LEAF ANATOMY AND CYTOGENETICS ANALYSES OF SELECTED ACANTHACEAE SPECIES IN PENINSULAR MALAYSIA

BY

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ABSTRACT

Acanthaceae is one of the potential medicinal plant families that occurred in Peninsular Malaysia. A great contribution of the Acanthaceae was observed by the documentation of several Acanthaceae species in Malay Medical Manuscripts. However, the treatment of Acanthaceae subfamilies previously seems to have many changes including the placement of the genus *Avicennia* L. into the Acanthaceae. The placement of this genus somehow is made by molecular study without a strong justification from leaf anatomy, micromorphology and cytogenetics points of view. A systematic study thereby was conducted on ten selected species from nine genera of Acanthaceae from Peninsular Malaysia. This research aims to investigate the significance of leaf anatomy and micromorphology characteristics that can be used in the identification of Acanthaceae as well as determine the genome size data to support the systematic study of Acanthaceae. The leaf anatomical study involved several methods such as cross-section using a sliding microtome, leaf epidermal peeling, leaf clearing and observation under a light microscope. The leaf micromorphology method involved the observation of adaxial and abaxial surfaces under a Scanning Electron Microscope (Zeiss Model EVO 50). Cytogenetics study method involved the determination of genome size by using Flow Cytometry Method. The dichotomous key of species was constructed based upon leaf anatomical and micromorphological features gathered in this study. Phylogeny analysis was conducted based on leaf anatomical and micromorphological features by using Multivariate Statistical Package. The analysis of One-Way ANOVA based on genome size was done to investigate the interspecific variations among Acanthaceae species. Results revealed three common characteristics, fourteen variation characteristics and forty diagnostic characteristics of leaf anatomy and micromorphology among the Acanthaceae species. Results also revealed the separation of the genus *Avicennia* from other Acanthaceae species based on the constructed phylogeny tree. For the cytogenetics part, the genome size of Acanthaceae species was measured by using *Glycine max* cv. Polanka (2C=2.5pg) as external reference standard. Results revealed the genome size of Acanthaceae species ranged from 2.05 to 4.09 pg. The determination of genome size however was only obtained for six species studied, whilst the other four species were unable to represent a good peak of histogram. This might be due to the insufficient of nuclei quantities and interference of DNA staining during the analysis. The One-Way ANOVA of genome size showed a statistical difference, thus indicating the presence of interspecific variation among the Acanthaceae species. In conclusion, the leaf anatomy, micromorphology and genomes size data possessed taxonomic values and proven to be additional data for the identification and classification of Acanthaceae.

ملخص البحث

يبدو أن علاج الفصائل الفرعية Acanthaceae يحتوي في السابق على العديد من التغييرات بما في ذلك وضع جنس *Avicennia* L. في Acanthaceae. وبالتالي أجريت دراسة منهجية على عشرة أنواع مختارة من تسعة أجناس من Acanthaceae من شبه جزيرة ماليزيا. يهدف هذا البحث إلى التحقق من أهمية تشريح الأوراق، وخصائص الأشكال الدقيقة التي يمكن استخدامها في تحديد Acanthaceae وكذلك تحديد بيانات حجم الجينوم لدعم الدراسة المنهجية لـ Acanthaceae. تضمنت الدراسة التشريحية للأوراق عدة طرق مثل المقطع العرضي باستخدام مبضع منزلق، وتقشير جلد الأوراق، وتنظيف الأوراق، والمراقبة تحت المجهر الضوئي. تضمنت طريقة الشكل المجهري للأوراق مراقبة الأسطح المحورية تحت مجهر المسح الإلكتروني (Zeiss Model EVO 50). تضمنت طريقة دراسة علم الوراثة الخلوية تحديد حجم الجينوم باستخدام طريقة قياس التدفق الخلوي. تم إنشاء المفتاح ثنائي التفرع للأنواع بناءً على السمات التشريحية والميكرو مورفولوجية للأوراق التي تم جمعها في هذه الدراسة. كشفت النتائج عن ثلاث خصائص مشتركة، أربعة عشر خاصية تباين وأربعين خاصية تشخيصية لتشريح الأوراق والميكرو مورفولوجيا بين الأنواع Acanthaceae. بالنسبة لجزء علم الوراثة الخلوية، تم قياس حجم الجينوم لأنواع Acanthaceae باستخدام *Glycine max* cv. Polanka (2C = 2.5pg) كمعيار مرجعي خارجي. كشفت النتائج أن حجم الجينوم لأنواع Acanthaceae تراوح من 2.05 إلى 4.09 بيكوغرام. في نهاية المطاف، تمتلك بيانات تشريح الأوراق، وعلم الأشكال المجهرية، وحجم الجينوم قيمًا تصنيفية وأثبتت أنها بيانات إضافية لتحديد وتصنيف Acanthaceae.

