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continued on the back inside cover

Cover: The nine vultures of India, digital art made on Krita by Dupati Poojitha.

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COMMUNICATION

Leaf architecture of threatened *Aquilaria cumingiana* (Decne.) Ridley and *Aquilaria malaccensis* Lam. (Thymelaeales: Thymelaeaceae) using morphometrics analysis

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Abstract: Due to a very limited number of scientific studies on the morphology of the very closely related threatened species, *Aquilaria cumingiana* and *Aquilaria malaccensis,* it is very challenging to identify them thoroughly. The leaf architecture was studied in *A. cumingiana* and *A. malaccensis* of the family Thymelaeaceae. Quantitative and descriptive methods were used to assess 21 leaf-traits of *A. cumingiana* and *A. malaccensis*. The study indicated that 10 leaf traits, such as base shape, apex shape, secondary vein spacing, tertiary vein angle category, tertiary vein angle to primary, quaternary vein, venation pattern, laminar shape, base angle, and apex angle, are important for identifying, and distinguishing the leaf architecture of *A. cumingiana*, and *A. malaccensis*. This study highlights the importance of leaf morphology and venation patterns in identifying and differentiating *A. cumingiana* and *A. malaccensis*.

Keywords: Dendrogram, leaf apex, leaf base, leaf blade, leaf morphology, leaf shape, leaf venation, trichomes.

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Author contributions: RLRG—research design, paper conceptualization, data collection, data analysis, writing and editing the manuscript. CCE— paper conceptualization, data analysis and writing the manuscript. GRAL—research design, paper conceptualization, data analysis, writing and editing the manuscript, and corresponding journal submission.

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INTRODUCTION

Agarwood-producing species, specifically the *Aquilaria* spp. in the Thymelaeaceae family, are primarily distributed in the Asian region (Li et al. 2023; Xie et al. 2024; Bora et al. 2025). The *Aquilaria* genus has 21 species, of which 13 species are reported to be agarwood producers (Lee & Mohamed 2016; Xie et al. 2024). They produce agarwood in their trunks and primary branches due to wounding by worms, lightning or wind-broken branches, natural microbial or fungal infections, or infections that are artificially induced by drilling holes, cutting the bark, and injecting chemicals (Jim 2015; Azren et al. 2019; Wang et al. 2020).

The infection court of the fungal infection of *Aquilaria* spp. is in the heartwood, where *Aquilaria* spp. would generate a high commercial value (Zhang et al. 2024). The increase in levels of trade over the past decade has resulted in overexploitation throughout the range of this species (Chowdhury et al. 2024; Xie et al. 2024). Despite the challenges, such as illegal harvesting in the wild, it is difficult to cultivate *A. malaccensis* due to its sensitivity index in terms of survival rate and environmental conditions where this species is compatible (Kharnaior & Thomas 2021; Latifah et al. 2024).

Aquilaria cumingiana and *A. malaccensis* are two closely related species of the family Thymelaeaceae. Globally, *A. malaccensis* was categorized as 'Critically Endangered' while *A. cumingiana* was categorized as 'Vulnerable' in the IUCN Red List (Harvey-Brown 2018). These *Aquilaria* sp. are considered as a problematic species in terms of species identification due to lack of scientific studies on species identification. Using leaf architecture is one way of baseline identification of the species (Mercado et al. 2024). Leaf architecture refers to the form and position of elements in leaf structure, including venation pattern, marginal configuration, and leaf shape. Maulia & Susandarini (2019) reported that venation patterns show significant differences in leaf architecture that distinguish the closely related species of *Aquilaria.*

In the present study, the leaf architecture in *Aquilaria cumingiana* and *A. malaccensis* was examined. This study aimed to evaluate the role of leaf architecture in species identification of *A. cumingiana* and *A. malaccensis* growing in Mindanao areas. To date, there is no published report on the characterization of leaf architecture of *A. cumingiana* and *A. malaccensis* as useful taxonomic evidence, especially for species identification.

