LEAF ARCHITECTURE OF THE AVERRHOA COLLECTION AT BOGOR BOTANICAL GARDEN

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Received: 11/01/2025, Revised: 30/01/2025, Approved: 02/02/2025

ABSTRACT

The Bogor Botanical Gardens (KRB) is one of Indonesia's key conservation sites, protecting a wide range of plant species, including the economically and ecologically valuable genus Averrhoa. This study aims to identify the morphological characteristics of the leaves of three Averrhoa species (A. bilimbi, A. carambola, and A. dolichocarpa) as a field identification tool. Morphological observations were conducted using visual inspection and digital microscopy. Data analysis involved Principal Component Analysis (PCA) and UPGMA cluster analysis. The results revealed that adaxial side coloration and lamina shape exhibit distinct traits among species, making them reliable identifiers for the genus Averrhoa. Other characteristics, such as abaxial side coloration and leaf margin, showed minimal variation. These findings demonstrate that leaf architecture can be used to identify Averrhoa species, contributing to taxonomy and biodiversity conservation. Further studies are recommended to expand the sample size and explore genetic correlations.

Keywords: Averrhoa, leaf architecture, PCA, biodiversity conservation

INTRODUCTION

The Bogor Botanical Gardens (KRB) is one of Indonesia's key plant conservation sites, playing a critical role in preserving biodiversity. Covering an area of 87 hectares at an elevation of 235–250 meters above sea level, the Bogor Botanical Gardens (KRB) serves as an ex-situ conservation site dedicated to the cultivation and preservation of diverse plant species, contributing to the protection of Indonesia's flora (Adhitya *et al.*, 2014). As a country with the highest biodiversity in the world, Indonesia bears significant responsibility for supporting global conservation efforts (Ramadhanti *et al.*, 2021). Accurate plant identification is crucial for biodiversity management, particularly in protecting rare and endangered species (Schmeller *et al.*, 2008; Jalonen *et al.*, 2014).

One of the key genera in Indonesia included in KRB's plant collection is *Averrhoa*. This genus, part of the Oxalidaceae family, comprises four main species: *Averrhoa carambola* (starfruit), *Averrhoa bilimbi* (cucumber tree), *Averrhoa dolichocarpa*, and *Averrhoa leucopetala* (Novianti and

Astuti, 2019). Starfruit (*A. carambola*), a species widely found in Indonesia, holds significant economic and ecological potential. It is commonly cultivated in home gardens for culinary purposes and as shade plants (Suriani and Sari, 2014). It thrives in tropical and subtropical regions, with some species in temperate climates (Raihandhany and Ramadian, 2021).

Species identification within the *Averrhoa* genus often relies on traits such as fruits, bark, or leaves. However, fruits are not always available, and bark characteristics can be challenging to distinguish between species, making leaves a more practical and consistent alternative for identification. Vegetative organ analysis, such as leaves, is frequently used in plant identification due to its distinctive features, which facilitate species recognition (Nasution, 2016; Felix *et al.*, 2020). Nonetheless, research on the morphological characteristics of leaves to differentiate species within this genus remains limited, presenting an opportunity to develop more effective identification methods.

This study focuses on exploring the morphological characteristics of leaves as tools for identifying three main *Averrhoa* species: *A. bilimbi*, *A. carambola*, and *A. dolichocarpa*. The study aims to differentiate these species and understand their taxonomic relationships by combining visual morphological observations with statistical analyses such as Principal Component Analysis (PCA) and cluster analysis. Leaf-based morphological identification offers a practical approach for documenting species, especially rare or seasonal species, that are challenging to observe through generative organs like fruits. The findings from this research could also enhance the documentation of plant collections at KRB. Understanding leaf architectural characteristics is expected to contribute to taxonomy and provide a scientific foundation for sustainable conservation efforts of tropical species.

