



Investigating the Taxonomic Value of Leaf Architecture in *Ixora* and *Psychotria* (Rubiaceae) Found in the Bogor Botanic Gardens' Living Collections

Winda Larasati¹, Niken Kusumarini¹, Baiq Farhatul Wahidah^{1*}, Rizmoon Nurul Zulkarnaen³, Muhammad Rifqi Hariri²

¹Biology Study Program, Faculty of Science and Technology, Universitas Islam Negeri Walisongo, Semarang, Jl. Walisongo No.3-5, Tambakaji, Semarang, Central Java 50185

²Research Center for Biosystematics and Evolution - National Research and Innovation Agency, Jl. Raya Jakarta-Bogor Km 46, Cibinong, Bogor, West Java 16911

³Environmental and Life Science Departement, Faculty of Science, Universiti Brunei Darussalam, Jl Tungku Link, BE 1410, Brunei Darussalam

* Corresponding author

e-mail: baiqfarhatulwahidah@walisongo.ac.id

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ABSTRACT

Ixora and *Psychotria* are two distinct genera within the Rubiaceae family found at the Bogor Botanic Gardens. Despite taxonomic differences at the genus and subfamily levels, these plants share certain characteristics that necessitate identifying similarities. This study aims to identify resemblances between *Ixora* and *Psychotria* by examining their leaf architecture and evaluating significant morphological characteristics across recognized variables. The data analysis objective is to identify commonalities in leaf architecture and key distinguishing characteristics. Species under investigation include *Ixora javanica* (Blume) DC., *Ixora paludosa* (Blume) Kurz, *Ixora coccinea* L., *Ixora chinensis* Lam., *Psychotria angulata* Korth., and *P. viridiflora* Reinw. ex Blume. Thirteen characters were used to identify shared traits and influential features. Research findings showed that both *Ixora* and *Psychotria* exhibit various characteristics. Cluster analysis indicated a strong correlation among subjects under investigation, primarily due to shared characteristics. Distinguishing features significantly contributing to differentiation are leaf abaxial surface color and leaf base morphology. It can be concluded that abaxial leaf surface color holds potential as a distinguishing characteristic for *Ixora*, while the leaf base exhibits distinguishing features for *Psychotria*.

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INTRODUCTION

Members of the family Rubiaceae are among the living botanical specimens conserved in the Bogor Botanic Gardens. The distribution of this family spans over all significant global regions, except Antarctica. It is particularly prevalent in the verdant woods found in lowland areas and elevations up to the mid-level range (Barbhuiya et al., 2014). The Rubiaceae, commonly referred to as the coffee family, holds the fourth position in terms of species diversity among families of flowering plants. The taxonomic group under consideration has a diverse array of organisms of about 13,000 distinct species. This group includes a wide range of plant habits, such as trees, shrubs, herbs,

geofrutices, myrmecophytes, epiphytes, and lianas (Alejandro & Liede, 2003). Ariati et al. (2019) reported that several Rubiaceae genera, which include species of *Psychotria* and *Ixora*, have been observed within the confines of the Bogor Botanic Gardens.

The Rubiaceae encompasses several genera, including *Psychotria* and *Ixora*. *Psychotria* is known to consist of approximately 2,000 species, as reported by Davis et al. (2001). On the other hand, *Ixora* is a genus that encompasses over 500 species, as documented by Mouly et al. (2009). The distribution of this latter genus is primarily in the tropical regions of Asia and Africa, with Southeast Asia exhibiting the highest levels of diversity (Tosh

et al., 2013). *Ixora* and *Psychotria* belong to distinct subfamilies, thereby necessitating the identification of shared characteristics between these taxonomic groups. The process of identification can be accomplished by utilizing vegetative features. The identification and categorization of plant species by taxonomists and systematists frequently neglect the consideration of leaf and other vegetative characteristics. This disregard is mostly attributed to the conventional belief that these properties exhibit significant phenotypic plasticity, which holds some validity, according to Medina et al. (2016).

