Faculty of Resource Science and Technology

Taxonomical and Antifungal Study of Selected *Mapania* spp. (Cyperaceae Juss.) from Sarawak

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Signature

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ABSTRACT

The largest state in Malaysia is Sarawak, with a total size of about 124,450 km^2. Several types of forests, including understory plants, cover 67% of the total area and are home to variety of fascinating flora. An important genus with a high endemism rate among Sarawak's flora continues to remain unfamiliar to many. In order to gain better understanding of this genus, taxonomical and antifungal study of the Sarawak *Mapania* were done. From August 2017, a series of field trips have been conducted throughout Sarawak. New localities were found and more than 100 samples had been collected. Majority of the species were found in lowland habitats, such as swamps and lowland Mixed Dipterocap Forest. A new species was described based on their vegetative and reproductive characteristics, viz. *M. multifolia* sp. nov. A complete field key to Sarawak *Mapania* collected was presented in this dissertation. In addition, the anatomical characteristics were also examined to add the information for delimiting the species. Morphologically, *Mapania* were divided into 2 major groups based on their leaf blade. The described anatomical characters were able to delimit the species within the groups. The anatomical characters in *Mapania* are linear with elongate cell, hexagonal, polygonal, and random shape of epidermal shape; sinuous and undulate pattern of anticlinal walls; anomocytic, diacytic, and tetracytic type of stomata; concave, concave with keel shape, flat, U shape, V shape, and narrowly V shape of petiole and mirib cross section. Although the anatomical characteristics were less meaningful for in species identification, *M. cuspidata* variety can be delimit based on the presence of cell inclusion. In addition, the screening of the antifungal activity also provides new information for this genus. Methanol crude extract obtained from leaves and root of 10 selected *Mapania* species were tested against four crop pathogenic fungi. Five root methanolic crude extracts and one leaves

methanolic crude extracts showed significant effect against *C. musae*, three leaves methanolic crude extracts and one root methanolic crude extracts showed significant effect against *P. oryzae* (isolates POSA 1) while two leaves methanolic crude extracts showed significant effect against *P. oryzae* (isolates POSA 2). Eventhough the results of the antifungal activity were considered as weak, the informations obtained benefits greatly in understanding and encouraged the conservation effort of this genus.

Keywords: Anatomical, Antifungal, Cyperaceae, crop pathogenic fungi, *Mapania*

Kajian Taksonomi dan Antikulat Mapania spp. Terpilih (Cyperaceae Juss.) dari Sarawak

ABSTRAK

Negeri terbesar di Malaysia ialah Sarawak, dengan saiz keseluruhan kira-kira 124,450 km^2. Beberapa jenis hutan, termasuk tumbuhan rendah, meliputi 67% daripada jumlah kawasan dan merupakan habitat kepada pelbagai flora yang menarik. Genus penting dengan kadar endemik yang tinggi di kalangan flora Sarawak asing di kalangan ramai. Untuk mendapatkan pemahaman yang lebih baik tentang genus ini, kajian taksonomi dan antikulat Mapania *Sarawak telah dilakukan. Sejak Ogos 2017, beberapa siri lawatan kerja lapangan telah dijalankan di seluruh Sarawak. Lokaliti baharu ditemui dan lebih daripada 100 sampel telah dikumpul. Majoriti species ditemui di habitat tanah rendah seperti paya dan hutan campuran dipterokap tanah rendah. Spesies baru diterangkan berdasarkan ciri-ciri vegetatif dan pembiakan, iaitu* M. multifolia *sp. nov. Kekunci lapangan yang lengkap untuk* Mapania *Sarawak yang dikumpulkan telah dibentangkan dalam disertasi ini. Di samping itu, ciri-ciri anatomi juga diperiksa untuk menambah maklumat untuk mengenalpasti perbezaan antara spesies. Secara morfologi,* Mapania *dibahagikan kepada dua kumpulan utama berdasarkan bilah daunnya. Karakter anatomi yang diterangkan dapat mengenalpasti spesies dalam kalangan kumpulan tersebut. Karakter anatomi dalam* Mapania *adalah sel epidermisnya berbentuk lurus dengan bentuk sel memanjang, heksagon, polygon dan rawak; dinding antiklinnya bercorak berliku dan beralun; jenis stomatanya anomositik, diasitik, dan tetrasitik; keratan rentas tangkai daun dan pelepahnya berbentuk cekung, cekung dengan bentuk lunas, rata, bentuk U, bentuk V, dan bentuk V yang sempit. Walaupun ciri-ciri anatomi kurang bermakna dalam pengecaman spesies, variasi* M. cuspidata *dapat dikenalpasti berdasarkan kehadiran sel inklusi. Selain*

