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# The Leaf Anatomy of *Ixora chinensis* Lam. and Its Systematic Significance

**Che Nurul Aini Che Amri[1,2]*, Rozilawati Shahari[1,2], Noor Syaheera Mohd Yunus[1,2], Nur Ain Binti Mohd Asri[1], Mohd Razik Midin[1,2], Mohd Fauzihan Karim[1,2]**

[1]Department of Plant Science, Kulliyyah of Science, International Islamic University Malaysia, 25200, Kuantan, Pahang, Malaysia

[2]Sustainable Agriculture and Green Technology Research Unit, Kulliyyah of Science, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia

*Corresponding Author Email: chenurulainicheamri@iium.edu.my

**ABSTRACT**

Rubiaceae is a well-known family of flowering plants or locally known as coffee family with almost 580 genera. *Ixora* L. is known as one of the genera under Rubiaceae family with approximately 544 species. The genus is native to tropical and subtropical regions worldwide. *Ixora chinensis* or locally known as 'Chinese ixora' is recognised as potential medicinal plants which has been used to treat diseases. The root and leaves parts from this species are used widely by Asian people due to it medicinal values. In Asian, the roots are used to relieve the stomach problems. Specifically, in Malaysia, a decoction of the root is used after childbirth for women. An anatomical study of *Ixora chinensis* from the Rubiaceae family have been conducted to identify the leaf anatomical characteristics of species studied that can be useful in species identification. The methods used were leaf clearing, epidermal peeling and sliding microtome cross-sectioning. The findings highlighted several characters that can be used to identify species *Ixora chinensis*. Key findings include the presence of mucilage cells, anticlinal walls of epidermis surface, cell inclusions such as druses in the petiole and midrib, and hypostomatic stomata. These characteristics can be used as additional data for species identification. Additionally, the presence of unique features like mucilage cells or specific stomatal arrangements may indicate adaptive responses to environmental conditions. In conclusion, leaf anatomical features can be useful tools for recognizing and classifying certain Rubiaceae species.

**Keywords**: Leaf anatomy, *Ixora chinensis*

**ABSTRAK**

Rubiaceae merupakan famili tumbuhan berbunga yang terkenal atau dikenali secara tempatan sebagai famili kopi dengan hampir 580 genus. *Ixora* L. dikenali sebagai salah satu genus di bawah famili Rubiaceae dengan hampir 544 spesies telah dikenalpasti. Genus ini berasal dari kawasan tropika dan subtropika di seluruh dunia. *Ixora chinensis* atau dikenali sebagai 'Chinese ixora' diiktiraf sebagai tumbuhan ubatan berpotensi yang telah digunakan untuk merawat penyakit. Bahagian akar dan daun daripada spesies ini digunakan secara meluas oleh orang Asia kerana ia mempunyai nilai perubatan. Di Asia, akarnya digunakan untuk melegakan masalah perut. Kajian anatomi daun ke atas famili Rubiaceae telah dijalankan untuk mengenal pasti ciri-ciri anatomi daun bagi spesies yang dikaji yang boleh digunakan dalam pengecaman spesies. Kaedah yang digunakan dalam kajian ini ialah penjernihan daun, siatan epidermis dan keratan rentas dengan menggunakan mikrotom gelongsor. Hasil kajian telah merekodkan beberapa ciri yang boleh digunakan untuk mengenal pasti spesies *Ixora chinensis*. Antaranya ialah kehadiran sel musilaj, dinding antiklin permukaan sel epidermis, hablur drus pada petiol dan tulang daun serta stomata ciri hipostomatik. Ciri-ciri ini boleh digunakan sebagai data tambahan untuk pengecaman spesies. Selain itu, kehadiran ciri unik seperti sel musilaj atau susunan stomatajuga menunjukkan tindakbalas penyesuaian terhadap keadaan persekitaran. Kesimpulannya, ciri anatomi daun boleh digunakan untuk pengecaman dan pengkelasan spesies bagi famili Rubiaceae.

**Kata kunci**: Anatomi daun, *Ixora chinensis*

**Corresponding author:*
***Che Nurul Aini Che Amri***
*Kulliyyah of Science,*
*International Islamic University Malaysia*
*Email:* ***chenurulainicheamri@iium.edu.my***

## 1. INTRODUCTION

Previous research by Simpson (2019) stated that Rubiaceae family contains majorly terrestrial plants comprising more than 13000 species distributed in at least 600 genera. This family is widely distributed in temperate, tropical and subtropical regions. The species under Rubiaceae family can be found in different forms, including trees, climbers, shrubs, annual or perennial herbs. Certain species of Rubiaceae family can be identified as medicinal plant and have important medicinal values, for instance in treating diarrhoea, headache, fever, wounds, eye problem, cancer, and typhoid (Ali et al. 2000; Jeruto et al. 2011).