METHOD

This research was conducted at the Bogor Botanical Gardens, and data analysis was conducted at the Research Center for Biosystematics and Evolution, BRIN. The observed species included *A. bilimbi, A. carambola,* and *A. dolichocarpa*. Leaf sampling was based on several justifications, considering ecological aspects, species representation, and the requirements of morphological analysis. Samples were collected from trees located in specific sites within the Bogor Botanical Gardens, namely plots VI.C.348, XVI.i.A.39-39a, and VI.C.310a, at 235–250 meters above sea level. The tools used in this study included pruning shears, a Dino-Lite digital microscope (AM9715 series), a computer, a ruler, black backdrop fabric, a smartphone camera, and writing instruments.

The research method involved observing and identifying the leaf architecture morphology of *Averrhoa* samples from the Bogor Botanical Gardens collection, followed by scoring and analyzing the leaf architecture traits of the studied species. The research was conducted in the following stages:

a. Determination of Averrhoa species

Averrhoa is part of the plant collection at the Bogor Botanical Gardens, with species distributed across several plots. Averrhoa plants have a tree-like habitus and compound leaves. The species whose leaf architecture traits were observed included *A. bilimbi*, *A. carambola*, and *A. dolichocarpa* (Table 1).

No.	Species	Origin	Location of sample
1.	A. bilimbi	Jawa	Vak. VI.C.348
2.	A. carambola	Kalimantan	Vak. XVI.i.A.39-39a
3.	A. dolichocarpa	Papua	Vak. VI.C.310a

Table 1. Species observed for their leaf architecture along with their plant origin and location

b. Leaf sampling

The collected leaves were carefully selected to ensure that only mature leaves with suitable phenotypes, representative of the observed species, were taken. Due to degradation, immature or overly old leaves may exhibit less accurate morphological traits, such as faded colour or altered shape. Five leaves were collected from the same tree for each sample to maintain accuracy and minimize variability between trees. Leaves were randomly selected from various tree parts to capture the natural morphological variation. By choosing leaves from specific locations and uniform environmental conditions, variability due to external factors was minimized, ensuring that the morphological differences truly reflect genetic variation between species rather than environmental differences. The leaf samples were then taken to the laboratory for further analysis.

c. Observation of leaf architecture characteristics

The leaves of the genus *Averrhoa* are compound leaves, which consist of several smaller leaflets connected to a single petiole (rachis) or main axis but collectively form a single leaf unit (Figure 1). Each leaf sample was observed for its leaf architecture characteristics using qualitative traits. The observed qualitative traits include the colour of the abaxial and adaxial surfaces, type of leaflet margin, type of leaflet venation, type of leaflet veins, type of leaflet apex, type of lamina, and type of leaflet base. The colour of the adaxial and abaxial leaf surfaces was observed visually. The types of leaflet lamina, apex, base, margin, venation, and veins were matched based on the literature from the Manual of Leaf Architecture by Ellis *et al.* (2009). The data from the observations were compiled in a Microsoft Excel worksheet.





Figure 1. A= A compound leaf shape; B = Observation of leaf apex and base (Ellis *et al.*, 2009).

The leaf apex constitutes 25% of the lamina. If the lamina has an apical extension (the distal tissue from the point where the primary vein ends), the apex covers all the distal tissue up to 0.75I_m, where I_m is the distance from the proximal tip to the distal tip of the central vein. The lamina shape is observed to determine typologies such as oval, lanceolate, or other characteristic forms. The evaluation of the leaf apex is based on categorizations such as acute, obtuse, or arcuate. Meanwhile, the leaf base covers the proximal 25% of the lamina. The leaf base is analyzed to identify its shape, such as rounded, acuminate, or arcuate. If the

lamina has a basal extension, the base includes all the proximal tissue up to 0.25I_m, where I_m is the distance from the proximal tip to the distal tip of the central vein (Ellis *et al.*, 2009).



Figure 2. A= Observation of leaf margin; B = Observation of leaf venation (Ellis *et al.*, 2009).