MATERIALS AND METHODS

Study area: Samples of plant materials were obtained from two provinces in Mindanao, Philippines. *A. cumingiana* leaf samples were collected from Davao Oriental, while *A. malaccensis* leaf samples were collected from Agusan del Sur (Image 1). These two species were later propagated in a backyard nursery situated in Makar, Baliok, Toril, Davao City, Davao del Sur, Philippines (Figure 1). Laboratory analysis of collected leaf material was performed at the Forestry Laboratory of the University of Mindanao, Matina Campus, Davao City, Davao del Sur, Philippines (Image 2). Data were analyzed on 01 August 2022.

Material collection

Materials used in this study were leaves from seedlings of *A. cumingiana* and *A. malaccensis* collected from the two provinces, Davao Oriental, and Agusan del Sur. There were 30 juvenile leaves of each species of *A. cumingiana* and *A. malaccensis* collected for the statistical data analysis. Some of the leaves was added to the herbaria collection for the taxonomical evidence. The leaves of each species were collected from different provenances. Foresters and a local parataxonomist confirmed the identification of tree species. The herbaria were deposited in the Department of Forestry of the University of Southeastern Philippines – Mabini Campus.

Leaf architecture traits

There were 21 leaf architectural traits employed in this study, covering both general morphological traits and detailed venation features. Traits such as base shape, apex shape, laminar shape, and angles (base and apex) describe the overall form of the leaf, while traits like tooth apex, lobation, marginal development, and leaf margin account for edge modifications. Venation–related traits, including primary to quaternary vein categories, vein spacing, and venation pattern, provide critical information on vascular architecture, which is highly diagnostic in distinguishing species. Additionally, the areole and laminar blade contribute to identifying structural variations at finer scales. These traits follow the standardized classification of leaf architecture proposed by Hickey (1973) and further refined in the Manual of Leaf Architecture by Ellis et al. (2009).

Measurement

The leaf architecture data were recorded based on manual leaf architecture (Table 1) with modifications and