Genus *Ixora* is presumably categorized as the second largest genus in Rubiaceae family after *Psychotria* (Mouly et al. 2009). *Ixora* species are tropical evergreen trees and shrubs which their centre of biodiversity is located at the Tropical Asia. Other than that, this genus could be also commonly found in subtropical climates. *Ixora chinensis* is native to Southeast China and Indo-China. Nonetheless, this species was introduced and cultivated in India, Pakistan, Malaysia, Indonesia, Thailand, Philippine and other tropical regions (Dontha et al. 2015). This species could be easily grown at riverbanks and sparse forests. In Malaysia, *Ixora chinensis* is locally known as "Jarum-jarum merah" or "Siantan hutan" (Lim 2014). Dontha et al. (2015) stated that *Ixora chinensis* is a shrub with many stems and glabrous branches, up to 2 m tall. The leaves are opposite, obovate oblong in shape, leathery, base acute to rounded, apex obtuse to subacute, petiole short, stipules deltoid and persistent, entire margin with dull green colour. The branchlets of inflorescence are opposite, red flowers with corolla tube 3-3.5 cm long, lobes circular-obovate, broadly rounded at apex and no fragrance. The fruit is globose and black.

The root and leaves parts from this species are used widely by Asian people due to its medicinal values. In Asian, the roots are used to relieve the stomach problems. Specifically, in Malaysia, a decoction of the root is used after childbirth for women. Other than that, in the Philippines, an infusion of the fresh flowers is reported can be remedy against incipient tuberculosis and hemorrhage. After that, in Indonesia, a decoction of the roots is used to treat bronchial disorders while a decoction of the flowers is prescribed for amenorrhea and hypertension.

Taxonomic data require complete sample data for plant classification and identification. Otherwise, a lack of sample data will result in incomplete and unsuccessful plant identifications. Anatomical characteristics are an important aspect and can be used for plant identification. As a result, study on anatomical parts of *Ixora chinensis* is valuable and crucial. Aside from that, studying anatomical features can help taxonomists and botanists identify and classify plants, particularly those with almost identical morphological characteristics. Additionally, anatomical data provide insights into phylogenetic relationships, helping to resolve taxonomic ambiguities and refine classification systems.

## 2. MATERIALS AND METHOD

Three replicates of *Ixora chinensis* were obtained from the IIUM Kuantan Campus, Pahang. Plant samples were collected and neatly put on newspapers. The plant samples were then wrapped in newspapers and pressed with a presser before being dried in an oven set to 55°C for two weeks. The dried samples were then mounted, labelled, and kept in the IIUM herbarium room for future reference. Collected samples were fixed in either spirit solution or Acetic Acid (AA) solutions. For AA solution, this fixative solution is made up of 70% ethanol and 30% acetic acid in a 1:3 ratio (Johansen 1940). Then, plant specimens were sliced into three sections: apex, middle, and base, which are attached

to the petiole. These specimens were stored in sample bottles. Then, two parts were selected and observed which are petiole and midrib. These parts were sectioned transversely in a range of thickness (15–30 μm) by using a sliding microtome, LEICA SM2010R.

The cut parts were immersed into a petri dish filled with bleach 'Clorox' solution for 10 minutes until the specimen cleared. The staining process involved two stages of staining solution which are Alcian blue and Safranin. Then, the stained specimens were dehydrated by a series of alcohol by precentages which are 50%, 70%, 95%, and 100% and lastly the specimen were mounted on microscope slides using Euparal solutions (Mohd Tajudin et al. 2022). The images were captured using acamera attached to a microscope and the images were analysed by using an imaging software (Cell^B). The adaxial and abaxial epidermis of the fresh leaves were scraped by using a sharp blade for the leaf epidermal peel method. After that, the cleared sample was immersed in Jeffrey solution for 5 minutes and was stained with Safranin and Alcian Blue. Lastly, the leaf sample was placed on a slide and covered with a cover slip and was observed under the light microscope.

For the leaf venation process, the leaf specimen was immersed in Basic Fuchsin solution (10% Basic Fuchsin and 10% KOH) in been stored in oven with temperature at 60°C for 1 week depending on the leaf thickness. After the leaf specimen were cleared, then the specimens were undergoing the dehydration process in alcohol series (50%, 70%, 95% and 100%), and lastly mounted properly on the slide by using Canada balsam. The slides were dried in an oven at 55°C before viewing under a light microscope.