The margin (edge) of the leaf is the outer edge of the lamina. Generally, leaf margins can be either smooth or serrated. The shape of the indentations (sinuses) can be acute or obtuse, and similarly, the prominent parts (angles) can be sharp or blunt (Hadisunarno, 2013). Leaf serrations are marginal projections with sinuses that protrude less than 1/4 of the distance to the leaf's central vein or main axis. Leaf serrations can be dentate, serrated, or crenate. Generally, the primary (1°) and secondary (2°) veins are the main structural veins of the leaf, while the tertiary (3°) veins are the largest veins that form a network. The venation of each leaflet was observed three times at different secondary vein intersections. The venation or veins of the leaf were observed at the secondary vein intersections at 4, 6, and 8, with secondary vein intersections counted from the base of the leaf.

d. Documentation of each leaf sample

Each leaf sample was documented using a smartphone camera and a Dino-Lite digital microscope (series AM7915). The leaves were photographed comprehensively, capturing the adaxial and abaxial sides and in detail for the tip, base, edge, and venation using the smartphone camera. Meanwhile, more detailed images of venation or tertiary veins were captured using the Dino-Lite microscope. The image scale was calibrated using a ruler as a reference.

e. Data analysis

Cluster analysis and morphological similarity of leaves among species within the genus *Averrhoa* were conducted using a principal component analysis (PCA) approach through online tools (https://www.statskingdom.com/pca-calculator.html) and the UPGMA dendrogram (http://genomes.urv.cat/UPGMA/index.php#examples). The compiled data were scored and further processed using the PCA and UPGMA dendrogram tools. This analysis aimed to identify the distinguishing characteristics of a species by simultaneously utilizing multiple variables, including traits, characteristics, or symbols, which were measured based on the relationships between these variables and the observed objects (Anshori *et al.*, 2018). This method also aimed to determine a species' most defining morphological traits and assess the degree of similarity among the tested species (Rosdayanti *et al.*, 2019).

RESULTS and DISCUSSIONS

Averrhoa is a member of the Oxalidaceae family, distributed in tropical America and Southeast Asia (Damayanti *et al.*, 2020). The three *Averrhoa* species—*A. carambola*, *A. bilimbi*, and *A. dolichocarpa*—are found in Indonesia with different utilization potentials. *A. carambola* and *A. bilimbi* are valued as fruits and spices, while *A. dolichocarpa* is a wild starfruit with significant ecological value (Rugayah and Sunarti, 2008). Additionally, chemical compounds such as flavonoids, saponins, and tannins found in starfruit contribute significantly as traditional medicinal materials, highlighting the importance of conservation and further research on the biodiversity of these plants (Rachmawati *et al.*, 2017; Luthfianto and Marfuah, 2022).

The diversity of these starfruit species can be differentiated through flower, inflorescence, fruit, and leaf characteristics (Rugayah and Sunarti, 2008). As one of the main vegetative organs, leaf characteristics are the focus of species identification because they are easy to observe and possess distinctive features that differentiate species (Felix *et al.*, 2020). This study used leaf morphology analysis to distinguish the three *Averrhoa* species. Each species showed variations in the shape of compound leaves, leaf margin type, the colour of the adaxial and abaxial sides, and venation patterns, which support the accuracy of species identification.

All three species have a compound leaf type. A compound leaf consists of several leaflets, each resembling a simple leaf. At the base of the leaflets (petiolules), a leaf joint (pulvinulus) usually allows the leaflets to fall off one by one. Due to the similarity between the leaflets and simple leaves, distinguishing them can sometimes be challenging, especially if the leaflets are large. Observing compound leaves involves analyzing the number, arrangement (such as pinnate or palmate), shape, size, colour, and characteristics of the leaflet margins, tips, and bases (Latifa, 2016). In contrast, observing simple leaves is more unadorned as it only involves a single lamina and petiole (Ulinniam and Indriyani, 2022). The leaf architecture of *A. bilimbi*, *A. carambola*, and *A. dolichocarpa* can be seen in Table 2.

