



Faculty of Resource Science and Technology

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

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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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ABSTRACT

The largest state in Malaysia is Sarawak, with a total size of about 124,450 km². 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 Dipterocarp 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 midrib 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 km². 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 tetrastitik; 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 diperolehi 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	Hydrochloric 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 km². 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 area approximately 124,450 km². 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 cemetery 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 (Metcalf, 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.

CHAPTER 2

LITERATURE REVIEW

2.1 Cyperaceae Juss.

Cyperaceae consists of 106 genera and approximately 5387 species which is ranked 3rd among the largest monocotyledons family and ranked 7th among the largest angiosperm family (Govaerts *et al.*, 2007). Although Cyperaceae have some similar appearances with Poaceae, some evidence are suggesting that it is more close relatives with the Juncaceae family (Bouchenak-Khelladi *et al.*, 2014).

According to Govaerts *et al.* (2007), *Carex* is the largest genus within the family of Cyperaceae which cover nearly three-quarters of the total species with an amount of 1781 species and is followed by *Cyperus* with the amount of 694 species. Cyperaceae can be found distributed in tropical regions such as Africa, Northern South America and other oceanic islands. Heywood *et al.* (2007) stated that the members of Cyperaceae are known to have triangular and solid culms with a closed sheathing leaf base as well as spikelet arrangement of inconspicuous flowers (Table 2.1).

Table 2.1: The Common Characteristics of Cyperaceae (Goetghebeur, 1998).

Parts of plant	Common characteristics
Root	Rhizomatous, stoloniferous or bulbiferous
Culms	Central and rarely lateral
Leaves	Closed sheathing leaf base and linear blade
Inflorescence	Inconspicuous flower arranged in spikelet, panicles but sometimes corymb, anthelate, spicate or capitate
Male flowers	The anthers appear to be elliptic or oblong to linear.
Stigma branches	Occur in two or three stigma branches.
Fruit	Commonly achenes (nutlets). It is usually in ovate, elliptic or obovate.

Currently, there are two subfamilies in Cyperaceae namely Mapanioideae and Cyperoideae (Govaerts *et al.*, 2007; Muasya *et al.*, 2009). Mapanioideae and Cyperoideae were classified based on their inflorescence structure where the structure is specialized into a unit termed ‘spicoid’ only for subfamily Mapanioideae.

Mapanioideae is a subfamily that comprises 11 genera with approximately 165 species. In this subfamily, it is divided into two tribes namely Hypolytreae with Chrysitricheae (Muasya *et al.*, 2009; Simpson *et al.*, 2003). These tribes were classified based on their pollen type and distribution. According to Simpson *et al.* (2003) who distinguished its pollen type, tribe Hypolytreae are known to be having the Mapania-type of pollen while tribe Chrysitricheae have pseudomonad pollen type. However, in terms of distribution, tribe Hypolytreae are pantropical and are more diverse in the centre of the Asia-Pacific region. In contrast, tribe Chrysitricheae are more diverse in Southern Hemisphere which occur in a various range of habitats like from tropical wet forests to swamps (Simpson *et al.*, 2003).

For genus *Mapania*, it is classified under tribe Hypolytreae together with *Hypolytrum* Pers. and *Scirpodendron* Zipp., *Diplasia* ex Kurz, *Principina* Uitten and *Paramapania* Uittien.

2.2 Genus *Mapania* Aubl.

Genus *Mapania* was first recognised by Aublet (1775) in his *Histoire des plantes de la Guiane Francoise*. The Penan people in Sarawak know it as ‘Ieee’ while Saban people in Sarawak called it ‘Ra’ah’. There are about 60-70 species distributed in tropical regions such as Africa, Northern South America and other oceanic islands. This species

occurred at lowland mixed dipterocarp forest, kerangas and swampy forest areas (Miraadila *et al.*, 2015). In Malaysia, it occurred in the area near rivers or streams. However, genus *Mapania* were divided into five sections which are *Mapania*, *Thoracostachyum*, *Pandanophyllum*, *Pycnocephala*, and *Mapaniopsis*. It is classified based on its geographical distribution. Each of these sections has unique characteristics (Table 2.2).

Table 2.2: The Unique Characteristics of Sections in *Mapania* (Simpson, 1992).

Sections	Unique characteristics
Mapania	The leaves were reduced to bladeless sheaths. Have six bracts and hard fruits.
Thoracostachyum	Have more or less paniculate inflorescence with six floral bracts in each spicoid. The fruits have a hard outer layer.
Pandanophyllum	Have lateral or central culms. Inflorescences are capitate or solitary spike. It has six floral bracts. Its fruits are succulent, detaching and disintegrating exocarp.
Pycnocephala	Inflorescence is mostly capitate, but rarely paniculate or rayed. Have four or six (rarely) floral bracts in each spicoid. It has a hard, thin, and not disintegrating exocarp.
Mapaniopsis	Inflorescence is open, paniculate with small spikes. Have four to five floral bracts in each spicoid. It has a hard, thin, and not disintegrating exocarp.

2.3 Morphology of Genus *Mapania*

Due to its appearance, *Mapania* is always being confused with grasses and pandan. However, their morphology varied from these two families in some respect. The general morphology of *Mapania* is: “Perennial with rhizomatous or stoloniferous roots that are stout and occasionally stilt-like on ascending rhizomes. Culms can be central or lateral, and (sub)scapose. Few to numerous lateral culms, with some basal leaves reducing to a sheath. Leaves are eligulate, sometimes pseudopetiole, and seldom reduced to a sheath. The inflorescence is seldom pseudolateral, capitate, corymbiform, subanthelate, or paniculate, and when on lateral culms, it is typically limited to a few spikelets. Primary bracts can be small (as on most lateral culms) or big and leaf-like (on most central culms). Spikelets with

a few to many spirally persistent glumes, each of which contains a flower. Flowers can be bisexual. Scales 4-6, basal pair lateral, ciliate-keeled, each with a single stamen, remaining 2-4 scales 0-2 with a stamen, rarely just one stamen per flower. Style 2-3-fid, no apparent base, not or slightly thickened, persistent or not. Thick lenticular or trigonous achene, often 3-ribbed, sometimes with a fleshy exocarp. Around 70 species, pantropical, mostly equatorial; tropical rainforest and open wetlands.” (Goetghebeur, 1998) (Figure 2.1).

Vegetative and inflorescence morphology are the two basic categories under which morphology studies in *Mapania* can be divided. Their habits, subsurface components, culms, and leaves were the involved vegetative characters. As the leaf and leaf apex are insufficient to distinguish between species, it is very hard for *Mapania* to identify species solely based on vegetative parts. Shabdin (2012) asserts that *Mapania* species can be divided into three groups depending on the morphology of their leaves, i.e. linear-oblong, linear (grass-like), and linear (pandan-like).



Figure 2.1: Illustration of *Mapania* sp. (Clarke, 1909)

The involucre bracts are the main emphasis of the inflorescence's morphology, which also includes spikes, spicoid bracts, spicoid and floral bracts. The glumaceous or foliaceous bract that encircled the spikes is known as the involucre bract. For instance, in *M. cuspidata*, the glumaceous involucre bract might occasionally have the same size and appearance as the spicoid bract (Simpson, 1992). In *Mapania*, there were four different

forms of inflorescence, i.e. solitary, paniculate, capitate, and sessile. The spike is a structure found in the tribes Cyperaceae and Scirpeae that is superficially similar to but not identical to the spikelet. The glumaceous bract subtending a spicoid and consisting of several to numerous spirally oriented spicoid bracts. In *Mapania* and other hypolytroid taxa, the fundamental flora component is the spicoid or pseudanthium. However, Simpson (1992) prefers the more useful and understandable term ‘spicoid’. In the spicoid, the scale-like bracts are referred to as floral bracts because they are close to the female flower and either contain male flowers or are empty.

Simpson (1992) also stated that based on the inflorescence structure, *Mapania* is closely related to genera *Hypolytrum* and *Paramapania*. *Mapania* can be distinguished for having four or six floral bracts in each spicoid while *Hypolytrum* is having only two floral bracts and rarely three floral bracts in each spicoid. In *Paramapania*, it can be distinguished by having five and rarely six floral bracts in each spicoid.

The *Mapania* fruit is the final component for inflorescence morphology. Both bicarpellate and tricarpetate fruit are present. There are two separate layers to the fruit. One anatropous seed is enclosed in a hard, rocky inner layer called the endocarp. While the exocarp, the outer layer, is either tough or succulent. Succulent exocarp are only present in species from Asia, Indomalaysia, Australia, and the Pacific Islands, and they are thought to aid in dispersal.

It is a challenge in identifying *Mapania* based on the vegetative characters only. Some dissection on the fruits and inflorescence are required to observe its taxonomic important characters. This is extremely difficult for Asian species as they showed a wide range of morphological variations. *Mapania* is known as a genus of herbaceous perennials, however, some particularly larger species are vegetatively similar to species *Pandanus*, for

example, *M. palustris*. Nevertheless, a closer examination discovered that the lowest part of the leaves margin in *Mapania* is smooth compared to the *Pandanus* which have stiff and sharp teeth (Simpson, 1992).

A number of the species' characteristics can be difficult to distinguish at first look. In an instant, even when mature, the inflorescence of *M. longiflora* C.B. Clarke is unique in that it does not appear to expand out and just the tip of each spike is visible. Because the bracts of this species are typically identical, it is easy to confuse it with immature *M. palustris* (Hassk. Ex Steud) F.-Vill. Not only that, it is related to *M. sessilis* Merrill (Simpson, 1992) that both have huge membranous spicoid bracts and flowering bracts. *M. squamata* (Kurz) C.B. Clarke resembles *M. palustris* (Hassk. Ex Steud) F.-Vill., especially when several spikes are present. Besides, *M. maschalina* Kern appears to be closely related to *M. micropandanus* Holttum (Simpson, 1992) since they share several characteristics, including an ascending rhizome along which the leaves are generated and a tiny sessile inflorescence that is partially or entirely hidden by leaf sheaths. *M. borneensis* Merrill (Simpson, 1992), like *M. richardsii* Uittien, has an abruptly constricted leaf apex that contrasts with a progressively narrower base. It is inevitable to get confused when some of the species shared similar characteristics. Most of the time, dissecting the inflorescence is the best option for identification. However, individual spikes are very hard to discern in some capitate species, especially as the inflorescence develops. Spicoids seen in the lower to the middle section of the spike does not appear to have staminate flowers in many species. It is impossible to ascertain whether the stamens are absent or disintegrated. As mentioned by Simpson (1992), stamens are ephemeral. It can last for only around 24 hours at most, and might easily disintegrate as the spike matured.

2.4 Ecology and Distribution of Genus *Mapania*

The majority of *Mapania* species are found in uncommon environments for Cyperaceae, especially the ground layer of tropical rainforests, where little light reaches the canopy (Miraadila *et al.*, 2015). They are frequently found in regions where the soil is unusually moist, muddy, or peaty, as well as in swampy depressions or along the side of pools or streams in flood-prone areas. Other species can be found in somewhat drier areas of the forest floor. About one or two species are found in more specialised forest environments, such as sandstone banks or cliffs. Section *Thoracostachyum* is found in more open places, notably on swamp borders and occasionally in up to 1m of water (Simpson, 1992).

Mapania species can be found in tropical rainforests and along open swamps with altitudes ranging from 50 m to 1650 m above sea level (Smith *et al.*, 2009). It is found mostly below 1000 m, but few species go beyond the range. *M. borneensis* and *M. palustris*, for example, may be found up to 1700 m a.s.l. on Mt. Kinabalu in Sabah, whereas *M. macrocephala* can be found up to 2000 m a.s.l. in New Guinea (Kern, 1974).

Many species occur very seldom in a particular region, and many ostensibly ideal environments are devoid of any species. They can be found alone or in tiny clusters of several plants. *M. tenuiscapa*, for example, dominates the forest undergrowth on wetter soils in Pasoh Forest Reserve in Peninsular Malaysia, but *M. sumatrana* is the dominant plant species in a vast, open expanse of the swamp near a river at Luagan Lalak in Brunei (Simpson, 1992). The table shows the distribution of *Mapania* in Sarawak according to the divisions (Table 2.3).

Table 2.3: The Distribution of *Mapania* According to Division in Sarawak (Simpson, 1992; Miraadila & Shabdin, 2016; Miraadila *et al.*, 2016a; Miraadila *et al.*, 2016b; Shabdin *et al.*, 2018).

No.	Species	Division in Sarawak
1.	<i>M. angustifolia</i> Uittien, 1935	Kapit and Miri
2.	<i>M. ballehensis</i> Miraadila, Shabdin & Meekiong, 2016	Kapit
3.	<i>M. bancana</i> (Miq.) Benth. & Hook.f. ex B.D. Jackson var. <i>bancana</i> , 1985	Kuching, Sri Aman, Bintulu and Miri
4.	<i>M. bauensis</i> Meekiong, Shabdin & Miraadila, 2016	Kuching
5.	<i>M. borneensis</i> Merrill, 1917	Kapit and Miri
6.	<i>M. caudata</i> Kuk, 1931	Kuching, Bintulu, Kapit, Miri and Limbang
7.	<i>M. cuspidata</i> (Miq.) Uittien var. <i>cuspidata</i> , 1939	Kuching, Serian, Bintulu, Kapit and Miri.
8.	<i>M. cuspidata</i> (Miq.) Uittien var. <i>angustifolia</i> (Uittien) Uittien, 1939	Kuching, Sri Aman and Limbang
9.	<i>M. cuspidata</i> (Miq.) Uittien var. <i>merayap</i> Miraadila, Shabdin & Meekiong, 2016	Kuching
10.	<i>M. cuspidata</i> (Miq.) Uittien var. <i>petiolata</i> (C.B. Clarke) Uittien, 1939	Kuching, Sibu, Miri, Limbang and Kapit
11.	<i>M. debilis</i> C.B. Clarke ex Ridl., 1906	Kuching. Sarikei, Bintulu, Kapit and Miri
12.	<i>M. enodis</i> (Miq.) C.B. Clarke, 1908	Kuching, Sri Aman and Miri
13.	<i>M. foxworthyi</i> Merrill, 1916	Kuching
14.	<i>M. graminea</i> Uittien, 1936	Sibu and Kapit
15.	<i>M. hidiriana</i> Miraadila, Shabdin & Meekiong, 2016	Miri
16.	<i>M. hispida</i> D.A. Simpson, 1992	Kuching, Miri and Limbang
17.	<i>M. kadimiana</i> Shabdin, Meekiong & Miraadila, 2016	Sarikei
18.	<i>M. kipas</i> Miraadila, Shabdin & Meekiong, 2016	Kapit
19.	<i>M. latifolia</i> Uittien, 1935	Sri Aman, Kapit and Miri
20.	<i>M. lentalifii</i> Miraadila, Shabdin & Meekiong, 2016	Sri Aman
21.	<i>M. longiflora</i> C.B. Clarke, 1908	Bintulu, Kapit, Miri and Limbang
22.	<i>M. lorea</i> Uittien, 1936	Kuching, Sri Aman, Bintulu, Kapit and Limbang

Table 2.3 continued

23. <i>M. maschalina</i> Kern, 1963	Sarikei and Kapit
24. <i>M. meditensis</i> D.A. Simpson, 1992	Kuching, Sri Aman, Kapit and Miri
25. <i>M. meekiongii</i> Miraadila & Shabdin, 2016	Kapit
26. <i>M. micropandanus</i> Holttum, 1947	Samarahan and Sarikei
27. <i>M. mirae</i> Shabdin & Meekiong, 2018	Kapit
28. <i>M. monostachya</i> Uittien, 1935	Kapit and Miri
29. <i>M. multiflora</i> Shabdin, 2013	Limbang
30. <i>M. obscuriflora</i> D.A. Simpson, 1992	Kapit
31. <i>M. palustris</i> (Hassk. Ex Steud) F.-Vill. var. <i>palustris</i> , 1882	Kuching, Serian, Sri Aman, Mukah, Kapit and Miri
32. <i>M. richardsii</i> Uittien, 1935	SriAman, Kapit and Miri
33. <i>M. rumputensis</i> Shabdin & Meekiong, 2016	Sri Aman
34. <i>M. sapuaniana</i> Shabdin, 2013	Kapit
35. <i>M. sembilingensis</i> Miraadila, Shabdin & Meekiong, 2016	Limbang
36. <i>M. sessilis</i> Merrill, 1922	Bintulu and Kapit
37. <i>M. spadicea</i> Uittien, 1936	Sibu and Miri
38. <i>M. squamata</i> (Kurz) C.B. Clarke, 1908	Kuching and Kapit
39. <i>M. sumatrana</i> (Miq.) Benth. subsp. <i>sumatrana</i> , 1910	Kuching and Miri
40. <i>M. tenuiscapa</i> C.B. Clarke, 1894	Kuching and Kapit
41. <i>M. unimasiana</i> Shabdin, Meekiong & Miraadila, 2016	Bintulu
42. <i>M. walichii</i> C.B. Clarke, 1894	Kuching, Sarikei, Bintulu, Kapit and Miri
43. <i>M. zinnirahea</i> Miraadila & Meekiong, 2016	Sibu

Mapania species which are endemic in Borneo are *M. longiflora*, *M. angustifolia*, *M. lorea*, *M. debilis*, *M. maschalina*, *M. obscuriflora*, *M. richardsii*, *M. borneensis*, *M. latifolia* and *M. hispida*. However, *M. foxworthyi*, *M. graminea*, *M. sapuaniana* and *M. multiflora* were considered as hyper endemic in Borneo. In Sarawak, these species are distributed in Kuching, Samarahan, Sri Aman, Sarikei, Sibuan, Mukah, Bintulu, Kapit, Miri, Limbang and Lawas except for *M. multiflora* which can only be found in Limbang (Miraadila *et al.*, 2015).

According to the International Union for Conservation (IUCN) Red List of Threatened Species, both *M. enodis* (Chadburn, 2016) and *M. longiflora* (Romand-Monnier & Chadburn, 2013) are on the verge of extinction as a result of the loss of their habitat owing to logging.

2.5 Anatomical Study of *Mapania*

Plant anatomy is useful study to distinguish sterile specimen up to the genus level. The anatomical characteristics may vary among the species, hence, it can help to support the data available. Cyperaceae was among the popular family that is used for anatomical study. The most popular genera for the anatomical study were *Carex* and *Cyperus*.

The leaf structure studies (Pfeiffer, 1927; Starr & Ford, 2001) have proven taxonomically informative within Cyperaceae at both genus and species levels. Some of these anatomical investigations have documented the presence of unique characteristics in *Mapania* (or its correlated genus *Hypolytrum*) that are uncommon in Cyperaceae. For example, tetracytic stomata are randomly distributed on the leaves, non-conical or missing silica structures, and spherical, monoporate pollen grains (pseudomonads) (Goetghebeur,

1998; Alves *et al.*, 2002; Coan *et al.*, 2010). Due to the genus's limited range, data on the anatomy of vegetative organs in *Mapania* are currently scarce and limited to a few species, *M. pyncocephala*, *M. sylvatica*, *M. macrophylla*, *M. pycnostachya*, and *M. effusa* (Metcalf, 1971; Alves *et al.*, 2002; Silva *et al.*, 2014; Silva *et al.*, 2019).

Silva *et al.* (2014) conducted the study to investigate the importance of anatomical leaf features for characterization between three species of *Mapania* from the Amazon forest which are *M. sylvatica*, *M. macrophylla*, and *M. pycnostachya*. There are a few anatomical characteristics that distinguished between the section *Mapania* (*M. sylvatica*) and *Pyncocephala* in terms of basal leaves or cataphylls and involucral bracts. Silva *et al.* (2019) also conducted a study on root, rhizome and scapes anatomy of Amazonian species namely *M. sylvatica*, *M. effusa*, *M. macrophylla*, *M. pyncocephala*, and *M. pycnostachya*. For scapes anatomy study, it shows variable morphoanatomical characteristics between these five species. For example, in terms of the presence of aerenchyma, it seems that it was only present in *M. effusa*, *M. pyncocephala*, and *M. pycnostachya*. The presence of aerenchyma is important for the adaptations of plants in humid forests. For root and rhizome anatomy, only three species were selected which are *M. sylvatica*, *M. macrophylla*, and *M. pycnostachya*. The rhizome anatomy study shows uniform characteristics in the genus. However, the presence of collateral vascular bundle in the vascular cylinder can be highlighted as it is also present in *Hypolytrum*. In root anatomy, it shows no anatomical characters of taxonomic importance and the morphoanatomical variations between species are primarily adaptations to their respective environment. The results were presented in Table 2.4.

Table 2.4: The Results of Previous Anatomical Study (Silva *et al.*, 2014; Silva *et al.*, 2019).

Taxa	Mesophyll in involuclral bracts						Reference	
	Aerenchyma	Fiber bundles	Phenolic idioblasts	Size of vascular bundle	Vascular bundles arrangement	Vascular bundles sheath extensions		
<i>M. sylvatica</i> (section <i>Mapania</i>)	Inconspicuous	Absent	Present	Same sized	One level	Absent	Silva <i>et al.</i> , 2014	
<i>M. macrophylla</i> (section <i>Pycnocephala</i>)	Conspicuous	Close to the epidermis	Absent	Small and large alternated	Two level	Present		
<i>M. pycnostachya</i> (section <i>Pycnocephala</i>)	Conspicuous	Close to the epidermis	Present	Small and large alternated	Two level	Present		
	Scapes						Silva <i>et al.</i> , 2019	
	Shape in cross section	Hypodermis	Clorenchyma	Peripheral vascular bundles	Aerenchyma	Phenolic idioblasts		Amyliferous parenchyma
<i>M. effusa</i> (section <i>Mapaniopsis</i>)	Trigonous	One-layered	Compact	Small and in one row	Interrupted in peripheral region and also present in central region	Abundant and scattered		Absent

Table 2.4 continued

<i>M. macrophylla</i> (section <i>Pycnocephala</i>)	Trigonous	2-3 layered	Sparse cells	Small and in 2-3 rows	Absent	Abundant and scattered	Absent
<i>M. pycnocephala</i> (section <i>Pycnocephala</i>)	Trigonous	One-layered	Braciform cells	Small and in one row	Continuous in peripheral region	Absent	Absent
<i>M. pycnostachya</i> (section <i>Pycnocephala</i>)	Trigonous	One-layered	Braciform cells	Small and only at scape edges	Continuous in peripheral region	Few and scattered	Absent
<i>M. sylvatica</i> (section <i>Mapania</i>)	Terete or subtrigonous	One-layered	compact	Large and smaller alternate; in one row	Absent	Abundant and mostly concentrated in peripheral region	Abundant and mostly concentrated in peripheral region
	Thick roots						
	Rhizodermis		Middle cortex		Metaxylem vessels		
<i>M. sylvatica</i> (section <i>Mapania</i>)	Thick-walled cells		10-12 layers of thick-walled cells + layers of thin-walled cells with amyloplasts			2-3 rings	
<i>M. macrophylla</i> (section <i>Pycnocephala</i>)	Thin-walled cells		4-6 layers of slightly thick to thick –walled cells + aerenchyma			1 ring	

Table 2.4 continued

<i>M.</i> <i>pycnostachya</i> (section <i>Pycnocephala</i>)	Thin-walled cells	4-6 layers of slightly thick to thick –walled cells + aerenchyma	1 ring
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2.6 Uses of Cyperaceae and Genus *Mapania*

Cyperaceae are being used traditionally for papermaking, weaving baskets and mats, as well as building rafts and houses. There were some known important wetland starchy root crops like chufa (*Cyperus esculentus*) and water chestnut (*Eleocharis dulcis*). Cyperaceae are also important for the environment as they contribute to filtering water in wetlands and stabilising the soil while their fruit is a food source for birds and other animals. *Cyperus prolifer* and *C. papyrus* were often being cultivated for it is attractive as garden plants (Huxley, 1992).

There are some species in Cyperaceae that are used as medicine. *Kyllingia triceps* was used for treating diabetes. The tubers and rhizome of *C. articulatus*, *C. iria*, and *C. longus* were used as a stimulant for heart, tonic, and carminative. Apart from that, *Scirpus kysoor* and *S. grossus* were used for treating diarrhoea and vomiting while *S. articulatus* were used as purgative.

Mapania is known to have ethnobotanical importance by the local people. For example, *M. palustris* was used for mats and basket making (Burkill, 1935; Simpson, 1992). In Liberia, the local people used *M. linderi* for thatching to cover their homes from the rain and to prevent the water from entering their homes (Burkill, 1985). The leaves of *M. cuspidata* were used in treating fever (Burkill, 1935; Kern, 1974; Simpson, 1992). In Sarawak, Fasihuddin and Laily (1999) discovered that Iban ethnic group burned the leaves or roots of *M. cuspidata* and mixed with coconut oil to treat poisonous caterpillar stings while Kadayan ethnic group drank the root decoction as a poison antidote. According to Ibrahim and Hamzah (1999), *M. cuspidata* leaves is used to treat diarrhoea while the root decoction is used for post-natal treatment. *M. bancana* was reported to be used as a medicinal plant in Sabah, but the application was unknown (Simpson, 1992). A Dyak Tribe

of Central Kalimantan, Ngaju have been using the root of *M. enodis* to treat diarrhoea (Setyowati *et al.*, 2005).

2.7 Secondary Metabolites in Cyperaceae

The phytochemical content between Cyperaceae and Poaceae are somewhat similar. According to Hari Babu and Savithramma (2014), Cyperaceae contains secondary metabolites such as alkaloids, flavonoids, lignins, phenols, quinones, coumarins, saponins, steroids, tannins and terpenoids. Harris and Hartley (1980) discovered that Cyperaceae starchy seeds are silica accumulators, and their vegetative parts stored kestose- and isokestose- type oligosaccharides, and esterified β -coumaric and ferulic acids with non-lignified cell walls. Cyperaceae also have strong tendencies to accumulate flavones and C-glycoflavones.

In the study conducted by Strout *et al.* (2013), the blue iridescence form on the upper surface of *M. caudata* leaves is due to the combination of helical fibres and the accumulation of silica nanoparticles. However, some have even proposed that iridescence may be a type of herbivore defence that has not yet been identified.

The phytochemical studies of *Mapania* done by Shabdin (2012) showed that leaf flavonoids were of the same class as found previously in Australian Cyperaceae. Proanthocyanidins could only be detected in one species, *M. cuspidata*, and here they provided a difference among the three varieties of this species studied var. *petiolata*, var. *cuspidata* and var. *angustifolia*. Extracts of *M. cuspidata* var. *petiolata* were pink-red, suggesting the presence of cyanidin after hydrolysis, while the extracts of var. *cuspidata* were orange-red, suggesting the presence of pelargonidin after hydrolysis. The hydrolysed

extracts of var. *angustifolia* on the other hand were colourless and thus this variety does not contain proanthocyanidins. An anthocyanidin, presumably pelargonidin, was found in only one species, *M. enodis*. This is very interesting as anthocyanidins have rarely been found as aglycone in leaf material and never pelargonidin.

In the plant defence system, these secondary products play a crucial role against threats such as fungi, bacteria, and nematodes, as well as restricting herbivores from feeding the plant (Mazid *et al.*, 2011). For example, flavonoids. From pigmentation to a plant defence mechanism, flavonoids, which are one of the main groups of plant phenolics, play different functions. To shield their foliage from UV damage, plants form anthocyanins as well as colourful water-soluble flavonoid pigments. They can act against a wide range of microorganisms due to their ability to complex with extracellular and soluble proteins and bacterial cell wall proteins (Tiku, 2018).

2.8 Previous Antimicrobial Study Using *Mapania*

Previously Wiart *et al.* (2004) conducted an antimicrobial screening of plants including *M. cuspidata* in the state of Perak, Peninsular Malaysia. From the study, *M. cuspidata* root, bark and leaves extracts were tested against five human pathogenic bacteria (*Bacillus cereus*, *B. subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* & *Staphylococcus aureus*) and human pathogenic fungi (*Candida albicans*). The result shows that the root extracts only show an inhibition when tested against *B. cereus*. Both bark and leaves extracts show inhibition when tested against *B. cereus* and *P. aeruginosa*.

2.9 Crop Pathogenic Fungi

Most crop pathogenic fungi have branching filamentous bodies called mycelium and this mycelium produces numerous branches with each branch called hypha. With a uniform thickness within 1-5 μ m in diameter, its shape is tubular. The mycelium is partitioned into cells by cross wall which contains one or two nuclei called septa. In terms of reproduction, fungi primarily reproduce via spores which consists of one or more cells (Agrios, 2009).

Practically, most crop pathogenic fungi spend part of their lives on their host vegetation and element within the soil or in plant particles in the soil. This caused the crop to be easily infected by crop pathogenic fungi. The majority of the crop pathogenic fungi are heavily dependent on the prevailing temperature and humidity conditions or the availability of water in the environment to survive and grow (Agrios, 2005). These fungi are recognised microorganisms in nature for harming or eliminating plant crops.

There are a variety of symptoms that occur to the plants when infected by fungi. One of the symptoms is necrosis symptoms where the fungi caused local or general necrosis on plant tissues and caused stunting on plant organs. Examples of symptoms are leaf spots, anthracnose, blight, scab, and canker. Other than that, there are symptoms associated with excessive enlargement or development and distortions of plant parts namely clubroot, galls, warts, leaf curls and witches'-brooms. There are also a few additional groups of symptoms recognized that are caused by fungi such as mildew, rust, smut and wilt (Agrios, 2005).