The application of leaf architecture may serve as a valuable method for identifying and distinguishing closely related taxa. The field of study discussed here holds significant importance in ecology, plant systematics, paleobotany, and conservation efforts (Keating, 2009). Leaf architecture, commonly known as leaf venation pattern, has been utilized as a significant tool for species identification (Baroga & Buot, 2014). The study conducted by Antonio and Buot (2021) utilized leaf architecture as a means to characterize and distinguish between two infraspecific taxa of *Dioscorea esculenta* (Lour.) Burkill. The findings of that study suggested that both varieties can be identified and classified as distinct taxa. The investigation of leaf architecture in *Ixora* and *Psychotria* is of paramount importance in the present study, as the use of leaf architecture as a means to establish their relationship has not yet been explored. The use of leaf architecture proves to be a highly suitable approach for discerning the similarities between *Ixora* and *Psychotria*. This is due to the fact that these plant species do not consistently exhibit flowering, thereby requiring reliance on vegetative traits for their accurate identification. Among these vegetative characteristics, leaf architecture emerges as a particularly valuable feature. Given the aforementioned description, it is imperative to investigate leaf architecture comprehensively to ascertain the resemblances between *Ixora* and *Psychotria*, predicated upon their leaf design and the

significance of essential attributes across diverse discovered qualities.

MATERIALS AND METHODS

Material

The collection of leaf samples for observing leaf architecture was conducted using a purposive sampling strategy. This involved the deliberate selection of healthy and fully developed leaves (Baroga & Buot, 2014). The present investigation involved the examination of four species of *Ixora* and two species of *Psychotria*, which were selected as samples from the collections of the Bogor Botanic Gardens (Table 1). A sample size of 10 leaves was gathered and thereafter observed for each individual species.

Morphological and Leaf Architecture Observation

A morphological and architectural examination of the leaf was conducted, focusing on 13 distinct characteristics, which comprised 11 qualitative qualities and two quantitative traits. The qualitative qualities that were observed by Baltazar & Buot (2019) included leaf form, apex, base, and margin. Another study conducted by Laraño & Buot (2010) examined leaf symmetry. Additionally, Meinata et al. (2021) investigated the venation type, namely primary, secondary, and tertiary veins. In the meantime, the qualitative characteristics under consideration were the dimensions of leaf length and width. The observation of leaf venation was conducted using the Dino-Lite Edge/SMP AM7915 series microscope, accompanied by the DinoCapture 2.0 software version 1.5.41. The standard utilized for leaf color observation was the Royal Horticultural Society color chart.

Analysis

The data analysis was conducted using the NTSYS version 2.02i software (Loutfy et al., 2005). The UPGMA algorithm was utilized for clustering in the data analysis process. The process of grouping based on similarity values was conducted using the SAHN function.

Table 1. List of *Ixora* and *Psychotria* for architectural leaf observation

Species	Vak No	Origin
<i>Ixora javanica</i> (Blume) DC.	V.D. 29	Java
<i>Ixora paludosa</i> (Blume) Kurz	V.E.61	Java
<i>Ixora coccinea</i> L.	V.D.68-68a	SE. Asia
<i>Ixora chinensis</i> Lam.	III.L.97	China
<i>Psychotria angulata</i> Korth.	III.L.28-28a	Sumatra
<i>Psychotria viridiflora</i> Reinw. ex Blume	III.L.104	Sumatra: Bangka

Furthermore, in order to demonstrate the distinctions among specific species of *Ixora* and *Psychotria*, a PCA was performed utilizing MINITAB version 19 (Maulia & Susandarini, 2019). To ensure unbiased data analysis in PCA visualization, qualitative characteristics present in every species and quantitative qualities were excluded from the scoring process.

RESULTS AND DISCUSSION

This study examined 13 leaf characteristics, consisted of qualitative (Tables 2 and 3) and

quantitative (Table 4) characters, in leaves from 6 species, including *Ixora javanica* (Blume) DC., *I. paludosa* (Blume) Kurz, *I. coccinea* L., *I. chinensis* Lam., *Psychotria angulata* Korth., and *P. viridiflora* Reinw. ex Blume. The use of morphological parameters in this study revealed a considerable spectrum of variability across various variables, including leaf shape. This variability may potentially be linked to genetic factors. The dimensions of leaves exhibited noticeable diversity, particularly with regard to their length

Table 2. Qualitative Leaf Architecture Characteristics of *Ixora* and *Psychotria* in Bogor Botanic Gardens Collection