No	Leaf Character	A. bilimbi	A. carambola	A. dolichocarpa
1	Adaxial Side Color	Green	Dark Green	Green
2	Abaxial Side Color	Light Green	Light Green	Light Green
3	Leaflet Lamina Shape	Oblong	Ovate	Oblong
4	Leaflet Tip Type	Acuminate	Acuminate	Acuminate
5	Leaflet Base Type	Rounded	Rounded	Rounded
6	Leaflet Margin Type	Entire	Entire	Entire
7	Leaflet Venation Type	Pinnate	Pinnate	Pinnate
8	Vein Type	Weak	Weak	Weak
	71	brochidodromous	brochidodromous	brochidodromous

Table 2. Leaf architecture of Averrhoa

The observations of 15 samples from 3 *Averrhoa* species—*A. bilimbi, A. carambola*, and *A. dolichocarpa*—showed similarities in several characteristics. These similarities include the light green colour of the abaxial side of the leaves, the entire (smooth) leaf margins, pinnate venation, weak brochidodromous leaf veins, acuminate leaf tips, and rounded leaf bases. However, there were also variations among the species, such as differences in the adaxial side colour, which ranged from green to dark green, and variations in the lamina shape, ranging from ovate to oblong.

Based on the observations (Figure 3), in *A. bilimbi*, the leaflets are light green on the abaxial side and green on the adaxial side, with an oblong (elongated) lamina shape. In *A. carambola*, the leaflets are light green on the abaxial side and dark green on the adaxial side, with an ovate (oval) lamina shape. Meanwhile, in *A. dolichocarpa*, the leaflets are light green on the abaxial side and green on the adaxial side, with an oblong (elongated) lamina shape. Research by Mardhatillah & Djuita (2022) explains that the sweet starfruit (*A. carambola*) has compound leaves with an ovate (egg-shaped) form. According to Siagian (2023), the leaves of *A. bilimbi* are pinnate compound leaves that are oblong (elongated) in shape, with the upper side being green and the underside light green. Swandono (2021) also states that the leaflets of *Averrhoa* can vary from oval to elongated in shape.



Figure 3. Comparison of the overall appearance of leaves from the three *Averrhoa* species, (a) *A. bilimbi*, (b) *A. carambola*, and (c) *A. dolichocarpa*.

Based on the comparison of the adaxial leaflet tip types of the three species (Figure 4), it was found that *A. bilimbi*, *A. carambola*, and *A. dolichocarpa* share the same characteristic of having acuminate (pointed) leaflet tips. This result aligns with the observations of Yanti & Vera (2019), who also found that *Averrhoa* leaves are compound leaves with acuminate leaflet tips. Additionally, according to Puji (2017), the upper surface of the *Averrhoa* leaflets tends to have pointed or sharp tips.



Figure 4. Comparison of the leaflets' adaxial tip types of the three *Averrhoa* species, (a) *A. bilimbi*, (b) *A. carambola*, and (c) *A. dolichocarpa*.



Figure 5. Comparison of the leaflets' adaxial margin types of the three *Averrhoa* species, (a) *A. bilimbi*, (b) *A. carambola*, and (c) *A. dolichocarpa*.

The comparison of the adaxial leaflet margin types of the three species (Figure 5) shows that *A. bilimbi*, *A. carambola*, and *A. dolichocarpa* share the same characteristic, which is an entire (smooth) leaflet margin. This observation aligns with the findings of Mardhatillah & Djuita (2022), who stated that the sweet starfruit (*A. carambola*) has compound leaves with entire leaflet margins. Puji (2017) also revealed that the surface of *Averrhoa* leaflets, especially the upper side, generally has entire (smooth) margins.

The comparison of the adaxial leaflet base types of the three species (Figure 6) shows that *A. bilimbi, A. carambola,* and *A. dolichocarpa* share the same characteristic, which is a rounded (circular) leaflet base. This observation follows the findings of Swandono (2021), who stated that *Averrhoa* leaves are odd-pinnate compound leaves with rounded (circular) leaflet bases.



Figure 6. Comparison of the leaflets' adaxial base types of the three *Averrhoa* species, (a) *A. bilimbi*, (b) *A. carambola*, and (c) *A. dolichocarpa*.