2.9.1 *Colletotrichum musae*

Anthrachnose was caused by the fungi *Colletotrichum* species. These species are favoured in warm weather and frequent rain environment (Roberts *et al.*, 2012). *Colletotrichum* species is one of the major fungi associated with seedling diseases in Malaysia (Zakaria & Bailey, 2000). This species is an important post-harvest apple fruit pathogen in temperate regions. It is one of the most destructive tropical fruit diseases in the world that reduces fruit quality and post-harvest life (Ploetz, 1998). However, *Colletotrichum* species can infect more than one host and may be present more than one *Colletotrichum* species in one host. *C. gleosporioides* and *C. acutatum* are causing the anthracnose disease in pepper where *C. acutatum* cause anthracnose in immature pepper fruits while *C. gleosporioides* occur on rice pepper fruit (Roberts *et al.*, 2012). Nevertheless, this pathogen also caused the brown needle disease of pines which are the most important forest tree disease in Malaysia.

Colletotrichum species which are isolates from banana and guava are known as *C. musae* (Sutton & Waterston, 1970). It is the most common disease associated with anthracnose during the ripening of bananas. *C. musae* is not only infecting its fruit, but it also can infect bracts, petioles, flowers and leaves of banana plants (Jones & Slabaugh, 1998). There is a slight difference in morphology between *C. musae* and *C. gleosporioides*. For example, the shape and colour of conidia as well as appressoria. *C. musae* have brown cylindrical or ellipsoidal conidia while *C. gleosporioides* have hyaline cylindrical conidia. Meanwhile, *C. musae* has dark brown irregularly lobe appressoria while *C. gleosporioides* have brown obovate to clavate appressoria (Zakaria *et al.*, 2009).

Banana fruits are typically harvested before ripening for international trade and processed at a relatively low temperature during the transport and marketing process. Long-distance transport and a prolonged period of storage on the market can make bananas susceptible to the occurrence of the diseases (Thompson & Burden, 1995). Although different symptoms have been reported, some of which may occur during transportation and storage as the symptomless bananas are still being imported and left untreated during the inspection times (Lim *et al.*, 2002). Several species of *Colletotrichum* are responsible for the banana anthracnose. *C. musae*, is the most commonly associated species. It is not only recorded in Malaysia (Sakinah *et al.*, 2014), but also recorded in many banana-growing regions such as India (Thangavelu *et al.*, 2004), Sri Lanka (Abayasekara *et al.*, 2013), Thailand (Udayanga *et al.*, 2013), and Brazil (Vieira *et al.*, 2017). It is one of the most significant and widely spread diseases of ripening and matured bananas. It is also especially associated with wastage during handling and transport following injuries in the form of scratches and other wounds incurred by the fruit (Wardlaw, 1934). The diseases are not only occurred during handling and transportation, but they also occurred during the growing season in the field as they can infect at any time (Simmonds & Mitchell, 1940). Due to this, it caused losses in the local markets.

Rabari *et al.* (2018) investigated the antifungal effect of essential oil from *Cyperus scariosus* against *C. gloeosporioides* by using standard disc diffusion assay. The result showed moderate antifungal activity with zone of inhibition between 5 mm and 20 mm. Ramli *et al.* (2022) revealed that the crude methanol extracts from aerial parts of *F. miliaceae* and *F. dichotoma* inhibited *Colletotrichum* spp. at 42.27% and 38.33% respectively. The minimum inhibitory concentration (MIC) and minimum fungicidal

concentration (MFC) of both plant extracts towards *Colletotrichum* spp. was also recorded at 1.25 mg/mL in the study.

2.9.2 *Fusarium solani*

Fusarium solani is a highly resistant fungus isolated from soil and plant debris. It is a fungus that can produce three types of asexual spores known as macroconidia, microconidia and chlamydospores. It can be spread via air and water. On top of that, these pathogens were favoured by high moisture soil. It is possible to distinguish *F. solani* into 50 subspecific lineages and most of them have not been formally further identified (O'Donnell, 2000). *F. solani* are a well-known plant pathogen that causes different types of disease in a large variety of plants where at least 111 species of plants from 87 genera were infected by it (Kolattukudy & Gamble, 1995). The symptoms of *F. solani* can be developed rapidly in cool weather and causes diseases such as root and fruit rot of *Cucurbita* spp., root and stem rot of pea, “sudden death” syndrome of soybean, foot rot of bean and dry rot of potato tubers (Zaccardelli *et al.*, 2008). Moreover, this has caused a problem in the production of soybean in North and South America (Aoki *et al.*, 2003). In addition, opportunistic infections of humans and other animals are increasingly associated with them, leading to systemic infections with a high mortality rate (Krcmery *et al.*, 1997). It also localized infections on skin and other body parts (Gugnani *et al.*, 1976; Gupta *et al.*, 2000).

Several reports have been made by Nik Masdek (1982), Nik Masdek and Abd Karim (1985), Yusup (1985), and Salleh *et al.* (1988) on the *Fusarium* rootrot disease and stem rot disease affecting tobacco (*Nicotiana tabacum* L.) caused by *F. solani* in Malaysia. However, Malaysia or specifically Sabah are not the only country where *F. solani* were

being listed as a causal agent of tobacco (Williams & Liu, 1976). Other tobacco-growing countries such as Australia (Simmonds, 1966), Brunei (Peregrine & Ahmad, 1982), and Papua New Guinea (Shaw, 1984) are also affected by these pathogens. According to Azmi (1991), in peninsular Malaysia, the root rot disease caused by *F. solani* and Fusarium wilt caused by *F. oxysporum* were widely spread in the tobacco-growing region mainly Melaka, Kelantan, Terengganu, and Negeri Sembilan states.

Palm oil is the primary industry in Malaysia which contributed 37.7% to the value-added of the Agriculture Sector in 2019 (Department of Statistics Malaysia, 2020). Malaysia is the second-largest supplier of palm oil with an average fresh fruit bunches (FFB) yield of 16.73 tonnes per hectare in the year 2020 (Malaysian Palm Oil Board, 2020). However, there were reports that crown disease frequently occurs in young palm during the early stages of planting (Monge *et al.*, 1994; Corley & Tinker, 2003). According to Chinchilla (2008), the symptoms of crown disease showed by rachis bending, the breaking and rooting of some spears and growing leaves. As a function of the severity of crown disease, the yield of fruit bunches was a decline with proof of the highest loss as at that age the canopy is not yet closed, so, the reduced crown diameter of the affected palms must affect light interception and therefore photosynthetic development due to the bending of the leaves (Breure & Soebagjo, 1991). Among the most common microorganisms isolated from the symptoms of the disease were *F. solani* and *F. oxysporum* (Monge *et al.*, 1994).

El- Mergawi *et al.* (2018) conducted a study using methanolic extract from *C. conglomerates* against *F. solani*. The antifungal activity of *C. glomerates* was studied by using the agar medium contact assay (in vitro). The results showed a low antifungal

activity when tested against *F. solani*. Adel *et al.* (2020) also investigated the antifungal activity of *C. rotundus* against *F. solani*. The results revealed that the methanol extract showed moderate antifungal activities against the tested phytopathogenic fungi. Preliminary screening of antifungal activity using dual culture test conducted by Ramli *et al.* (2022) showed that the crude methanol extracts from aerial parts of *F. miliaceae* and *F. dichotoma* inhibited *Fusarium* spp. at 37.34% and 29.32% respectively. The study also investigated the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of *F. miliaceae* and *F. dichotoma* towards *Fusarium* spp. suspension by using two-fold dilution method. The results showed that the lowest concentration of methanol extract that able to inhibit *Fusarium* spp. is 2.5 mg/mL.

2.9.3 *Pyricularia oryzae*

Pyricularia oryzae is plant-pathogenic fungi that cause serious disease affecting rice. *P. oryzae* is an ascomycete and is classified under the family Magnaporthaceae. “Pyricularia” refers to the pyriform shape of the conidia (Bussaban *et al.*, 2005). Perithecia are the location where the asci are founded. The mycelium of this fungus is septate. This pathogen is favoured in a moderate temperature, high levels of nitrogen fertilizer, and high moisture regions. Rice blasts affect the leaves, causing white to grey or reddish-brown diamond-shaped lesions with reddish to the brown border (Hajano *et al.*, 2011). The lesions can enlarge, coalesce and disrupt the whole leaves. *P. oryzae* not only attack the leaves but the collar, necks, panicles, pedicels and seeds as well. According to Chuwa *et al.* (2014), the blast panicle results in a total loss of yield either by causing the grain to be partially unfilled or causing the panicle to split.

P. oryzae conidia are found in the air throughout the tropics during the year. It is produced and released by the fungus during periods of relatively high humidity. It became airborne and adhered strongly through sticky mucilage that produces at its tip when landing on rice plants. The conidia can germinate when the surface of the rice leaf or stem is wet. Then, the appressorium is formed by the germ tube through which the fungus penetrates or reaches plant surfaces through stomata (Agrios, 2005b).

Malaysia's Self-Sufficiency Level (SSL) in rice production currently stands at 71.6% of the Malaysian population (Department of Statistics Malaysia, 2020). In 2019, the total planted area in Malaysia was 684,416 hectares which produced 2,912,203 tonnes of rice grain and generated approximately 1,876,922 tonnes of white rice (DOA, 2019). Not only the national definition of self-sufficiency level was the ability of a specific country to provide sufficient food in local production, but also the capacity and affordability to acquire and maintain sufficient nutritious food without any obstacles (Chamhuri *et al.*, 2014).

One of the challenges of growing rice, however, is pests and diseases that are capable of causing tremendous economic losses and reducing the national Self-sufficiency level. Rice field that is infected by blast diseases could reduce the yield by 50% (Saad *et al.*, 2004). In addition, the rice blast pathogens can live for at least two crop seasons on rice residues in the field (Raveloson *et al.*, 2018). However, since the pathogens have the potential to adapt and later emerge into new and more virulent pathotypes, rice blast disease has become more difficult to control (Nurul Nahar *et al.*, 2020). The proliferation of dominant pathotypes that have the potential to adapt and grow new virulent pathotypes could render these varieties susceptible to blast disease (Missman & Zakaria, 2019).

Although several blast resistance varieties have been released in Malaysia, the degree of blast resistance has deteriorated from year to year (Missman & Zakaria, 2019). Thus, there is still a need for fungicides for immediate control especially in disease-prone epidemic-dealing areas (Saad & Supaad, 1982).

Hu *et al.* (2001) conducted a screening bioassay detecting morphological deformation of mycelia germinated from conidia of *P. oryzae*. The aqueous and ethanol extracts of *C. iria* whole plants and *C. rotundus* root were prepared to test for their antifungal activity against *P. oryzae*. The extracts were unable to inhibit conidia germination and mycelium growth.

CHAPTER 3

MATERIALS AND METHODS

3.1 Sampling Sites

Mapania species were collected from various localities in Sarawak through four field trips (Figure 3.1) from August 2017 until April 2018. *Mapania* species were observed and photographed on their vegetative and reproductive parts including their habits and habitats. The detailed observations on every component of species identification was based on the method as reported by Simpson (1992) such as habit, rhizome, leaf, inflorescence, spike and fruit. The measurements were taken with a measuring tape and a digital clipper, and the images were taken digitally. Flowers and seeds' morphologies were examined using a hand lens or USB digital microscopy. The identified species were annotated and illustrations of newly identified species and designations of their nomenclature types were provided. The morphological characteristics of each species collected were observed and measured according to the description in Table 3.1. The whole parts of the plant were harvested for the study. The number of samples collected were based on the distribution of species in the area. For herbarium specimen, three samples per species were collected and three pieces of leaves and petiole were taken from each specimen for Anatomical study, while 10 to 15 samples per species were collected for Antifungal study.

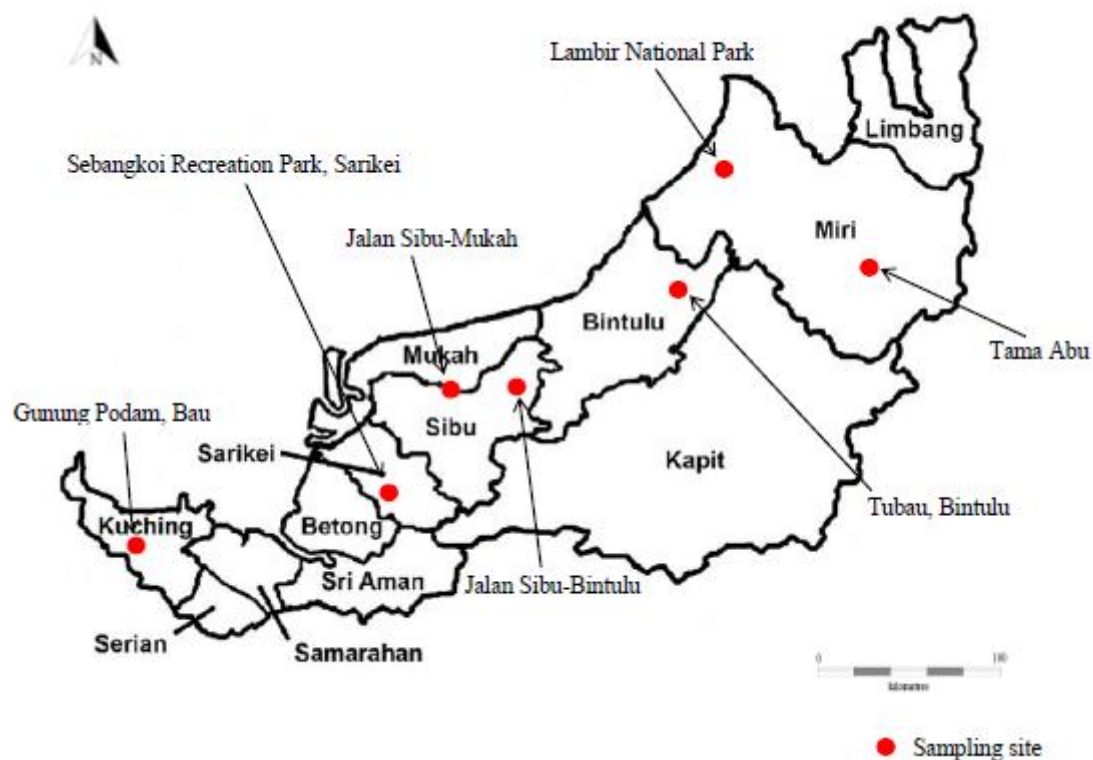


Figure 3.1: Map of Sarawak Showing Marked Sampling Site

Table 3.1: Plant Parts and Characters Observed for Each Specimen Encountered.

Parts of the plant	Characters
Whole plant	Habit Height
Leaves	Leaf blade: Shape (linear/linear-oblong/oblong) Apex (acute/acuminate/cuspidate/filiform) Bases (acute/acuminate) Margin (entire/serrulate) Venation (parallel; 1-nerved/3-nerved) Pseudopetiole: (present/ absent) (Length & diameter) Surface feature (glabrous/hispid) Length Width Colour (abaxial & adaxial)

Table 3.1 continued

Inflorescence	Types (solitary/capitate/sessile/paniculate)
	Culm: (Central/lateral)
	Shape (rounded/angular/trigonous)
	Presence (glabrous/scabrid/hispid)
	Size
	Spike: No. of spike
	Shape (lanceolate/elliptic/rounded)
	Size
	Involucral bract: Type (glumaceous/foliaceous)
	No. of bract(s)
	Spicoid bract: Colour
	Shape
	Size
	Glabrous/hispid
	Floral bract: No. of spicoid
	Shape
	Size
	Colour
	Fruit: (bicarpellate/tricarpellate)
	Shape (ellipsoid/obovoid/lageniform/ovoid/subpyriform/ subglobose/pyriform/oblanceoloid)
	Colour
	Size
	Apex (conical/apiculate/elongated/acuminate/triangular/ acute/obtuse)
	Base (stipitate/rounded/conical/truncate)
	Exocarp (succulent/hard)
	Costae (absent/present)
Root	Types (rhizomatous/stoloniferous)
	Length
	Diameter

3.2 Specimens Preparation

3.2.1 Herbarium Specimen

All samples collected were tagged with the collection number and prepared according to the standard herbarium protocols (Bridson & Forman, 1992).

Based on the standard protocol, the samples were preserved with 70% alcohol in a heavy-duty polyethene bag for a week. After a week, the preserved samples were taken out and placed in between dry newspapers. The samples were then stacked and arranged properly on the metal press and tightened using a rope. It was left to dry in an oven at 60 °C laboratory for two weeks. After the drying process, the specimens were glued and sewn on the mounting paper to prevent them from falling apart. The specimen label was prepared and glued on the mounting paper.

3.2.2 Anatomical Study

Mature leaves of the plants were selected and prepared according to standard protocol by Cutler (1978) with a little modification where the leaves surfaces were scraped manually rather than boiled it with 3% nitric acid. The preparations of the anatomical study were as followed.

3.2.2.1 Preparation for Epidermal Peel

Mapania leaves were cut at the middle parts of the lamina were cut approximately by 1 cm x 1 cm (Figure 3.2). The leaves were scraped thoroughly using surgical blades to remove the leaf surface until it is cleared and transparent. Then, they were cleaned using a brush to remove excess debris until it becomes a clean tissue.

The tissue was soaked in a petri dish containing 70% Clorox until it decolourized. Then, the tissue was rinsed with distilled water to remove debris on the tissue before staining procedures.

After rinsing, the tissue was soaked with Safranin in a petri dish for 3 minutes. Thereafter, the tissue underwent a series of alcohol (ethanol) 50%, 70%, 80%, 90%, and 100% for permanent slide preparations. Then, the stained tissue was placed on clean slides

and glued using Canada balsam before covering it with a cover-slip. The prepared slide was dried in an oven at 60 °C for three days or more until it was fully dried. Lastly, the slide was observed under a light compound microscope and the image was taken and analysed.



Figure 3.2: Part of the Leaf Blade Taken for Epidermal Cell and Stomata Observation

3.2.2.2 Preparation of Stomata of the Leaves

The leaves were scraped using a surgical blade and brush to obtain clean tissue. The clean tissue was soaked with 70% Clorox in a petri dish until decolourized. Then, the tissue was rinsed with distilled water to remove debris before staining procedures.

Afterwards, the tissue was soaked in a petri dish containing Safranin for 3 minutes. Subsequently, the tissue underwent a series of alcohol (ethanol) 50%, 70%, 80%, 90%, and

100% for permanent slide preparations. The stained tissue was glued on a clean slide using Canada balsam and covered with a cover-slip. Lastly, the permanent slide was observed using a light compound microscope. The images were taken and analysed. The stomatal index (Salisbury, 1927) and stomatal density (Poole & Kürschner, 1999) were counted using the following formula (Equation 3.1 & 3.2) and the data were tabulated.

Stomatal index (SI) is the number of stomata expressed as a percentage of the total number of cells per unit area:

$$\text{Stomatal Index (SI)} = \frac{S}{E+S} \times 100 \quad \text{Equation 3.1}$$

SI - Stomatal index

S - No. of stomata per unit area

E – No. of epidermal cells per unit area;

Stomatal density (SD) is the number of stomata per unit area:

$$\text{Stomatal Density (SD)} = \frac{\text{Total number of stomata}}{A=\pi r^2} \quad \text{Equation 3.2}$$

SD – Stomatal density

A – Area (mm²)

π – 3.142

r – radius (mm²)

3.2.2.3 Preparation for Transverse Section of Petiole and Midrib

Free hand petiole and midrib sections were obtained by using a surgical blade (Figure 3.3). The cross-sections were soaked with 70% Clorox until it decolourized. Then, it is rinsed with distilled water and transferred to a petri dish containing Safranin for 3 minutes. After 3 minutes, the cross-sections were rinsed and stained with Alcian Blue for 3 minutes. The cross-sections have then undergone a series of alcohol (ethanol) 50%, 70% and a few drops of hydrochloric acid (HCl), 80%, 90%, and 100% for 5 minutes each. Next, the cross-sections were placed on a clean slide and glued using Canada balsam before covering with a cover-slip. The slide is left to dry in an oven at 60 °C. Lastly, the slide was observed under a light compound microscope and the image was captured and analysed.

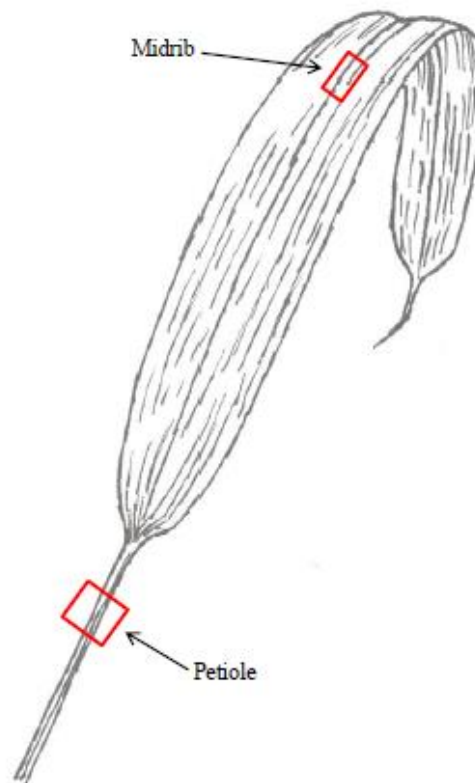


Figure 3.3: Parts of the Midrib and Petiole Taken for the Transverse Section Observation

3.2.3 Antifungal Assay

The antifungal assay was prepared according to the procedure described by Abu Bakar *et al.* (2018) with modification where the crude extract used is extracted from *Mapania* leaves and root. The preparations were as follows:-

3.2.3.1 Samples Preparation

The ten selected *Mapania* species, viz. *M. caudata*, *M. cuspidata* var. *petiolata*, *M. debilis*, *M. enodis*, *M. kadimiana*, *M. multifolia* sp. nov., *M. squamata*, *M. unimasiiana*, *M. walichii* and *M. zinnirahea* were collected with 10 to 15 samples per species and washed thoroughly with tap water. After washing with tap water, the samples were rinsed with distilled water to remove any contaminants. The samples were patted dry and air-dried for two weeks. Next, the leaves and roots were separated and blended into powder form. Inflorescence were not included as it is limited in number.

3.2.3.2 Preparation of Crude Extracts

Approximately 200 g of powdered sample were soaked with methanol to extract polar compounds mainly flavonoid in a conical flask. The conical flask was covered with aluminium foil and shaken for three days at room temperature. After three days, the mixture was filtered using Whatman No.1 filter paper. The filtered extract was evaporated using a rotary evaporator with a water bath at 50 °C to obtain the methanol crude extract. The pure crude extract was weighed to obtain the dried weight and the percentage of extraction yield was calculated using Equation 3.3.

$$\text{Extraction Yield (\%)} = \frac{\text{Weight of extract after evaporating solvent}}{\text{Dry weight of the sample}} \times 100 \quad \text{Equation 3.3}$$

3.2.3.3 Preparation of Fungi for Fungal Growth Inhibition Test

Four types of fungi were chosen for the fungal growth inhibition test namely *Colletotrichum musae*, *Fusarium solani*, and *Pyricularia oryzae* (isolates POSA 1 & POSA 2) due to its availability. *F. solani* was provided by Agriculture Research Centre, Semenggok (Department of Agriculture, Sarawak) while *C. musae* (Kong & Vu, 2019) and *P. oryzae* isolate POSA 1 and POSA 2 (Hussin *et al.*, 2020) were from Universiti Malaysia Sarawak. To grow *C. musae* and *F. solani*, Potato Dextrose Agar (PDA) was used. For *P. oryzae*, the medium used was Oat Meal Agar (Hussin *et al.*, 2020). A plug of each fungus was obtained from the stock and transferred on the agar.

3.2.3.4 Fungal Growth Inhibition Test

The procedures were prepared according to Abu Bakar *et al.* (2018). Media containing 1% Dimethyl sulfoxide (DMSO) was used as a control treatment while three different concentrations of methanolic crude extracts (100 µg/ml, 50 µg/ml & 25 µg/ml) were prepared for the assay. Then, 20 ml media were poured into the petri dish and by using a cork borer, a plug of fungi (6 mm Ø) taken from 7 day old culture (*C. musae* & *F. solani*) and 14 day old culture (*P. oryzae*) was taken from the peripheral of the culture and put at the centre of the petri dish. At the bottom of the petri dish, two lines intersecting at the centre were drawn (Figure 3.4). The assay for each pathogenic fungus was performed with a total of four treatments with 10 replicates of each treatment. The assay was repeated twice.

The growth of mycelium was marked and measured daily. Until the fungi were fully occupied by the petri dish, the experiment was monitored and recorded daily. The result of the average length of the mycelium grow per day were calculated (Equation 3.4 &

3.5). The average length of the mycelium growth at different concentrations of methanolic crude extracts for each *Mapania* species were analysed using One Way Anova (SPSS 21).

$$\text{Colony Fungal Growth (D)} = \frac{(\text{Axis A} + \text{Axis B} + \text{Axis C} + \text{Axis D})}{4} \quad \text{Equation 3.4}$$

$$\text{Growth Rate} = \frac{[(D2 - D1) + (D3 - D2) + (D4 - D3) + \dots]}{N - 1} \quad \text{Equation 3.5}$$

Where N is the number of days of growth of the pathogen.

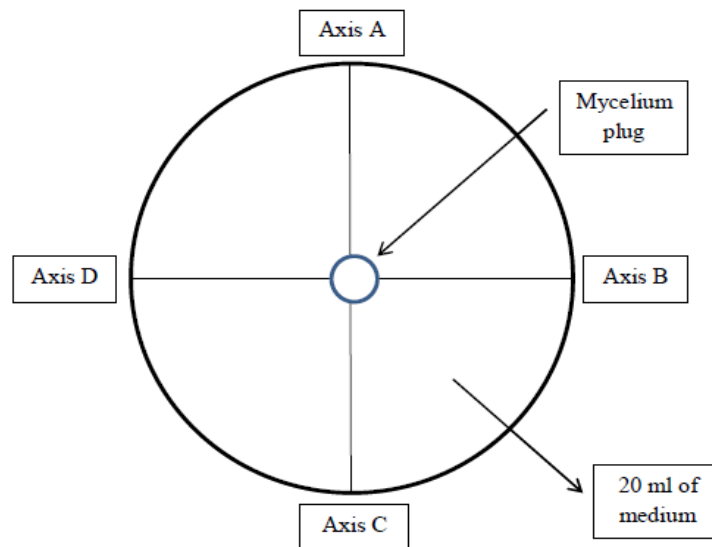


Figure 3.4: Overview of Agar Plate

CHAPTER 4

ANATOMICAL STUDY

4.1 Selected *Mapania* Species for Anatomical Study

Anatomical study is important as it has the value and significance for a key of differentiation in order level to genus level such as epidermal (Stace, 1965). It also can assist in the identification of sterile plants (Meltcalfe, 1969). For this study, 11 out of 15 *Mapania* species were selected for anatomical study as the remaining species were fragile and not being able to process for analysis. Based on morphological characters, only 4 out of 11 species collected were having pseudopetiole. Thus, three species were selected for the anatomical feature of the cross-section of the petiole. Meanwhile, 7 species were selected for cross-section midrib.

4.1.1 Epidermal Cell

In monocotyledons, the epidermal cell usually showed 4-6 sided or more sided and the anticlinal walls were either straight, undulate (curvy) or sinuous (curvier). Generally, both surfaces of *Mapania* species are glabrous, with the absence of trichomes or any special ornamentation.

Table 4.1: Characteristics of an Epidermal Cell of Selected *Mapania* Species of Sarawak.

Taxa	Shape in surface view		Anticlinal walls		Average size (µm)
	Abaxial	Adaxial	Abaxial	Adaxial	
<i>M. caudata</i>	polygonal	polygonal	Undulate	Undulate	71.64 x 37.32
<i>M. cuspidata</i> var. <i>cuspidata</i>	linear, elongate cells	linear, elongate cells	Sinuous	Sinuous	76.8 x 28.84

Table 4.1 continued

<i>M. cuspidata</i> var. <i>petiolata</i>	linear, elongate cells	linear, elongate cells	Sinuuous	Sinuuous	85.36 x 31.36
<i>M. kadimiana</i>	hexagonal	hexagonal	Undulate	Undulate	59.56 x 50.36
<i>M. lorea</i>	hexagonal	hexagonal	Sinuuous	Sinuuous	76.32 x 35.12
<i>M. meditensis</i>	random	hexagonal	Undulate	Undulate	45.47 x 51.25
<i>M. multifolia</i> sp. nov.	random	hexagonal	Sinuuous	Sinuuous	58.05 x 25.45
<i>M. palustris</i>	hexagonal	hexagonal	Undulate	Undulate	53.44 x 38.7
<i>M. spadicea</i>	hexagonal	random	Undulate	Undulate	53.32 x 48.3
<i>M. unimasiana</i>	random	hexagonal	Undulate	Undulate	58.39 x 45.07
<i>M. walichii</i>	hexagonal	hexagonal	Undulate	Undulate	67.72 x 49.96

From the result, the 11 selected *Mapania* species showed two different patterns of anticlinal walls (Table 4.1). Only four species showed sinuous anticlinal walls namely *M. cuspidata* var. *cuspidata* (Figure 4.1B), *M. cuspidata* var. *petiolata* (Figure 4.1C), *M. lorea* (Figure 4.1E), and *M. multifolia* sp. nov. (Figure 4.1H). This feature was also reported in section *Pycnocephala*, namely *M. macrophylla* and *M. pycnostachya* (Silva *et al.*, 2014). The other 7 species showed a contradictory type of undulate anticlinal walls.