Species	Leaf Characters						
	Shape	Apex	Base	Margin	Adaxial Colour	Abaxial Colour	Symmetry
<i>Ixora javanica</i> (Blume) DC.	Oblanceolate to lanceolate	Acute	Rounded to obtuse	Entire	Dark Yellowish Green	Moderate Yellowish Green	Symmetrical
<i>Ixora Paludosa</i> (Blume) Kurz	Lanceolate	Acute	Attenuate	Entire	Greyish Olive Green	Moderate Olive Green	Symmetrical
<i>Ixora coccinea</i> L.	Oblanceolate to lanceolate	Acuminate	Obtuse	Entire	Dark Yellowish Green, Moderate olive green, Moderate olive green	Moderate Yellowish Green	Symmetrical
<i>Ixora Chinensis</i> Lam.	Lanceolate	Acute to Acuminate	Cuneate	Entire	Moderate yellow green, Moderate Yellowish Green, Greyish Olive Green, Olive Green, Dark Greyish, Greyish Olive Green to Moderate Yellowish Green	Moderate Yellowish Green, Moderate yellow green, Moderate Yellowish Green, Greyish olive green, Dark Greyish to Moderate yellowish green	Symmetrical
<i>Psychotria angulata</i> Korth.	Oblanceolate	Attenuate, Acuminate	Attenuate	Entire	Strong Yellow Green to Moderate Yellowish Green	Strong Yellow Green to Strong Yellow Green	Symmetrical
<i>Psychotria viridiflora</i> Reinw. ex Blume	Oblanceolate	Attenuate	Cuneate	Entire	Greyish Olive Green, Moderate Yellowish Green, Deep Yellowish	Moderate Yellowish Green to Moderate yellow green	Symmetrical

Table 3. Qualitative Leaf Venation Characteristics of *Ixora* and *Psychotria* in Bogor Botanic Gardens Collection

Species	Type Venation	Type Venation Primer	Type Venation Secondary	Type Venation Tertiary
<i>Ixora javanica</i> (Blume) DC.	Excurent	Pinnate	Brochidodromous	Composite
<i>Ixora paludosa</i> (Blume) Kurz	Branching	Pinnate	Brochidodromous	Admedial
<i>Ixora coccinea</i> L.	Excurent	Pinnate	Brochidodromous	Composite
	Branching			Admedial
	Excurent	Pinnate	Brochidodromous	Alternate
	Branching			Percurrent
				Tertiary
				Fabric,
				Regular
				Reticulate
				Tertiary
				Fabric,
				Straight
<i>Ixora chinensis</i> Lam.	Excurent	Pinnate	Brochidodromous	Composite
	Branching			Admedial
<i>Psychotria angulata</i> Korth.	Excurent	Pinnate	Brochidodromous	Straight
	Branching			
<i>Psychotria viridiflora</i> Reinw. ex Blume	Excurent	Pinnate	Brochidodromous	Straight
	Branching			

Table 4. Quantitative Leaf Architecture Characteristics of *Ixora* and *Psychotria* Collections at Bogor Botanic Garden

Species	Leaf Length	Leaf Width
<i>Ixora javanica</i> (Blume) DC.	11,85 cm	4,25 cm
<i>Ixora paludosa</i> (Blume) Kurz	14,88 cm	4,42 cm
<i>Ixora coccinea</i> L.	4,91 cm	1,74 cm
<i>Ixora chinensis</i> Lam.	10,48 cm	3,94 cm
<i>Psychotria angulata</i> Korth.	12,15 cm	4,87 cm
<i>Psychotria viridiflora</i> Reinw. ex Blume	13,57 cm	5,13 cm

and width. According to Syukur et al. (2015), qualitative qualities are subject to environmental influences and are regulated by genes of a straightforward nature.

Quantitative features are phenotypic characteristics that arise from complex growth processes that are under the control of several genes (Karuniawan et al., 2017). According to Parkhurst & Loucks (1972), leaf size and form are subject to genetic factors. There is clear evidence of significant variances among different species coexisting within a shared environment. The initial stages of leaf shape development are characterized by a concise process of morphogenesis, which is predominantly driven by the establishment of secondary veins (Dengler & Kang, 2001).