The comparison of leaf venation and venation types in *Averrhoa* across the three species (Figure 7) shows that all three species share a pinnate venation type and weak brochidodromous venation (parallel). The pinnate venation type is characterized by the arrangement of the leaf veins on both sides of the leaf blade, with a single main vein extending from the base to the tip of the leaf, from which smaller veins branch off to the left and right. *Averrhoa* leaves are compound and pinnate, with leaflets arranged on either side of the main leaf petiole, typically having smaller leaflets (Wulandari, 2021). Sulasmi & Rachmawati (2013) explain that dicot plants generally have net-like venation. Still, there are exceptions where the leaf veins are arranged parallel, as seen in *Averrhoa* leaves, which feature secondary veins with a weak brochidodromous type (Hilwan & Maulidiyan, 2022).



Figure 7. Comparison of leaf vein and venation types of *Averrhoa* leaflets, (a) *A. bilimbi*, (b) *A. carambola*, and (c) *A. dolichocarpa*.

Cluster analysis using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram is used to group species based on qualitative characteristics of leaf architecture. According to Rachmatin (2014), cluster analysis aims to group individuals into distinct groups so that individuals within the same group are similar while individuals from different groups do not share similarities.

Cluster analysis was conducted through the Dendrogram UPGMA website, which generated a dendrogram grouping individuals based on the genetic similarity of the three species using eight qualitative characteristics of leaf architecture. The UPGMA method is the oldest and most commonly used method in phylogenetic tree reconstruction. The principle of this method is to combine individuals or organisms into one group based on the smallest pairwise distance between organism pairs. With a (CP) value of 1, this dendrogram accurately represents the pairwise distance data between species. The clustering displayed in the dendrogram indicates differences in specific characteristics among *A. bilimbi*, *A. carambola*, and *A. dolichocarpa* (Figure 8) (Susilo *et al.*, 2022).



Figure 8. Dendrogram of *Averrhoa* species based on leaf architecture characteristics, (A.b) *A. bilimbi*, (A.c) *A. carambola*, and (A.d) *A. dolichocarpa*.

Principal Component Analysis (PCA) is one of the most commonly used methods for dimensionality reduction. This statistical technique can help reduce the dimensionality of a data set. PCA is a multivariate analysis method that uses linear transformation and is often used to

extract important information from large data sets and analyze variables' structure (Ritonga & Muhandhis, 2021). PCA aims to extract key information from the data and organize it into new variables known as principal components. Additionally, PCA summarises data by reducing the number of existing variables (Khikmah, 2021).

Parameter	PC ₁	PC ₂	PC ₃	PC ₄	PC ₅	PC ₆	PC ₇	PC ₈
Eigenvalue	2	0	0	0	0	0	0	0
% of Variance	100	0	0	0	0	0	0	0
Cumulative (%)	100	100	100	100	100	100	100	100

Table 3. Eigenvalues variasi karakter arsitektur daun Averrhoa

The eigenvalues in the Principal Component Analysis (PCA) are presented in Table 1. Generally, the assessment is considered good if the cumulative variance proportion is within 70-90% (Rasyid *et al.*, 2021). In this table, only the first principal component (PC1) has an eigenvalue of 2, while the eigenvalues for the other components, from PC2 to PC8, are 0. These results indicate that all the variance in the data can be fully explained by the first principal component, which is also reflected in the variance percentage of 100% for PC1. The other components do not explain any variance, with a variance percentage of 0%. It suggests that PC1 can fully represent the variance between species, while the other components do not contribute meaningfully to the data variability. The cumulative percentage shows that the total variance explained remains 100% for all components, emphasizing that only PC1 is relevant in explaining the data variability. In contrast, the other components do not make a meaningful contribution.



The Scree Plot is an important Principal Component Analysis (PCA) tool. It determines the number of principal components that should be retained in the analysis. In this plot, the eigenvalue of each principal component is mapped to identify the point where the contribution of the components starts to decline significantly. Based on the Scree Plot (Figure 9), the other components have very low or zero eigenvalues, reinforcing the decision only to consider PC1 as the significant component in the analysis.