There are a few species that showed a different shape of the epidermal cell on both surfaces. In terms of the shape of the epidermal walls from a surface view, some species contain different types of shapes on one surface. Some of the species showed a mixture of pentagonal (5 sided cells), hexagonal (6 sided cells), and polygonal (which are more than 6 sided cells) on one surface. Thus, as a result, it is labelled as random. In this case, it showed in adaxial surfaces of *M. spadicea* (Figure 4.1G) while in abaxial surfaces of *M. unimasiana* and *M. multifolia* sp. nov. (Figure 4.1H). Most of the species showed hexagonal shape of the epidermal cell on both surfaces. Only *M. cuspidata* var. *cuspidata* (Figure 4.1B) and *M. cuspidata* var. *petiolata* (Figure 4.1C) have linear and elongate cell shape while *M. caudata* (Figure 4.1A) has polygonal cell shape. The occurrence of cell

inclusion on the epidermis of *M. cuspidata* var. *cuspidata* (Figure 4.1B), which is lacking in var. *petiolata*, is an intriguing discovery. Cell inclusions are non-living cell structures that include pigment granules, lipid droplets, or crystals.

The average size of the epidermal cell was 45.47 - 85.36 μm x 25.45 - 51.25 μm . The smallest average size was *M. meditensis* while *M. cuspidata* var. *petiolata* has the largest epidermal cells among the 11 selected species.

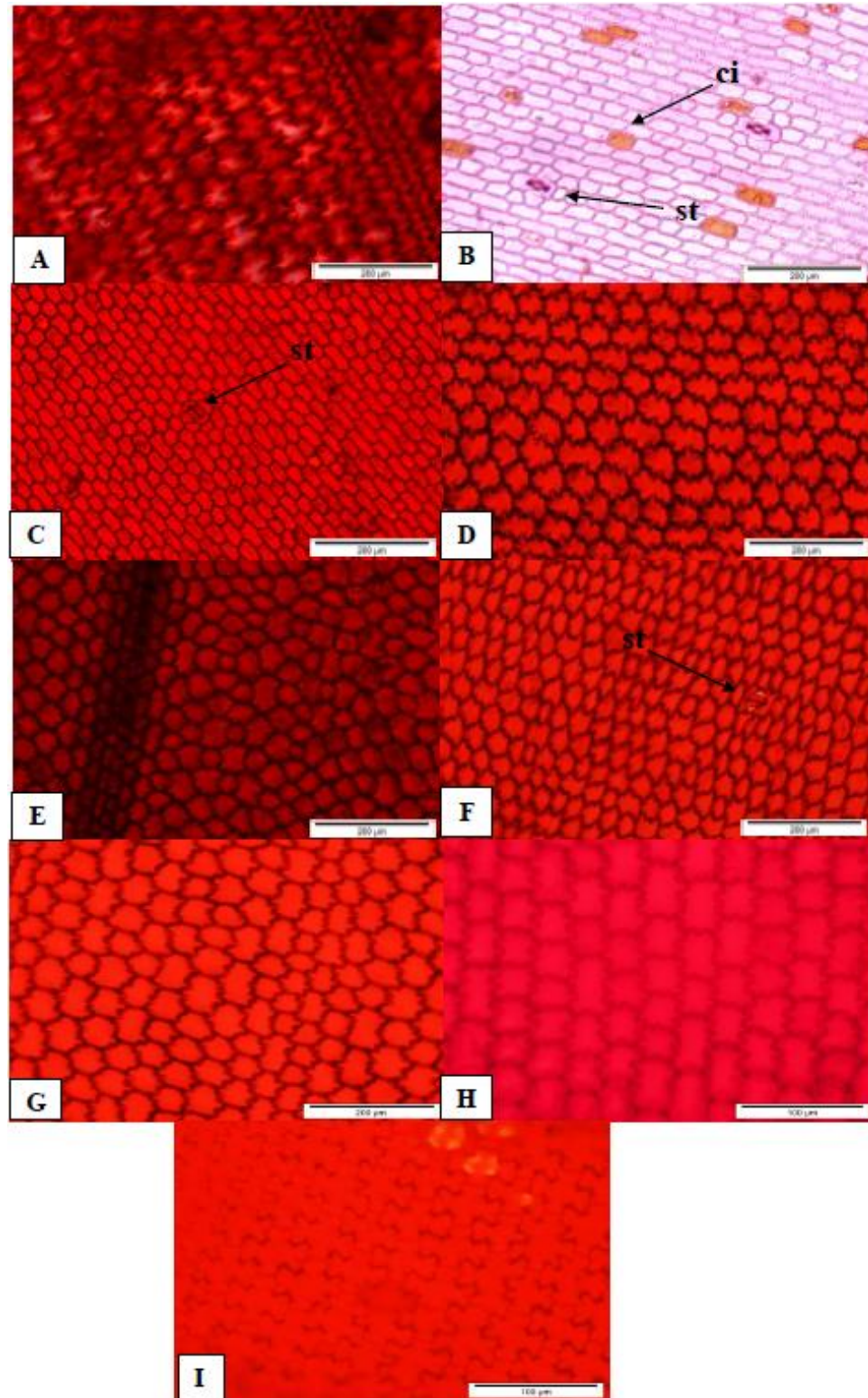


Figure 4.1: The View of the Adaxial Surface. **A.** *M. caudata*. **B.** *M. cuspidata* var. *cuspidata*. **C.** *M. cuspidata* var. *petiolata*. **D.** *M. kadimiana*. **E.** *M. lorea*. **F.** *M. palustris*. **G.** *M. spadicea*. **H.** *M. multifolia* sp. nov. **I.** *M. mediterranea*. Abbreviations: **st** - stomata, **ci** - cell inclusion. Magnification: **A-G** = 20×; **H & I** = 40×. Scale bar: **A-G** = 200µm; **H & I** = 100µm

4.1.2 Stomata

According to Cutler *et al.* (2008), the two layers of the epidermis can be differentiated by the presence of stomata. Stomata normally present similarly on both abaxial and adaxial surfaces in monocotyledon and are commonly arranged in parallel rows (Singh & Srivastava, 2011). Leaves may have stomata on both abaxial and adaxial (amphistomatic), or only adaxial surface (hyperstomatic) and only on abaxial surface (hypostomatic) (Cutler, 1978; Cutler *et al.*, 2008). Stomata is a small element in the epidermal tissue system (Singh & Srivastava, 2011). The stomata pores help to regulate the opening and closing of the stomata which the key influence is water movement (Cutler *et al.*, 2008). The stomatal complex is composed of stoma pores, guard cells and subsidiary cells or also known as accessory cells (Cotthem, 1970). The four main types of stomata are anomocytic (irregular-celled), anisocytic (unequal-celled), diacytic (cross-celled), and paracytic (parallel-celled) (Metcalf & Chalk, 1950). Among these 11 selected *Mapania* species, most of them are hypostomatic except for *M. cuspidata* var. *cuspidata* (Figure 4.2B), *M. cuspidata* var. *petiolata* (Figure 4.2C), and *M. meditensis* (Figure 4.2I) which are amphistomatous leaves.

Table 4.2: Characteristics of Stomata of Selected *Mapania* Species of Sarawak.

Taxa	Types of stomata	Stomata size (µm)	Stomatal index (%)	Stomatal density (mm ²)
<i>M. caudata</i>	tetracytic	35.8 x 36.28	6.8	38
<i>M. cuspidata</i> var. <i>cuspidata</i>	anomocytic	50.84 x 39.04	9.1	76
<i>M. cuspidata</i> var. <i>petiolata</i>	anomocytic	43.24 x 38.44	10.5	100
<i>M. kadimiana</i>	tetracytic	54.0 x 69.56	8.4	53
<i>M. lorea</i>	tetracytic	46.36 x 34.72	10.9	69
<i>M. meditensis</i>	anomocytic	36.8 x 34.1	14.5	92
<i>M. multifolia</i> sp. nov.	anomocytic	44.6 x 27.0	9	53
<i>M. palustris</i>	diacytic	67.28 x 89.72	2.7	7
<i>M. spadicea</i>	anomocytic	49.92 x 42.09	11.8	61

Table 4.2 continued

<i>M. unimasiana</i>	anomocytic	43.35 x 33.67	15.8	92
<i>M. walichii</i>	tetracytic	72.36 x 59.8	11.2	115

The average size of stomata was 35.8 - 72.36 μm x 27.0 – 89.72 μm . According to Metcalf (1969), nearly all species stomata in Cyperaceae are paracytic and rarely tetracytic. However, in *Mapania*, there are three types of stomata observed namely anomocytic, tetracytic, and diacytic (Table 4.2). Most of the species are anomocytic. The other species which are *M. caudata* (Figure 4.2A), *M. kadimiana*, *M. lorea* (Figure 4.2E), and *M. walichii* (Figure 4.2D) types of stomata appear to be tetracytic except for *M. palustris* (Figure 4.2F) which bear a diacytic type of stomata.

Based on the stomatal index results, it seems that *M. unimasiana* has the highest stomatal index which is 15.8%. Contradictory to this species, *M. palustris* has the lowest stomatal index (2.7%) among the 11 selected species. However, in terms of stomatal density, the species with the highest stomatal density is *M. walichii* (115 mm^2) while *M. palustris* (7 mm^2) has the lowest stomatal density.

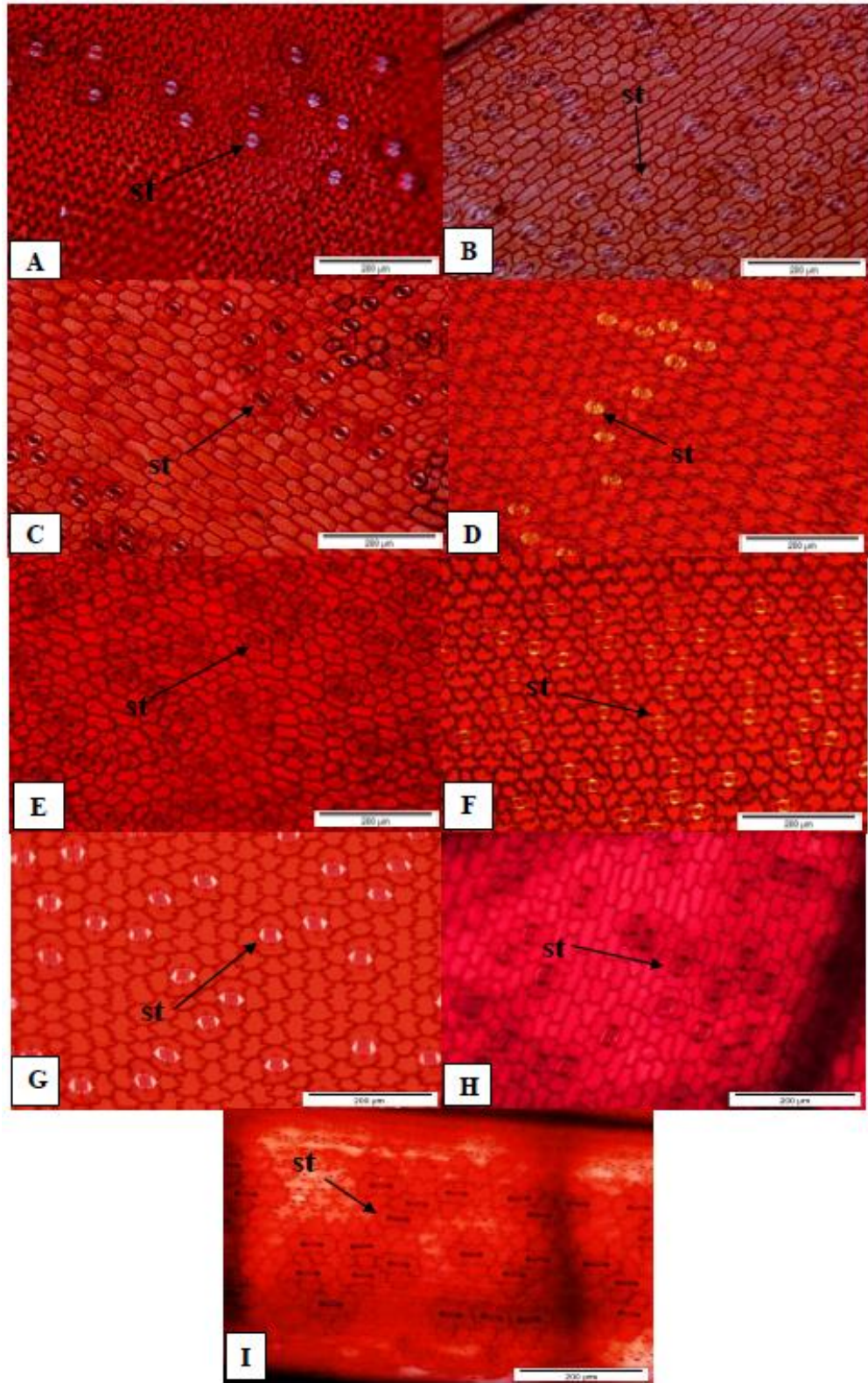


Figure 4.2: The View of the Abaxial Surface. **A.** *M. caudata*. **B.** *M. cuspidata* var. *cuspidata*. **C.** *M. cuspidata* var. *petiolata*. **D.** *M. walichii*. **E.** *M. lorea*. **F.** *M. palustris*. **G.** *M. spadicea*. **H.** *M. multifolia* sp. nov. **I.** *M. meditensis*. Abbreviations: st - stomata. Magnification: A-I = 20×. Scale bar: A-I = 200µm

4.1.3 Cross-section of Petiole and Midrib

Petiole shape

Based on the morphology of these 11 selected species, only five of the species have pseudopetiole and three were selected for petiole cross-section observation as the remaining species were ruptured when being processed. They have various shapes of petiole abaxial outline and adaxial outline. According to Melcalfe & Chalk (1950), in taxonomical analysis, petioles may have some significant differences. Howard (1963) also reported that the characteristics of petioles can be used for identification in family, generic or in rare cases at the species level.

Table 4.3: Characteristics of Petiole Cross-section of Selected *Mapania* Species of Sarawak.

Taxa	Outline shape		Average size (µm)
	Abaxial	Adaxial	
<i>M. cuspidata</i> var. <i>cuspidata</i>	Concave with keel shape	V-shape	1323.35 x 911.97
<i>M. cuspidata</i> var. <i>petiolata</i>	Concave	U-shape	1429.16 x 2153.70
<i>M. meditensis</i>	Concave	U-shape	911.65 x 1892.20

The four basic shapes of petiole were observed, namely, U-shaped, V-shaped, concave, and concave with a keel (Table 4.3). From the three selected species, only *M. cuspidata* var. *cuspidata* (Figure 4.3A) abaxial outline is concave with keel while the other two species were concave (Figure 4.3). *M. cuspidata* var. *petiolata* (Figure 4.3B) and *M. meditensis* (Figure 4.3C) shared the same abaxial and adaxial outline which is concave (abaxial) and U-shaped (adaxial). For *M. cuspidata* var. *cuspidata* (Figure 4.3A), its adaxial outline is V-shaped. From Table 4.3, it seems that *M. cuspidata* var. *petiolata* has

the thickest petiole (1429.16 x 2153.70 μm) and *M. meditensis*. has the thinnest petiole (911.65 x 1892.20 μm).

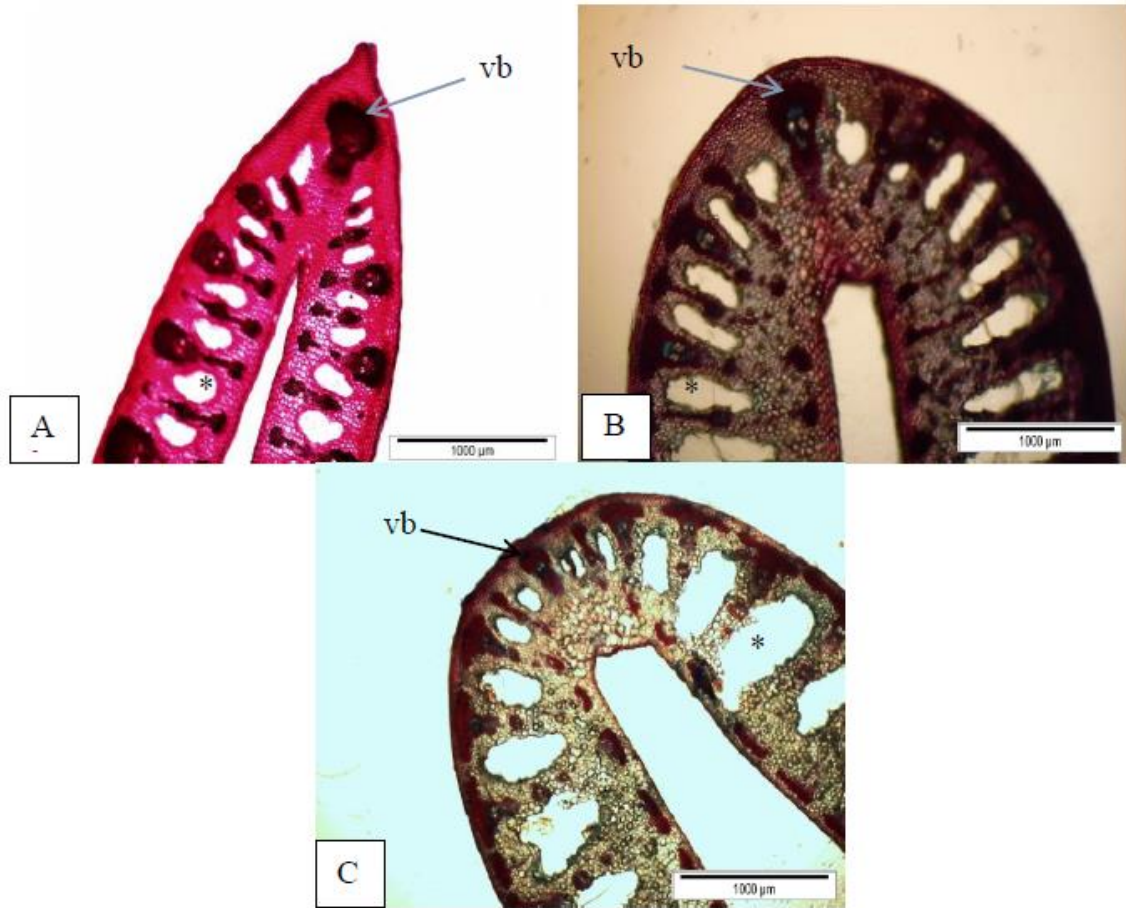


Figure 4.3: *Mapania* Petiole Cross-section. **A:** *M. cuspidata* var. *cuspidata*, **B:** *M. cuspidata* var. *petiolata*, and **C:** *M. meditensis*. Abbreviations/symbol: * - air chamber, **vb** - vascular bundles. Magnification: **A-D** = 4 $\times$. Scale bars: **A-D** = 1000 μm

Midrib

The midrib outline was observed and identified according to the terminology described by Croat & Bunting (1979). The 7 selected *Mapania* species showed various abaxial and adaxial midrib outlines (Table 4.4).

Table 4.4: Characteristics of Midrib Cross-section of Selected *Mapania* Species of Sarawak.

Taxa	Outline shape		Layers of bulliform cell	Average size (µm)
	Abaxial	Adaxial		
<i>M. caudata</i>	Concave	Flat	2	435.70 x 286.37
<i>M. cuspidata</i> var. <i>cuspidata</i>	V-shape	Flat	3	767.60 x 277.64
<i>M. cuspidata</i> var. <i>petiolata</i>	Concave	U-shape	3	446.77 x 567.19
<i>M. meditensis</i>	V- shape	U-shape	4	439.22 x 313.95
<i>M. multifolia</i> sp. nov.	V-shape	Flat	4	1000.24 x 316.27
<i>M. spadicea</i>	Concave with keel shape	V-shape	2	562.35 x 201.12
<i>M. walichii</i>	V-shape	U-shape	3	579.63 x 265.12

From the observation, abaxial midrib outline with keel was detected in one species which is *M. spadicea* (Figure 4.4C). Some species shared the same midrib outline namely *M. cuspidata* var. *cuspidata* (Figure 4.4B) and *M. multifolia* sp. nov. (Figure 4.4D). These two species showed a V-shape abaxial and flat adaxial midrib outline. Apart from them, two species shared the same V-shape abaxial midrib outline but contradictory to them, their adaxial midrib outline were U-shape (*M. meditensis* & *M. walichii*). The remaining two species namely are *M. caudata* and *M. cuspidata* var. *petiolata* shared the same concave shape abaxial midrib outline but different adaxial midrib outlines which are flat, U-shape, and V-shape respectively. The thickness of the midrib was measured and the result shows that *M. multifolia* sp. nov. has the thickest midrib (1000.24 x 316.27 µm) while *M. caudata* has the thinnest midrib (435.70 x 286.37 µm) among the 7 species (Table 4.4).

There was also the presence of bulliform cells on each of the species. Bulliform cells appearance can be differentiated by large bubble-shaped cells. There are 4 layers of bulliform cells were present on the midvein region of the adaxial surface in *M. meditensis*

(Figure 4.4A) and *M. multifolia* sp. nov. (Figure 4.4D), three layers in *M. cuspidata* var. *cuspidata* (Figure 4.4B), *M. cuspidata* var. *petiolata*, and *M. walichii*. Meanwhile, *M. caudata* and *M. spadicea* (Figure 4.4C) only have two layers of bulliform cells present on the adaxial surface.

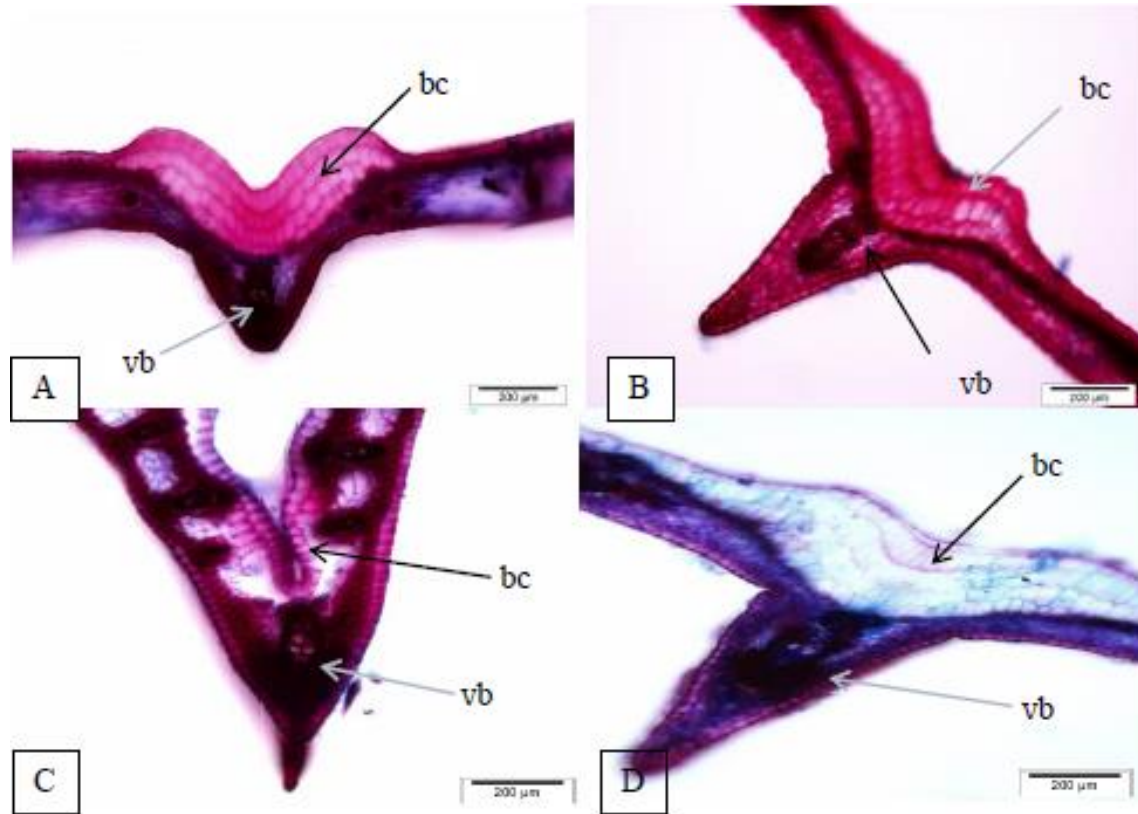


Figure 4.4: The Midrib Cross-section of *Mapania* Species. **A:** *M. meditensis*, **B:** *M. cuspidata* var. *cuspidata*, **C:** *M. spadicea*, and **D:** *M. multifolia* sp. nov. Abbreviations: **bc** - bulliform cells, **vb** - vascular bundles. Magnification: **A-D** = 4×. Scale bars: **A-D** = 200µm

4.2 Discussion

Based on the result, the leaf anatomical features were discovered to be of significant diagnostic value in identifying certain species of *Mapania*. It is important to remember that these anatomical characteristics are more useful when combined with other traits than when used alone (Table 4.5).

The selected 11 species were morphologically different and the investigation of anatomical characters were based on their leaf blade. Morphologically, *M. cuspidata* var. *cuspidata*, *M. cuspidata* var. *petiolata*, *M. meditensis*, *M. multifolia* sp. nov. and *M. unimasiana* leaves blade are oblong shape or commonly known as having a petiolate leaf. The other six species, viz. *M. caudata*, *M. kadimiana*, *M. palustris*, *M. lorea*, *M. spadicea* and *M. walichii* leaf blade are more to linear.

The noteworthy anatomical characteristics to compared among the petiolate leaf blade and linear leaf blade are the epidermal cell shapes, i.e. linear and elongated, hexagonal, polygonal and random (mixture of pentagonal, hexagonal & polygonal). Apart from that, two types of anticlinal walls patterns were observed namely undulate and sinuous. Although Silva *et al.* (2014) reported *M. macrophylla* and *M. pycnostachya*, which under section *Pycnocephala*, to have sinuous anticlinal wall pattern, seven species shows contradictory result from the feature reported eventhough they are of the same genus. The type of stomata was also varied depending on the species, i.e. anomocytic, diacytic or tetracytic. Another parameters involving the outline of the petiole and midrib were varied for each species.

When comparing the anatomical leaf surface of the five petiolate leaf blade species, it is clear that the leaf shape of *cuspidata* varieties are different from the other three species, i.e the abaxial and adaxial epidermal cells shape are linear and elongated.

Furthermore, cell inclusion that has been discovered in *M. cuspidata* var. *cuspidata* can be utilised to distinguish between the varieties. To further determine the content of this cell inclusion, histology studies are needed. The leaf shape of the three remaining petiolate leaf blade species (*M. meditensis*, *M. unimasiana* & *M. multifolia* sp. nov.) mostly hexagonal and random. However, the anticlinal wall (sinuous) of *M. multifolia* sp. nov. can be used to distinguished it from other two remaining petiolate leaf blade species which mainly undulated. Unfortunately, the data for petiole and midrib of *M. unimasiana* are still lacking so it is challenging to further differentiate between *M. meditensis* and *M. unimasiana* as their epidermal cell and stomata characters are the same.

Apart from that, it is easy to distinguished *M. caudata* from the other five linear leaf blade species based on its blue-sheen leaf appearance. Upon investigation on its leaf anatomy, the polygonal epidermal cell shape helps to compared to other linear leaf blade species could be of supportive diagnostic value that delimits the species based on the morphology description. Moreover, the sinuous anticlinal wall pattern of *M. lorea* helps to differentiate from the other five linear leaf blade species. When comparing *M. palustris* from the other linear leaf blade species, the type of stomata can be utilised as it is the only species which have the diacytic type. To further delimit the three remaining linear leaf-blade species, the type of stomata for *M. kadimiana* (tetracytic) and *M. spadicea* (anomocytic) for comparing. Meanwhile, the midrib outline shape for *M. walichii* (abaxial: V-shape; adaxial: U-shape) are compared with *M. spadicea* (abaxial: Concave with keel; adaxial: V-shape). It is difficult to compare between *M. kadimiana* and *M. walichii* as they shared the same anatomical characteristics and *M. kadimiana* are also lacking in data for midrib outline shape.

The results of leaf anatomical assessment, which included the nature of the leaf epidermal on both sides, as well as transverse sections of the midrib and petiole were meaningful in delimiting the species. Although the anatomical approach provides additional traits that aid in the identification of plant species, further sampling of the entire genus is required to confirm this.

Table 4.5: The Summary of Anatomical Characters for 11 Selected Species.

Taxa	Shape in surface view		Anticlinal wall		Stomata	Petiole outline shape		Midrib outline shape	
	Abaxial	Adaxial	Abaxial	Adaxial		Abaxial	Adaxial	Abaxial	Adaxial
<i>M. caudata</i>	Polygonal	Polygonal	Undulate	Undulate	Tetracytic	AO	AO	Concave	Flat
<i>M. cuspidata</i> var. <i>cuspidata</i>	Linear, elongate cells	Linear, elongate cells	Sinuuous	Sinuuous	Anomocytic	Concave with keel shape	V-shape	V-shape	Flat
<i>M. cuspidata</i> var. <i>petiolata</i>	Linear, elongate cells	Linear, elongate cells	Sinuuous	Sinuuous	Anomocytic	Concave	U-shape	Concave	U-shape
<i>M. kadimiana</i>	Hexagonal	Hexagonal	Undulate	Undulate	Tetracytic	AO	AO	AO	AO
<i>M. lorea</i>	Hexagonal	Hexagonal	Sinuuous	Sinuuous	Tetracytic	AO	AO	AO	AO
<i>M. meditensis</i>	Random	Hexagonal	Undulate	Undulate	Anomocytic	Concave	U-shape	V-shape	U-shape
<i>M. multifolia</i> sp. nov.	Random	Hexagonal	Sinuuous	Sinuuous	Anomocytic	AO	AO	V-shape	Flat
<i>M. palustris</i>	Hexagonal	Hexagonal	Undulate	Undulate	Diacytic	AO	AO	AO	AO
<i>M. spadicea</i>	Hexagonal	Random	Undulate	Undulate	Anomocytic	AO	AO	Concave with keel shape	V-shape
<i>M. unimasiana</i>	Random	Hexagonal	Undulate	Undulate	Anomocytic	AO	AO	AO	AO
<i>M. walichii</i>	Hexagonal	Hexagonal	Undulate	Undulate	Tetracytic	AO	AO	V-shape	U-shape

*AO – indicate absent organ due to its morphological character or rupture due to its fragile nature while undergoing processing.