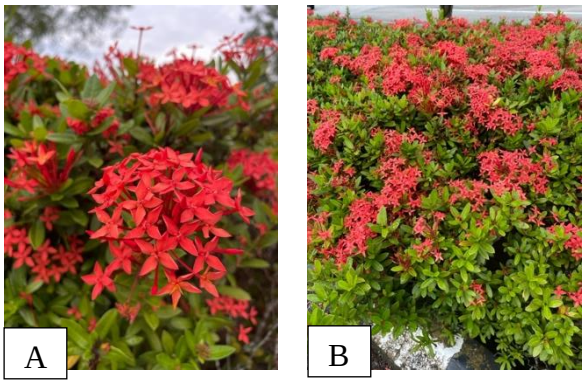


Figure 1 (A&B). Morphological characteristics of *Ixora chinensis*.

## 3. RESULTS AND DISCUSSION

**Cross Section of Petiole**

**Epidermal cell:** One layer cell with (1:1) ratio of height:width. **Vascular tissue:** Closed vascular system with continuous ring of vascular bundle; two additional vascular bundles located at the above right and left of the main vascular bundle near each wing (Figure 2A). S**clerenchyma cell:** Absent. **Parenchyma cell:** 10-11 layers of parenchyma cells. **Collenchyma cell:** 2-3 layers of collenchyma cells under the epidermis of abaxial and adaxial. **Idioblast tannin:** Present in parenchyma cortex and vascular bundle. **Mucilage cell:** Present at the parenchyma cells. **Crystal oxalate:** Present (druses). **Trichome:** Present (simple unicellular trichome (short, pointed end) (Figure 2D).

**Cross Section of Midrib**

**Epidermal cell:** One layer cell with (1:1) ratio of height:width. **Vascular tissue:** Main vascular bundle: Closed vascular system with continuous ring of vascular bundle; Central vascular bundle: Opened vascular system with continuous ring of vascular bundle (Figure 1B). **Sclerenchyma cell:** Densely scatted at the below phloem tissue of the main vascular bundle and additional vascular bundle. **Parenchyma cell:** 7-8 layers of parenchyma cells. **Collenchyma cell:** 2-3

layers of collenchyma cells under the epidermis of abaxial and adaxial. **Idioblast tannin:** Present in parenchyma cortex and vascular bundle. **Mucilage cell:** Present at the parenchyma cortex. **Crystal oxalate:** Present (druses) (Figure 2C). **Trichome:** Present (simple unicellular trichome (short, pointed end).

**Leaf Epidermis**

**Anticlinal wall of adaxial epidermis:** Straight to wavy (Figure 2F). **Anticlinal wall of abaxial epidermis:** Straight to wavy. **Stomata:** Hypostomatic; only present on abaxial epidermis. Type; paracytic (Figure 2E). Stomata size; abaxial: W=19.33 µm, H=27.93 µm (width: min= 16.40 µm, max=22.26 µm; height: min=23.66 µm, max=32.19 µm). **Trichome:** Present (simple unicellular trichome (short, pointed end).

**Leaf Venation (Figure 2G & 2H)**

**Main venation:** Majority open and minority close. **Marginal venation:** Complete. **Tracheid ending:** Swollen.

The results of this study showed that the leaf anatomical traits play an important role in identifying *Ixora chinensis*. Liu (2021) define epidermal cells as live parenchyma cells that can be found in various morphology but mostly are often characterized either rectangular or irregular flat. The epidermis structures consist of rectangular or irregular flat cells, half-moon guard cells, and some species have hairy epidermal cells. There are two common ratio that have been identified for the epidermal cells which are 1:1 or 1:2. Species studied showed ratio for epidermal cells in cross section which is 1:1 (height:width). Research by Nurul-Aini (2016) explain the importance of the features of the epidermal layer that are useful in identification of *Justicia* species for the Acanthaceae family.

Previous research that has been done by Pakravan et al. (2007) identified the numbers and size of mucilage cells are varies depend on the species and genera. Mucilage cells are easily distinguished from other cells especially in cross sections of the petiole and midrib because they have a distinct size compared with the parenchyma cells. Result of this study showed the presence of mucilage cells in petiole and midrib in *Ixora chinensis*. This result also supported previous research that has recorded mucilage cells in Rubiaceae family (Vieira et al. 2001). Mucilage cells contain either chloroplast, starch grains, or cluster of calcium oxalate crystals. Based on this study, crystal oxalates have been found in of *Ixora chinensis* which identified as druses. Vadivu et al. (2009) also discovered druses in in the lamina of *Ixora coccinea*. Research by Nurul-Aini (2011) stated that the presence of calcium oxalates can be used to identify species in *Microcos* and *Grewia* species. Other than that, the result of this study showed the presence of idioblast tannin in parenchyma cortex and vascular bundle. Idioblast tannin also can be found at various parts in plants.