Figure 10. The tendency of species group distribution based on leaf architecture characteristics: (a) adaxial leaflet colour, (b) abaxial leaflet colour, (c) leaflet lamina, (d) leaflet margin, (e) leaflet venation, (f) leaf veins, (g) leaflet tip, and (h) leaflet base.

The distribution of species groups was based on leaf architectural characteristics with various attributes, including the colour of the adaxial leaflet, the colour of the abaxial leaflet, leaflet lamina, leaflet margin, leaflet venation, vein structure, leaflet tip, and leaflet base. A deeper interpretation of the PCA results shows that the colour of the adaxial side of the leaf and the lamina shape are the main characteristics contributing to the most significant variance in grouping the Averrhoa species. The significance of these characteristics is evident from their distinctly different data distribution (Figure 10). In contrast, other characteristics, such as the colour of the abaxial side, leaf margin type, and leaf venation type, exhibit minimal variation, making them less relevant for species differentiation. This pattern suggests that the variation between species in these aspects is relatively low, providing insufficient information to distinguish one species from another.

The combination of PCA and the dendrogram reinforces the importance of multivariate analysis in identifying the most informative morphological traits. Dominant characteristics such as adaxial colour and lamina shape distinguish species more clearly and provide a foundation for species grouping that can be utilized in taxonomic studies and conservation efforts. These findings align with the notable colour and leaf structure differences among the studied species. Table 4 provides a quantitative summary of the results of the analysis. Therefore, this analysis not only offers insights into the morphological diversity of the leaves but also can aid in managing and conserving species based on their physical characteristics.

Table 4. Comparison of adaxial color an	d lamina shape between Averrhoa species
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Morphological Character	A. bilimbi	A. carambola	A. dolichocarpa
Adaxial Color	Green	Dark Green	Green
Lamina Shape	Oblong	Ovate	Oblong

The study by Rosdayanti *et al.* (2019) and Luthfianto & Marfuah (2022) found that the leaf architectural characteristics of *Averrhoa* exhibit variation in both adaxial and abaxial colours, as well as leaf margin and tip types, which are consistent with the results of this research. The finding that adaxial colour plays a more dominant role in distinguishing species also aligns with existing literature, highlighting the striking differences in leaf colour among *Averrhoa* taxa. Additionally, variation in lamina shape and leaf venation type is commonly used for species grouping, which is reflected in this study's findings.

While some characteristics, such as abaxial color and leaf tip type, show limited variation among species, these findings are consistent with previous studies that indicate the primary differences in *Averrhoa* species identification are often found in adaxial colour and lamina shape (Khikmah, 2021). Therefore, the results of this study reinforce existing knowledge and contribute to mapping morphological characteristics that can be utilized for the taxonomic identification and conservation of *Averrhoa* species.

CONCLUSIONS AND RECOMMENDATIONS

The leaf architectural characteristics of *Averrhoa* species can be reliably used to distinguish between species by examining various morphological aspects, including the shape and colour of the adaxial and abaxial sides, leaf lamina, leaf margin, venation, veins, leaf tip, and leaf base. Based on cluster analysis and Principal Component Analysis (PCA), these traits have effectively differentiated species, such as Averrhoa bilimbi and Averrhoa carambola. However, some overlapping or similar traits were observed among the species, particularly regarding leaf tip and vein patterns. The most significant contributors to the grouping of leaf architecture include the adaxial leaf surface's colour and the leaf lamina's structure, which showed clear differentiation between species. Despite some overlap, these characteristics provide a valuable framework for the taxonomic classification of *Averrhoa*.

The observed morphological characteristics can serve as a foundation for taxonomic studies of *Averrhoa*. Further research should focus on genetic analysis to complement the morphological data, helping to clarify species differentiation significantly where traits overlap. Expanding the sample size across different populations will also strengthen the findings.

ACKNOWLEDGEMENT

Gratitude is extended to the Bogor Botanical Garden, the National Research and Innovation Agency (BRIN), and the Arcadia Millennium Seed Bank Partnership RBG Kew for providing the facilities and infrastructure used during the research activities. Special thanks are also given to the academic advisors and colleagues who assisted throughout the research. May the results of this study benefit the readers.

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