CHAPTER 5

ANTIFUNGAL STUDY

5.1 Antifungal Study

Ten selected *Mapania* species, viz. *M. caudata*, *M. cuspidata* var. *petiolata*, *M. debilis*, *M. enodis*, *M. kadimiana*, *M. multifolia* sp. nov., *M. squamata*, *M. unimasiana*, *M. walichii* and *M. zinnirahea* were selected to test the antifungal effect of their methanol crude extract at different concentrations (25 µg/ml, 50 µg/ml & 100 µg/ml) towards the growth of *Colletotrichum musae*, *Fusarium solani*, and *Pyricularia oryzae* (isolate POSA 1 & POSA 2). The methanolic crude extracts were extracted from leaves and roots of the 10 selected *Mapania* species. Thus, a total of 20 crude extracts were obtained.

5.1.1 Extraction Yield of the 10 *Mapania* Species

Figure 5.1 shows the extraction of the ten selected *Mapania* spp. leaf and root samples. The extraction yield varied from 0.52% to 5.47% for leaf samples and 1.65% to 4.39% for root samples. A total of 20 crude extracts were tested against four crop pathogenic fungi (*C. musae*, *F. solani* & *P. oryzae* isolate POSA 1 & POSA 2). From this current study of the 10 selected *Mapania* species, five species (*M. caudata*, *M. enodis*, *M. kadimiana*, *M. unimasiana* & *M. walichii*) had their leaf crude extract showing inhibition effect on the tested crop pathogenic fungi. Another five species namely *M. debilis*, *M. enodis*, *M. kadimiana*, *M. squamata* and *M. unimasiana* had their root crude extract showing inhibition effect on the tested crop pathogenic fungi in this current study (Table 5.1).

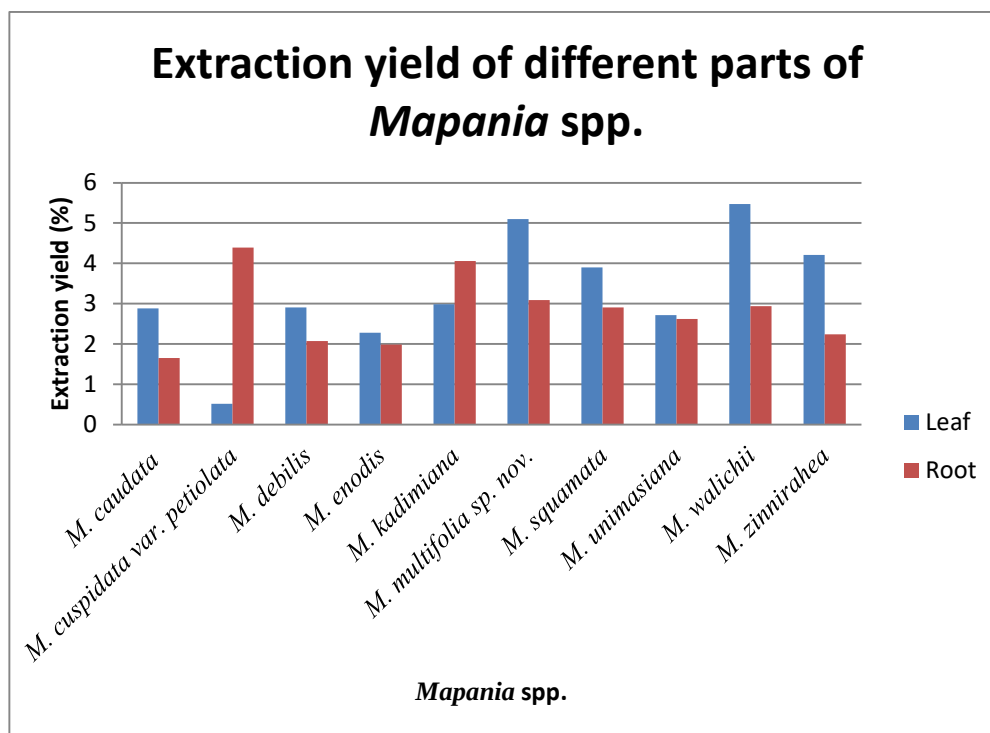


Figure 5.1: Extraction Yield from Leaf and Root Samples of Ten Selected *Mapania* spp.

Table 5.1: The Overall Antifungal Assay Results for This Current Study.

Taxa	Plant Parts	<i>C. musae</i>	<i>F. solani</i>	<i>P. oryzae</i> (isolate POSA1)	<i>P. oryzae</i> (isolate POSA 2)	Total
<i>M. caudata</i>	Leaves	-	-	-	√	1
	Root	-	-	-	-	0
<i>M. cuspidata</i> var. <i>petiolata</i>	Leaves	-	-	-	-	0
	Root	-	-	-	-	0
<i>M. debilis</i>	Leaves	-	-	-	-	0
	Root	√	-	-	-	1
<i>M. enodis</i>	Leaves	-	-	√	-	1
	Root	√	-	-	-	1
<i>M. kadimiana</i>	Leaves	√	-	-	-	1
	Root	√	-	-	-	1
<i>M. multifolia</i> sp. nov.	Leaves	-	-	-	-	0
	Root	-	-	-	-	0
<i>M. squamata</i>	Leaves	-	-	-	-	0
	Root	√	-	√	-	2
<i>M. unimasiana</i>	Leaves	-	-	√	√	2
	Root	√	-	-	-	1
<i>M. walichii</i>	Leaves	-	-	√	-	1
	Root	-	-	-	-	0
<i>M. zinnirahea</i>	Leaves	-	-	-	-	0
	Root	-	-	-	-	0

Table 5.1 continued

	Total	6	0	4	2	12
* “√” =The methanolic crude extract inhibited the growth of a pathogen at a certain concentration (p<0.05), “—” = No inhibition, Total = Total of methanolic crude extracts having significant growth inhibition effect						

5.1.2 Methanolic Crude Extracts Against *Colletotrichum musae*

Out of the 20 methanolic crude extracts tested on *C. musae*, six crude extracts showed growth inhibition effect on *C. musae* at various concentrations level. From the six crude extracts, it is from five *Mapania* species. One crude extract was from leaf and five crude extracts is from root. From the five species, one *Mapania* species (*M. kadimiana*) had its leaf and root crude extract having inhibition effect. Four *Mapania* species had only their leaf crude extract showing inhibition effect.

From the six crude extracts, three root crude extracts (*M. enodis*, *M. kadimiana* & *M. unimasiana*) showed inhibition effect at the lowest concentration (25 µg/ml). Two crude extracts (*M. debilis* root crude extract & *M. kadimiana* leaf crude extract) showed inhibition effect at 50 µg/ml. For *M. squamata* root crude extract, the growth of *C. musae* was retarded when tested at the highest treatment concentration (100 µg/ml) (Table 5.2). The effective concentrations of crude extracts of *Mapania* delayed the growth of *C. musae* by less than one day to reach full plate (8.0 cm Ø) as compared to the corresponding control plate (Table 5.3).

Table 5.2: The Growth Rate (cm/day) of *C. musae* Cultured on Different Concentration of Methanolic Crude Extracts From 10 selected *Mapania* Species.

Taxa	Plant parts	Fungus Growth Rate (cm/day)			
		<i>Colletotrichum musae</i>			
		Treatment (µg/ml)			
		C (1% DMSO)	25	50	100
<i>M. caudata</i>	Leaf	0.688 ± 0.034 ^a	NA	NA	0.700 ± 0.017 ^a

Table 5.2 continued

	Root	0.611 ± 0.087^a	0.657 ± 0.078^a	0.632 ± 0.088^a	0.606 ± 0.099^a
<i>M. cuspidata</i>	Leaf	NA	NA	NA	NA
var. <i>petiolata</i>	Root	NA	NA	NA	NA
<i>M. debilis</i>	Leaf	0.644 ± 0.046^a	0.653 ± 0.039^a	0.635 ± 0.027^a	0.643 ± 0.050^a
	Root	0.554 ± 0.032^a	0.533 ± 0.013^a	0.504 ± 0.011^b	0.479 ± 0.014^c
<i>M. enodis</i>	Leaf	0.648 ± 0.054^a	0.645 ± 0.005^a	0.642 ± 0.009^a	NA
	Root	0.677 ± 0.045^a	0.644 ± 0.038^b	0.622 ± 0.028^b	0.628 ± 0.036^b
<i>M. kadimiana</i>	Leaf	0.686 ± 0.045^a	0.623 ± 0.094^{ab}	0.610 ± 0.086^b	NA
	Root	0.696 ± 0.048^a	0.541 ± 0.023^b	0.480 ± 0.022^c	NA
<i>M. multifolia</i>	Leaf	0.569 ± 0.019^a	0.549 ± 0.021^a	0.569 ± 0.026^a	NA
sp. nov.	Root	0.544 ± 0.016^a	0.556 ± 0.014^a	0.547 ± 0.009^a	0.555 ± 0.017^a
<i>M. squamata</i>	Leaf	NA	NA	NA	NA
	Root	0.623 ± 0.029^a	0.617 ± 0.016^{ab}	NA	0.591 ± 0.021^b
<i>M. unimasiana</i>	Leaf	0.591 ± 0.105^a	0.621 ± 0.087^a	0.645 ± 0.060^a	0.613 ± 0.034^a
	Root	0.663 ± 0.051^a	0.477 ± 0.222^b	NA	NA
<i>M. walichii</i>	Leaf	NA	NA	NA	NA
	Root	0.616 ± 0.014^a	0.625 ± 0.008^a	0.621 ± 0.008^a	0.628 ± 0.007^a
<i>M. zinnirahaea</i>	Leaf	0.677 ± 0.044^a	0.703 ± 0.028^a	0.699 ± 0.029^a	0.703 ± 0.033^a
	Root	NA	NA	NA	NA

*Different letters indicate a significant difference in fungal growth rate on media infused with different concentrations of methanolic crude extracts ($p < 0.05$); NA indicate not available as the experiment were contaminated.

Table 5.3: The Significant Colony Fungal Growth of *C. musae* on the Plates Containing Crude Extracts from *Mapania* Species That Have Antifungal Properties.

Taxa	Plant parts	Effective concentrations	Additional days for <i>C. musae</i> to reach full plate (8.0 cm Ø)
<i>M. debilis</i>	Root	50 µg/ml and 100 µg/ml	Less than 1 day
<i>M. enodis</i>	Root	25 µg/ml, 50 µg/ml and 100 µg/ml	Less than 1 day
<i>M. kadimiana</i>	Leaf	50 µg/ml	Less than 1 day
	Root	25 µg/ml and 50 µg/ml	Less than 1 day
<i>M. squamata</i>	Root	100 µg/ml	Less than 1 day
<i>M. unimasiana</i>	Root	25 µg/ml	Less than 1 day

*The number of additional days needed to reach full plate (8.0 cm Ø) in comparison to the corresponding control plate.

5.1.3 Methanolic Crude Extracts Against *Fusarium solani*

From 20 extracts tested, only seven crude extracts data were able to collect while 13 other crude extracts were contaminated during the procedures even after a round of repetition. There was no significant difference in the growth rate of *F. solani* when tested with the leaf crude extract of *M. caudata* and *M. unimasiana* when compared to the corresponding control treatment. *M. caudata*, *M. cuspidata* var. *petiolata*, *M. enodis*, and *M. kadimiana* root crude extract also has no significant difference when compared to control (Table 5.4).

Table 5.4: The Growth Rate (cm/day) of *F. solani* Cultured on Different Concentration of Methanolic Crude Extracts From 10 Selected *Mapania* Species.

Taxa	Plant parts	Fungus Growth Rate (cm/day)			
		<i>Fusarium solani</i>			
		Treatment (µg/ml)			
		C (1% DMSO)	25	50	100
<i>M. caudata</i>	Leaf	0.436 ± 0.020 ^a	0.457 ± 0.024 ^a	0.449 ± 0.026 ^a	0.443 ± 0.032 ^a
	Root	0.445 ± 0.020 ^a	0.437 ± 0.025 ^a	0.441 ± 0.023 ^a	0.444 ± 0.027 ^a
<i>M. cuspidata</i> var. <i>petiolata</i>	Leaf	NA	NA	NA	NA
	Root	0.349 ± 0.003 ^a	0.351 ± 0.003 ^a	0.350 ± 0.005 ^a	0.353 ± 0.005 ^a
<i>M. debilis</i>	Leaf	NA	NA	NA	NA
	Root	NA	NA	NA	NA
<i>M. enodis</i>	Leaf	NA	NA	NA	NA
	Root	0.357 ± 0.014 ^a	0.355 ± 0.015 ^a	0.361 ± 0.011 ^a	0.355 ± 0.017 ^a
<i>M. kadimiana</i>	Leaf	NA	NA	NA	NA
	Root	0.487 ± 0.010 ^a	0.492 ± 0.014 ^a	0.488 ± 0.011 ^a	0.488 ± 0.011 ^a
<i>M. multifolia</i> sp. nov.	Leaf	NA	NA	NA	NA
	Root	0.355 ± 0.009 ^a	0.364 ± 0.013 ^a	0.356 ± 0.009 ^a	0.363 ± 0.012 ^a
<i>M. squamata</i>	Leaf	NA	NA	NA	NA
	Root	NA	NA	NA	NA
<i>M. unimasiana</i>	Leaf	0.423 ± 0.056 ^a	0.431 ± 0.076 ^a	0.453 ± 0.062 ^a	0.445 ± 0.078 ^a
	Root	NA	NA	NA	NA

Table 5.4 continued

<i>M. walichii</i>	Leaf	NA	NA	NA	NA
	Root	NA	NA	NA	NA
<i>M. zinniraha</i>	Leaf	NA	NA	NA	NA
	Root	NA	NA	NA	NA

*Different letters indicate a significant difference in fungal growth rate on media infused with different concentrations of methanolic crude extracts ($p < 0.05$); NA indicate not available as the experiment were contaminated even after repetition.

5.1.4 Methanolic Crude Extracts Against *Pyricularia oryzae* (isolates POSA 1 and POSA 2)

Isolates POSA 1

There were four crude extracts showed inhibition towards the growth of *P. oryzae* isolate POSA 1. From the 10 *Mapania*, three had their leaf crude extract and one *Mapania* species had its root crude extracts having inhibition effect against the growth of *P. oryzae* isolate POSA 1.

The leaf crude extract of *M. unimasiana* and *M. walichii* inhibited the growth of *P. oryzae* isolate POSA 1 starting from lowest treatment concentrations (25 µg/ml). The leaf crude extract of *M. enodis* and root crude extract of *M. squamata* only showed inhibition on the isolate at 50 µg/ml (Table 5.5). The effective concentrations of crude extracts of *Mapania* delayed the growth of isolate POSA 1 by less than 1 day to reach full plate (8.0 cm Ø) as compared to the corresponding control plate (Table 5.7).

Table 5.5: The Growth Rate (cm/day) of *P. oryzae* (isolate POSA 1) Cultured on Different Concentration of Methanolic Crude Extracts From 10 Selected *Mapania* Species.

Taxa	Plant parts	Fungus Growth Rate (cm/day)			
		<i>Pyricularia oryzae</i> (isolates POSA 1)			
		Treatment (µg/ml)			
		C (1% DMSO)	25	50	100
<i>M. caudata</i>	Leaf	0.410 ± 0.050 ^a	0.441 ± 0.036 ^a	0.431 ± 0.045 ^a	0.441 ± 0.045 ^a
	Root	0.401 ± 0.018 ^a	NA	NA	0.400 ± 0.033 ^a

Table 5.5 continued

<i>M. cuspidata</i> var. <i>petiolata</i>	Leaf	0.437 ± 0.026 ^a	0.438 ± 0.020 ^a	0.452 ± 0.028 ^a	0.442 ± 0.035 ^a
	Root	0.437 ± 0.026 ^a	0.440 ± 0.019 ^a	0.434 ± 0.013 ^a	0.423 ± 0.023 ^a
<i>M. debilis</i>	Leaf	0.413 ± 0.054 ^a	0.371 ± 0.038 ^a	0.406 ± 0.049 ^a	0.379 ± 0.052 ^a
	Root	0.382 ± 0.010 ^a	0.369 ± 0.028 ^a	0.386 ± 0.014 ^a	0.377 ± 0.025 ^a
<i>M. enodis</i>	Leaf	0.421 ± 0.015 ^a	0.420 ± 0.034 ^a	0.402 ± 0.014 ^b	NA
	Root	0.400 ± 0.014 ^a	0.411 ± 0.015 ^a	NA	0.402 ± 0.013 ^a
<i>M. kadimiana</i>	Leaf	0.409 ± 0.028 ^a	0.418 ± 0.029 ^a	0.429 ± 0.035 ^a	0.433 ± 0.028 ^a
	Root	0.384 ± 0.024 ^a	0.364 ± 0.026 ^a	0.366 ± 0.032 ^a	0.374 ± 0.025 ^a
<i>M. multifolia</i> sp. nov	Leaf	NA	NA	NA	NA
	Root	0.454 ± 0.018 ^a	0.470 ± 0.015 ^a	0.461 ± 0.025 ^a	0.468 ± 0.020 ^a
<i>M. squamata</i>	Leaf	0.426 ± 0.055 ^a	0.434 ± 0.041 ^a	0.442 ± 0.057 ^a	0.428 ± 0.045 ^a
	Root	0.489 ± 0.035 ^a	0.454 ± 0.035 ^{ab}	0.421 ± 0.063 ^b	NA
<i>M. unimasiana</i>	Leaf	0.468 ± 0.063 ^a	0.415 ± 0.026 ^b	0.414 ± 0.013 ^b	0.411 ± 0.023 ^b
	Root	0.458 ± 0.074 ^a	0.429 ± 0.037 ^a	0.432 ± 0.030 ^a	0.445 ± 0.036 ^a
<i>M. walichii</i>	Leaf	0.485 ± 0.063 ^a	0.433 ± 0.031 ^b	0.436 ± 0.027 ^b	0.442 ± 0.028 ^b
	Root	0.454 ± 0.070 ^a	0.439 ± 0.036 ^a	0.449 ± 0.024 ^a	0.436 ± 0.040 ^a
<i>M. zinnirahea</i>	Leaf	NA	NA	NA	NA
	Root	0.413 ± 0.012 ^a	NA	0.434 ± 0.009 ^a	0.433 ± 0.026 ^a

*Different letters indicate a significant difference in fungal growth rate on media infused with different concentrations of methanolic crude extracts ($p < 0.05$); NA indicate not available as the experiment were contaminated even after repetition.

Isolates POSA 2

P. oryzae isolate POSA 2 were tested with 20 methanolic crude extracts. From the 10 *Mapania* species, leaf crude extract of two *Mapania* species showed inhibition effect. *M. caudata* and *M. unimasiana* leaf extract inhibited the growth of *P. oryzae* isolate POSA 2 in culture at the lowest treatment concentrations (25 µg/ml) (Table 5.6). The effective crude extracts of *M. caudata* and *M. unimasiana* delayed the growth of isolate POSA 2 by one day to reach full plate (8.0 cm Ø) as compared to the corresponding control plate (Table 5.7).

Table 5.6: The Growth Rate (cm/day) of *P. oryzae* (isolate POSA 2) Cultured on Different Concentration of Methanolic Crude Extracts From 10 Selected *Mapania* Species.

Taxa	Plant parts	Fungus Growth Rate (cm/day)			
		<i>Pyricularia oryzae</i> (isolates POSA 2)			
		Treatment (µg/ml)			
		C (1% DMSO)	25	50	100
<i>M. caudata</i>	Leaf	0.583 ± 0.052 ^a	0.509 ± 0.043 ^b	NA	NA
	Root	0.499 ± 0.006 ^a	0.498 ± 0.023 ^a	0.511 ± 0.042 ^a	0.496 ± 0.042 ^a
<i>M. cuspidata</i> var. <i>petiolata</i>	Leaf	0.423 ± 0.033 ^a	0.452 ± 0.033 ^a	0.442 ± 0.044 ^a	0.443 ± 0.048 ^a
	Root	0.423 ± 0.033 ^a	0.447 ± 0.044 ^a	0.444 ± 0.047 ^a	0.452 ± 0.060 ^a
<i>M. debilis</i>	Leaf	NA	NA	NA	NA
	Root	0.644 ± 0.046 ^a	0.653 ± 0.039 ^a	0.635 ± 0.027 ^a	0.643 ± 0.050 ^a
<i>M. enodis</i>	Leaf	0.486 ± 0.025 ^a	0.534 ± 0.037 ^a	0.525 ± 0.037 ^a	0.522 ± 0.061 ^a
	Root	0.525 ± 0.060 ^a	0.523 ± 0.053 ^a	0.520 ± 0.052 ^a	0.543 ± 0.060 ^a
<i>M. kadimiana</i>	Leaf	NA	NA	NA	NA
	Root	0.500 ± 0.043 ^a	0.504 ± 0.050 ^a	0.538 ± 0.031 ^a	0.533 ± 0.066 ^a
<i>M. multifolia</i> sp. nov.	Leaf	0.412 ± 0.016 ^a	0.404 ± 0.025 ^a	0.421 ± 0.015 ^a	NA
	Root	NA	NA	NA	NA
<i>M. squamata</i>	Leaf	0.607 ± 0.061 ^a	0.577 ± 0.061 ^a	0.580 ± 0.042 ^a	0.608 ± 0.056 ^a
	Root	NA	NA	NA	NA
<i>M. unimasiana</i>	Leaf	0.554 ± 0.086 ^a	0.504 ± 0.038 ^b	NA	NA
	Root	0.578 ± 0.086 ^a	0.556 ± 0.043 ^a	0.559 ± 0.047 ^a	0.549 ± 0.038 ^a
<i>M. walichii</i>	Leaf	0.515 ± 0.024 ^a	0.507 ± 0.061 ^a	0.510 ± 0.040 ^a	0.518 ± 0.028 ^a
	Root	0.494 ± 0.045 ^a	0.492 ± 0.043 ^a	0.501 ± 0.045 ^a	0.529 ± 0.052 ^a
<i>M. zinnirahea</i>	Leaf	NA	NA	NA	NA
	Root	0.499 ± 0.006 ^a	0.514 ± 0.043 ^a	0.490 ± 0.025 ^a	0.509 ± 0.035 ^a

*Different letters indicate a significant difference in fungal growth rate on media infused with different concentrations of methanolic crude extracts ($p < 0.05$); NA indicate not available as the experiment were contaminated even after repetition.

Table 5.7: The Significant Colony Fungal Growth of *P. oryzae* on the Plates Containing Crude Extracts From *Mapania* Species That Have Antifungal Properties.

Taxa	Plant parts	Isolate	Effective concentrations	Additional days for <i>P. oryzae</i> to reach full plate (8.0 cm Ø)
<i>M. caudata</i>	Leaf	POSA 2	25 µg/ml	1 day
<i>M. enodis</i>	Leaf	POSA 1	50 µg/ml	Less than 1 day
<i>M. squamata</i>	Root	POSA 1	50 µg/ml	Less than 1 day
<i>M. unimasiana</i>	Leaf	POSA 1	25 µg/ml, 50 µg/ml and 100 µg/ml	Less than 1 day
		POSA 2	25 µg/ml	1 day
<i>M. walichii</i>	Leaf	POSA 1	25 µg/ml, 50 µg/ml and 100 µg/ml	Less than 1 day

*The number of additional days needed to reach full plate (8.0 cm Ø) in comparison to the corresponding control plate.

5.2 Discussion

In this study, the results demonstrated a wide range of antifungal activities of the crude extract from ten selected *Mapania* species against crop pathogenic fungi. It was previously documented that *Mapania* has some antimicrobial properties that can be used to treat fever (Burkill, 1935; Kern, 1974; Simpson, 1992). The effectiveness of this plant, however, was only known to local people and little knowledge was proved in scientific studies. A previous research study was conducted to test the antifungal activity of *M. cuspidata* against human pathogenic fungus (Wiart *et al.*, 2014). The result showed that both bark and leaves extract inhibited the human pathogenic fungus (*Pseudomonas aeruginosa*). This previous study outcome is useful in providing baseline information to initiate the screening of antifungal activity for *Mapania* species to get a better insight of this genus.

Based on the results, several extracts showed significant inhibition on the growth of fungi tested at different concentrations (25 µg/ml, 50 µg/ml & 100 µg/ml). The crude extracts from different plant parts were effectively retarded the growth of *C. musae* but not towards *P. oryzae* tested in this study. This species-dependent effect can be observed for the root crude extract of *M. unimasiensis* which inhibited the growth of *C. musae* but the leaf extract inhibited *P. oryzae* isolate POSA 1 and POSA 2 (Table 5.2, Table 5.5 & Table 5.6).

Apart from that, the isolate specificity effect can be seen when *Mapania* crude extracts were tested against *P. oryzae* isolate POSA 1 and POSA 2. The results showed that certain extracts were highly effective when tested on one of the isolates but not to the other. *M. enodis* leaves crude extract was able to inhibit *P. oryzae* isolate POSA 1 but unable to inhibit *P. oryzae* isolate POSA 2 (Table 5.5 & Table 5.6). The isolate specific

effect of the crop pathogenic fungi are significant, implying that when searching for a potent antimicrobial agent, it is advisable to look for one that is effective against not only a broad species of crop pathogenic fungi, but also against majority of its isolates or genotypes (Brauer *et al.*, 2019; Edouarzin *et al.*, 2019).

A common feature of certain pesticides is the stimulatory activity of extracts at low concentration and their inhibitory effect at high concentration (Fedtke, 1982). From the results, *M. debilis* root crude extracts inhibited the growth of *C. musae* at 100 µg/ml compared to 25 µg/ml (Table 5.2). This result showed that the antimicrobial activity of the crude extracts became more effective by increasing the concentration of the extracts and, in addition, the concentrations depended on the inhibition activity of the extracts (Dellavalle *et al.*, 2011). This result is in line with the study by Banso *et al.* (1999), who also found that further growth inhibition was shown by higher concentrations of antimicrobial substances.

The various inhibitory effect of *Mapania* methanolic crude extracts was probably due to the interactions of compounds present when tested against the four different fungi. Davicino *et al.* (2007), mentioned that in antimicrobial activity of plant extracts, it might be due to the synergistic effect of several compounds that are in a small proportion in the plant and not due to the action of a single active compound. This might be due to various compounds interfering with the behaviour of each other within the crude extracts (Dellavalle *et al.*, 2011). However, further testing to determine the composition of the compound needed to be conducted to confirm this.

Different parts of tissue in plants produce an array of active compounds (Rosenthal & Janzen, 1979; Goodwin & Mercer, 1983). In Cyperaceae, several metabolites such as flavonoids, saponins, phenolic acids, iridoids, alkaloid, linolenic and myristic has been

identified from tubers of *Cyperus rotundus*. Some of the fungicidal activities are due to these metabolites (Sivapalan, 2013). The volatile oil found in the leaves and seeds of *C. rotundus* is rich in bacterial and fungicidal compounds (Nima *et al.*, 2008). From the ten selected *Mapania* sp., more leaves crude extracts having inhibition effect when tested against *C. musae*. While when tested against *P. oryzae*, more root crude extracts having inhibition effect.

Typically, polar solvents such as hot water, methanol, ethanol, acetone or ethyl acetate were used to extract flavonoids or phenolic acids. In this study, the ten selected *Mapania* species were extracted using methanol which aimed to extract flavonoids or phenolic acids which are known to contribute for plant defence system (Harborne & Williams, 2000). Plants create flavonoids, which are hydroxylated phenolic chemicals, in response to microbial diseases (Dixon *et al.*, 1983; Nabavi *et al.*, 2018). Flavonoids, due to their physical and biochemical properties, not only protect against harmful abiotic substances, but also make it simpler to communicate with other plants and bacteria. Various flavonoids have biological activities such as antimicrobial (flavonoids & phenolic acid esters) (Janićijević *et al.*, 2007; Scarano *et al.*, 2018). It was also mentioned that phenolic acids are secondary metabolites produced by plants as a part of their antimicrobial defence system with the ability to kill bacteria or limit their growth (Liu *et al.*, 2020). A study conducted by Al-Huqail *et al.* (2019) showed that the presence of certain phenolic and flavonoid compounds may be associated to the antibacterial activity of the aqueous extract from *Acacia saligna* flowers against the three mould species examined (*Fusarium culmorum*, *Rhizotonia solani* & *Penicillium chrysogenum*).

However, many factors, such as the age of the organism, the process of extraction (Elzaawely & Tawata, 2011; Nguyen & Le, 2018) and the time of collecting plant

materials (Aladag *et al.*, 2009) affect the existence of an active principle in plants (Qasema & Abu-Blan, 1996; Khan, 2006). With these, the quantity and quality of these active compounds depend on the species of plants, the plant tissue being research, and environmental factors (Demo & Oliva, 2008; Webster *et al.*, 2008). This might be the reason why some of the crude extract from the selected *Mapania* sp. showed no significant effect against crop pathogenic fungi.