Asri et al. (2023) explained the importance of vascular bundles arrangement in petiole and midrib cross section in identification of *Morinda citrifolia*. The pattern of vascular bundles that have been identified in the petiole of *Ixora chinensis* is closed vascular system with continuous ring of vascular bundle with two additional vascular bundles located at the above right and left of the main vascular bundle near each wing. Meanwhile, closed vascular system with continuous ring of vascular bundle have been recorded in midrib cross section. Opened vascular system with continuous ring of vascular bundle also have been identified in central vascular bundle of midrib for species studied. Metcalfe and Chalk (1950) stated that the structure of vascular bundle have taxonomic value that can be used in plant identification.

Matured sclerenchyma cells are consisted of dead cells with heavily thickened walls which contain lignin and high cellulose content for about 60% to 80%. Sclerenchyma cells are well-known for their role to give structural support in the plants. There are two types of cell wall in sclerenchyma cells which are primary wall and secondary walls. The secondary wall is very thick and more lignified compared to primary wall (Carrillo-lopez & Yahia, 2019). There are two main types of sclerenchyma cells which are sclereids and fibers (Lopez & Barclay, 2017). Fibers are very elongated cells which mainly can be found in stems, roots, and vascular bundles in leaves while sclereids can be found in periderm, cortex, pith, xylem, phloem, leaves, and fruit. The presence of sclerenchyma cells was observed in midrib of *Ixora chinensis*. Based on a study by Kocsis and Borhidi (2003), there were distribution of sclerenchyma cells observed in Rubiaceae plant species.

Trichomes located on outer epidermal cells in the plants, and they are morphologically diverse appendages present on the epidermis surfaces of most of the plants. According to Zhang et al. (2020), trichomes have many roles in plants such as play a buffering role in the interaction between plants and biotic or abiotic stresses, act as a physical barrier against external invasion, reducing plant heat losses, increasing the resistance of plants to cold damage and drought, lastly protecting plant tissues from ultraviolet light insects. Other than that, trichomes provided a model system to study the regulation of plant cell differentiation. Trichomes can be divided into two types either unicellular or multicellular. Results of this study reported the presence of trchomes in *Ixora chinensis*. Research by Kanakhara et al. (2017) has showed the present of trichomes in petiole and midrib in genus *Ixora* studied.

Early research by Dilcher (1974) discovered on the type and structure of leaf arrangement has great implications for the relationship between taxonomy and phylogenetic study. A study on the leaf venation has taxonomic value and can be used for the identification of several species in the genus *Corchorus* and *Grewia* (Sharma 1991). The presence study reported the type of main venation is majority open and minority close while complete type of ultimate marginal venation can be observed in species studied. Besides, Sehgal and Paliwal (2008) stated that anatomical research on the leaf venation characteristics have important implications for identifying *Euphorbia* species.

Previously, Ao (2006) identified that the variation characteristics for the pattern of anticlinal walls could be either sinuous or straight. Noraini et al. (2019) stated that the epidermis of dicotyledonous plants has generally occurred in polygonal-shaped cells, while the epidermis of monocotyledonous plants occurred as parallel elongated shaped. The pattern of anticlinal walls for *Ixora chinensis* has been recorded as straight to wavy in abaxial and adaxial epidermis. Siti Maisarah et al. (2021) mentioned the significance of the anticlinal wall pattern which can be used to identify *Ruellia repens* species. Other than that, stomata play crucial role in controlling physiological activities like photosynthesis, respiration and transpiration in plants (Hong et al., 2018). Stomata occurs in vascular plants where it commonly can be found on leaves and on stems, but it rarely occurs (Kirkham, 2014). There are some significantly different characteristics of stomata that can be observed in different plants such as density, size and shape of stomata. Finding of this study recorded paracytic stomata (both subsidiary cells positioned parallel to long axis of guard cells) with hypostomatic type in leaf epidermis of *Ixora chinensis*. The hypostomatic type is defined as stomata

present only on the abaxial epidermis while absent on the adaxial epidermal surface (Asri et al. 2023).