APPROVAL PAGE

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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This thesis is dedicated to my dear self for keeping strong throughout the bittersweet of this journey

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LIST OF ABBREVIATIONS

°C	Celcius
µL	Microlitre
µm	Micrometre
ANOVA	Analysis of variance
bp	Base pair
ca	circa
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FCM	Flow cytometry
g	Gram
ha	hectare
HCl	Hydrochloric acid
kb	Kilo base
KCL	Potassium chloride
Mbp	Megabase pair
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulphate
mL	Mililitre
mm	Milimetre
MPOB	Malaysian Palm Oil Board
MVSP	Multivariate Statistical Package
Na_2EDTA	Sodium EDTA
NaCl	Sodium chloride
pg	Picogram
pH	Negative logarithm of hydrogen ion concentration

PI	Propidium iodide
PVP-40	Polyvinylpyrrolidone-40
RNA	Ribonucleic acid
RNAse	Ribonuclease
SEM	Scanning electron microscope
SEM	Standard error of mean
SPSS	Statistical Package for The Social Science
UKM	Universiti Kebangsaan Malaysia
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
UPM	Universiti Putra Malaysia

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Planet Earth is a unique space endowed with a rich variety of life forms including plants and animals. Pullaih et al. (2015) emphasized the estimation of more than 50 million species of plants and animals, including invertebrates and microorganisms that existed on Earth. In fact, each life form starting from unicellular primary producers to complexly built higher plants and animals possessed its functions. The plant is one of the important life forms and has a crucial role in human life such as for the sake of food source, shelter, spiritual or medicinal purposes. The foremost role of plants in the medicinal field is through the involvement of plant-based drugs which provide new remedies to mankind. To be surprised, 25% of ingredients in the prescribed modern medicine are directly or indirectly derived from the higher plants (Bandaranayake, 2006; Khan et al., 2017). Somehow, plants are not only important for humankind but also for ecological balance. The showy colors of bracts and nectars in the floral composition of plants are significantly useful for the ecological part since many different classes of pollinators from bees, butterflies, hawkmoths, hummingbirds, sunbirds and bats depend upon the nectar and pollen to survive (Adjonohoum et al., 1996).

Malaysia is a country of Southeast Asia located just north of the equator, straddling between 0°51'N and 7°25'N with a total landmass of 329, 847 square kilometers (Saw & Chung, 2015). According to Conservation International, Malaysia is considered one of the 17 designated 'megadiversity' countries of the world (Suhaimi et al., 2001). The flora and fauna of Malaysia are exceedingly rich and unique which contribute to important parts of biodiversity. The botanical history of Peninsular Malaysia started with the first British settlement in the early 1800s in Penang, where the island is significant for the spice trade. Burkill (1927) presented a full list of the collectors together with the details of their backgrounds. About 12, 500

species of flowering plants and more than 1, 100 species of ferns including fern allies conservatively estimated in Malaysia (Suhaimi et al. 2001). Saw and Chung (2015) also mentioned that Malaysia has an estimated number of 15, 000 species of vascular plants. From this number, Ridley's (1922-1926) successfully documented 7, 183 species whereas Turner's (1997) catalog recorded 8, 198 species including ferns allies, especially focussed on Peninsular Malaysia. To be noticed, one of the families with the largest number of genera and species occurred in the Acanthaceae family with 29 genera and 158 species respectively recorded in Flora of Malaya (Turner, 1997).

Scotland et al. (1995) recognized Acanthaceae as a large pantropical family of mainly herbs and shrubs belongs to the order Lamiales. Somehow, the members of Acanthaceae are climbers or liana such as in the genera of *Thunbergia* and *Mendoncia*, whilst a few species are woody plants as in the genera of *Graptophyllum* and *Sanchezia* (Carlquist, 1988; Scotland & Vollesen, 2000). The Acanthaceae family consists of approximately 240 genera and 3250 species throughout the world (Wasshausen & Wood, 2004). The four main regions of distribution are Malaysia and Indonesia, Brazil, Africa and Central America (Durkee, 1996). The systematic position of Acanthaceae is discussed relatively according to recent molecular studies, pollen morphology, corolla aestivation and other potentially informative morphological homologs (Scotland & Vollesen, 2000). Lindau (1895) recognized four subfamilies of Acanthaceae based on the retinacula characteristics which are; Nelsonioideae, Thunbergioideae, Mendoncioideae and Acanthoideae. The genera from Nelsonioideae, Thunbergioideae and Mendoncioideae do not possess retinacula fruits compared to Acanthoideae. Bremekamp (1965) excluded genera that lack retinacula fruits and therefore divided the Acanthaceae into two groups which are; Acanthoideae and Ruellioideae. However, a new finding by Schwarzbach and McDade (2002) reported that the genus *Avicennia* has a sister relationship with the Acanthaceae based on the floral molecular analysis. Thorne and Reveal (2007) came out with an updated classification and agreed on the placement of Avicennioideae into Acanthaceae. Recently, this up-to-date classification of Acanthaceae categorized this family into four subfamilies which are Nelsonioideae, Thunbergioideae, Acanthoideae and Avicennioideae (Stevens, 2017; Manzitto-Tripp et al., 2021).