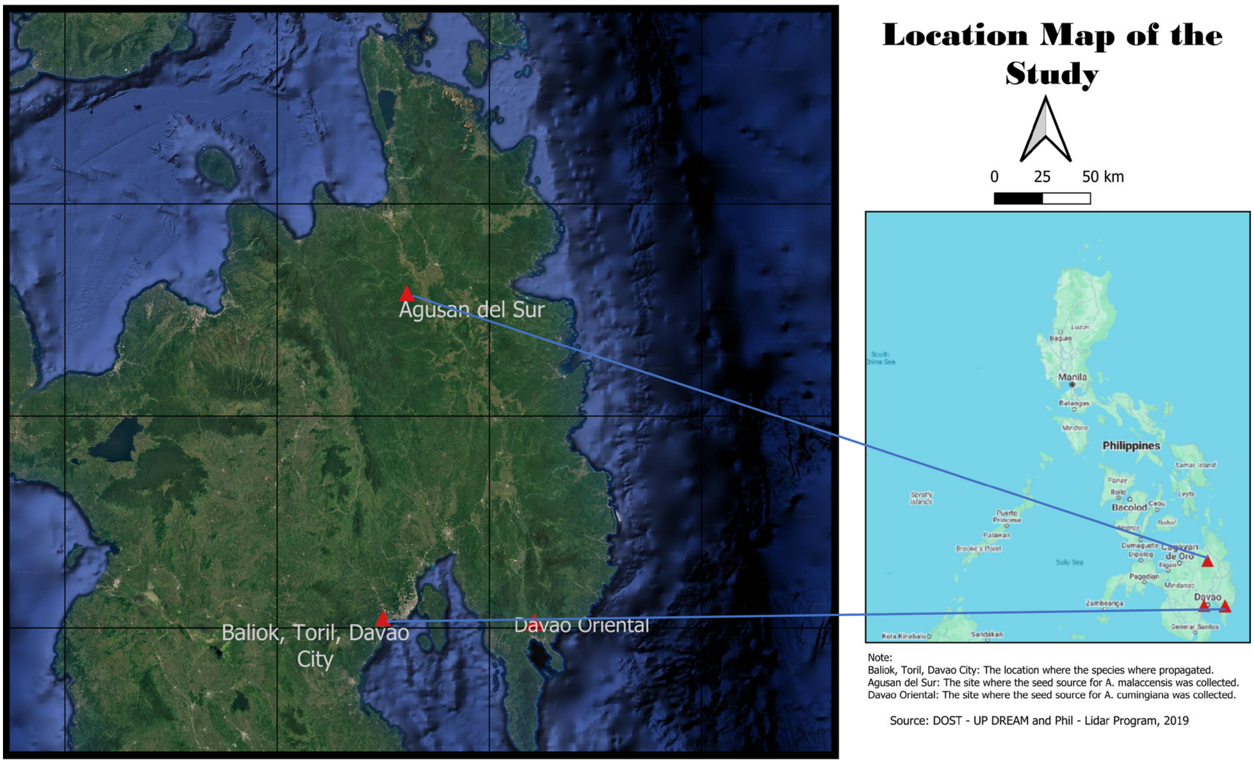

Image 1. The location map shows where the Aquilaria species propagated and the areas where the species were collected.

several additional traits developed by the Smithsonian Institution (1999). The general morphological traits (laminar shape, base, apex, margin, lobation, leaf size, and area) of *A. cumingiana and A. malaccensis* were measured using ruler, calipers, and image analysis (Hickey 1979). Venation traits were examined under compound OptiLab microscope camera for digital image capturing.

Analysis: Evaluating the leaf architecture in *A. cumingiana and A. malaccensis* was analyzed to cluster analysis using the PAST (Paleontological Statistics) software version 3.23 to determine the hierarchical relationships among the different species variations.

RESULTS AND DISCUSSION

Leaf architecture of *Aquilaria cumingiana*

Leaves of *A. cumingiana* were alternate and simple in terms of leaf attachments (Image 3a). Laminar shape was lanceolate, with laminar size varying 754–5,600 mm (Image 3b). The leaves are symmetrical, glabrous, cuneate, entire, acute both in leaf shape, base angle, apex shape, and apex angle (Image 3a–e). The leaf texture was smooth and shiny, light green in colour, while the leaf margin was untoothed, and no distinguished lobation (Image 3). The leaf venation was pinnate, weak in primary vein size, regular polygonal reticulate, vein spacing increasing towards the base (Image 3). The primary venation is straight to slightly curved (Image 3f–g,i), the secondary venation is festooned semi-craspedodromous, secondary vein angle uniform (Image 3g), and the tertiary venation is opposite percurrent (Image 3h–m). The areolation and the quaternary venation were not observed. The marginal development was arranged in a looped formation (Image 3i). There were variations in midrib width, marginal vein width, and the blade class. Trichomes in the laminar area were observed, but strong evidence is required (Image 3i–j).

Leaf architecture of *Aquilaria malaccensis*

Aquilaria malaccensis displays its variation in terms of leaf architecture as compared to *A. cumingiana.* The leaves of *A. malaccensis* were alternate, simple, lanceolate, symmetrical, acute, obtuse, acuminate, entire, glabrous, untoothed, and no lobation (Image 4a–e). The venation characteristics of *A. malaccensis* are pinnate, weak, reticulodromous, straight to slightly curved for the primary vein course, with irregular venation spacing (Image 4f). The secondary vein category is semi-craspedodromous, the tertiary vein is categorized as random, while the quaternary vein is dichotomizing

