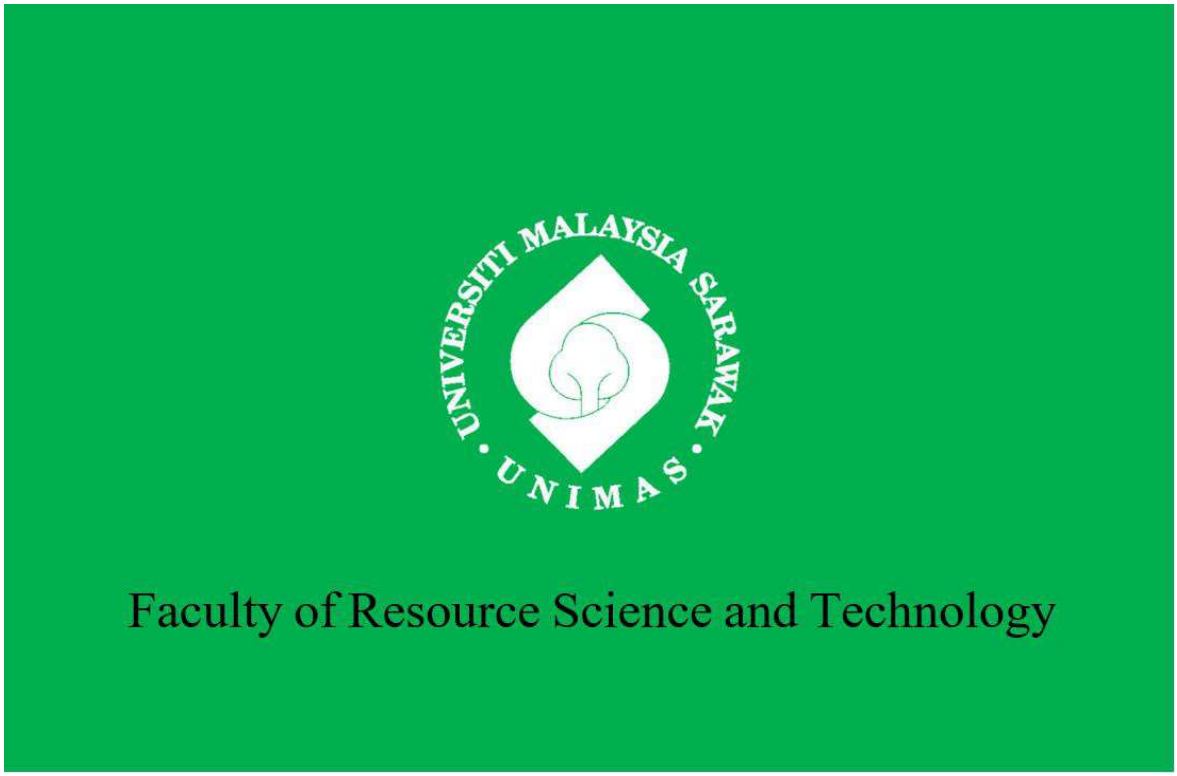


# Screening of Medicinal Plants against *A. flavus*

Syamimi Izzati Binti Hussin
(73251)

Bachelor of Science with Honours
(Resource Biotechnology)
2022

# Screening of Medicinal Plants against *A. flavus*

Syamimi Izzati Binti Hussin

A thesis submitted in partial fulfilment of the Requirement of The Degree Bachelor of Science with Honours
(Resource Biotechnology)

**SUPERVISOR: DR. ROSMAWATI BINTI SAAT**

Programme of Resource Biotechnology
Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2022

**UNIVERSITI MALAYSIA SARAWAK**

Grade: _____

| **Please tick (√)** | |
|---|---|
| Final Year Project Report | √ |
| Masters | |
| PhD | |

**DECLARATION OF ORIGINAL WORK**

This declaration is made on the 14th day of June 022.