The present study employed PCA to examine the leaf architecture characteristics of *Ixora* and *Psychotria*, focusing on 13 specific characteristics. The results of the analysis revealed distinct and influential characteristics that differentiate between the two genera. Specifically, the color of the leaf abaxial surface was identified as the primary influential characteristic ($PC1 = 0.498$), while the

leaf base emerged as the secondary differentiating characteristic ($PC2 = 0.735$) (Table 5). The characteristics mentioned above can be utilized as differentiating criteria between *Ixora* and *Psychotria*, as ascertained from the values of eigenvalues and eigenvectors. The PCA results indicated that the cumulative diversity contribution accounted for a substantial proportion, specifically 87.7%. The contribution observed in this study was found to be composed of 60.9% from Principal Component 1 ($PC1$) and 26.8% from Principal Component 2 ($PC2$), as visually represented in Figure 1. The findings aligned with the perspective put forth by Jeffers (1967), who posited that eigenvalues of 1.0 and eigenvector values surpassing 0.7 are regarded as influential values.

Ixora and *Psychotria* are two distinct genera that are classified under separate subfamilies. The subfamily Ixoroideae encompasses the genus *Ixora*, whereas the subfamily Rubioideae includes the genus *Psychotria*. The dissimilarities in genus and subfamily gave rise to variations in traits that serve to distinguish between the two. According to Rahmawati et al. (2016), the degree of similarity in

traits across living species is directly proportional to the proximity of their relationship, whereas a lower number of similarities in characteristics suggests a more distant relationship.

The analysis of substantial characteristics has been conducted using PCA. Moreover, it is imperative to perform an analysis that

categorizes entities exhibiting significant similarities into distinct clusters while simultaneously emphasizing substantial dissimilarities (Han et al., 2006). The degree of morphological and leaf architecture similarity between *Ixora* and *Psychotria* is 54%, as illustrated in Figure 2. This finding aligned with the assertion

Table 5. Eigen vector value for each principal component

Variable	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Leaf Shape	-0,322	0,539	-0,664	-0,226	0,288	0,175
Leaf Apex	0,460	0,284	-0,218	-0,157	-0,770	0,208
Leaf Base	0,108	0,735*	0,315	0,434	0,051	-0,397
Tertiary Venation	0,464	-0,244	-0,561	0,134	0,155	-0,607
Adaxial Leaf Colour	0,458	0,168	0,307	-0,722	0,379	-0,063
Abaxial Leaf Colour	0,498*	0,001	-0,055	0,444	0,393	0,630