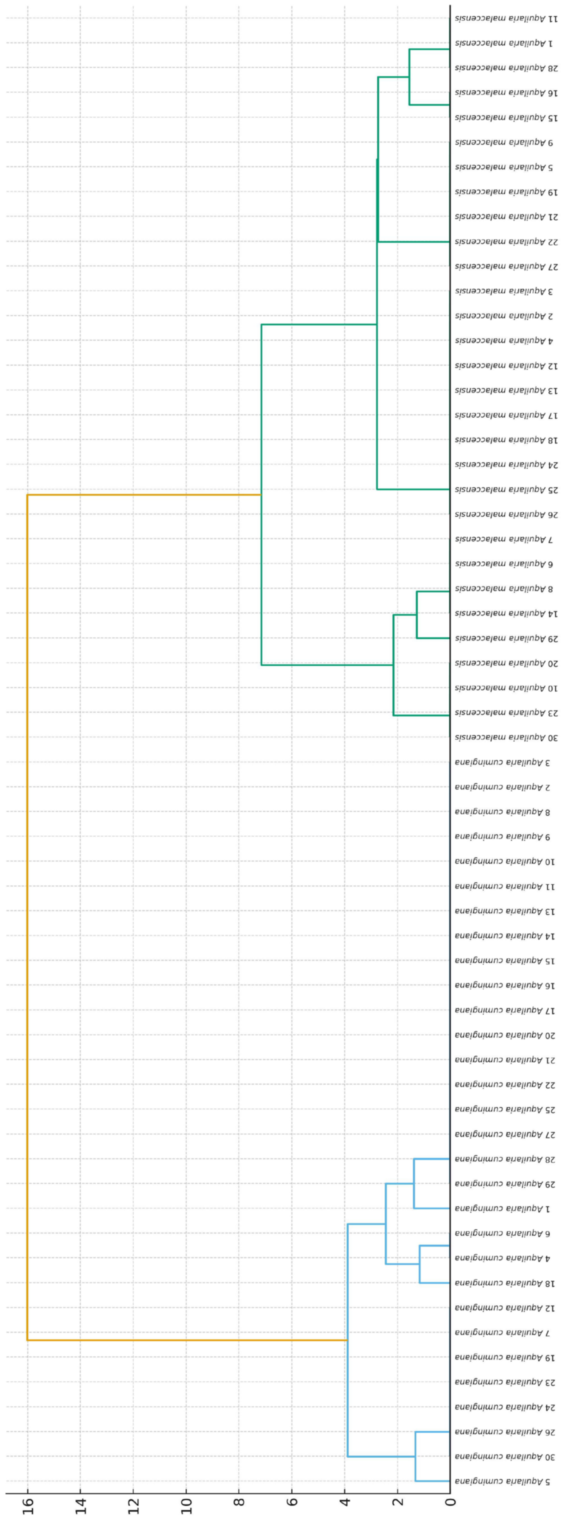

Figure 1. Dendrogram of *Aquilaria cumingiana* (left) and *A. malaccensis* (right) samples based on 21 leaf architectural traits used in this study, using PAST (Paleontological Statistics) software version 3.23.

(Image 4). The areolation was not observed, while the marginal development was looped (Image 4h–k). There was a notable occurrence of trichomes in the below leaf surface (Image 4i). This result has a similarity assessment to the study of Maulia & Susandarini (2019) on the leaf architecture of *A. malaccensis.*

Variations between *Aquilaria cumingiana* and *Aquilaria cumingiana*

The dendrogram (Figure 1) clearly distinguishes *A. cumingiana* from *A. malaccensis* based on 21 leaf architectural characteristics, with *A. cumingiana* forming a compact cluster that reflects its morphological uniformity, while *A. malaccensis* displays broader sub-clustering, indicative of greater intraspecific variation. The correlation (Figure 2) further shows that only 10 traits strongly influenced this clustering, particularly base shape, apex shape, and venation-related traits such as secondary, tertiary, and quaternary vein categories, while other traits like leaf margin, lobation, and tooth apex contributed little to species identification. These results highlight that venation and lamina form are the most reliable diagnostic features for separating the two *Aquilaria* sp.

Summary of key findings

The comparative study of leaf architecture in *A. cumingiana* and *A. malaccensis* is important for their morphological and taxonomic identification. These species have smooth texture and pinnate venation that includes festooned semi-craspedodromous secondary veins, and a symmetrical, and lanceolate lamina. The stable morphological profile suggested by the invariant features in the sample over different times could be the result of the adaptation to an ecological niche.

Aquilaria malaccensis has higher leaf variability. The secondary venation, mostly dichotomous, but there is also random tertiary venation with possible irregular spacing, arc venation, and other morphological plasticity, is a testament to its greater morphological plasticity. The presence of trichomes under the *A. malaccensis* leaves (as opposed to the smooth surface of *A. cumingiana*) could also be an adaptation to different environmental pressures.