**Student's Declaration:**

I, Syamimi Izzati Binti Hussin (73251), Faculty of Resource Science and Technology

(PLEASE INDICATE STUDENT'S NAME, MATRIC NO. AND FACULTY) hereby declare that the work entitled, Screening of Medicinal Plants against *A. flavus* is my original work. I have not copied from any other students' work or from any other sources except where due reference or acknowledgement is made explicitly in the text, nor has any part been written for me by another person.

15th June 2022
Date submitted

Syamimi Izzati Binti Hussin (73251)
Name of the student (Matric No.)

**Supervisor's Declaration:**

I**,** Rosmawati binti Saat**,** hereby certify that the work entitled, Screening of Medicinal Plants against *A. flavus* was prepared by the above named student, and was submitted to the "FACULTY" as a partial fulfillment for the conferment of Bachelor of Science with Honours (Resource Biotechnology), and the aforementioned work, to the best of my knowledge, is the said student's work.

Received for examination by: Dr Rosmawati binti Saat Date: 15th June 2022

I declare this Project/Thesis is classified as (Please tick (√)):

☐ **CONFIDENTIAL** (Contains confidential information under the Official Secret Act 1972)*** RESTRICTED** (Contains restricted information as specified by the organisation where research was done)*****

☒ **OPEN ACCESS**

**Validation of Project/Thesis**

I therefore duly affirmed with free consent and willingness declared that this said Project/Thesis shall be placed officially in the Centre for Academic Information Services with the abide interest and rights as follows:

- This Project/Thesis is the sole legal property of Universiti Malaysia Sarawak (UNIMAS).
- The Centre for Academic Information Services has the lawful right to make copies for the purpose of academic and research only and not for other purpose.
- The Centre for Academic Information Services has the lawful right to digitise the content to for the Local Content Database.
- The Centre for Academic Information Services has the lawful right to make copies of the Project/Thesis for academic exchange between Higher Learning Institute.
- No dispute or any claim shall arise from the student himself / herself neither third party on this Project/Thesis once it becomes sole property of UNIMAS.
- This Project/Thesis or any material, data and information related to it shall not be distributed, published or disclosed to any party by the student except with UNIMAS permission.

Student's signature_____ (15TH June 2022)

Supervisor's signature: _____ (15TH June 2022)

Current Address:

_____

_____

Notes: ***** If the Project/Thesis is **CONFIDENTIAL** or **RESTRICTED**, please attach together as annexure a letter from the organisation with the period and reasons of confidentiality and restriction.

[The instrument was duly prepared by The Centre for Academic Information Services]

## ACKNOWLEDGEMENT

First of all, all praise be to Allah, as He has given me strength and guidance throughout the completion of this research. Next, I want to give my earnest gratitude to Dr. Rosmawati Saat, my supervisor, for her valuable efforts, support, help and feedback as I am fulfilling this project. I am very pleased for the golden chance to do this wonderful research. I want to convey my gratefulness particularly to my parents, Mr. Hussin bin Simpol and Mdm. Sarimah binti Rahmat, for being my backbone and for endless support during the completion of this study. Furthermore, I want to thank all my laboratory colleagues for all the assistance and support that I have received. Last but not least, I would like to give my appreciation to all that has helped me throughout the completion this research.

Screening of Medicinal Plants against *A. flavus*

Syamimi Izzati Binti Hussin

Resource Biotechnology Programme
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

## ABSTRACT

*Aspergillus flavus* is one of the most common fungi that produce carcinogenic aflatoxins. The plants' bioactive components were already been used to lower the risk of carcinogenicity and mutagenicity of aflatoxin through inhibition of fungal growth and aflatoxin contamination. Antimicrobial compounds in plants are unstable and their effectiveness is affected by several factors such as the number of microorganism present, the concentration of antimicrobial agent used and the storage temperature. One of the important factors that could enhance the effectiveness of antimicrobial activity in plants is the concentration of antimicrobial agent used. In this study, the plant extracts' antifungal activity against *A. flavus* at various concentrations is evaluated via agar well diffusion method. Based on the results, 10 mg/ml of *P. odorata* has the lowest percentage of inhibition (35.7 %) meanwhile 90 mg/ml of both *Z. mauritiana* and *P. odorata* have the highest percentage of inhibition (85.7 %) against *A. flavus.*