Findings on anatomical and micromorphological characteristics of Acanthaceae by Nurul-Aini (2013) and Noor-Syaheera et al. (2015) however, revealed different anatomical and micromorphological characteristics of *Avicennia* compared to other Acanthaceae members. Present, the treatment of Acanthaceae lineages still has many changes, unclear and questionable, and therefore researchers should give more attention and emphasize the systematic position of Acanthaceae. Consequently, comprehensive studies might be significant to clarify the Acanthaceae lineages. For that reason, plant anatomy and cytogenetics parts are significant to support the taxonomic study of plants. Plant anatomy is an important fundamental field that provides such great importance details on the inner parts of the plants. According to Noraini et al. (2019), plant anatomy is one of the additional tools used in taxonomic studies specifically for the identification and classification of plants. Fahn (1967) even mentioned that without the aid of the anatomy part, the phylogenetic relationships between various plant groups cannot be fully understood. Also, plant anatomy plays a significant role to have a better understanding on the physiological part of the plants and their relationship with the environment (Metcalfe, 1963). This present research hence focuses on the investigation of the leaf anatomy and micromorphology on ten selected Acanthaceae species in Peninsular Malaysia.

Meanwhile, the cytogenetics part which involved the Flow Cytometry Method (FCM) is also another important tool that helps to refine the existence of classification in plants. It is expected to provide additional data and evidence especially to better understand the Acanthaceae lineages. FCM typically involved the analysis of the genome size of the plants. Even though the application of FCM is reported to be less frequently applied in the plant science industry, a high number of publications based on the estimation of nuclear DNA content information are published to date (Loureiro et al., 2007). The genome size data obtained from FCM is important for various fields of research including ecology, evolution and taxonomy (Bennett & Leitch, 2005). The estimation of genome size by FCM in Acanthaceae thereby served as a good additional parameter and supportive evidence, especially in the taxonomic study. Also, the anatomical and cytogenetics parts of Acanthaceae have been sporadically recorded in Peninsular Malaysia. Therefore, this research project is considered a good platform and significant to provide additional data and support the systematic treatment of the Acanthaceae, specifically for the species of Peninsular Malaysia.

1.2 PROBLEM STATEMENT

The Acanthaceae members are familiarized with the presence of unique fruit types, by which their seeds are borne on retinacula structures (Nurul-Aini et al., 2018). However, taxonomists faced difficulties to identify and classify species in the Acanthaceae, especially when there are incomplete parts of plant specimens obtained from the field samplings such as the absence of flowers and fruits. In other words, the morphological characteristics alone might invalidate the process of identification and classification of plants if there is incomplete data gained, especially the absence of flowers and fruits. Besides, the Acanthaceae is also recognized with the occurrence of cystoliths that are visible with the magnifying lenses as rod-shaped, especially in the epidermis surfaces of the leaves. Hence, it is very significant to have an additional tool to provide information about the inner part of the plants. The anatomical and micromorphological tool is one of the most reliable tools to confirm the existence of cystoliths and other important cell structures of the Acanthaceae members. Besides, the anatomical and micromorphological tools have also important to better understand the adaptation of plants to their habitats and environment.

Also, previous studies reported the changes in the systematic position of the Acanthaceae such as in the treatment of its subfamilies. Schwarzbach and McDade (2002) even assumed that the genus *Avicennia* which previously has been treated under the family Avicenniaceae has a sister relationship with the Acanthaceae based on the floral character shared between *Avicennia* and Thunbergioideae, but this statement is still uncertain and debatable. Surprisingly, modern studies found that the taxonomic placement of the genus *Avicennia* into the Acanthaceae by the analysis of chloroplast and nuclear ribosomal DNA sequences (Thorne & Reveal, 2007). However, Nurul-Aini (2013) and Noor-Syaheera et al. (2015) reported on the differences of the anatomical and micromorphological features of *Avicennia* and Acanthaceae and therefore more future works and studies are needed to clarify the systematic issue of the Acanthaceae. In fact, the conflicting issues in the systematic position of the Acanthaceae are still unclear and yet, comprehensive and systematic studies of Acanthaceae in Peninsular Malaysia are still lacking. Therefore, this research provides several tools for systematic studies including leaf anatomical, micromorphological and the contribution of a cytological analysis (FCM). The