Cluster analysis of 21 traits of leaf form revealed clear taxonomic separation between *A. cumngiana* and *A. malaccensis*. From this, it could be concluded that the variation in *A. malaccensis* is driven to a greater extent, suggesting that genetics or environment has a greater effect on the morphology of these specimens. These findings underscore the importance of leaf architecture

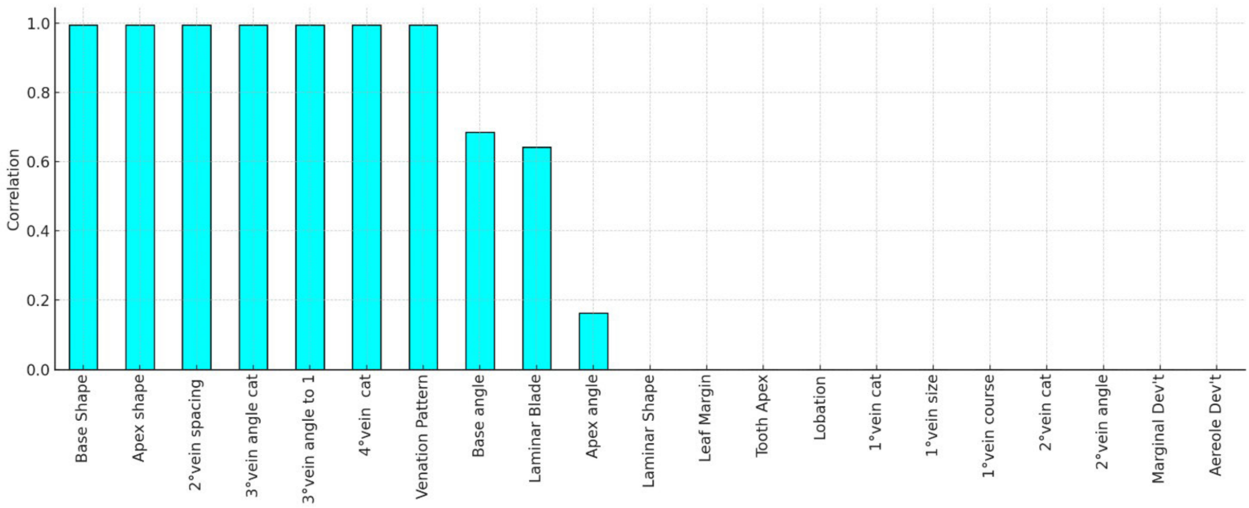

Figure 2. Correlation variation in *Aquilaria cumingiana* and *A. malaccensis*, using PAST (Paleontological Statistics) software version 3.23.

Image 2. Examination of venation pattern using photo microscope (Olympus CH40). © RL Germo.

in distinguishing closely related species, particularly where morphological similarities blur taxonomic boundaries.

The fixed differences were observed in 10 characteristics, including laminar blade, base angle, and apex angle between both species. These dissimilarities suggest that these characteristics could serve as diagnostic markers for taxa identification. While the fixed nature of other traits reinforces the genealogical relationship among these species, morphological divergence may result from ecological divergence but may reflect genetic divergence.

CONCLUSION

This study underscores the relevance of a comprehensive leaf architectural study toward the identification of closely related species in the genus *Aquilaria*. The study suggests that *A. malaccensis* is more morphologically variable compared to *A. cumingiana* and is likely to have a broader ecological amplitude or population genetic diversity. In contrast, the stable morphology observed in *A. cumingiana* suggests a stable taxonomic relationship that may be dictated by particular environmental demands. These findings serve as original data for taxonomic identification and for the conservation and sustainable management of these economically valuable agarwood-producing species.