**Key words:** Carcinogenic aflatoxins, mutagenicity, maceration method, agar well diffusion.

## ABSTRAK

Aspergillus flavus *adalah salah satu kulat paling biasa yang menghasilkan aflatoksin yang karsinogenik. Sebatian bioaktif dalam tumbuhan telah digunakan untuk mengurangkan risiko karsinogenik dan mutagenasi oleh aflatoksin dengan menghalang pertumbuhan kulat dan pencemaran aflatoksin. Sebatian antimikrob dalam tumbuhan adalah tidak stabil dan keberkesanannya dipengaruhi oleh beberapa faktor seperti bilangan mikroorganisma yang ada, kepekatan agen antimikrob yang digunakan dan suhu penyimpanan. Salah satu faktor penting yang boleh meningkatkan keberkesanan aktiviti antimikrob dalam tumbuhan ialah kepekatan agen antimikrob yang digunakan. Dalam kajian ini, aktiviti antikulat ekstrak tumbuhan terhadap* A. flavus *pada kepekatan berbeza dinilai melalui kaedah pencairan telaga agar. Hasil daripada kajian ini didapati, 10 mg/ml* P. odorata *mempunyai peratusan perencatan yang paling rendah (35.7 %) manakala 90 mg/ml kedua-dua* Z. mauritiana *dan* P. odorata *mempunyai peratusan perencatan tertinggi (85.7 %) terhadap* A. flavus*.*

***Kata kunci:*** *Aflatoksin karsinogenik, mutagenasi, kaedah maserasi, pencairan telaga agar.*

TABLE OF CONTENT

DECLARATION OF ORIGINAL WORK ..... iii
ACKNOWLEDGEMENT ..... iii
ABSTRACT ..... iv
TABLE OF CONTENT ..... v
LIST OF TABLES ..... vi
LIST OF FIGURES ..... vii
LIST OF ABBREVIATION ..... viii
CHAPTER 1: INTRODUCTION ..... 1
CHAPTER 2: LITERATURE REVIEW ..... 3
2.1 *Aspergillus flavus* ..... 3
2.2 Medicinal Plants ..... 5
2.2.1 *Ziziphus mauritiana* ..... 5
2.2.2 *Persicaria odorata* ..... 6
2.3 Antifungal Compounds ..... 7
2.4 Plant Extraction Method ..... 9
CHAPTER 3: METHODS & MATERIALS ..... 10
3.1 Collection and preparation of plant materials ..... 10
3.2 Collection of fungal strains (*A. flavus*) ..... 10
3.3 Plant extraction ..... 10
3.4 Antifungal activity assay ..... 11
CHAPTER 4: RESULTS ..... 12
4.1 The results of agar well diffusion method for negative control ..... 12
4.2 The results of agar well diffusion method for positive control ..... 13
4.3 The results of agar well diffusion method for *Z. mauritiana* extract ..... 13
4.4 The results of agar well diffusion method for *P. odorata* extract ..... 14
CHAPTER 5: DISCUSSION ..... 17
CHAPTER 6: CONCLUSION ..... 19
CHAPTER 7: REFERENCES ..... 20
CHAPTER 8: APPENDICES ..... 23
APPENDIX A ..... 23

LIST OF TABLES


| Table | | Page |
|---|---|---|
| 1 | The average inhibition zone of negative and positive controls against *A. flavus* | 14 |
| 2 | The differences of percentage of inhibition between *Z. mauritiana* and *P. odorata* extracts against *A, flavus* at three different concentrations via agar well diffusion method | 15 |