itu, saringan aktiviti antikulat juga memberikan maklumat baharu untuk genus ini. Ekstrak mentah methanol yang diperoleh daripada daun dan akar sepuluh spesies Mapania *terpilih telah diuji terhadap empat kulat patogenik tanaman. Lima ekstrak akar mentah methanol dan satu ekstrak daun mentah methanol menunjukkan kesan ketara terhadap* C. musae, *tiga ekstrak daun mentah methanol dan satu ekstrak akar mentah methanol menunjukkan kesan ketara terhadap* P. oryzae *(isolasi POSA 1) manakala dua ekstrak daun mentah methanol menunjukkan kesan ketara terhadap* P. oryzae *(isolasi POSA 2). Walaupun hasil aktiviti antikulat dianggap sebagai lemah, maklumat yang diperolehi sangat bermanfaat dalam memahami dan menggalakkan usaha pemuliharaan genus ini.*

Kata kunci: *Anatomi, antikulat, Cyperaceae, kulat patogenik tanaman, Mapania*

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LIST OF ABBREVIATIONS

BO	Herbarium Bogoriense (Indonesia Institute of Science)
BRUN	Brunei National Herbarium
cm	Centimetre
cm/day	Centimetre Per Day
diam.	Diameter
DMSO	Dimethyl Sulfoxide
g	Gram
GPS	Global Positioning System
HCl	Hydrocholoric Acid
holo	Holotype
HUMS	Herbarium of Universiti Malaysia Sarawak
iso	Isotype
IUCN	International Union for Conservation of Nature and Natural Resource
i.e	Id Est
K	Royal Botanic Gardens Kew
KEP	Forest Research Institute Malaysia
m	Metre
MDF	Mixed Dipterocarp Forest
MIC	Minimum Inhibitory Concentration
MFC	Minimum Fungicidal Concentration
mg/mL	Miligram Per Mililitre
mm	Milimetre

mm^2	Milimetre Square
m a.s.l.	Meter above sea level
no.	Number
OMA	Oatmeal Agar
PDA	Potato Dextrose Agar
SAN	Herbarium of Forest Department, Sandakan, Sabah
SAR	Herbarium of the Sarawak Forestry Department
SD	Stomatal Density
SI	Stomatal Index
sp.	Species (singular)
sp. nov.	Species Nova
spp.	Species (plural)
SPSS	Statistical Package for the Social Sciences
SSL	Self-Sufficiency Level
UNIMAS	Universiti Malaysia Sarawak
USB	Universal Serial Bus
UV	Ultraviolet
var.	Variety
viz.	Videlicet
µm	Micrometre
µm/ml	Micrometre Per Mililitre
%	Percent
°C	Degree Celsius
<	Less Than

CHAPTER 1

INTRODUCTION

1.1 Study Background

Borneo, is a third-largest island in the world with a total area of approximately 748,168 km^2. This island comprises of Sarawak, Sabah, Brunei, and Kalimantan. It is mostly covered by dense rainforests with a vast population of flora and fauna. Borneo is acknowledged as the centre of plant diversity in the world (Soepadmo & Wong, 1995). It is estimated to contain about 15,000 species of plants.