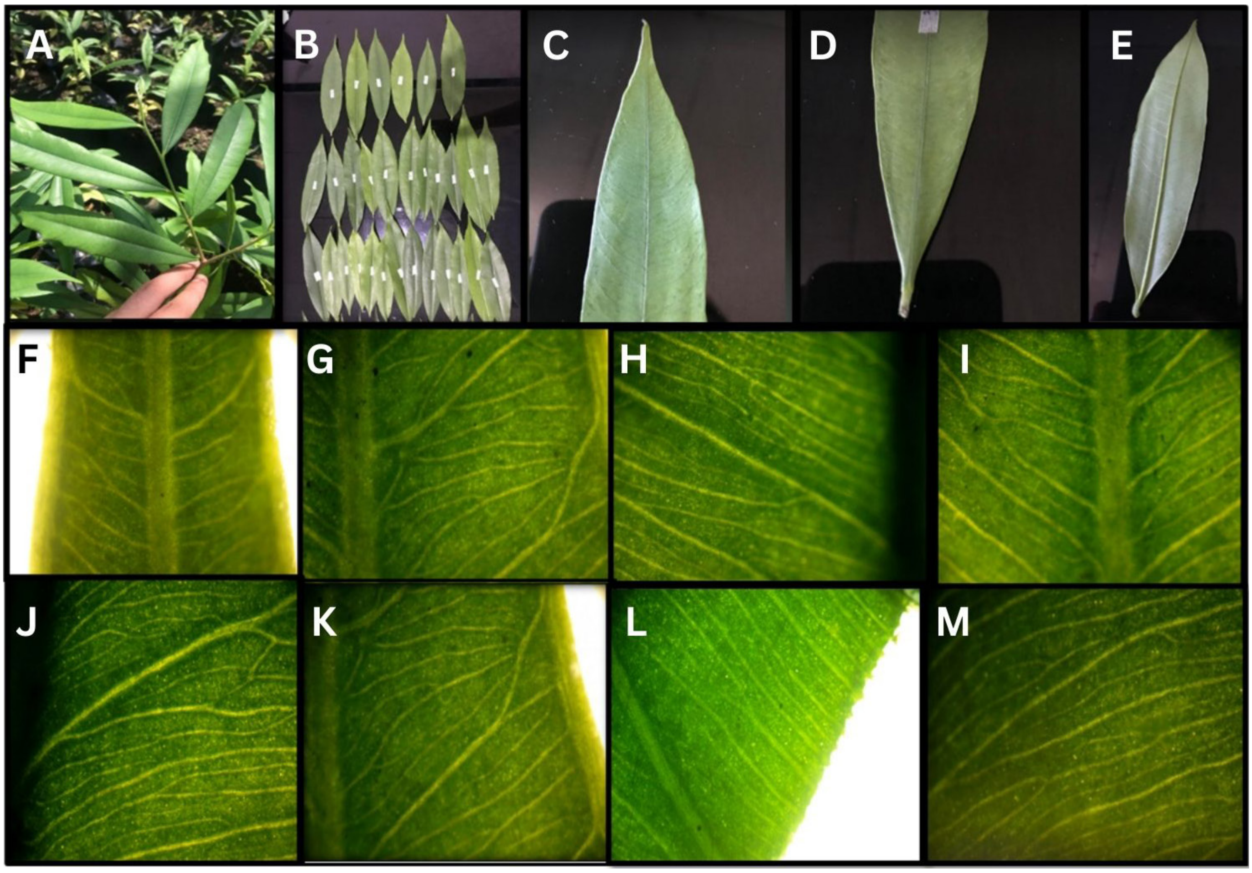

Image 3. *Aquilaria cumingiana*: a—leaf composition | b—leaf shape | c—leaf apex | d—leaf base | e—leaf margin and leaf surface coverings | f—primary vein | g—secondary vein | h—tertiary vein | i—vein spacing | j—leaf venation | k—marginal leaf venation | l—trichomes | m—basal venation arrangement. © RL Germo.

These morphological differences should be further explored in terms of their ecological and genetic basis using more molecular approaches and by sampling more habitat types in the future. Indeed, exploring the environment where trichome and venation patterns develop could also help in deciphering the adaptive strategies of these species.