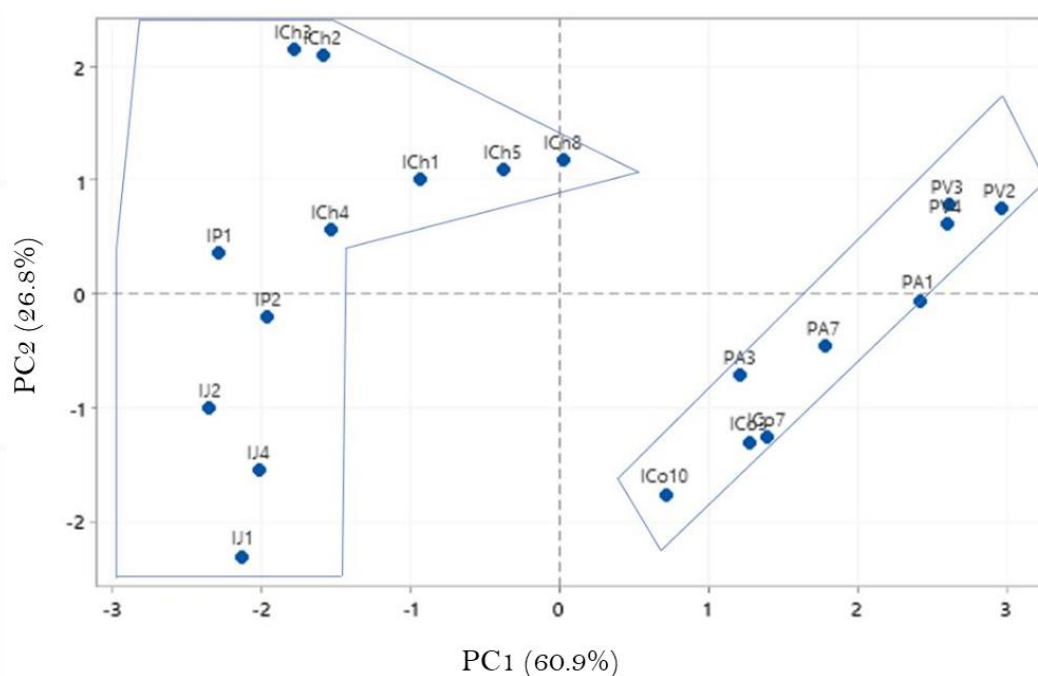


Figure 1. Principal component analysis based on 6 leaf characters on *Ixora* and *Psychotria* showing an almost similar characters on *Ixora coccinea* and *Psychotria* compared to other *Ixora* species. IJ=*Ixora javanica* (Blume) DC., IP=*Ixora paludosa* (Blume) Kurz, ICo=*Ixora coccinea* L., ICh=*Ixora chinensis* Lam., PA=*Psychotria angulata* Korth., PV=*Psychotria viridiflora* Reinw. ex Blume

made by Hasanuddin & Fitriana (2014), wherein they posit that a similarity index exceeding 51% indicates a significant level of relatedness among species within the same group. The categorization of *Ixora* species can be classified into three distinct clusters. The initial cluster comprises *Ixora javanica* (Blume) DC and *Ixora paludosa* (Blume) Kurz. The

second cluster exclusively consists of *Ixora chinensis* Lam. Lastly, *Ixora coccinea* L. is found to be unexpectedly grouped together with *Psychotria angulata* Korth. and *Psychotria viridiflora* Reinw. ex Blume in the same clade. *Ixora coccinea* and *Psychotria* are two plant species that exhibit discernible characteristics, particularly in the leaf

base region of *Ixora coccinea*. These distinguishing features serve as reliable characteristics for differentiation between *Ixora coccinea* and *Psychotria*. The similarities seen between *Ixora* and *Psychotria* suggested a significant degree of relatedness

between the two species. According to Hasanuddin and Fitriana (2014), species that exhibit tight links tend to possess a greater number of common traits, whereas species with more distant relationships tend to exhibit a smaller number of shared traits.