Futhermore, the inhibition of the crop pathogenic fungi tested were seen to be delayed by less than 1 day or 1 day to reach full plate (8.0 cm Ø) as compared to the corresponding control plate (Table 5.3 & Table 5.7). The slowing down of growth rate of the tested crop pathogenic fungi in *in vitro* may not be useful in terms of reducing crop pathogen populations and managing crop disease development in the field. The increase of the concentration could be considered to increase the inhibition effect. However, it solution is not sustainable or cost-effective for subsequent field application.

Some *Mapania* crude extracts showed no inhibition effect when tested against crop pathogenic fungi. The possibility might be due to the low concentration (Hamburger & Hostettmann, 1991; Zavala *et al.*, 1997) or absent of antifungal active substances (Farnsworth & Bingel, 1977; Gordon *et al.*, 1980). In this study, the investigation of minimum inhibitory concentration and screening of the compounds were not conducted, thus, it is difficult to determine the active compounds that act as antifungal agent.

In conclusion, the results obtained from this study has added a new information to the genus. Although the screening of the antifungal activity of ten selected *Mapania* species against three crop pathogenic fungi are consider weak, the results benefit greatly in understanding this underrated genus. As the antifungal value were documented, its indirectly helps in initiating the conservation effort of this genus.

CHAPTER 6

TAXONOMIC TREATMENT

6.1 Fieldwork

A total of 15 *Mapania* species were collected and recorded from various localities in Sarawak through several field trips (Figure 6.1). The localities and GPS coordinates of the location were recorded. The samples were then selected for different studies (Table 6.1). Eleven *Mapania* species were selected to undergo anatomical study which observed their epidermal cell, stomata, and cross-section of petiole and midrib. For the antifungal study, methanol crude extract from 10 selected *Mapania* species was tested against four crop pathogenic fungi (*Colletotrichum musae*, *Fusarium solani* & *Pyricularia oryzae* (isolates POSA 1 & POSA 2)).

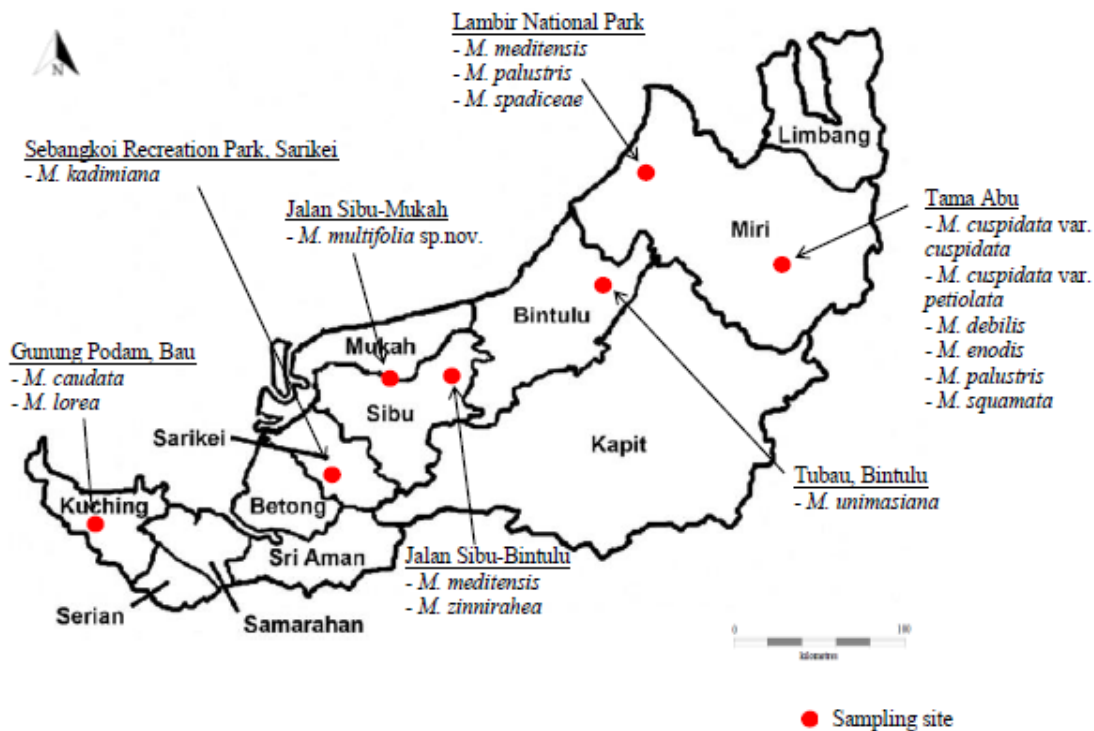


Figure 6.1: The Map of Sarawak Shows Marked Sampling Sites with *Mapania* Species

Table 6.1: The Localities and GPS Coordinates for *Mapania* Species Collected and The Type of Study Used.

Taxa	Localities	Coordinates	Study
<i>M. caudata</i>	Gunung Podam, Bau	1° 25' 44.4'' N 110° 12' 13.86 E	Anatomy and antifungal
<i>M. cuspidata</i> var. <i>cuspidata</i>	Tama Abu, Miri	3° 33' 37.4'' N 115° 19' 37.2'' E	Anatomy
	Gunung Podam, Bau	1° 25' 44.4'' N 110° 12' 13.86 E	
<i>M. cuspidata</i> var. <i>petiolata</i>	Tama Abu, Miri	3° 33' 37.4'' N 115° 19' 37.2'' E	Anatomy and antifungal
	Gunung Podam, Bau	1° 25' 44.4'' N 110° 12' 13.86 E	
<i>M. debilis</i>	Tama Abu, Miri	3° 33' 37.4'' N 115° 19' 37.2'' E	Antifungal
<i>M. enodis</i>	Tama Abu, Miri	3° 33' 37.4'' N 115° 19' 37.2'' E	Antifungal
<i>M. kadimiana</i>	Sebangkoi Recreation Park, Sarikei	1° 57' 34.128'' N 111° 25' 59.772'' E	Anatomy and antifungal
<i>M. lorea</i>	Gunung Podam, Bau	1° 25' 44.4'' N 110° 12' 13.86 E	Anatomy
<i>M. meditensis</i>	Lambir National Park, Miri Jalan Sibu-Bintulu	4° 11' 53.736'' N 114° 2' 34.116'' E 2° 39' 38.9'' N 112° 40' 24.1'' E	Anatomy
<i>M. multifolia</i> sp. nov.	Jalan Sibu-Mukah	2° 44' 57.0'' N 112° 20' 30.9'' E	Anatomy and antifungal
<i>M. palustris</i>	Lambir National Park, Miri	4° 11' 53.736'' N 114° 2' 34.116'' E	Anatomy
	Tama Abu, Miri	3° 33' 37.4'' N 115° 19' 37.2'' E	
<i>M. spadicea</i>	Lambir National Park, Miri	4° 11' 53.736'' N 114° 2' 34.116'' E	Anatomy
<i>M. squamata</i>	Tama Abu, Miri	3° 33' 37.4'' N 115° 19' 37.2'' E	Antifungal
<i>M. unimasiana</i>	Tubau, Bintulu	3° 08' N 113° 4' E	Anatomy and antifungal
<i>M. walichii</i>	Jalan Sibu-Bintulu	2° 39' 38.9'' N 112° 40' 24.1'' E	Anatomy and antifungal
<i>M. zinnirahea</i>	Jalan Sibu-Bintulu	2° 39' 38.9'' N 112° 40' 24.1'' E	Antifungal

6.2 Description of Genus

Mapania Aublet

Hist. Pl. Guiane Fr. 1: 47 t. 17 (1775).; Clarke, C.B. in Hooker, J.D., *Flora of British India* 6: (1894a); Ridley, H.N. *Journal of Straits Branch Asiatic Society*, 44: (1905), Uittien, H., *Rescueil Travaux Botaniques Neerlandais* 32: 193 (1935b); Kern, J.H. *Blumea*, 12: (1962), Burkill, H.M., *The Useful Plants of West Tropical Africa*, (1985), Simpson, D.A. *A Revision of the Genus Mapania (Cyperaceae)*: 34 (1992). Shabdin, Z. *Evolution and biochemical diversity of Mapania (Cyperaceae) from Southeast Asia*: (2012) (unpublished). **Type:** *Mapania sylvatica* Aublet. Guiane Francoise, Orapuensibus, Aublet s.n. (holo: P).

Description: Moderate to very robust, rhizomatous, rarely caespitose or stoloniferous, perennial herbs. Rhizome horizontal or ascending, sometimes covered by fibrous remains of old leaf sheaths. Roots coarse, sometimes stilt-like, particularly on ascending rhizome. Cataphylls subtending lateral culms only, widely ovate to lanceolate, obtuse to acute, the upper ones with at least part of the margins adnate at first and completely sheathing the culm, later splitting. Culm central or lateral arising from leaf axils, 1 only when central, 2-several when lateral, terete to triquetrous, sometimes very short. Leaves usually basal, $\pm$ 3-ranked, rarely 1-2 cauline, comprising blade, sheath and often a pseudopetiole, rarely reduced to a bladeless sheath; leaf-blade narrowly linear to elliptic, apex very gradually to abruptly narrowed, acute, acuminate or cuspidate, base very gradually to abruptly narrowed, subcoriaceous to thickly coriaceous, 1-3 nerved with many indistinct to distinct secondary nerves, flat to deeply inverse w-shaped in cross section, becoming conduplicate towards sheath when pseudopetiole absent, smooth to distinctly septate-nodulose when dry, margins entire to densely serrulate, rarely the nerves densely serrulate; pseudopetiole

comprising two halves of the lamina narrowed and folded together at the midrib, margins sometimes enrolled, usually serrulate; basal leaf sheaths with two halves of lamina folded together, open adaxially, narrowly lanceolate to ovate when flattened, very gradually to abruptly narrowed into pseudopetiole where present, coriaceous keel rounded, margins membranous, cauline leaf sheath tubular; ligule absent. Involucral bracts foliaceous on central culms, glumaceous or rarely foliaceous on lateral culms; foliaceous bracts 2-several, linear to elliptic, apex gradually to abruptly narrowed, acuminate to obtuse, sometimes with pseudopetiole, rarely with a sheath; glumaceous bracts several, lanceolate to ovate, acute to obtuse, indistinctly to distinctly many-nerved. Inflorescence axillary or terminal, spike(s) terminal, rarely pseudolateral, paniculate, the ultimate branches subtending small clusters of spikes, or with up to 9 unbranched rays each subtending globose capitula, or capitate with 2-many spikes, or with a solitary terminal spike; spike ovate to linear-oblong, composed of few to numerous, tightly imbricate, spirally arranged glumaceous bracts (spicoid bracts), each subtending a much reduced pseudanthium (spicoid); spicoid bracts lanceolate to ovate, acute to rounded, membranous to coriaceous, glabrous to hispid at apex and on margins, indistinctly to distinctly many-nerved; spicoid composed of 4 or 6 floral bracts of which (1-) 2-3 (-4) subtend staminate flowers and 0-3 are empty, the spicoid terminated by a female flower; (exception for *M. unimasiana*, where the staminate and pistillate flowers are in different spicoid) floral bracts free or sometimes loosely connate, membranous, lower 2 bracts usually strongly keeled, linear to oblong when flattened, acute to obtuse, keel acute, narrowly winged or wingless, ciliate, ciliate-hispid, denticulate-hispid, hispid or glabrous, upper bracts narrowly linear to oblong, acute to obtuse, flat to $\pm$ keeled, glabrous to ciliate-hispid or denticulate-hispid; staminate flowers each comprising 1 stamen; anther oblong to linear, latrorsely dehiscent, without

extended connective tip; filament filiform, somewhat flattened; stigma branches 2-3; style elongate, rarely persistent. Fruit globose, obovoid to subpyriform, rarely conical or lageniform, sometimes with a prominent conical spongy apical portion, exocarp hard to succulent, rarely drupaceous, thick or thin, the succulent type disintegrating and often detaching with age, smooth to papillose, rugose or tuberculate, with 0-3 lateral costae, endocarp stony. Embryo of *Carex*-type.

6.3 Key to the species of *Mapania* in Sarawak

1. Plants stoloniferous, spikes solitary 2
 - Plants rhizomatous, spikes solitary, sessile, paniculate or capitate 5
2. Leaves sheath purplish/ maroon, spikes oblong or oblanceolate *M. zinnirahea*
 - Leaves sheath green/ light green, spikes elliptic or lanceolate 3
3. Young shoot salmon-pink to reddish, spikes brown, chocolate brown *M. cuspidata*
 - Young shoot green/light green, spikes green, greenish yellow or light brown 4
4. Leaves more than 80 *M. multifolia* sp. nov.
 - Leaves less than 80 *M. meditensis*
5. Leaves with distinct pseudopetiole between leaf blade and sheath *M. unimasiana*
 - Leaves without distinct pseudopetiole, the leaf blade gradually narrowed into sheath ... 6
6. Leaf blade very dark green often with metallic blue sheen *M. caudata*
 - Leaf-blade light green to dark green 7
7. Spike solitary, 0.8-1.6 cm length, 0.3-0.7 cm diameter 8

- Spike capitate, more than 1.5 cm length, ≥ 0.5 cm diameter 12
8. Leaves sheath chocolate-brown, culm angular, scabrid ***M. spadicea***
- Leaves sheath not so, culm hispid or cylindrical, glabrous 9
9. Spikes globose or subglobose, spicoid bract margin brown ***M. walichii***
- Spikes elliptic, oblong or lanceolate, spicoid bract margin not distinct 10
10. Spike elliptic, fruits ellipsoid ***M. debilis***
- Spike elongated-lanceolated, fruits obovoid or lageniform 11
11. Spikes elliptic- subglobose, 1.5-1.9 cm length, fruits ellipsoid to obovoid, 4.7-6 x 2.5-3.8 mm ***M. lorea***
- Spikes elliptic- conical, 2-3.5 cm length, fruits lageniform, 5-9 x 2.5-4.0 mm ***M. enodis***
12. Leaves sheath chocolate-brown ***M. kadimiana***
- Leaves sheath not so 13
13. Spicoid bract large subtending 2/3 or full of spike ***M. squamata***
- Spicoid bract small, spike expose ***M. palustris***

6.4 Species Treatment

6.4.1 *Mapania caudata* Kuk

Repertorium Specierum novarum regni vegeabilis, 29 (1931): 201, Simpson, D.A. *A Revision of the Genus Mapania (Cyperaceae)*: 120 (1992). **Type**: MALAYSIA, Sarawak, Kapit, Upper Rejang River, 1929, J. Clemens & M.S. Clemens, 21359 (holo: B, iso: K).

Synonym: *Mapania petiolate* C.B. Clarke var. *pumila* Uittien, *Rescueil Travaux Botaniques Neerlandais* 33 (1936): 33; *M. cuspidata* (Miq.) Uittien var. *pumila* (Uittien) Uittien, *J. Arn. Arb.* 20 (1939): 214.

Description: Moderately robust, rhizomatous; **rhizome** 1-5 mm diam. **Cataphylls** ovate to lanceolate, 0.2-4.5 x 0.1-0.2 cm, rounded to acute. **Culms** several, erect or arching, lateral, 3-8.5 cm x 0.4-1.4 mm, $\pm$ terete, glabrous, greenish. **Leaves** basal, up to 57 cm long; leaf-blade linear to linear-oblong, 16-30 x 0.7-2 cm, apex abruptly narrowed, obtuse, long-cuspidate, base gradually narrowed into pseudopetiole, coriaceous, very dark green often with metallic blue sheen, distinctly paler beneath, 3-nerved, secondary nerves indistinct to distinct, flat in cross-section, strongly septate-nodulose when dry, margins entire to serrulate; pseudopetiole 2-11 x 0.3-0.5 cm; sheath narrowly lanceolate, 1-6 x 0.5-0.8 (-1.2) cm, apex very gradually narrowed, sometimes indistinct from leaf-blade, coriaceous, brownish. **Involucral bracts** several, glumaceous, ovate to lanceolate, 0.4-1 x 0.2-0.5 cm, basal bract longest, acute to subobtuse, somewhat spreading in mature inflorescence, coriaceous, brownish, glabrous, nerves distinct. **Inflorescence** terminal, solitary; spike elliptic to obovate with maturity 0.9-1.4 x 0.3-0.5 cm, obtuse, mid-brown; spicoid bracts lanceolate to ovate-lanceolate, 6.7-8.5 x 1.6-2.2 mm, subobtuse, somewhat coriaceous, mid-brown, glabrous, nerves indistinct to distinct; floral bract 6, free, lowest 2 bracts linear; 6.7-8.7 x 0.7 mm, acute, mid-brown, keel wingless, hispid, upper bract linear, 6.7-

8.7 x 0.5 mm, flat to slightly keeled, glabrous; staminate flowers 3 per spicoid, anther linear, 4 mm long, filaments up to 10 mm long; stigma branches 3; style up to 5 mm long.

Fruit ellipsoid, sometimes obscurely trigonous, 3.7-6 x 2-2.4 mm, apex apiculate, base stipitate; exocarp succulent, thin, dull mid- to dark brown, costae absent.

Leaf micromorphology: Polygonal shaped of epidermal cell on both surface. Undulate anticlinal wall pattern on both surface, 71.64 x 37.32 μm . Stomata type is tetracytic, 35.80 x 36.28 μm . The stomatal index and stomatal density is 6.8% and 38 mm^2 respectively. The outline of its midrib is concave on the abaxial and flat on the adaxial.

Ecology and Distribution: moist soil in deep shade of lowland mixed dipterocarp forest. Peninsular Malaysia and Borneo.

Specimens examined: MALAYSIA, Sarawak, Bintulu Division, Jln Bintulu-Bakun, Sg. Kelabai, MDF, near stream, Dec 2014, *Miraadila, Meekiong & Burhanuddin*, MY 0044 (HUMS); Miri Division, Suai, Wilma Plantation, fragmented forest, May 2015, *Hidir M.*, MY 0077 (HUMS); Bau Division, G. Podam, MDF, Dec 2017, *Ivy, Shabdin & Meekiong*, IG 0024 (HUMS); Kapit, Belaga, Sg. Pileran, MDF, ridge top, 570 m a.s.l., Oct 1993, *L.G. Saw*, FRI 39915 (SAR).

Notes: Easily recognized in the field by the blue-green metallic sheen leaves, similar to *M. mirae* but readily distinguished by the plant size. *M. mirae* is a miniature plant with less than 30 cm in height.

Conservation status: Currently not threatened.



Figure 6.2: *Mapania caudata* Kuk. IMAGE: Ivy GERALYN NANGAN

6.4.2 *Mapania cuspidata* (Miq.) Uittien

Journal of Arnold Arboretum 20 (1939): 213, Simpson, D.A. *A Revision of the Genus Mapania (Cyperaceae)*: 111 (192). **Type:** INDONESIA, Sumatera, Korthals s.n. (holo: L).

Synonym: *Lepironia cuspidata* Miq. *Flora of Indie Batavia Supplement* (1861): 603; *Pandanophyllum zippelianum* Kurz., *Natuurkundig Tijdschrift voor Nederlandsch-Indie N.I.*: 224 nom. nud.; *Lepistachya paremorsa* Zipp. – ex Kurz., *Journal Asiatic Society Bengal*, 33 (2) (1869): 82; *Lepironia humilis* Miq., *Illustration* 61 (1870): 61 t. 23; *Mapania humilis* F.-Vill., *Nova Appalachian* (1882): 309; *M. lucida* N.E. Brown, *Illustration Horticulture* 32 (1885): 77 t. 309; *M. triquetra* Ridl., *Journal of Strait Branch of Royal Asiatic Society*, 41 (1904): 51; *M. polyphylla* Merr. *Philippines Journal of Science*

11 (1916): Bot. 54.; *M. inopinata* Uittien, *Rescueil Travaux Botaniques Neerlandais*, 33 (1936): 151; *M. petiolata* C.B. Clarke var. *cuspidata* (Miq.) Uittien, *Rescueil Travaux Botaniques Neerlandais* 33 (1936): 282; *M. stolonifera* Uittien, *Rescueil Travaux Botaniques Neerlandais* 33 (1936): 279.

Description: Moderately robust to very robust, rhizomatous; **rhizome** 5-11 mm diam., stilt roots sometimes present. **Cataphylls** ovate to lanceolate, 0.4-5 x 0.2-0.5 cm, acute to obtuse. **Culms** several, erect, lateral, 1.5-29 cm x 0.7-2.7 mm. terete, glabrous, green. **Leaves** basal, up to 130 cm long or longer; leaf-blade linear, linear-oblong or oblong, (16-) 20-98 x 1.9-6.5 cm, apex abruptly narrowed, acute to rounded, cuspidate to long-cuspidate, base gradually to abruptly narrowed into pseudopetiole; coriaceous, mid- to dark green, young emerging leaves salmon pink to pinkish red or underside, 3-nerved, secondary nerves distinct, septate-nodulose when dry, margins entire to scabrid near apex; pseudopetiole 9.5-39 x 0.4-1.4 cm; sheaths ovate to lanceolate, 4.5-145 x 1.5-3 cm apex narrowed or abruptly narrowed, green, reddish or mid- to dark reddish brown. **Involucral bracts** several, glumaceous, ovate, 0.6-1.4 x 0.1-0.8 cm, acute, spreading in mature inflorescence, coriaceous, green to brown, sometimes reddish at base, glabrous, nerves indistinct to distinct. **Inflorescence** terminal, composed of 1 (-3) spikes; spikes elliptic to half globose when mature, 1.5-2.9 x 0.5-1.4 cm, mid-brown; spicoid bracts ovate to lanceolate-ovate. 7.2-12 x 4.5-55 mm, obtuse, coriaceous, glabrous, mid-brown, nerves indistinct; floral bracts 6, free, lowest 2 bracts linear, 7.6-11.5 x 1.4-1.8 mm, acute, mid-brown, keel wingless, moderately hispid, upper bracts linear, 7.6-11.5 x 0.5-1 mm, acute, flat to slightly keeled, glabrous; staminate flowers 3 per spicoid, anther linear, 4 mm long, cream, filaments up to 1.5 mm long; stigma branches 3; style 4-5 mm long, dark reddish, brown. **Fruit** ellipsoid to obovoid-ellipsoid, (4-) 5.2-7.6 x 2.5-4 mm, apex apiculate, base

stipitate; exocarp succulent, thin without sculpturing, dull light green to mid-brown, costae absent.

Leaves micromorphology: Both surfaces of the epidermal cell are linear and elongated. Sinuous anticlinal wall pattern on both surfaces, 76.8 – 85.36 x 28.84 – 31.36 μm . Stomata type is anomocytic, 43.24 – 50.84 x 38.44 – 39.04 μm . The stomatal index and stomatal density is 9.1% - 10.5% and 76 – 100 mm^2 respectively. For *M. cuspidata* var. *cuspidata*, the outline of its petiole is concave with keel on the abaxial and V-shape on the adaxial while the outline of the midrib is V-shape on the abaxial and flat on the adaxial. For *M. cuspidata* var. *petiolata*, the outline for both of its petiole and midrib is concave on the abaxial and U-shape on the adaxial.

Ecology and Distribution: The habitat for this species ranges from semi mangrove, peat swamp, mixed dipterocarp forest, kerangas and up to submontane forest to 1400 m altitude. *M. cuspidata* is one of the most common and widespread species that many specimens can be found in the herbaria. This species is distributed well in the Southeast Asia includes Thailand, Peninsular Malaysia, Singapore, Sumatera, Java, Borneo, Brunei Darussalam, Philippines, Maluku, Irian Jaya, Sulaweis, Papua New Guinea, Solomon Islands and New Hebrides.

Specimen examined: MALAYSIA, Sarawak. **(1) *Mapania cuspidata*** (Miq.) Uttien var. *angustifolia*: Kapit Division, Btg Balui, Bukit Kumbong, upper MDF, 950 m a.s.l., Feb 1992, Runi, Lai, Awang, Jugah et al., S. 60042 (SAR); Kuching Division, Bako National Park, Lintang Trail, kerangas forest, Oct 2014, Miraadila & Meekiong, MY 0030 (HUMS); Miri Division, Mulu National Park, Ulu Matong, 1050 m a.s.l., Nov 1990, Yii & Runi, S. 60540 (SAR); Marudi, Mulu National Park, Oct 1991, Runi, Yii & Rantai, S. 63561 (SAR). **(2) *Mapania cuspidata*** (Miq.) Uttien var. *cuspidata*: Kuching Division, Matang, Matang

Wildlife Centre, lowland MDF, *Meekionget al.*, MY 0012 (HUMS); Limbang Division, Bukit Sembiling, Hill MDF, Dec 2014, *Miraadila, Meekiong & Burhanuddin*, MY 0064 (HUMS); Miri Division, Tama Abu Range, MDF, Aug 2017, *Ivy, Shabdin & Meekiong*, IG 0006 (HUMS); Serian Division, Tebedu, Bukit Tubeh, logging area, MDF, 700 m a.s.l., Jun 1996, *Jamree et al.*, S. 73278 (SAR) **(3) *Mapania cuspidata*** (Miq.) Uittien var. ***merayap*** Meekiong, Shabdin & Miraadila: Kuching Division, Matang, Jln Matang – Lundu, roadside, kerangas forest, April 2014, *Meekiong et al.*, MY 0013 (HUMS); Jan 2009, *Shabdin & Meekiong*, ZINN 87 (HUMS); *Shabdin & Meekiong*, ZINN 86 (HUMS), **(4) *Mapania cuspidata*** (Miq.) Uittien var. ***petiolata***: Bintulu Division, Jln Bintulu – Bakun, Sg. Kelabai, MDF, near stream, Dec 2014, *Miraadila, Meekiong & Burhanuddin*, MY 0043 (HUMS); Kuching Division, Sematan, G. Pueh, Hill MDF, Feb 2014, *Miraadila et al.*, MY 007 (HUMS); April 2015, *Miraadila et al.*, MY 0072, MY0074 (HUMS); Jln Bau – Lundu, Sg. Mutut, kerangas forest, Oct 2014, *Miraadila et al.*, MY 0022 (HUMS); Lundu, G. Gading, MDF, Dec 2008, *Shabdin & Meekiong*, ZINN 80 (HUMS); Sri Aman Division, Tapang Rumpit Waterfall, Kerangas forest, Jan 2009, *Shabdin & Meekiong*, ZINN 81 (HUMS); Miri Division, Tama Abu Range, MDF, Aug 2017, *Ivy, Shabdin & Meekiong*, IG 0008 (HUMS).

Vernacular name: Pandan tikus (Mal.), Rumpit serapat (Mal.)

Notes: Simpson (1992) recognised three varieties, viz. var. *cuspidata*, var. *petiolata* and var. *angustifolia* base on the leaves characteristics while Miraadila *et al.* (2016b) described a new variety, viz. var. *merayap* (key as below).

Key to varieties of *M. cuspidata*

1. Leaf blade oblong or elliptic-oblong, up to 8 times longer than wide; sheath broadly ovate var. ***cuspidata***

 Leaf blade linear or linear-elliptic, usually more than 8 times longer than wide **2**
2. Leaf blade usually more than 3.5 cm wide, the apex obtuse to rounded var. ***petiolata***

 Leaf blade up to 3.5 cm wide, apex acute **3**
3. Plant rhizomatous; inflorescence always at the rhizome, involucre bracts exposed var. ***angustifolia***

 Plant stoloniferous; inflorescence from axillary, involucre bract obscured by the sheaths var. ***merayap***

Conservation status: Currently not threatened. However, it is believed that the population in the wild are decreasing due to over collecting by locals for traditional medicine i.e postnatal treatment and fever.



Figure 6.3: *Mapania cuspidata* (Miq.) Uittien **A.** *M. cuspidata* (Miq.) Uittien var. *petiolata* (C. B. Clarke) Uittien. **B.** *M. cuspidata* (Miq.) Uittien var. *cuspidata*. IMAGE: Ivy Geralyn Nangan

6.4.3 *Mapania debilis* C.B. Clarke ex Ridl.

Journal of Straits Branch of Royal Asiatic Society, 46 (1906): 227, Simpson, D.A. A *Revision of the Genus Mapania (Cyperaceae)*: 93 (1992). **Type:** MALAYSIA, Sarawak, Matang, Ridley 12344 (holo: K).

Synonym: *Mapania gracilipes* Merr., *Journal of Straits Branch of Royal Asiatic Society*, 85 (1992): 158; *Mapania flagellaris* Uittien, *Rescueil Travaux Botaniques Neerlandais* 32 (1935): 198 f.3.