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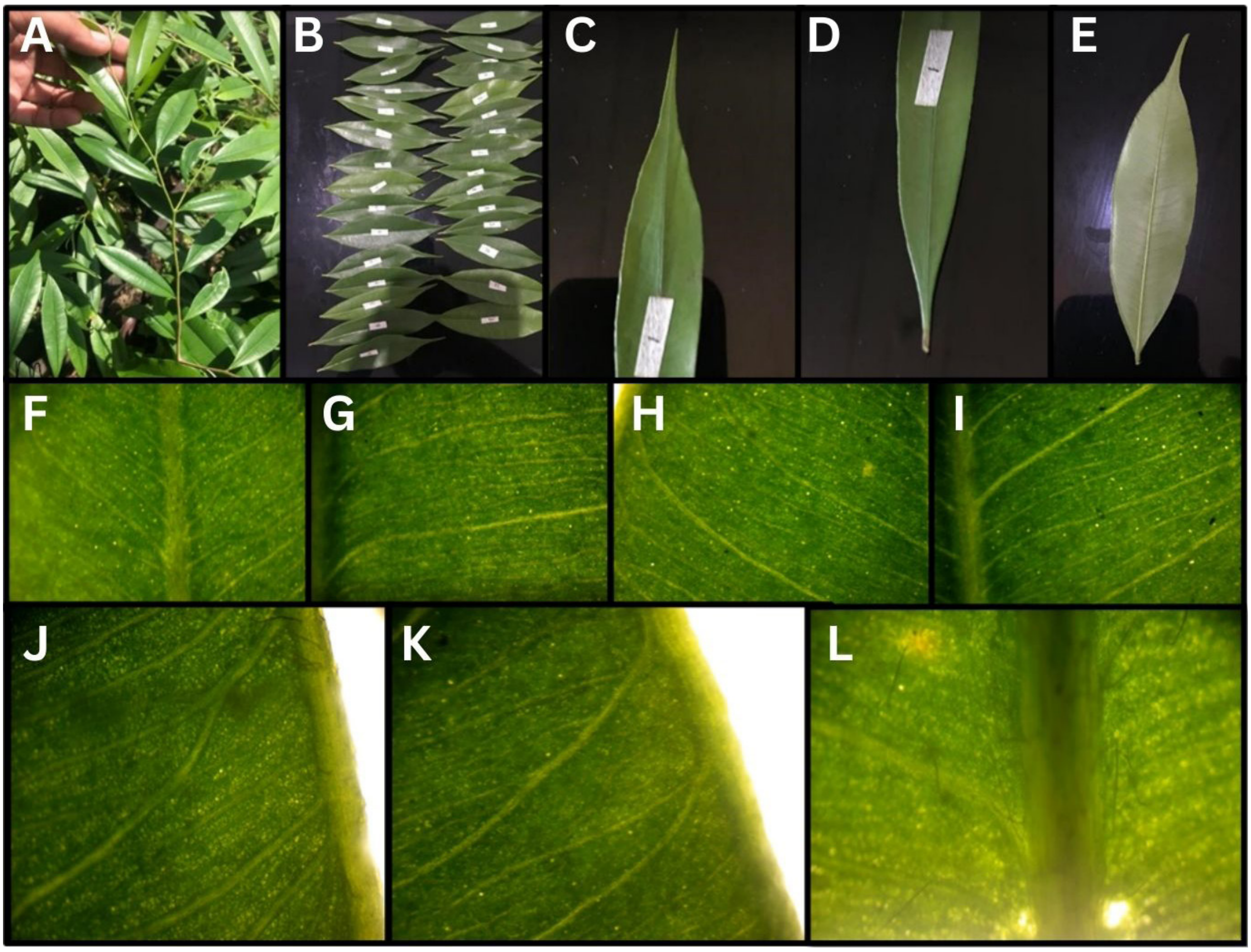

Image 4. ***Aquilaria malaccensis*: a—leaf composition | b—leaf Shape | c—leaf Apex | d—leaf Base | e—leaf margin and leaf surface coverings | f—primary vein | g—secondary vein | h—tertiary & quaternary vein | i—vein spacing | j—pattern of leaf venation | k—marginal leaf characteristics | l—trichomes. © RL Germo.**

Image 5. Seeds of: a—*Aquilaria cumingiana* | b— *Aquilaria malaccensis*. © First Pirico Farmers Association Inc.

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