## LIST OF FIGURES


| Figure | | Page |
|---|---|---|
| 1 | *Ziziphus mauritiana.* | 5 |
| 2 | *Ocimum basilicum.* | 6 |
| 3 | *Persicaria odorata.* | 7 |
| 4 | No zone of inhibition of *A. flavus* on three replicates of agar plates that serve as negative control. | |
| 5 | Zone of inhibition of *A. flavus* on three replicates of agar plates with 70 % ethanol used as positive control. | 12 |
| 6 | Zone of inhibition of *A. flavus* on three replicates of agar plates with different concentration of *Z. mauritiana* (10, 50, 90 mg/ml). | 13 |
| 7 | Zone of inhibition of *A. flavus* on three replicates of agar plates with different concentration of *P. odorata* (10, 50, 90 mg/ml). | 13 |
| 8 | The inhibition zone of Z. *mauritiana* and *P orodata* extracts against *A. flavus.* | |

## LIST OF ABBREVIATION

| | |
|---|---|
| ℃ | Degree Celsius |
| mm | Millimeter |
| µm | Micrometer |
| µL | Microliter |
| m | Meter |
| cm | Centimeter |
| GC | Gas Chromatography |
| PDA | Potato Dextrose Agar |
| mg/ml | Milligram per millimeter |
| g | Gram |
| rpm | Revolution per minute |
| DMSO | Dimethyl-sulfoxide |

# CHAPTER 1

# INTRODUCTION

Numerous types of fungi can be found in abundance all around us particularly in air and soil, contaminated stored-food products and agricultural commodities. Omidpanah *et al*. (2015) reported that one of the most common fungi that produce carcinogenic aflatoxins is *Aspergillus flavus*. Besides, production of aflatoxin B1 by *A. flavus* is highly poisonous, carcinogenic and mutagenic. According to Saleem *et al*. (2017), the adverse effects of contaminated food and feed by aflatoxin to human and livestock health such as anemia, immunity suppression, jaundice, gastrointestinal dysfunction, liver damage and reduced feed utilization had gained the global significant. On the other hand, aflatoxin contamination or aflatoxin-producing fungi caused negative impact on the profitability and marketing of many agricultural products.

Medicinal plants have been used as the foundation in medicinal therapy throughout most of human history and today such medicinal plants are still broadly utilized by many people throughout the world to treat various types of health problems in many different situations. Plants provide significant opportunities for the discovery of novel therapeutic medicines with some of them being produced as nutraceuticals (prevent mycotoxicosis) and bio-fungicides (prevent contamination) (Dikhoba *et al*., 2019). Makhuvele *et al*. (2020) stated that plants produced secondary metabolites that are used as a defence mechanism to protect from insects, pathogenic microorganism as well as harsh climatic circumstances. Plus, they can also prevent animals and human diseases caused by microbes or toxins affiliated with them because of their antibacterial characteristics.

Several approaches including the application of atoxigenic fungi to suppress fungal strains which are toxigenic, have been used to regulate contamination of aflatoxin for both

pre-harvest and post-harvest. Synthetic fungicides are the most preferred approach to control contamination by aflatoxin for pre- and post-harvest. However, due to extensive periods of degradation, development of toxigenic fungal strains that are resistance mostly to synthetic fungicides and negative environmental consequences had led to finding an alternative which are less expensive and eco-friendly (Dikhoba *et al*., 2019). The use of bio-organic fungicides could be a good alternative to reduce the toxicity and genotoxicity of aflatoxins. Plant bioactive compounds have been utilised in food and feed additives to inhibit the growth of fungal and contamination by aflatoxins therefore lowering the risk of aflatoxins' carcinogenicity and mutagenicity (Makhuvele *et al*., 2020).