Description: Moderately robust to robust, caespitose to shortly rhizomatous; **rhizome** 2-6 mm diam. **Cataphylls** ovate to narrowly lanceolate, 0.2-4.5 x 0.18-0.2 cm, acute to obtuse. **Culms** several, $\pm$ erect, lateral, 5.6-22 cm x 0.8-1.5 mm, subterete to subtrigonus, glabrous, greenish. **Leaves** basal, up to 105 cm or longer; leaf-blade narrowly into sheath, coriaceous, bright green to bluish green, 1-3 nerved, flat to inverse w-shaped in cross-section, often septate-nodulose when dry, margins entire to coarsely scabrid, particularly

near apex; pseudopetiole absent; sheath linear-lanceolate, 4-7 x 0.4-0.6 cm, apex very gradually narrowed, coriaceous, greenish brown to dark brown. **Involucral bracts** several, glumaceous, lanceolate to ovate, 0.5-1 x 0.2-0.6 cm basal bract longest, acute, subcoriaceous, greenish to mid-brown or dark reddish brown, glabrous, nerves indistinct to distinct. **Inflorescence** axillary, terminal, solitary, elliptic to subglobose when mature, 0.7-1.4 x 0.65-1.2 cm brown, composed of (1-) 3-5 spikes; spikes elliptic, 0.5-1.1 x 0.2-0.5 cm, acute, $\pm$ distinct; spicoid bracts oblong, 5.2-6.5 x 1.6-3 mm, obtuse to rounded, subcoriaceous, mid-brown, glabrous, nerves indistinct; floral bracts 6, free, lowest 2 bracts linear – lanceolate, 4.5-6.5 x 0.9-1.2 mm, acute, mid-brown, keel very narrowly winged, denticulate-hispid, upper bracts linear, 4.5-6.3 x 0.3-0.5 mm, acute, flat to slightly keeled, glabrous; staminate flowers 3 per spicoid, anthers linear, 1.5-2.2 mm white; filaments up to 6.5 mm long; stigma branches 3; style 2.5-3 mm long. **Fruit** ellipsoid, 2.9-3 x 1.5 mm, apex shortly apiculate, base broadly stipitate; exocarp succulent, thin, often rugulose when dry; dull dark brown, costae absent.

Ecology and Distribution: Shaded slopes often by streams or rivers, 800-1450 m altitude. Endemic to Borneo.

Species examined: MALAYSIA, Sarawak, Bintulu Division, Jln Bintulu-Bakun, Sg. Kelabai, MDF, near stream, Dec 2014, *Miraadila, Meekiong & Burhanuddin*, MY 0042 (HUMS); Kuching Division, Jln BAu – Lundu, Sg. Mutut, kerangas forest, Oct 2014, *Miraadila et al.*, MY 0023 (HUMS); Sematan, G. Pueh, Hill MDF, April 2015, *Miraadila et al.*, MY 0071 (HUMS); Miri Division, Tama Abu Range, MDF, Aug 2017, *Ivy, Shabdin & Meekiong*, IG 0005 (HUMS).

Notes: Can be recognized by narrow leaves and small inflorescence. Similar to *M. tenuiscapa*, but differ by the leaves appearances. *Mapania tenuiscapa* usually with soft and bright green leaves.

Conservation status: So far recorded from few places in Sarawak and thought to be widespread but not common. Proposed as Least Concern (LC).

6.4.4 *Mapania enodis* (Miq.) C.B. Clarke

Kew Bulletin, Additional Series 8: 53, (1908), Simpson, D.A. *A Revision of the Genus Mapania (Cyperaceae)*: 81 (1992). **Type:** INDONESIA. Sumatera, Palembang, Danoh Tjaloh Muri, *Teysmann* 3685 (holo: BO, U).

Synonym: *Lepironia enodis* Miq. *Sum.* (1861): 263, 603; *L. foliosa* Miq. *Sum.* (1861): 263, 603; *Pandanophyllum uniuquelianum* Kurz., *Journal of Asiatic Society Bengal*, 38 (1869): 81; *Mapania longa* Ridl., *Journal Straits Branch of Royal Asiatic Society*, 23 (1981): 14 nom. nud.; Ridl. ex C.B. Clarke in Hook.f. *Flora of British India*, 6 (1894): 683; *Mapania tumida* Uittien, *Gardens' Bulletin Straits S.*, 10 (1939): 182; *M. zeylanica* sensu Ridl. & Winkler, *Botanische Jahrvuchern*, 44 (1910): 525 non C.B. Clarke.

Description: Robust, rhizomatous; **rhizome** 11-12 mm diam. **Cataphylls** widely ovate to lanceolate, 0.7-9 x 0.4-1 cm, broadly obtuse to acute. **Culms** several, erect, lateral, 20-87 cm x 0.9-2.3 (-3) mm, terete, glabrous, greenish. **Leaves** basal, up to 110 cm long; leaf-blade narrowly linear, 66-98 x 1-2.2 cm, apex very gradually narrowed, acuminate, base very gradually narrowed into sheath, coriaceous, dark green or bluish-green, paler beneath, 1-nerved, rarely indistinctly 3-nerved, secondary nerves indistinct, flat to shallowly inverse w-shaped in cross-section, septate-nodulose when dry, margins entire to sparsely serrulate;

pseudopetiole absent; sheath lanceolate, 5-13 x 1.2-1.8 cm, apex gradually narrowed, coriaceous, dark green to brownish. **Involucral bracts** several, glumaceous, ovate-lanceolate to lanceolate, (0.7 -) 1-1.5 x 0.4-0.6 cm, basal bracts longest, acute, shortly mucronate, coriaceous, greenish, glabrous, nerves indistinct. **Inflorescence** axillary, spikes terminal, composed of 1 (-5) spikes; spikes elliptic to conical, 2-3.5 x 0.6-1.3 cm, acute, greenish to dark brown; spicoid bracts lanceolate to oblong-lanceolate, 7.3-10 x 2.5-3.5 mm, obtuse, membranous light brown, becoming striate with dark brown towards apex, glabrous, nerves indistinct; floral bracts 6, free, lowest 2 bracts linear-lanceolate, 7-10 x 0.8-1 mm, acute, light brown, becoming mid- to dark brown at apex, keel wingless or very narrowly winged, hispid, upper bracts linear to linear-lanceolate, 7-10 x 0.5-0.6 mm, acute, flat to somewhat keeled, glabrous; staminate flowers 3- per spicoid, stamens not seen; stigma branches 3; style 6-8mm long. **Fruit** lageniform, (5-) 6-9 x 2.5-4 mm, apex long-apiculate, base stipitate, constricted between stipe and body of fruit; exocarp succulent, thin, without sculpturing, dull mid-brown, costae absent.

Ecology and Distribution: Primary forest, often at damp and wet places near banks or rivers, 50-1100 m altitude. Borneo, Peninsular Malaysia, Peninsular Thailand, Singapore and Sumatra.

Specimens examined: MALAYSIA, Sarawak, Lawas Division, Jln Lawas-Sipitang, roadside, old secondary forest, Dec 2014, *Miraadila, Meekiong & Burhanuddin*, MY 0054 (HUMS); Miri Division, Tama Abu Range, MDF, Aug 2017, *Ivy, Shabdin & Meekiong*, IG 0012 (HUMS).

Notes: Can be distinguished by its lageniform fruits.

Conservation status: Common and widely distributed in Sarawak. Not threatened.



Figure 6.4: *Mapania enodis* C. B. Clarke ex Ridl. **A.** Plant Habit. **B.** Inflorescence. IMAGE: Ivy Geralyn Nangan.

6.4.5 *Mapania kadimiana* Shabdin, Meekiong & Miraadila

Borneo Journal of Resource Science & Technology 5(2) (2015). **Type:** MALAYSIA, Borneo, Sarawak, Sarikei Division, Sebangkoi Recreation Park, mixed dipterocarp forest, near to the river, 18 Dec 2014, Miraadila, M.I., Baharuddin & Meekiong, K., MY 0036 (holo: SAR, iso: Herbarium Universiti Malaysia Sarawak, SAN).

Synonym: none

Description: Robust, rhizomatous; **rhizome** 13-15 mm diameter. **Culm** solitary, more or less erect or lateral, 5027 x 0.5-0.9 cm, terete, mostly scabrid, medium greenish. **Leaves** basal, crowded and strongly 3-ranked, up to 155 cm long; leaf blade narrowly linear, 122-145 x 2-2.5 cm, apex gradually narrowed, acuminate with long acumen, 8-10 cm long, base very gradually narrowed into sheath, coriaceous, medium green, 1-nerved to

indistinctly 3-nerved, secondary nerves indistinct to distinct, strongly inverse w-shaped in cross-section, margins densely serrulate, pseudopetiole absent, sheaths lanceolate, 6.2-10 x 1-2 cm, apex very gradually narrowed, thickly coriaceous, shiny, mid- to dark chocolate brown. **Involucral bract** 2-4, glumaceous, lanceolate, 20-24 x 3-5 mm, apex acuminate, dark brownish. **Inflorescence** axillary, up to 10 per plants or more. Spikes sessile terminal capitulum, up to 4 spikes, seldom with solitary; spikes rounded to lanceolate, 10-20 x 5-8 mm, acute to obtuse, or subobtuse, greenish brown; spicoid bract lanceolate, 5-8 x 6-8 mm, subobtuse, subcoriaceous, greenish brown, light brown along the margin edge, faintly with hairs, nerves indistinct; floral bract 6, free, lower 2 bracts linear, 4-5 x 0.6-1 mm, acute, mid brown, keeled, wingless, hispid to ciliate hispid, upper bract linear-lanceolate, 3.8-4.6 x 0.4-0.8 mm, acute flat to keeled, transparent light brown, staminate flowers 4 per spicoid, anther linear, 6 mm, stigma branches 2, c. 2 mm long, whitish; style 4 mm long. **Fruit** obovoid, 5-6 x 2-3 mm, apex with extended conical, keeled, base shortly stipitate, exocarp hard, rugose at the upper part and with small tubercles at lower part, brownish at the lower part and become darker toward the apex and apex blackish.

Leaves micromorphology: Hexagonal shape on both epidermal cell surface. Anticlinal wall pattern is undulate on both surfaces, 59.56 x 50.36 μm . Stomata type is tetracytic, 54.0 x 69.56 μm . The stomatal index and stomatal density is 8.4% and 53 mm^2 respectively.

Ecology and Distribution: At present this species is recorded from the type locality only. This species thrives well as lithophytes on the rock surface or on the slopes at the wet and damp places, especially near to the stream or river.

Vernacular name: Daun Meing (Iban, Sarikei)

Materials examined: Sarawak, Sarikei Division, Sebangkoi Recreation Park, mixed dipterocarp forest, near to the river, 18 Dec 2014, *Miraadila, M.I., Baharuddin & Meekiong, K.*, MY 0036 (type specimen); Sept 2017, *Ivy, Shabdin & Meekiong*, IG 0020 (HUMS).

Notes: This new species is very much similar to *M. spadicea*, a species described by Uittien from Temburong, Brunei by having strongly 3-ranked leaves and the leaf sheaths being tightly imbricate and distinctly dark chocolate brown. *M. kadimiana* however differs from *M. spadicea* by having 3 spikes with sessile terminal capitulum. Our initial studies suggested that this new species might be associated with alluvial soils. The populations at the type locality mostly are lithophytes on the rock surface and so far never been discovered at other habitats. From far sight the species might be confused with *Pandanus* sp. that sympatrically inhabits the area.

Conservation status: At present the species are growing in a protected forest which is being utilise for recreational purposes. Until further discovery being made as there are yet many localities to be explored, we would propose this species to as not threaten.

6.4.6 *Mapania lorea* Uittien

Rescueil Travaux Botaniques Neerlandais 33 (1936): 150, Simpson, D.A. *A Revision of the Genus Mapania (Cyperaceae)*: 84 (1992). **Type:** INDONESIA, Sumatra, Reopart I, Bruinier 17 (holo: BO, iso: L).

Synonym: none

Description: Robust, rhizomatous; **rhizome** 5-14 mm diam. **Cataphylls** widely ovate to lanceolate, 0.4-4 x 0.4-0.5 cm, broadly obtuse to acute. **Culms** 1-3 per plant, ± erect,

lateral, 7-26 (-30) cm x 0.8-1.8 mm, $\pm$ terete, glabrous, greenish. **Leaves** basal, up to 100cm long; leaf-blade narrowly linear, 55-91 x 1-1.8 cm, apex very gradually narrowed, acuminate, base very gradually narrowed into sheath, thickly coriaceous, dark green, paler beneath, 1-nerved, secondary nerves indistinct, $\pm$ flat to shallowly inverse w-shaped in cross-section, septate-nodulose when dry, margin densely serrulate; pseudopetiole absent; sheath narrowly lanceolate, 6-7 x 1-1.2 cm, apex very gradually narrowed, dark green becoming dark, somewhat shiny brown toward base. **Involucral bracts** several, glumaceous, ovate, 0.7-1 x 0.3-0.5 cm, basal bract longest, subacute to obtuse, glabrous, coriaceous, greenish at first, becoming dark brown when matured, glabrous, nerves $\pm$ distinct. **Inflorescence** axillary, spike terminal, solitary; spike elliptic to subglobose, 1.5-1.9 x 0.7-1.4 cm, obtuse to rounded, dark reddish brown; spicoid bracts lanceolate to oblong-lanceolate, 7-9 x 3-5 mm, rounded, subcoriaceous to coriaceous, light brown at base becoming densely striate with dark reddish brown at apex, glabrous, nerves indistinct; floral bracts 6, free, lowest 2 bracts linear-lanceolate, 7-8 x 1-1.2 mm, acute, light brown, striate with dark reddish brown towards apex and along keel, keel wingless, densely denticulate-hispid near apex, upper bracts linear to linear-lanceolate, 6.8-8 x 0.7-1.2 mm, flat to $\pm$ keeled, glabrous; staminate flowers 3 per spicoid, anthers linear, 4 mm long, filaments up to 8 mm long; stigma branches 3; style 4-5 mm long. **Fruit** ellipsoid to obovoid, 4.7-6 x 2.5-3.8 mm, apex acute to subobtuse, rarely very shortly apiculate, base stipitate; exocarp succulent, thin, without sculpturing to slightly wrinkled, dull mid- to dark brown, costae absent.

Leaf micromorphology: Epidermal cell shape for *M. lorea* is hexagonal on both surfaces. The anticlinal wall pattern for both surfaces is sinuous. The average size of the epidermal

cell is 76.32 x 35.12 μm . Stomata type is tetracytic, 46.36 x 34.72 μm . The stomatal index and density are 10.9% and 69 mm^2 respectively.

Ecology and Distribution: swampy forest, often by stream, 100-700 m altitude.

Specimens examined: MALAYSIA, Sarawak, Kapit Division, Nanga Gat, Upper Rejang River, *J. & M.S. Clemens*, No. 21850 (SAR); Kuching Division, Sematan, Samunsam Wildlife Sanctuary Extention, kerangas forest, Oct 2014, *Meekiong et al.*, MY 0026 (HUMS); Sri Aman Division, Tapang Rumpit, kerangas forest, Dec 2014, *Miraadila, Meekiong & Burhanuddin*, MY 0031 (HUMS); Bau Division, G. Podam, MDF, Dec 2017, *Ivy, Shabdin & Meekiong*, IG 0028 (HUMS).

Notes: Frequently found submerged in the water or rheophytic on the rock surface by the streams. The tips of the bracts are not enclosing the spike apex, sometimes deflexed and developed pineapple top-like structure. This species is locally known as *pandan tekura* or 'tortoise' pandan by the local people in Sri Aman Division because the inflorescences or spikes are eaten by the tortoises.

Conservation status: Widely distributed in Sarawak, but not common. Not threatened.



Figure 6.5: *Mapania lorea* Uittien. IMAGE: Ivy Geralyn Nangan

6.4.7 *Mapania meditensis* D.A. Simpson

A Revision of the Genus Mapania (Cyperaceae): 116 (1992). **Type:** BRUNEI DARUSSALAM, Tutong, Medit River, Medit Swamp, 3 Nov 1991, *Simpson et al.*, 2515 (holo: K, iso: BRUN, L).

Synonym: none

Description: Robust, rhizomatous; **rhizome** 0.5-1 cm diam., often ascending. **Cataphylls** not seen. **Culms** absent. **Leaves** basal, up to 65 cm long; leaf-blade linear-oblong to oblong, 15-37 x 2.5-5.1 cm, apex abruptly narrowed, cuspidate, broadly obtuse to rounded, base abruptly narrowed into pseudopetiole, coriaceous, mid-green, paler below, shiny, young emerging leaves pale green on underside, 3-nerved, secondary nerves $\pm$ distinct, $\pm$ flat in cross-section, indistinctly septate nodulose when dry, margins entire to serrulate; pseudopetiole 10-23 x 0.6-0.8 cm; sheath ovate, ovate-lanceolate, 3-7 x 1.5-4 cm, apex

abruptly narrowed, coriaceous, greenish towards apex to shiny mid-brown below.

Involucral bracts several, glumaceous, ovate to ovate-lanceolate, 1.2-2 x 0.9 cm, basal bract shortest, obtuse, coriaceous, shiny, mid-brown, glabrous, nerves indistinct.

Inflorescence sessile, solitary; spike lanceolate, 2-5.5 x 1cm, usually hidden within leaf sheath, often indistinctly 3-sided when young, pale green, or brown, often with pinkish tinge; spicoid bracts ovate-lanceolate to lanceolate, 21-46 x 1.2-3.2 mm, acute, coriaceous, the lowest one somewhat keeled, sometimes hispid on keel, pale green or brown with pinkish tinge, somewhat translucent, nerves indistinct; floral bracts 6, free, lowest 2 bracts narrowly lanceolate, 18-44 x 0.8-1.2 mm, acute, mid-brown, keel wingless to narrowly winged, sparsely denticulate-hispid, upper bracts linear to narrowly lanceolate, 18-44 x 0.3-1 mm, acute, glabrous, flat; staminate flowers 3 per spicoid, anthers linear, 3 mm long, whitish, filaments up to 22 mm long; stigma branches 3; style up to 22 mm long. **Fruit** narrowly ellipsoid to ellipsoid, sometimes slightly compressed, 7.5-10.5 x 4.5-5 mm, apex very slightly apiculate, base stipitate; exocarp succulent, thin, without sculpturing, dark brown, costae absent.

Leaf micromorphology: Epidermal cell shape for *M. meditensis* are different on abaxial and adaxial. Mixture of hexagonal and polygonal on abaxial, and hexagonal on adaxial, but similar anticlinal walls for both, which is undulate. The average size of the epidermal cell is 45.47 - 66.25 x 46.35 – 51.25 μm . Stomata type is anomocytic, 36.80 – 37.52 x 34.1 - 35.2 μm . The stomatal index and density are 10.8% - 14.5% and 84 – 92 mm^2 respectively. The petiole outline shape on the abaxial is concave, while on the adaxial is narrowly U-shape. The outline for its midrib is V-shape on the abaxial and U-shape on the adaxial.

Ecology and Distribution: Lowland mixed dipterocarp forest up to mountain forest, in swampy ground and stream sides, 20-1730 m altitude. Borneo, Peninsular Malaysia and Peninsular Thailand.

Specimens examined: MALAYSIA, Sarawak, Kapit Division, Sg. Bena, wet sloping ground near river, April 1980, *B.L. Burtt*, *B. 12977* (SAR); Kuching Division, Sematan, Samunsam Wildlife Sanctuary Extention, kerangas forest, Oct 2014, *Meekiong et al.*, *MY 0027* (HUMS); Lawas Division, Lawas-Sipitang Road, logging road, MDF, Dec 2014, *Miraadila, Meekiong & Burhanuddin*, *MY 0051* (HUMS); Limbang Division, Jln Pandaruan, Sg. Semabat, old secondary forest, Dec 2014, *Miraadila, Meekiong & Burhanuddin*, *MY 0061* (HUMS); Miri Division, Lambir Hills National Park, MDF, Waterfall Trail, Dec 2014, *Miraadila, Meekiong & Burhanuddin*, *MY 0047* (HUMS); Sibul Division, Jln Sibul – Bintulu, roadside, MDF, Sept 2017, *Ivy, Shabdin & Meekiong*, *IG 0019* (HUMS).

Notes: In common with *M. meekiongii* in appearance, *M. meditensis* can be differentiated by the sessile inflorescence that is obscured by the sheaths, while *M. meekiongii* has long peduncle and capitate inflorescence.

Conservation status: Widespread in Southeast Asia and common throughout. It is believed that the populations will be decreasing in years to come because of habitat loss and over harvesting for local medicinal purposes.



Figure 6.6: *Mapania meditensis* D. A. Simpson. **A.** Plant Habit. **B.** Inflorescence. IMAGE: Ivy Geralyn Nangan.

6.4.8 *Mapania palustris* (Hassk. Ex Steud.) F.-Vill. var. *palustris*

Kew Bulletin Additional Series 8: 53 (1908), Simpson, D.A. *A Revision of the Genus Mapania (Cyperaceae)*: 66 (1992). **Type:** INDONESIA. Sumatra, Palembang, Danoh Tjaloh Muri, *Teysmann* 3685 (holo: BO, U).

Synonym: *Pandanophyllum palustre* Hassk. Ex Steud., *Syn Plantarum Glumaceae*. (1855): 134; *P. zeylanicum sensu* Kues., *Journal Asiatic Society Beng.* 38 (1869): 80 non Thwaites; *Lepironia palustris* (Hassk. ex Steud.) Miq., *Illustration* (1871): 63, t.25; *Mapania kurzisensu* Merr., *Philippines Journal of Science*, 2 (1907): 422 non C.B. Clarke; *M. albescens* C.B. Clarke, *Kew Bulletin Additional Series* 8 (1908): 54; *M. banahaensis* Elmer, *Leaf Philippines Botany* 2 (1909): 574; *M. macrocephala sensu* Ridl., *Trans Linnean Society Botany* 9 (1916): 245 non (Gaudich) K. Schum.; *M. affinis* Merr., *Journal Straits Branch of Royal Asiatic Society*, 85 (1922): 157; *M. grandiceps* Kuk, *Bot. Jahrb.* 59 (1924): 55; *M. longiflora sensu* H. Pfeiffer, *Mitteilungen aus dem Institut für Allgemeine*

Botanik Hamburg, 7 (1928): 173 non C.B. Clarke; *M. javana* Uittien, *Rescueil Travaux Botaniques Neerlandais* 33 (1936): 287.

Description: Robust to very robust, rhizomatous; **rhizome** 5-20 mm diam. **Cataphylls** ovate to lanceolate, 1-16 x 0.6-2.8 cm, acute to obtuse. **Culms** 1-4 per plant, erect, lateral, 7-73 cm x 1.6-5.5 mm, terete to trigonous, glabrous to densely hispid, greenish to reddish brown. **Leaves** basal, up to 260 cm or longer; leaf-blade linear, 68-206 x 1.5-4.7 cm, apex gradually or very gradually narrowed, acuminate to long acuminate, base gradually or very gradually narrowed into sheath, coriaceous to thickly coriaceous, mid-green, somewhat paler beneath, indistinct to distinctly 3-nerved with distinct secondary nerves, flat to inverse w-shaped in cross-section, septate-nodulose when dry, margins entire to serrulate, pseudopetiole absent; sheath linear, 9-22 x 1.4-3.9 cm, apex very gradually narrowed, coriaceous to thickly coriaceous, reddish brown to dark chestnut brown, somewhat shiny in robust specimens. **Involucral bracts** several, glumaceous, ovate, ovate-lanceolate or oblong, 0.7-3.4 x 0.5-2.8 cm, acute to subobtuse, coriaceous, greenish to reddish brown, purplish brown or blackish brown, glabrous to hispid on margins and midrib of lower bracts, nerves indistinct to distinct. **Inflorescence** axillary, spike terminal, globose, 1.7-5 x 1.7-5 cm, light to mid-brown or somewhat reddish brown, composed of (1-) 2-20 or more, often densely packed spikes; spikes elliptic, 1-3 x 0.5-1 cm, acute to obtuse, usually indistinct; spicoid bracts oblong to lanceolate, 6-14.5 x 1.6-2.2 mm, subobtuse to obtuse, sometimes split, coriaceous to thickly coriaceous, light to mid-brown, sometimes reddish brown, glabrous, nerves indistinct; floral bracts 6, free, lowest 2 bracts linear to linear-lanceolate, 6-14 x 1-1.4 mm, acute, light to mid-brown, keel wingless, densely hispid, upper bracts linear, 7.5-14 x 0.4-0.6 mm, acute, flat to somewhat keeled, glabrous; staminate flowers 3 per spicoid, anthers linear, 2.8-4 mm, white, filaments up to 14 mm

long; stigma branches 3, up to 4 mm long; style 5-8 mm long. **Fruit** ellipsoid to obovoid, 3.5-5 x 2.3-3.5 mm, apiculate, base shortly stipitate; exocarp succulent, thin, without sculpturing, dull mid- to dark brown, costae absent.

Leaf micromorphology: Both surfaces of epidermal cell are hexagonal. The anticlinal wall pattern is undulate on both surfaces. The average size of the epidermal cell is 53.44 x 38.70 μm . Stomata type is diacytic, 67.28 x 89.72 μm . The stomatal index and stomatal density are 2.7% and 7 mm^2 respectively.

Ecology and Distribution: Damp, shady areas in primary forest and secondary forest, 50-1700 m altitude. Borneo, Java, Maluku, New Guinea, New Hebrides, Peninsular Malaysia, Philippines, Singapore, Solomon Island, Southern Peninsular Thailand and Sumatra.

Specimens examined: MALAYSIA, Sarawak, Kuching Division, Sematan, G. Pueh, Hill MDF, Feb 2014, *Miraadila et al.*, MY 006 (HUMS); Sematan, G. Pueh, Hill MDF, April 2015, *Miraadila et al.*, MY 0075 (HUMS); Lawas Division, Lawas-Sipitang Road, logging road, MDF, Dec 2014, *Miraadila, Meekiong & Burhanuddin*, MY 0050 (HUMS); Limbang Division, Jln Pandaruan, Sg. Semabat, old secondary forest, Dec 2014, *Miraadila, Meekiong & Burhanuddin*, MY 0062 (HUMS); Miri Division, Lambir Hills National Park, MDF, Waterfall Trail, Dec 2014, *Miraadila, Meekiong & Burhanuddin*, MY 0046 (HUMS); Tama Abu Range, MDF, Aug 2017, *Ivy, Shabdin & Meekiong*, IG 0010 (HUMS).

Notes: Easily distinguished from others non petiolate leaves by the numerous number of capitate inflorescences.

Conservation status: Not threatened. This species is among the most common species found in almost all habitats including peat swamp, lowland mixed dipterocarp, kerangas, semi-mangrove and foothill of limestone forest.



Figure 6.7: *Mapania palustris* (Hassk. Ex Steud) F.-Vill. var. *palustris*. **A.** Plant Habit. **B.** Inflorescence. IMAGE: Ivy Geralyn Nangan

6.4.9 *Mapania spadicea* Uittien

Rescueil Travaux Botaniques Neerlandais 33: 150 (1936), Simpson, D.A. *A Revision of the Genus Mapania (Cyperaceae)*: 89 (1992). **Type:** INDONESIA, Borneo, Kalimantan, West Kutai, Long Petak, 16 Sept 1925, *Endert 3348* (holo: BO, iso: K, L).

Synonym: none

Description: Robust, rhizomatous; **rhizome** 12-18 mm diam. **Cataphylls** ovate to lanceolate, 0.6-4 x 0.2-1 cm, obtuse to acute. **Culms** 1-several per plants, more or less erect or lateral, 3.5-23 cm x 2.9-4 mm, terete, mostly scabrid, greenish. **Leaves** basal, crowded and strongly 3-ranked, up to 147 cm long, leaf-blade narrowly linear, 98-140 cm x 0.9-1.4 cm, apex very gradually narrowed, acuminate, base very gradually narrowed into

sheath, thickly coriaceous, dark green, 1-nerved to indistinctly 3-nerved, secondary nerve indistinct to distinct, strongly inverse w-shaped in cross-section, smooth to indistinctly septate-nodulose when dry, margins densely serrulate, pseudopetiole absent, sheath lanceolate, 5.5-8 x 0.8-1.8 cm, apex very gradually narrowed, thickly coriaceous, somewhat shiny, mid- to dark chocolate brown. **Involucral bract** several, glumaceous, lanceolate, 1.4-3 x $\pm$ 0.8 cm, acute, coriaceous, greenish at first becoming dark brown when matured, somewhat scabrid, nerves indistinct. **Inflorescence** axillary, spike terminal, solitary, spike elliptic to lanceolate, 2.5-5 x 1-1.3 cm, acute to subobtuse, brown; spicoid bracts lanceolate, 13-14 x 2.2-4 mm, obtuse, subcoriaceous, often splitting from the base, mid-brown, glabrous, nerves indistinct; floral bracts 6, free, lowest 2 bracts linear to linear lanceolate, 12.8-13.7 x 0.3-0.7 mm, acute, flat to somewhat keeled, sometimes sparsely hispid on margin; staminate flowers 3 per spicoid, anther linear, 5-8 mm long, stigma branches 3, style 6 mm long. **Fruit** ellipsoid, 5-5.5 x 2.5-2.7 mm, apex triangular, base conical-stipitate; exocarp succulent, thin without sculpturing, slightly wrinkled, dull mid-brown with 2 indistinct lateral costae.

Leaf micromorphology: Epidermal cell shape for *M. spadicea* are different on abaxial and adaxial. Hexagonal on abaxial and mixture of hexagonal and polygonal on adaxial, but similar anticlinal walls for both, which is undulate. The average size of the epidermal cell is 53.32 x 48.30 μm . Stomata type is anomocytic, 49.92 x 42.09 μm . The stomatal index and density are 11.8% and 61 mm^2 respectively. The midrib outline on the abaxial is concave with keel while on the adaxial is V-shape.

Ecology and Distribution: mixed dipterocarp forest, on the slope in the gully, clay loam soils. Borneo (previously recorded only from Brunei and Kalimantan); a new record for Sarawak.

