flow properties. This analysis serves as a benchmark for the authentication of herbal drugs, aiding in the detection of adulteration and ensuring standardization. It highlights the importance of globally recognized guidelines for assessing the quality of medicinal plants and calls for the development of comprehensive monographs to ensure their standardization.

The inorganic and heavy metal analysis of the sample reveals the presence and absence of several elements that are crucial for understanding its chemical composition and potential health implications. The analysis indicates the presence of essential elements like calcium, iron, potassium, and sulphate. Calcium and iron are vital for biological functions, with calcium being crucial for bone health and iron playing a significant role in oxygen transport. Potassium is essential for maintaining fluid balance and nerve function, while sulphate is involved in various metabolic processes. The absence of harmful heavy metals like arsenic, lead, mercury, and aluminium indicates the sample's safety, as these metals are linked to severe neurological and cardiovascular damage. This lack of mercury, a known neurotoxin, further confirms its non-toxic nature. However, the overall absence of harmful heavy metals suggests that the sample may be safe for consumption or use in formulations, provided the essential nutrients present are utilized effectively [19,20].

CONCLUSION

Numerical taxonomy and DNA barcoding are effective tools for understanding species relationships, supporting medicinal plant discovery, and promoting sustainable biodiversity conservation. The present study highlights the pharmacognostic and physicochemical characteristics of *Averrhoa bilimbi* leaves Linn. These findings can serve as a valuable reference, offering diagnostic tools for standardization and identification of adulterants in future studies or applications. This information will be instrumental for further research and revalidation of its Phylogenetical approach and medicinal uses. The microscopic features identified could support the establishment of micromorphological standards, in alignment with WHO guidelines, for authenticating the original drug.

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