## 4. CONCLUSION

This study highlights the significant of leaf anatomical aspects of *Ixora chinensis*, providing valuable insights for botanists and taxonomist as well as pharmacologists. The leaf anatomical characteristics of *Ixora chinensis* may provide valuable information for taxonomic studies and further research, particularly for potential medicinal applications. The anatomical features such as the presence of druses and idioblast tannin, the pattern of anticlinal walls, stomata types as well as the vascular bundle pattern in petiole and midrib are important and can be useful as an additional data in identification of *Ixora chinensis*.

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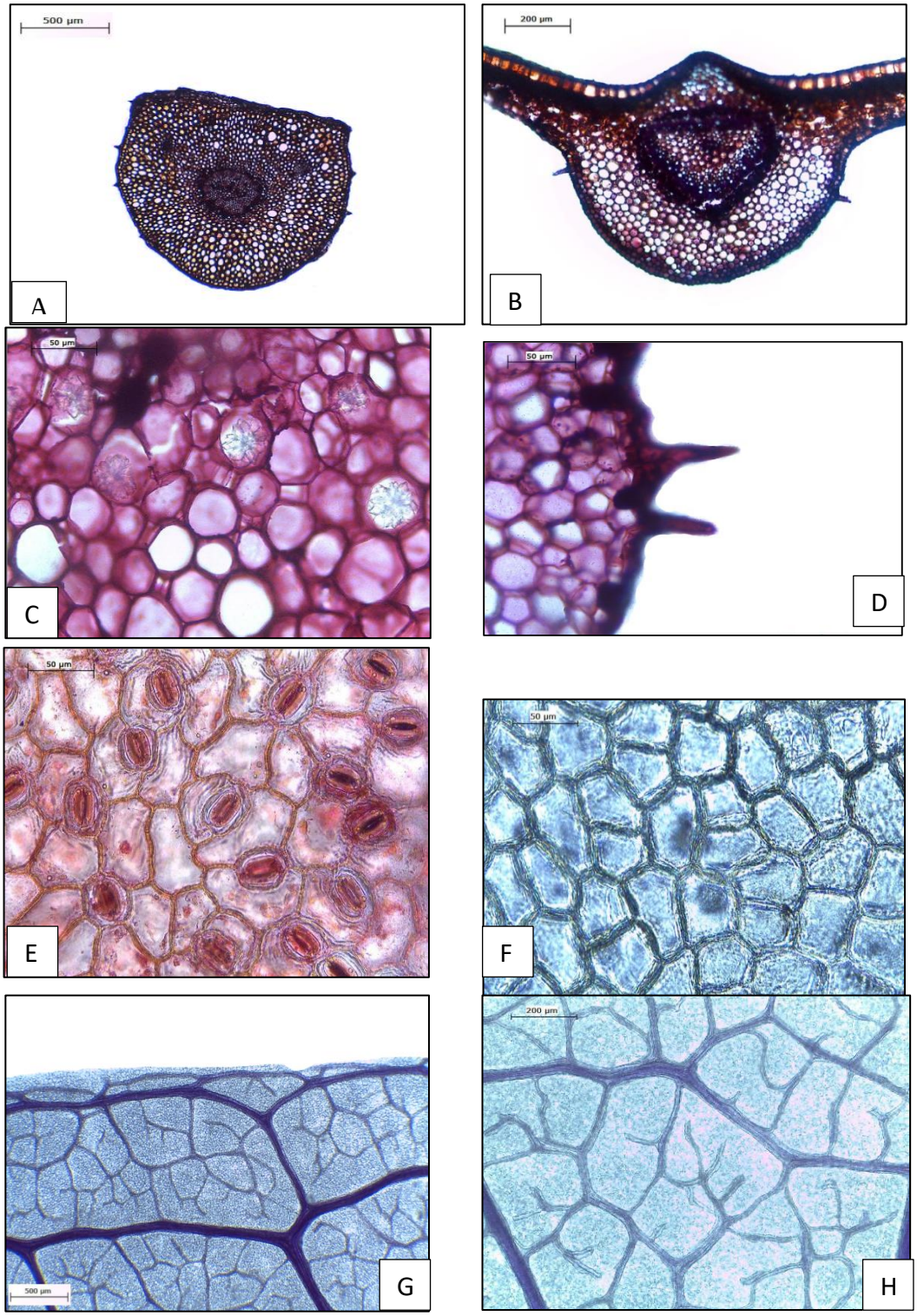


Figure 2: *Ixora chinensis*. A) Cross Section of Petiole. B) Cross Section of Midrib. C) Druses. D) Trichomes. E) Stomata. F) Adaxial Anticlinal Wall. G&H) Leaf venation.
Scale: A,G) 500 µm. B,H) 200 µm. C-F) 50 µm.