Specimens examined: MALAYSIA, Sarawak, Sibü Division, Sg. Apeng, Sibü – Bintulu Road, *M.I. Miraadila, B. Borhanudin & K. Meekiong, MY 0068* (HUMS); Miri Division, Lambir National Park, primary lowland forest, *Ivy, Shabdin & Meekiong, IG 0016* (HUMS).

Notes: This species is characterised particularly by its strongly 3-ranked leaves, the leaf sheaths being tightly imbricate and distinctly dark chocolate brown which is similar to the newly described species, *M. kadimiana*. However, the latter species easily distinguished by the spikes number, 3-4 whereas *M. spadicea* with solitary spike.

Conservation status: The populations at Sg. Apeng, Sibü – Bintulu Road is close to the roadside and the area is surrounded by the agricultural activities. We believe the populations might be gone very soon. However, since the species was original has described from Kalimantan, we believed that the species might be found elsewhere in Sarawak.

6.4.10 *Mapania squamata* (Kurz) C.B. Clarke

Kew Bulletin Additional Series 8: 53 (1908), Simpson, D.A. *A Revision of the Genus Mapania (Cyperaceae)*: 76 (1992). **Type:** INDONESIA, Java, Zippelius s.n. (holo: BO).

Synonym: *Pandanophyllum squamatum* Kurz, *Journal Asiatic Society Bengal*, 38 (1869): 80; *Lepironia squamata* (Kurz) Miq., *Illustration* (1870): 64, t.26; *Mapania heterocephala* Merr., *Journal Straits Branch of Royal Asiatic Society*, 85 (1922): 158; [*Mapania walichii* sensu Ridl. *Flora of Malay Peninsula (Monograph.)* 3 (1907): 105 non C.B. Clarke prop parte quoad specime Ridley 11475]

Description: Robust to very robust; **rhizome** 10-18 mm diam. **Cataphylls** ovate to lanceolate, 0.8-5 x 0.6-0.8 cm, acute. **Culms** several, spreading to erect, lateral, 1-10 (-14) cm x 2-3.5 mm, terete, glabrous, greenish to dark brown. **Leaves** basal, up to 150 cm long; leaf-blade linear, 103-144 x 2.4-4.5 cm, apex narrowed or gradually narrowed, acuminate, base very gradually narrowed into sheath, coriaceous, dark green, blue-green beneath, 3-nerved, secondary nerves indistinct, flat to inverse w-shaped in cross-section, indistinctly septate-nodulose when dry, margins serrulate; pseudopetiole absent; sheath lanceolate, 5.5-10 x 2.4-3.6 cm, apex gradually narrowed, coriaceous, dark green. **Involucral bracts** several, glumaceous, ovate to ovate-lanceolate, 1.3-2.1 x 0.8-1.2 cm, basal bract longest, acute to subobtusate, thickly coriaceous, mid- to dark green at first, usually becoming dark brown, glabrous, nerves mostly indistinct. **Inflorescence** axillary, spike terminal, composed of 1 (-5) spikes; spikes narrowly elliptic to elliptic, 2.5-3.2 x 0.7-2.4 cm, acute, greenish at first, becoming dark brown, distinct when more than one; spicoid bracts lanceolate to oblong-lanceolate, 1.3-18 x 5.5-6.5 mm, obtuse submembranous at base becoming dark brown at apex, glabrous, nerves indistinct; floral bracts 6, free, lowest 2 bracts linear, 13.5-17 x 1.2-1.6 mm, acute, dark brown, keel narrowly winged, sparsely denticulate-hispid towards apex, upper bracts linear-lanceolate; 12.5-16.5 x 0.9-1.5 mm, acute, flat to somewhat keeled, glabrous; staminate flowers 3 per spicoid, anthers linear, 5-6 mm long; filaments up to 17 mm long; stigma branches 3; style up to 10 mm long. **Fruit** ellipsoid to obovoid, 5-7 x 2.5-4 mm, apex shortly apiculate, base stipitate; exocarp succulent, thin, without sculpturing, green at first becoming dark brown, costae absent or sometimes indistinctly 3-costae.

Ecology and Distribution: Lowland mixed dipterocarp forest, 50-1200 m altitude. Borneo, Java, Peninsular Malaysia, Singapore and Sumatra.

Specimens examined: MALAYSIA, Sarawak, Kapit Division, Balleh, Nanga Balang, on shallow sandstone cliff face to edge of riverine forest, Jun 1969, *J.A.R. Anderson*, S. 28311, S. 28373 (SAR); Tama Abu Range, MDF, Aug 2017, *Ivy, Shabdin & Meekiong*, IG 0004 (HUMS); Serian Division, Tebakang, G. Bakat, growing on rocks along the Sg. Kukur, April 1983, *Dawang Awa & Ilias Paie*, S. 45845 (SAR)

Notes: The appearance of this species is closely similar to *M. longiflora* but readily distinguished by ovoid spike and shorter culm. Also might be confused with the two new species, *M. rumputensis* and *M. bauensis*. *M. rumputensis* easily distinguished by the presence of silvery wax on the lower surface of leaves and specifically found in kerangas forest, while the leaves of *M. bauensis* are hardly coriaceous and so far recorded only from the limestone habitat.

Conservation status: Not threatened. Common and widely distributed in northern west and central parts of Sarawak.

6.4.11 *Mapania unimasiana* Shabdin, Meekiong, & Miraadila

Folia malaysiana, 17(2): 18 (2016). **Type:** MALAYSIA, Borneo, Sarawak, Bintulu Division, Bintulu-Bakun Road, 38 miles, old secondary forest, wet and damp place along the stream, 21 Dec 2014, *Miraadila, Burhanuddin & Meekiong*, MY 0045A (holo – SAR; iso – Herbarium of Universiti Malaysia Sarawak, SAN).

Synonym: none

Description: Moderately robust, rhizomatous; **rhizomes** 2-5 mm diam. **Culms** absent. **Leaves** basal, up to 185 cm long; leaf blade linear, 145-170 x 4.5-5 cm, apex abruptly narrowed, cuspidate, broadly obtuse to rounded, base gradually narrowed into

pseudopetiole, coriaceous, light to medium green or yellowish green, 3-nerved, secondary nerves distinct, flat in cross-section, margin entire to scabrid near apex; pseudopetiole 30-38 x 2-2.3 cm. **Inflorescence** sessile, compose of 3-7 spikes, the spikes often remaining obscured by involucre bracts at maturity, spikes 4-4.2 x 2-2.5 cm, greenish yellow, pistillate flower numerous per spike, 20-30 enclosing staminate flowers located in the center in separate spicoids. **Involucre bracts** 7-9, or more, ovate to lanceolate shape, 2.7-3 x 1.3-2.2 cm, coriaceous, margin wrinkled, with thin light brown layer along, light to medium green or yellowish green. Spicoid 7-9 per spike, 6-8 consists pistillate flowers, only 1 with staminate flowers. Spicoid bracts lanceolate, 3-3.2 x 1.2-2 cm, coriaceous, greenish yellow, apex acute, light brown green, glabrous; floral bracts linear, 3.5-3.7 cm long, membranous, transparent whitish with light brown at the apex, inner floral bract 4, free, 6-6.2 x 3-3.2 mm, membranous, whitish with light brown at the apex. Each spicoid consists of 4-6 anthers, anther linear, 3.5-3.6 mm long, whitish, filament up to 2 mm long. Each spicoid consists of 3-4 stigmas, stigma branches 2 or 3 and rarely 4, 5-6.5 mm long, whitish. **Fruit** ellipsoid, 8-8.5 mm in length, 3-3.3 mm in width, surface with tubercles, apex acute, black.

Leaf micromorphology: Epidermal cell shape for *M. unimasisana* are different on abaxial and adaxial. Mixture of hexagonal and polygonal on abaxial, and hexagonal on adaxial but similar anticlinal walls for both, which is undulate. The average size of the epidermal cell 58.39 x 45.07 μm . Stomata type is anomocytic, 43.35 x 33.67 μm . The stomatal index and density are 15.8% and 92 mm^2 respectively.

Ecology and Distribution: Endemic to Borneo (Sabah and Sarawak).

Specimens examined: SABAH, Labuk and Sugut, Roadside of Telupid Road, 23 Oct. 1968, Shohei Kokawa & Mitsuro Hotta, No. 326 (SAN); SARAWAK, Bintulu Division,

Bintulu – Bakun Road, 38 Miles, old secondary forest, wet and damp place along the stream, 21 Dec 2014, MY 0045b (SAN), MY 0045c (KEP), MY 0045d (SAR), MY 0045e (HUMS); April 2018, Ivy, Shabdin & Meekiong, IG 0026 (HUMS); Kapit Division, Belaga, Ulu Batang Balui, Ulung Sungai Kabbor, mixed dipterocarp forest, 600 meters a.s.l., 29 Mar. 1989, Yii Puan Ching, S. 62351 (SAR).

Notes: This new species has sessile inflorescence that comprises of 3-7 spikes. Unlike others species, *M. unimasiana* has centred staminate flowers (4-6) that are enclosed by numerous pistillate flowers (20-30) making it unique among all the *Mapania* species.

Conservation status: At the moment, this species is considered not threatened due to the wide distribution (Sabah and Sarawak). The population at the type locality at present are protected by the local people as their water catchment and supplies area. Kabbor, mixed dipterocarp forest, 600 meters a.s.l., 29 Mar. 1989, Yii Puan Ching, S. 62351 (SAR).



Figure 6.8: *Mapania unimasiana* Shabdin, Meekiong & Miraadila. **A.** Plant Habit. **B.** Inflorescence. IMAGE: Ivy Geralyn Nangan

6.4.12 *Mapania walichii* C.B. Clarke

In: Hook.f., *Flora of British India*. 6: 682 (1894), Simpson, D.A. *A Revision of the Genus Mapania (Cyperaceae)*: 78 (1992). **Type**: SINGAPORE, Wallich 3541 (holo: K).

Synonym: none

Description: Robust, rhizomatous; **rhizome** 10-12 mm diam. **Cataphylls** ovate to broadly lanceolate, 1-3 x 0.8 cm, obtuse to acute. **Culms** 1 – several, spreading to erect, lateral, 8-28 cm x 1.3-2.2 (-3) mm, terete, glabrous, greenish to dark brown. **Leaves** basal, up to 170 cm long; leaf-blade narrowly linear, 72-160 x 1.3-2.2 cm, apex very gradually narrowed, acuminate, base very gradually narrowed into sheath, coriaceous, dark green, 3-nerved, secondary nerves indistinct, flat to inverse w-shaped in cross-section, indistinctly septate-nodulose when dry, margin serrulate; pseudopetiole absent; sheath lanceolate, 7-12 x 1.6-2 cm, apex gradually narrowed, coriaceous, dark green. **Involucral bracts** several, glumaceous, ovate to ovate-lanceolate, 1-1.5 x 0.4-0.7 cm, basal bract longest, subacute to obtuse, coriaceous, greenish at first, becoming dark brown when matured, glabrous, nerves mostly indistinct. **Inflorescence** axillary, spike terminal, solitary; spike elliptic to broadly elliptic, 2.5-4.2 x 1.2-1.6 cm, acute, dark brown; spicoid bracts lanceolate, 15-16 x 4-8 mm, obtuse, membranous at base becoming subcoriaceous at apex, light brown at base becoming dark brown at apex, keel wingless, hispid towards apex, upper bracts linear-lanceolate, 15-16 x 1-1.5 mm, acute, flat somewhat keeled, glabrous; staminate flowers 3 per spicoid, anthers linear, 8 mm long, filaments up to 15 mm long; stigma branches 3; style 9 mm long. **Fruit** broadly ellipsoid, 4.3-5.5 x 3.2-3.5 mm, apex apiculate, base stipitate; exocarp succulent, thin, without sculpturing greenish at first, becoming dark brown gradually, costae absent.

Leaf micromorphology: Both surfaces of epidermal cell are hexagonal. The anticlinal wall pattern is undulate on both surfaces. The average size of the epidermal cell is 67.72 x 49.96 µm. Stomata type is tetracytic, 72.36 x 59.8 µm. The stomatal index and stomatal density are 11.2 % and 115 mm² respectively. The outline of its midrib is V-shape on the abaxial and U-shape on the adaxial.

Ecology and Distribution: Primary forest and kerangas forest, deep shaded or often on granitic sand, 20-800 m altitude. Borneo, Peninsular Malaysia, Singapore and Sumatra.

Specimens examined: MALAYSIA, Sarawak, Bintulu Division, Jln Bintulu-Bakun, Sg. Kelabai, MDF, near stream, Dec 2014, *Miraadila, Meekiong & Burhanuddin, MY 0041* (HUMS); Kuching Division, Jln Bau – Lundu, Sg. Mutut, kerangas forest, Oct. 2014, *Miraadila et al., MY 0021* (HUMS); Sematan, Samunsam Wildlife Sanctuary Extention, kerangas forest, Oct. 2014, *Meekiong et al., MY 0025* (HUMS); Miri Division, Lambir Hills National Park, MDF, Waterfall Trail, Dec. 2014, *Miraadila, Meekiong & Burhanuddin, MY 0048* (HUMS); Mukah Division, Jln Sibu-Mukah, Mukah Water Treatment, MDF, Dec. 2014, *Miraadila, Meekiong & Burhanuddin, MY 0037* (HUMS); Sarikei Division, Sebangkoi Recreation Park, Hill MDF, near stream, Dec. 2014, *Miraadila, Meekiong & Burhanuddin, MY 0033* (HUMS); Sibu Division, Jln Sibu-Bintulu, Sg. Apeng, roadside, MDF, Dec. 2014, *Miraadila, Meekiong & Burhanuddin, MY 0067* (HUMS); Sept 2017, Ivy, *Shabdin & Meekiong, IG 0018* (HUMS).

Notes: *Mapania walichii* is the most common species and widely distributed in Sarawak and easily recognize by rounded or subglobose spikes.

Conservation status: Not threatened. Common throughout Sarawak.



Figure 6.9: *Mapania walichii* C. B. Clarke. **A.** Plant Habit. **B.** Inflorescence. IMAGE: Ivy Geralyn Nangan.

6.4.13 *Mapania zinnirahea* Miraadila & Meekiong

Folia malaysiana, 17(2): 21 (2016). **Type:** MALAYSIA, Borneo, Sarawak, Sibu Division, Sibu-Bintulu Road, Sg. Apeng, near to the roadside, 26 Dec 2014, *Miraadila, Burhanuddin & Meekiong*, MY 0031 (holo – SAR; iso – Herbarium of Universiti Malaysia Sarawak).

Synonym: none

Description: Moderately robust, shortly stoloniferous perennial; **rhizome** 2-4 cm long, 8-15 mm diam. **Cataphylls** 6-10, elliptic to lanceolate, 7.5-12 x 1.6-2.1 cm, maroon or brownish maroon. **Culms** 1- to several, erect, lateral, 5-20 cm x 2-4 mm, glabrous, brownish or maroon red. **Leaves** basal, up to 47.5 cm long; leaf blade oblong or linear oblong, 11-32.5 x 3-7.6 cm, apex abruptly narrowed or cuspidate with cauda c. 3-4 cm long, base gradually narrowed into a short pseudopetiole which widens again into the

sheath, coriaceous to subcoriaceous, papery when dried, upper surface greenish, dull, lower surface reddish purple with maroon nerves (in living material), 3-nerved, secondary nerves distinct, flat in cross-section, margins entire to serrulate or more less scabrid near apex; pseudopetiole 10-20 x 1.5-2.5 cm, sheath lanceolate, 4-6.5 cm x 4-15 mm, apex very gradually narrowed, coriaceous, reddish green to maroon. **Involucral bracts** 3-5, glumaceous, ovate to lanceolate, 2.5-4.5 x 0.6-0.8 cm, basal bract shorter, coriaceous; peduncle slender, 8-14 cm long. **Inflorescence** axillary, spike terminal, ovoid to lanceolate, composed of 1-4 spikes; spikes remaining $\pm$ obscured by floral bracts even at maturity, ovoid-lanceolate, 1.4-2.5 x 1-1.4 cm; spicoid bracts lanceolate, 1-1.2 x 0.6-1 cm, coriaceous, nerves indistinct; floral bracts 6, free, lowest 2 bracts linear to linear lanceolate, 5-8 mm long, keeled, wingless, hispid, upper bracts linear, flat to $\pm$ keeled, glabrous; staminate flowers 3 per spicoid, anthers linear, 2-2.6 mm long, stigma branches 3, style 5 mm long. **Fruit** not seen.

Ecology and Distribution: Remnant lowland mixed dipterocarp forest by the roadside, in gully, on wet place of swampy patch. Borneo, so far only recorded from the type locality at Sg. Apeng of Sibuan-Bintulu Road.

Specimens examined: MALAYSIA, Sarawak, Sibuan Division, Jln Sibuan-Bintulu, Sg. Apeng, roadside, MDF, Dec 2014, *Miraadila, Meekiong & Burhanuddin*, MY 0069 (holotype: SAR; iso: HUMS); Sept 2017, *Ivy, Shabdin & Meekiong*, IG 0017 (HUMS).

Notes: At the very first sight, this species resembles the recently new described species, *M. sapuaniana* from the Lanjak Entimau Wildlife Sanctuary (LEWS) however differs by inflorescences morphology. The spikes are ellipsoid whilst the later have angular spikes. This species grows exposed to sunlight close to the roadside.

Conservation status: This species has so far collected only from a single location in Sibuan Division. This species is considered rare as only few plants were spotted from the type locality. Considering the number of populations are very low, less than 20 plants growing localised to the particular area, we, therefore believe this species in Sarawak to be Vulnerable (VU D2) following the IUCN (2010) Categories and Criteria.



Figure 6.10: *Mapania zinnirahea* Miraadila & Meekiong. IMAGE: Ivy Geralyn Nangan

6.4.14 *Mapania multifolia* sp.nov. Ivy, Shabdin & Meekiong

Synonym: none

Description: Robust, rhizomatous, height 104-106 cm; stilt roots present. **Cataphylls** present, light green, 16-18 x 4.5-5 cm. Culms absent. Leaves numerous, more than 30 leaves; blade: basal, linear-oblong to oblong, 29.5 – 29.7 x 6.0 – 6.2 cm, apex acuminate, slightly serrulate, 2 cm toward the apex, base acute, coriaceous, margin entire at first,

slightly serrulate toward the apex, abaxial light green, adaxial dark green, 3-nerved, distinct secondary nerve. Leaf sheaths present, yellowish-green, margin pinkish, 5 cm, gradually develop into sheaths, not wavy. Pseudopetiole present, up to 9.5 cm, c. 3.6 mm diam., abruptly narrowed into pseudopetiole, shorter than leaves, channelled along. Inflorescence sessile, obscured within leaf sheaths, 2.8 x 1.3 cm, spike lanceolate, glabrous, spiked bract 13 x 4 mm. Fruit not seen.

Leaf micromorphology: Random shaped of the epidermal cell on abaxial, while hexagonal shaped on adaxial. Both surfaces have sinuous anticlinal walls, 58.05 x 25.45 μm . Stomata type is anomocytic, 44.6 x 27.0 μm . The stomatal index and stomatal density is 9% and 53 mm^2 respectively. The outline of its midrib is V-shape on the abaxial and flat on the adaxial.

Ecology and Distribution: Damp, shady areas in fragmented forest.

Specimens examined: MALAYSIA, Sarawak, Mukah Division, Jalan Sibul-Mukah, roadside, MDF, April 2018, Ivy, Shabdin & Meekiong, IG 0032 (HUMS)

Notes: At first sight, *M. multifolia* is closely similar to *M. cuspidata* and *M. meditensis* due to its distinct pseudopetiole. However, upon close examination, the inflorescence structure is different. It is sessile with long spicoid bracts and totally hidden within its leaf sheaths.

Conservation status: Currently, this species is only recorded from Mukah Division. This species probably endemic to the area, since we never encountered this species from any other localities.

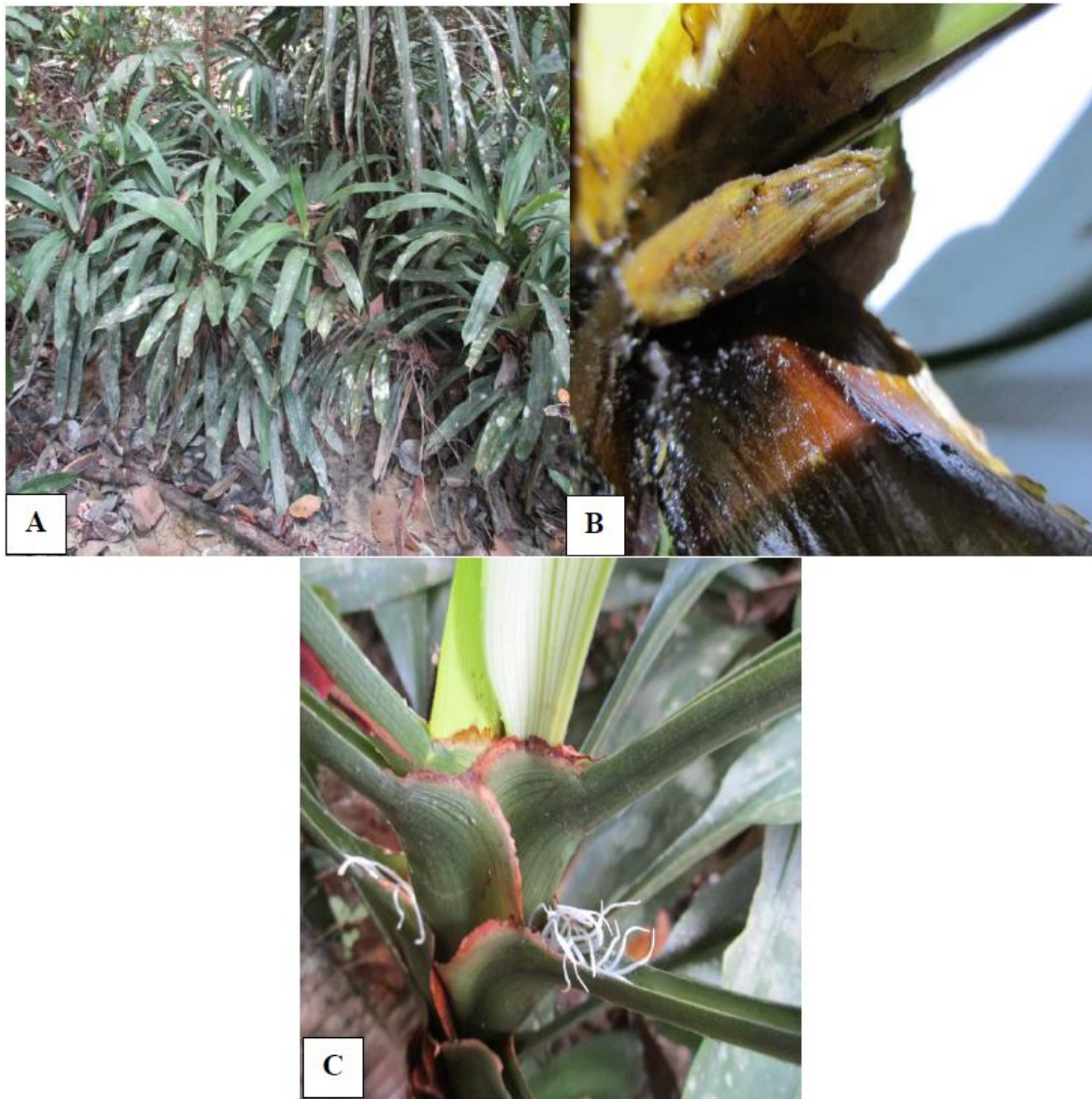


Figure 6.11: *Mapania multifolia* Ivy, Shabdin & Meekiong, sp. nov. **A.** Plant Habit. **B.** Inflorescence. **C.** Inflorescence hidden within the sheath. IMAGE: Ivy Geralyn Nangan



Figure 6.12: *Mapania multifolia* Ivy, Shabdin & Meekiong, sp. nov. **a.** Habit; **b.** detail of sheath margin; **c.** spike; **d.** spicoid; **f.** staminate flowers (stamens). [Drawing by Umie Naylisa]

6.5 Discussion

Fifteen species of *Mapania* were collected and recorded from various localities throughout Sarawak. Most of the species were collected from Tama Abu, Miri. The localities and GPS readings were recorded (Table 6.1). Living collections of selected *Mapania* species were planted in the Universiti Malaysia Sarawak Arboretum. Herbarium specimens collected were kept in Herbarium of Universiti Malaysia Sarawak (HUMS) and duplicates were deposited at SAR for future reference.

Up to date, 44 species of *Mapania* including one new species were recorded in Sarawak. The majority of the species are found in tropical rainforests with low light intensity. Some species require high humid condition such as open swamp, near the stream or riverbanks. As shown in Figure 6.1, *Mapania* species are widely spread from South to West of Sarawak. However, the distribution of certain *Mapania* species can only be found in one locality, for example; *M. zinnirahea*, *M. kadimiana* and *M. unimasiana*. *M. zinnirahea* can only be found exactly at the roadside of Jalan Sibu-Bintulu. As the sampling site currently is under the construction of the Pan Borneo project, it is predicted that the number of individuals might be getting lesser as of now. Meanwhile, *M. kadimiana* were only found near the stream at Sebangkoi Recreational Park. *M.unimasiana* was found at Tubau, Bintulu. The region is a fragmented forest surrounded by palm oil plantations and pipeline constructions.

As for *M. enodis*, it has been listed as threatened species by the IUCN Red List of Threatened Species. However, a previous study found that it occurred at Lawas (Miraadila, 2016) and the latest area found was in Tama Abu, Miri. As of now, two localities of *M. enodis* were noted.

One newly recognized species were also found during sampling. So far, the new species were only found in one locality each. *M. multifolia* sp. nov. was found at Jalan Sibumukah. The area where the species were found was a shady and muddy area of fragmented forest that is a favourable condition for *Mapania* to survive.

The assessment of *Mapania* species distribution is necessary. Certain localities might expose to logging activities to make way for road development or new township, hence, threatening the species population. Thus, further action can be taken to help conserve the species particularly *M. zinniraha*, where the number of population are very low.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

In the present study, a total of 15 *Mapania* species with a newly described species, *M. multifolia* sp. nov. were found throughout Sarawak. Eleven *Mapania* species were selected for anatomical study. Based on morphology, there are two types of leaf blades, i.e. petiolate leaf blade and linear leaf blade. The distinguishable characters between the species in terms of leaf surface and transverse sections are taxonomically significant thus far. The study which presented epidermal cells, stomata, and cross-sections of petiole and midrib support the identifications of the species. The 11 selected species showed a distinct three types of stomata, shape and pattern of the anticlinal wall, as well as the abaxial and adaxial outline of petiole and midrib. Most of the species studied were anomocytic type of stomata with a combination of hexagonal and random shape of the anticlinal wall except for *M. cuspidata* varieties which is linear and elongate cell. Tetracytic type of stomata can be found in four *Mapania* species studied with hexagonal shape of the anticlinal wall except for *M. caudata* (polygonal). Only one species was found with a diacytic type of stomata. However, the pattern of anticlinal wall is varied (undulated & sinuous) among the species studied. Based on the findings, the difference of the seven species studied can be seen in the cross-section of petiole and midrib. In the petiole cross-section, two species (*M. cuspidata* var. *petiolata* & *M. meditensis*) shared the same abaxial outline, but a different adaxial outline. The same goes in the midrib cross-section. Thus, the combinations of these characters, can help narrow down the species identification and make the evidence stronger

for taxonomy classification. In addition, the findings of cell inclusion in *M. cuspidata* var. *cuspidata* can also help distinguish between *M. cuspidata* varieties.

In antifungal study, among 20 crude extracts obtained, at least one crude extract were effective against *C. musae* and *P. oryzae* (isolates POSA 1 & POSA 2). The effective crude extract inhibited the growth of the crop pathogenic fungi but did not kill the fungi. However, the growth retardation of the crop pathogenic fungi varied between the crude extracts from different *Mapania* species and at different concentrations. Based on the findings, certain *Mapania* species crude extract was effective against *C. musae*, but not effective against *P. oryzae*. The isolate specificity effect also can be seen when *Mapania* crude extracts were tested against two different isolate. The *Mapania* crude extracts were effective when tested on one of the isolate but not effective to the other. The crude extract that induces the genotypic effect or isolate specificity effect in this study may not be meaningful in the field since an effective antimicrobial agent must be effective against the majority of its isolates or genotypes. Nevertheless, the discovery of its antifungal effect gives a better insight on this underrated genus and create awareness in conservation effort.

7.2 Recommendations

The anatomical information gained in this study is the first to be examined from Southeast Asia. *Mapania* species, particularly from section *Pandanophyllum*. More sampling of other species will give a better picture of the informative anatomical characters of the leaves, and may also be expanded to the inflorescence anatomical structures. To obtain more precise data to be mapped with other forms of data, phytochemical study should be conducted, i.e. the isolation of the bioactive compounds from the crude extracts may be meaningful in determining the main compound responsible

for the antifungal activity as well as the possible interactions of the active compounds. Besides, extraction with different solvents can also be considered to determine the bioactive compound that can be beneficial in the future.