Sarawak is located in the Northwest of Borneo island. It is the largest among the 13 states with an of area approximately 124,450 km^2. With its equatorial climate, it is diverse with flora and fauna. There are several types of forest covering Sarawak. The most dominant forest are hill mixed dipterocarp forest, peat swamp forest, and mangrove forest. Only minimum areas were covered by kerangas forest, montane forest, and limestone forest. The forest land in Sarawak was categorized into three types which are Permanent Forest Estates (Protected Forest, Forest Reserve & Communal Forest), Totally Protected Areas (National Parks, Wildlife Sanctuaries & Nature Reserves) and Stateland Forest (Forest Department Sarawak, 2020).

Within the vast forest in Sarawak, there exist a family of monocot plants namely Cyperaceae. Family Cyperaceae is among the common weeds that are ubiquitous but may be difficult to identify due to its similarity to the grass family (Poaceae) except their triangular-shaped stems that are characteristic. Although Cyperaceae and Poaceae were formerly thought to be related plant families (Cronquist, 1981), a new cladistics study combining genetic and morphological data shows that the Cyperaceae family is more

closely linked to the Juncaceae and Thurniaceae families (Chase *et al*., 2000). Cyperaceae, commonly known as sedges, are monocot flowering plants with small, primarily wind-pollinated (anemophilous) flowers. Cyperaceae have been overlooked as weeds, however research has revealed that the majority of Cyperaceae species play an essential role in both economic and environmental conservation (Simpson *et al.,* 2003).

Mapania is a sedge species from the Cyperaceae family which is distributed in tropical regions such as Africa, Northern South America and other Oceanic islands. In Sarawak, *Mapania* were considered abundant as the areas are suitable for its growth. *Mapania,* where several of its species provide significant benefits to the local people, for example, the local people used *M. cuspidata* leaves as traditional medicine for postnatal treatment and against fever (Simpson, 1992). Locally known as 'rumput serapat' or 'pandan tikus', Iban and Melanau community called it 'Daun meing' and 'daun sisiek' respectively. There are 43 taxa of *Mapania* initially recorded by Simpson (1992) in Sarawak, but the number has increased with addition of *M. sapuaniana* Shabdin (2013a), *M. multiflora* Shabdin (2013b), *M. meekiongii* (Miraadila & Shabdin, 2016), *M. kadimiana* Shabdin, Meekiong & Miraadila (Shabdin *et al.,* 2016). However, their studies so far focussed more on morphological characteristics, palynology and phytochemical constituents in *Mapania*. In terms of phytochemical research, the presence of anthocyanidins – of which *M. cuspidata* var. *cuspidata* has higher concentration – helps to shed light on the different *M. cuspidata* variations in addition to their morphological characteristics.

According to Nakajima *et al.* (1978) and Gamal *et al.* (2015), the majority of isolated compounds identified in Cyperaceae were phenolic compounds, stilbenes, flavonoids, phenolic acids, phenylpropanoids, terpenoids (sesqui- & triterpenes, sterols),

coumarins, and quinones. Based on this, it is predicted that *Mapania* have the same chemical constituents as the family has. Thus, Shabdin (2012) conducted a study and found two phenolic acid and six flavonoids exists in *Mapania.* Some of the pharmacological, insecticidal, and fungicidal activities are attributed to these metabolites (Harborne *et al*., 1999), for example, the flavonoids. According to available data, flavonoids are hydroxylated phenolic compounds that plants produce in response to microbial infections (Dixon *et al.,* 1983; Nabavi *et al*., 2018). It is a physiologically active compound with a wide variety of pharmacological activities (Mahomoodally *et al.,* 2005; Pandey, 2007). Because of their physical and biochemical characteristics, flavonoids not only defend against damaging abiotic elements, but also make it easier to interact with other plants and microbes. According to Janićijević *et al.,* (2007), different flavonoids exhibit various biological activities such as anti-inflammatory (kaempferol, quercetin & myricetin), antimicrobial (flavonoids & esters of phenolic acid), antibacterial activity (quercetin), and antioxidant (flavones & catechins). It has been suggested for used against human fungal diseases because they prevent the spore germination of plant pathogens (Harborne & Williams, 2000). Not to mention, phenolic acids also a secondary metabolite that plant make as a component of their antimicrobial defence system to restrict the growth of bacteria (Liu *et al*., 2020) as well as they possess an ability to demonstrated antifungal activity against *Candida* species (Teodoro *et al*., 2015).