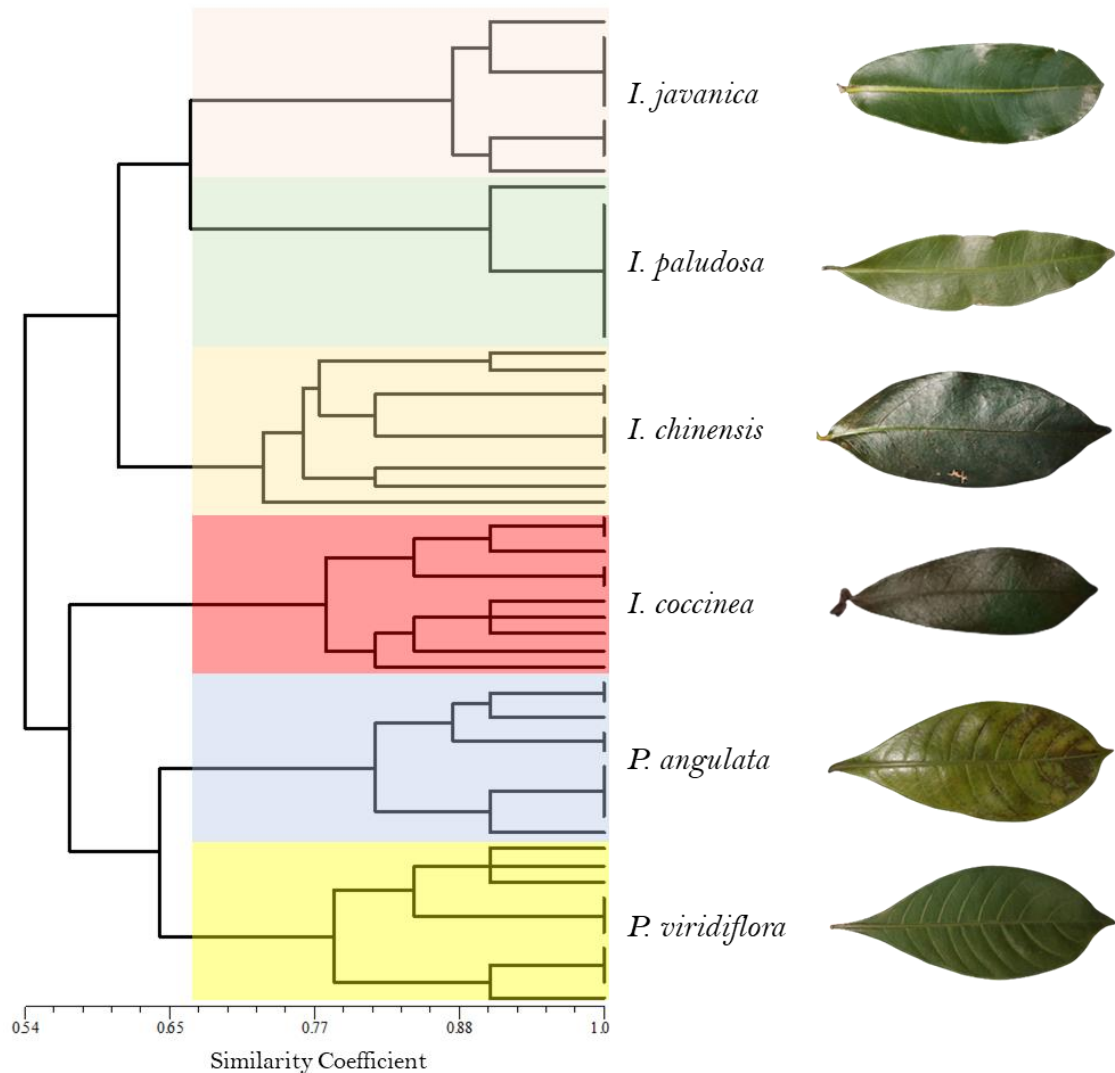


Figure 2. Cluster analysis using unweighted pair group method with arithmetic mean based on 13 leaf characters on *Ixora* and *Psychotria*

The study focused on the examination of venation patterns in the tertiary veins of *Ixora javanica* (Blume) DC., *Ixora paludosa* (Blume) Kurz, *Ixora coccinea* L., *Ixora chinensis* Lam., *Psychotria angulata* Korth., and *Psychotria viridiflora* Reinw. ex Blume (Figure 3). The main and secondary venation patterns observed on *Ixora* and *Psychotria* leaves exhibited similar characteristics (Table 4), specifically a pinnate arrangement with excurrent branching in both species, as well as brochidodromous secondary venation. The sole distinguishing characteristics among leaf venation

patterns are the tertiary veins, which include composite admedial (Figures 3A, B, C), alternating percurrent tertiary fabric (Figure 3D), regularly reticulate (Figure 3E), and straight (Figures 3 F, G, H). The leaf morphological attributes identified in this study are anticipated to serve as supplementary characteristics for distinguishing between *Ixora* and *Psychotria* species in field observations. The findings of Tan & Buot Jr (2018) aligned with our study, indicating that there is a notable increase in the diversity of venation categories beyond the tertiary vein level.