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APPENDICES

APPENDIX 1: THE NATIONAL PARKS AND NATURE RESERVES REGULATIONS 1999 FORM OF PERMIT


FIRST SCHEDULE
THE NATIONAL PARKS AND NATURE RESERVES
REGULATIONS 1999
FORM OF PERMIT
(Not Transferable)
(Regulation 5)

Permit No: **WL104/2018**

Permission is hereby given to **Ivy Geralyn Nangan Anak Melana**, Fakulti Sains dan Teknologi Sumber, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak
NRIC/Passport No: 941003135758

And the following persons, namely --- NRIC/Passport No:
1. Nil

To enter **Niah NP, Lambir NP, Similajau NP, Bako NP, Batang ai NP, Kubah NP, Gunung Gading NP, Gunung Lesung NP, Bungo Range NP, Santubong NP, Derek Krian NP, Gunung Apeng NP** in Sarawak and to:-

(a) ~~exercise a subsisting right/privilege namely~~
(b) ~~conduct a special activity, namely~~
(c) ~~conduct a research activity, namely~~
Taxonomical Study of Selected
Mapania spp. (Cyperaceae) from
Sarawak
(d) ~~conduct an expedition, namely~~
(e) ~~conduct a filming work, namely~~

Subject to the agreement/ terms and special conditions* as prescribed by the Controller.

Date of Issue: **25.09.2018**
Valid Until (date): **24.09.2019**

Fees paid (RM): **Waived entrance fee**

Signature:
Name: **HAJI AZAHARI BIN OMAR**

Official Stamp:
for Controller of National Parks & Nature Reserves

N.R.1. This permit shall be returned to the Controller or any authorized Park Officer upon its expiration, cancellation or withdrawal.

2. This permit must be produced for inspection at any time on demand by the Controller or any authorized Park Officer.
(Special conditions attached)

*Delete wherever inapplicable.

APPENDIX 2: THE ANTIFUNGAL ACTIVITY OF EFFECTIVE MAPANIA SP. CRUDE EXTRACT

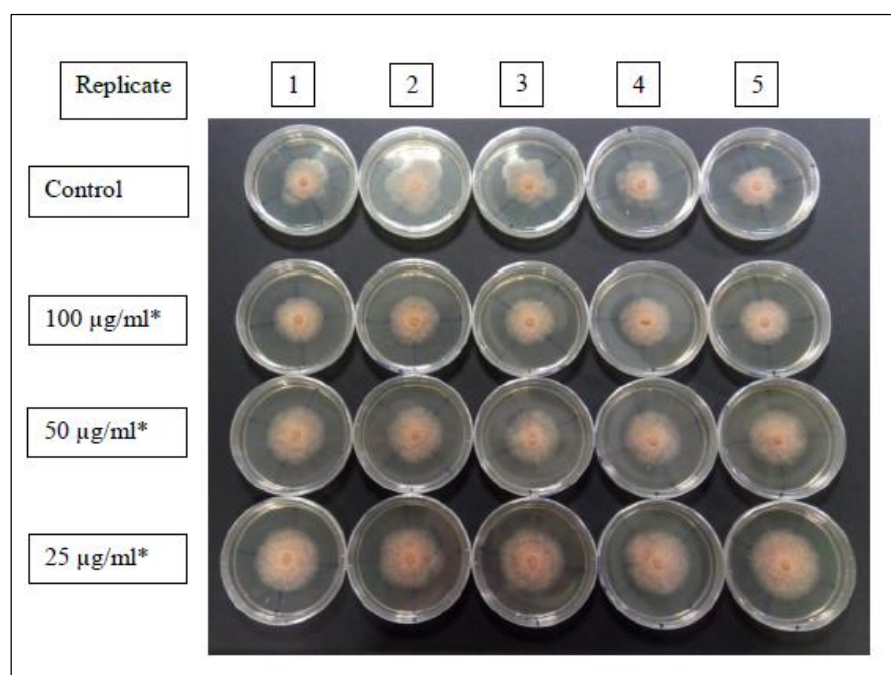


Figure 1: Antifungal activity of root from *M. debilis* methanolic crude extract against *C. musae* with different concentrations, where “*” indicates significant different from control medium only ($p < 0.05$).

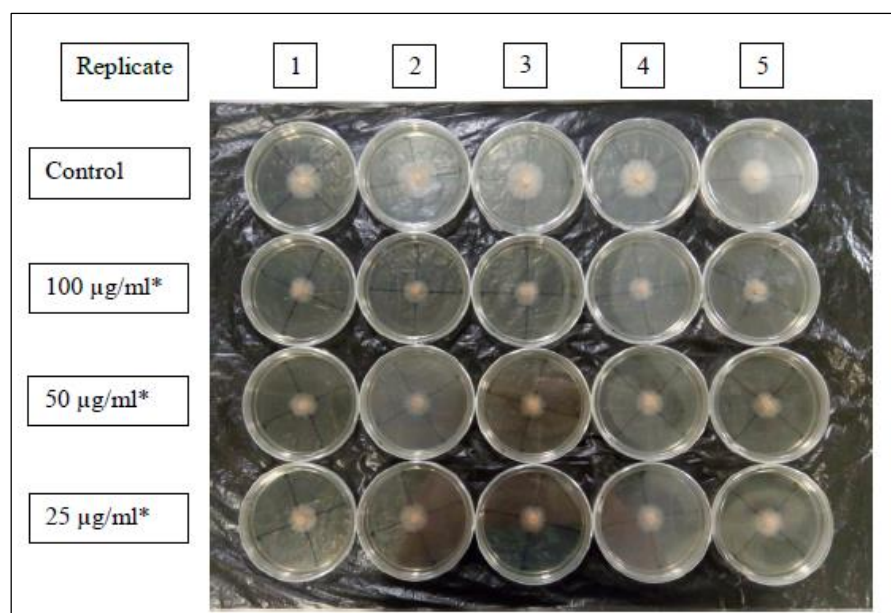


Figure 2: Antifungal activity of root from *M. enodis* methanolic crude extract against *C. musae* with different concentrations, where “*” indicates significant different from control medium only ($p < 0.05$).

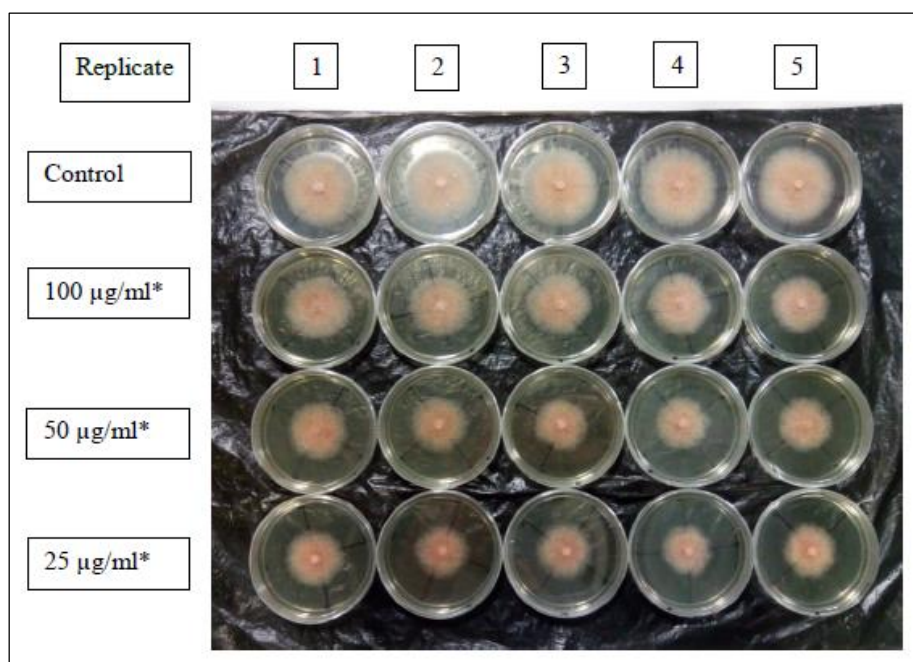


Figure 3: Antifungal activity of leaf from *M. kadimiana* methanolic crude extract against *C. musae* with different concentrations, where “*” indicates significant different from control medium only ($p < 0.05$).

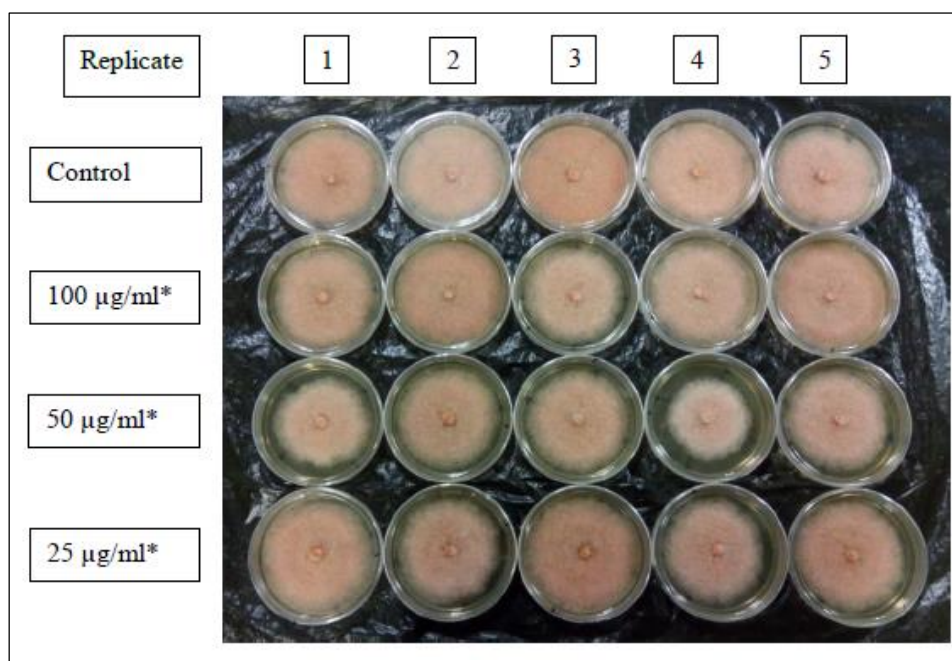


Figure 4: Antifungal activity of root from *M. kadimiana* methanolic crude extract against *C. musae* with different concentrations, where “*” indicates significant different from control medium only ($p < 0.05$).

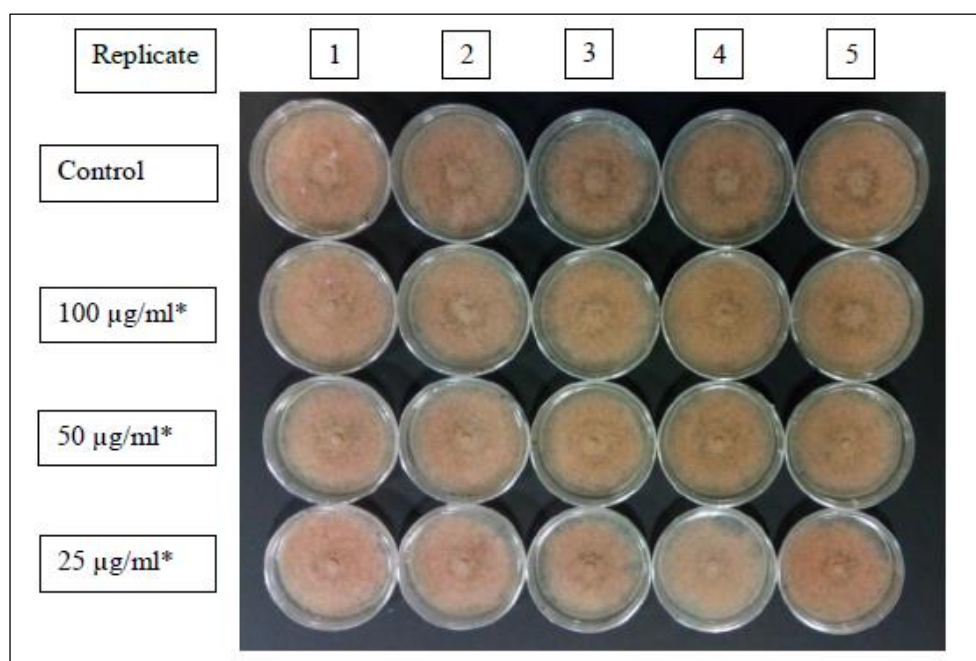


Figure 5: Antifungal activity of root from *M. squamata* methanolic crude extract against *C. musae* with different concentrations, where “*” indicates significant different from control medium only ($p < 0.05$).

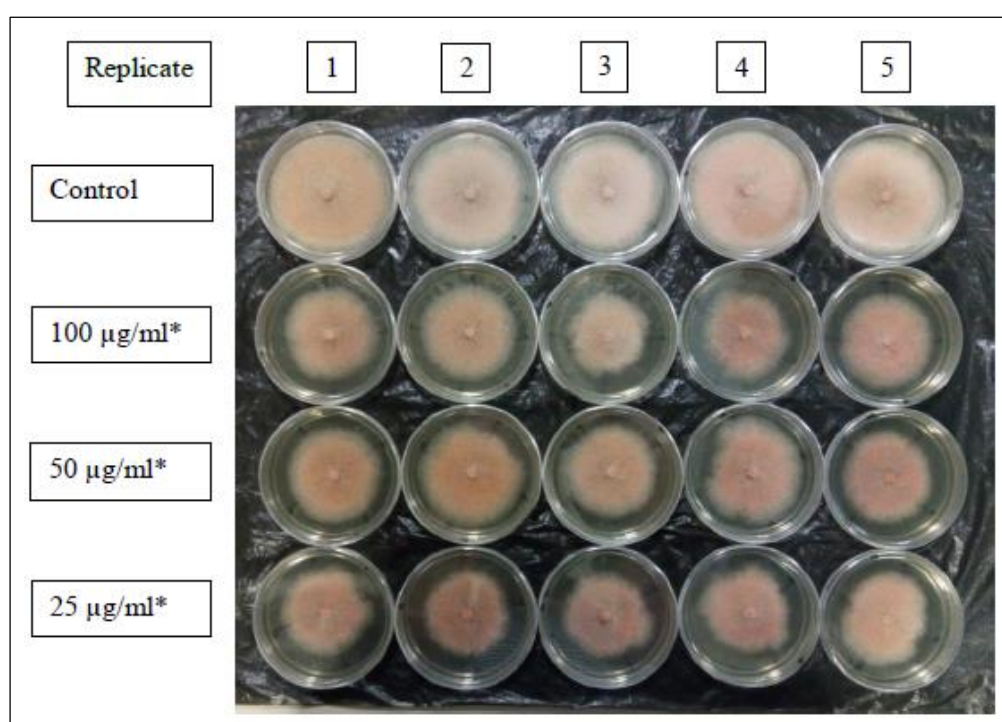


Figure 6: Antifungal activity of root from *M. unimasiana* methanolic crude extract against *C. musae* with different concentrations, where “*” indicates significant different from control medium only ($p < 0.05$).

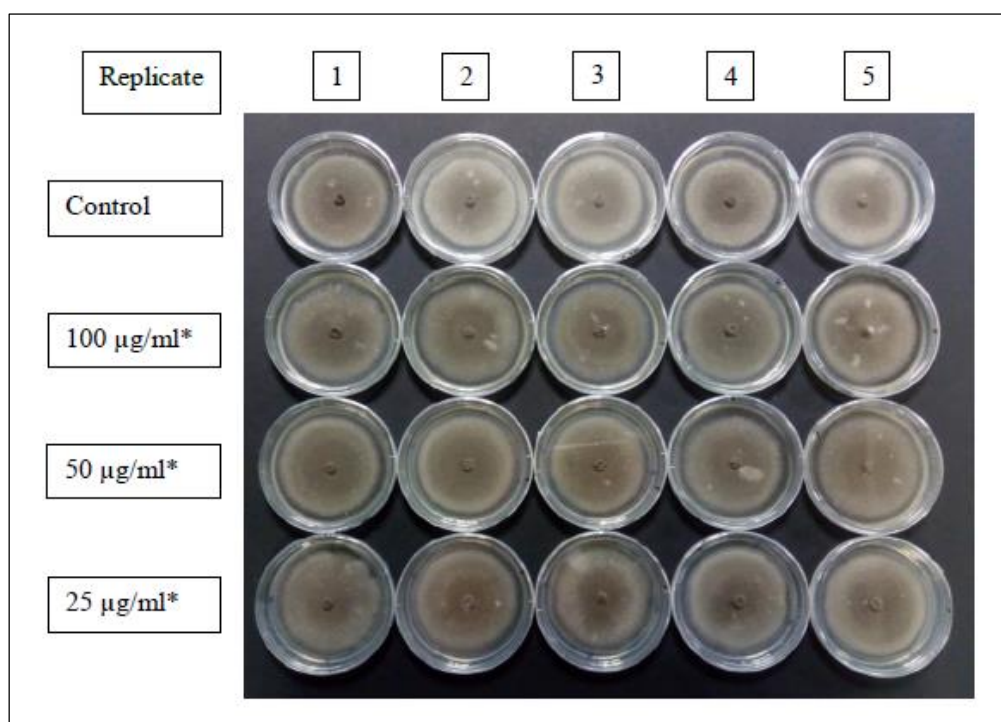


Figure 7: Antifungal activity of leaf from *M. enodis* methanolic crude extract against *P. oryzae* (isolate POSA 1) with different concentrations, where “*” indicates significant different from control medium only ($p < 0.05$).

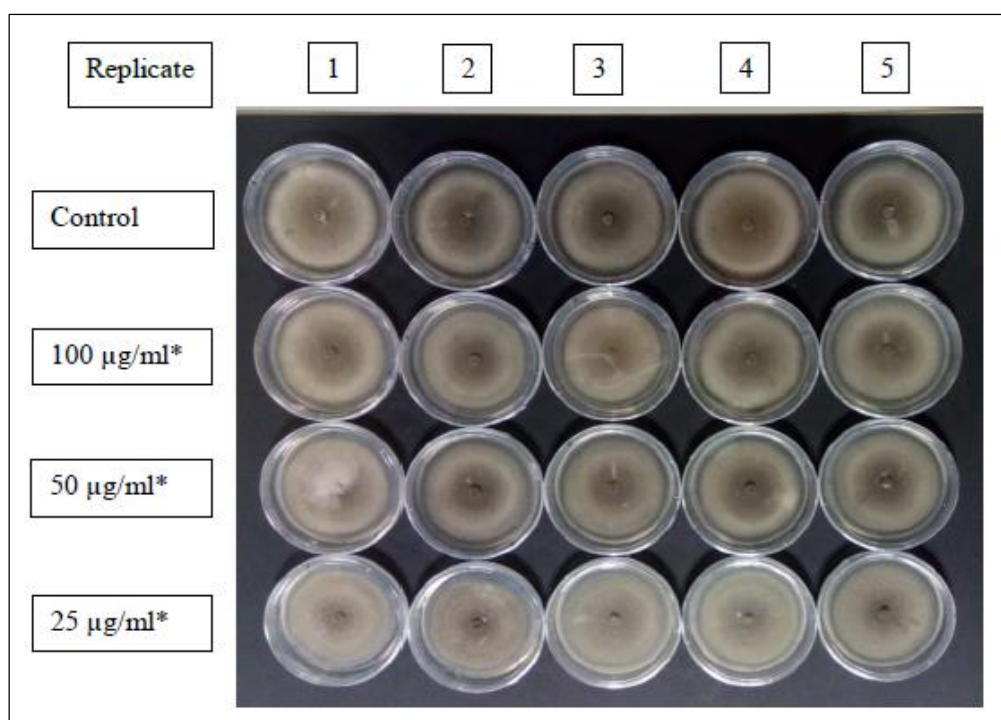


Figure 8: Antifungal activity of root from *M. squamata* methanolic crude extract against *P. oryzae* (isolate POSA 1) with different concentrations, where “*” indicates significant different from control medium only ($p < 0.05$).

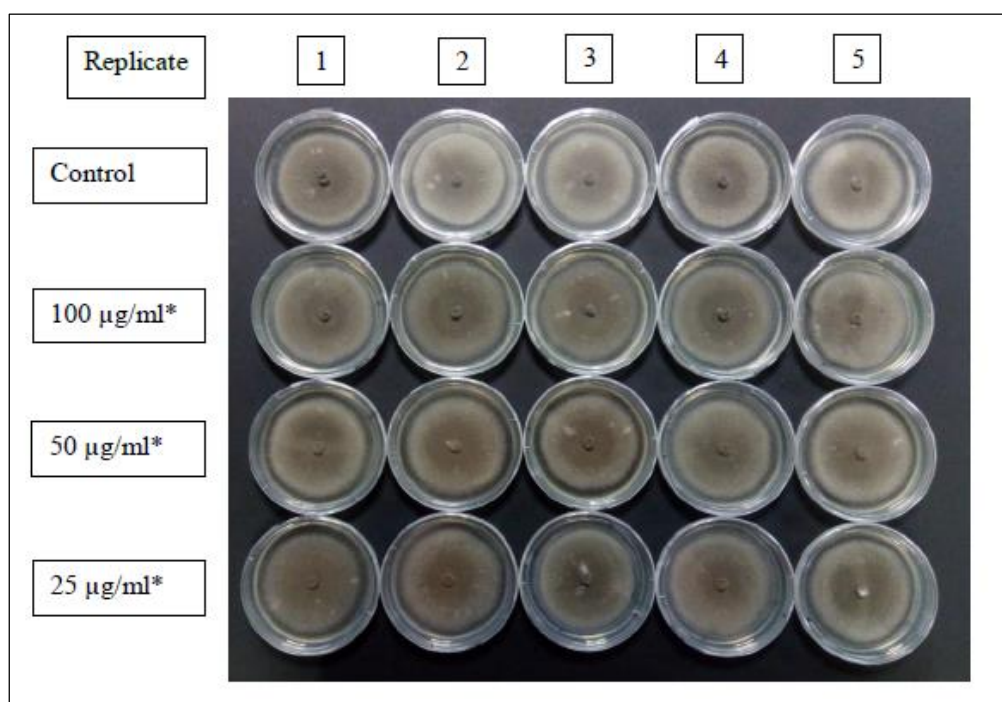


Figure 9: Antifungal activity of leaf from *M. unimasiana* methanolic crude extract against *P. oryzae* (isolate POSA 1) with different concentrations, where “*” indicates significant different from control medium only ($p < 0.05$).

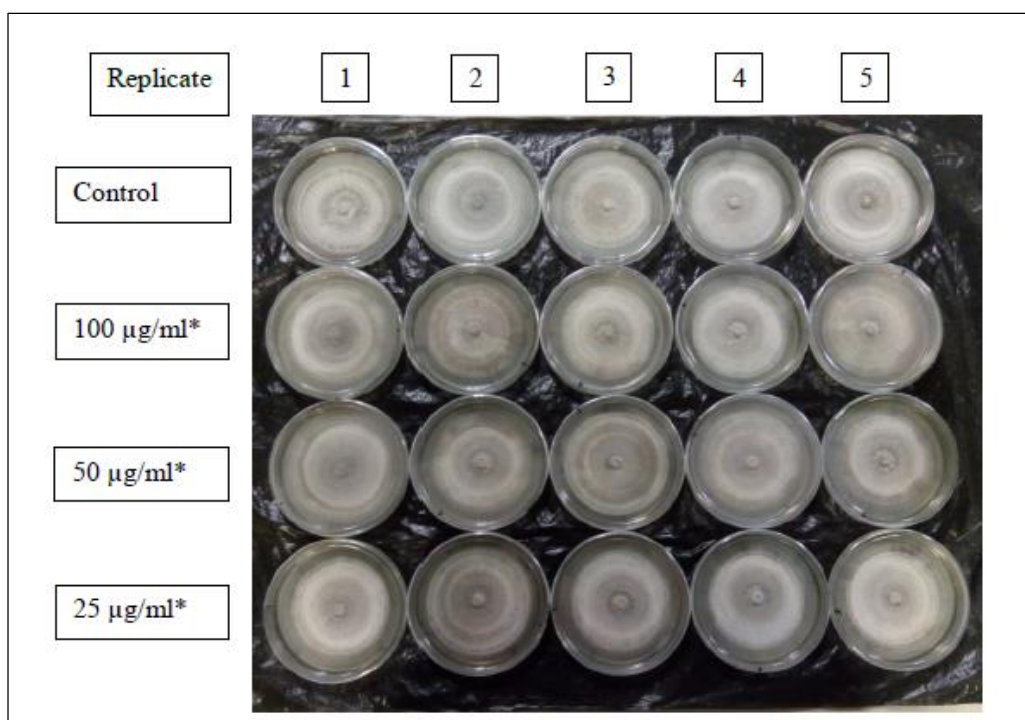


Figure 10: Antifungal activity of leaf from *M. walichii* methanolic crude extract against *P. oryzae* (isolate POSA 1) with different concentrations, where “*” indicates significant different from control medium only ($p < 0.05$).

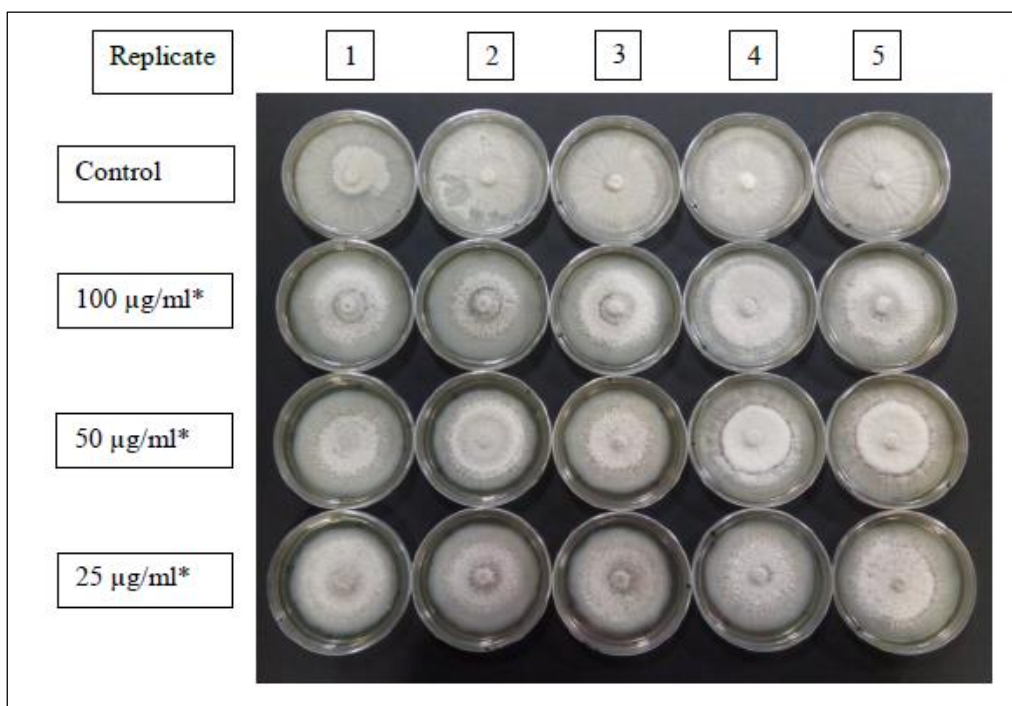


Figure 11: Antifungal activity of leaf from *M. caudata* methanolic crude extract against *P. oryzae* (isolate POSA 2) with different concentrations, where “*” indicates significant different from control medium only ($p < 0.05$).

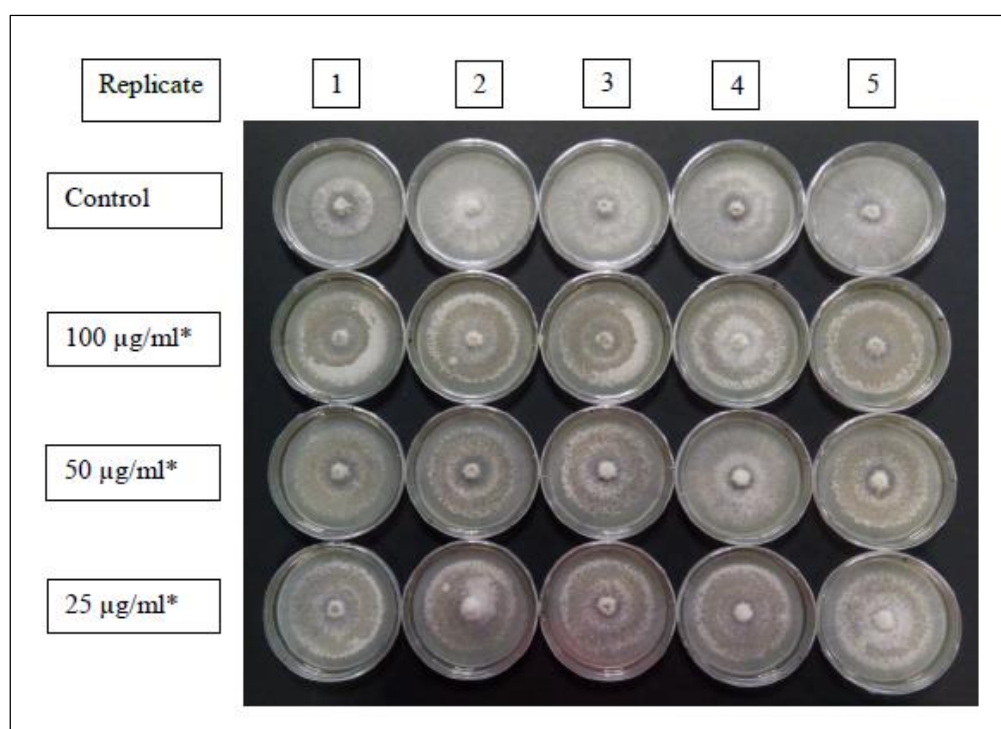


Figure 12: Antifungal activity of leaf from *M. unimasiiana* methanolic crude extract against *P. oryzae* (isolate POSA 2) with different concentrations, where “*” indicates significant different from control medium only ($p < 0.05$).