Unfortunately, rising tendency of reckless deforestation and land conversion for plantation and other developmental objectives became unavoidable, resulting in significant loss of many valuable species (WWF, 2020). For instant, the distribution of *M. sapuaniana* only recorded at Lanjak Entimau Wildlife Sanctuary in 2008, however, during the revisit in 2010, the area has been cleared up for cemetary used (Meekiong, pers. com, 23 September

2022). In many situations, economic exploitation takes precedence over scientific research, resulting in the extinction of previously unknown species. As a result, it is clear that there is an urgent need to address additional collecting and documentation, particularly on valuable and varied species in Sarawak.

In short, the purpose of this study is to reveal information on the anatomical study of *Mapania* species as well as to evaluate the methanol crude extracts in the role of inhibiting the growth of plant pathogenic fungi. In inclusion, the addition of micromorphology and antifungal information may give light to the identification of certain members of the species of *Mapania*, as it is necessary to assist in species delimitations. Perhaps in the future, potential species may be conserved and protected for other fields of study.

1.2 Problem Statement

The systematic research on the genus *Mapania*, were carried out by Simpson (1992), primarily using samples from herbariums. Shabdin (2012) then broadened the investigation's focus to encompass morphology, molecular DNA, and chemical compounds, focusing on two Southeast Asian plant species, *Pandanophyllum* and *Thoracostachym*. The systematic study was then further expanded the scope on morphology, palynology, chemical evidences particularly on Sarawak's *Mapania* (Miraadila, 2018).

While several morphological studies have been carried out, the problems with overlapping characters such as the leaves shape among *Mapania* species still occur, from which it can be seen that new characters are still in need in delimiting the problematic taxa such as *M. hidiriana* Miraadila, Shabdin & Meekiong (2016a) where it usually found with

the sterile or empty spike. It has been a common condition in the delimitation of *Mapania* species that overlapping characters and the absence of their inflorescences which always hinders and results in poor identifications (Shabdin, 2012). As proper identification is crucial when it comes to utilising any particular species for commercial uses, adding other potential anatomical characters is deemed promising to aid the whole identification process, particularly with difficult species. To aid the process of delimitation, adding new anatomical characters is seen to be helpful, especially when the inflorescence is absent (Metcalfe, 1969).

Although in recent years, the taxonomic work has been made, there is no comprehensive study on antifungal properties of *Mapania*. The subject of this important genus with a high endemism rate continues to be obscure to many. Eventhough being underrated locally, this genus deserves special attention. As *Mapania* is a significant part of the herb layer in tropical rainforests, this study is helpful in terms of conservation. Future researchers who are not familiar with this genus can benefit greatly from the results in order to gain a deeper understanding of this genus.

1.3 Research Question

This study is to aimed to answer:

i. What anatomical characters are useful to delimit *Mapania* species?

ii. Do the evaluated anatomical characters of the selected *Mapania* species are useful in species identification?

iii. Do the evaluation of antifungal study of the selected *Mapania* species are useful for conservation effort?

1.4 Objectives

The objectives of the study are:

i. To evaluate the anatomical characters of selected *Mapania* species in aiding species identification.

ii. To test the antifungal effect of methanol crude extract of 10 *Mapania* species on selected crop pathogenic fungi.