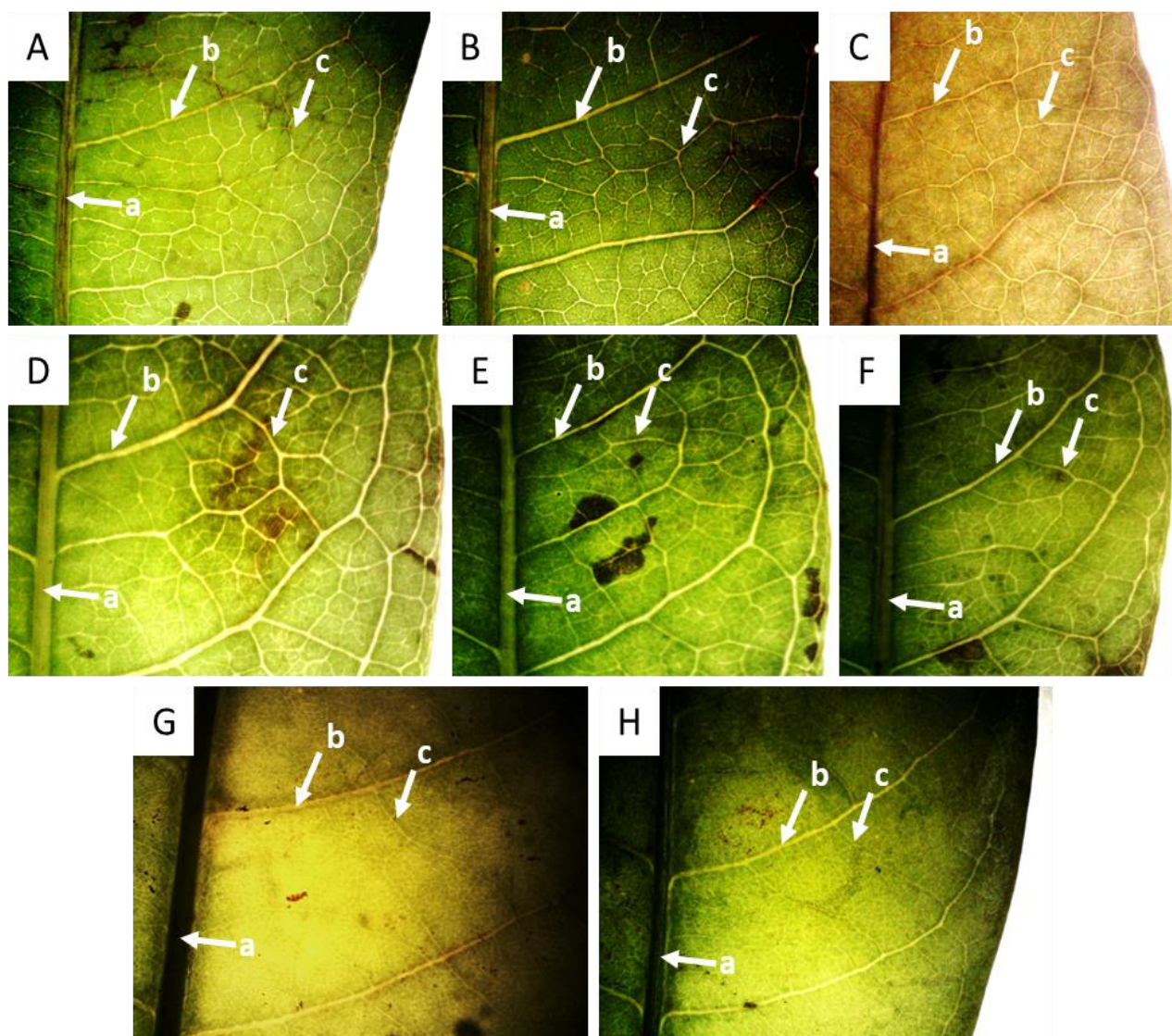


Figure 3. Leaf venation variation among of *Ixora* and *Psychotria*. A=composite admedial *Ixora javanica* (Blume) DC., B=composite admedial *Ixora paludosa* (Blume) Kurz, C=composite admedial *Ixora chinensis* Lam., D=alternating percurrent tertiary fabric *Ixora coccinea* L., E=regular reticulate *Ixora coccinea* L., F=straight *Ixora coccinea* L., G=straight reticulate *Psychotria angulata* Korth., and H=straight *Psychotria viridiflora* Reinw. ex Blume
Note: a=primary venation, b=secondary venation, and c=tertiary venation

Based on the research conducted by Maulia & Susandarini (2019), the results show that the cluster analysis exhibited distinct separation of samples into two distinct species. Seven characteristics were identified as differentiating factors between the two species, namely leaf shape, laminar size, petiole width, trichomes on the lamina, trichomes on the petiole, calcium oxalate density on the lamina, and length of raphid-type calcium oxalate. The results clearly differentiated between *Aquilaria malaccensis* Lam. and *Gyrinops versteegii* (Gilg) Domke. This is different from the findings of this study, as *Ixora coccinea* is still categorized within *Psychotria*.

CONCLUSION

The color of the leaf's abaxial surface may serve as a distinguishing feature for *Ixora*, whereas *Psychotria* has distinguishing characteristics found in the leaf base. The leaf colors may also be utilized further for species identification by additional analysis of digital color extraction. In field observations, the leaf morphological attributes identified in this study are anticipated to serve as supplementary characteristics for early field detection of *Ixora* and *Psychotria* species.

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