Natural compounds like plant extracts either as standardized extracts or as pure compounds, offer boundless opportunities for microbial growth control due to their chemical variability (Azwanida, 2015). However, antimicrobial compounds in plants are unstable and the effectiveness is affected by the number of microorganism present, the concentration of antimicrobial agent used and the storage temperature since compound diffusibility is associated with temperature (Negi, 2012). Therefore, this research was conducted to study the relationship between the plant extract concentration and the effectiveness of antifungal activity of plant extract against *A. flavus.* The significance of this study is to identify more types of medicinal plants that possess antifungal effect against *A. flavus* so that the production of bio-organic fungicides can be increased hence substituting the usage of synthetic fungicides. The objectives of this project are:

1. To examine the effectiveness of the selected medicinal plants against *A. flavus* at different concentrations and,
2. To determine which medicinal plant (*Ziziphus mauritiana* and *Persicaria odorata*) has the best antifungal property against *A. flavus.*

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 *Aspergillus flavus*

*Aspergillus flavus (A. flavus)* is a fungus that can commonly be found in soil and is mostly saprotrophic meaning it feeds on decaying plant tissue (Chang *et al*., 2014). *A. flavus* can be found in subtropical and temperate climates around the world and its populations in the soil can be determine by two important elements which are soil moisture and soil temperature (Bhatnagar *et al*., 2014). The optimal temperature for the growth of *A. flavus* is at 37 ℃ however the fungus can grow at the range of temperature between 12 ℃ to 48 ℃ (Hedayati *et al*., 2007). According to Klich (2007), after 7 days of growth on Czapek yeast extract in the dark with temperature of 2 5℃, *A. flavus* produces fast-growing colonies which are yellow-green in colour and their diameter are typically between 65-70 mm.

*A. flavus* can be classified into two phenotypic types which are L strain and S strain depending to its sclerotial size (Hedayati *et al*., 2007). Chang *et al*. (2014) described that the average sclerotial size of L strain is more than 400 μm while the sclerotial size of S strain is less than 400 μm. Isolates of L and S strains commonly produce B aflatoxins only (Bhatnagar *et al*., 2014) and under the same dark condition, isolates from S strain generate higher aflatoxin level, possess higher number of sclerotia and lesser conidial heads meanwhile isolates from L strain generate lower aflatoxin level, produce small number of sclerotia and greater number of conidial heads (Chang *et al*., 2014).

*A. flavus* plays important ecological role as it serves as a nutrient recycler in the soil and it is aided by animal and plant waste (Hedayati *et al*., 2007). Bhatnagar *et al*. (2014)

stated that *A. flavus* is able to degrade lignin as well as become a possible agent to substitute chemical pesticides or in other words as insect pathogen which may help to reduce pest numbers. *A. flavus* is pathogenic to plants, animals as well as humans. In plants, *A. flavus* can grow and form aflatoxin in almost every crop seed such as peanuts, cotton and corn (Klich, 2007). Although *A. flavus* colonization does not always affect yield, however it does result in economic losses due to aflatoxin contamination of seed (Amaike & Keller, 2011). On the other hand, *A. flavus* is an opportunistic fungus that affects both animals and humans especially those with impaired immune systems (Chang *et al*., 2014). In humans, *A. flavus* induce a broad range diseases, from reaction of hypersensitivity to invasive infections related to angioinvasion (Hedayati *et al*., 2007). *A. flavus* may cause diseases like aspergillosis (allergic, saprophytic colonizing and invasive) and aflatoxicosis (disease caused by aflatoxin poisoning) (Amaike & Keller, 2011).

Production of aflatoxin is influenced by various of factors including fungus species, environment, and substrate and three types of elements which influence aflatoxin production are biological, physical and nutritional factors (Bhatnagar *et al*., 2014). According to Klich (2007), managing aflatoxin contamination can be accomplished by controlling either the production of fungus or aflatoxin. Correct conditions of storage following harvest which is outside of *A. flavus* growth requirement will help to prevent contamination (Bhatnagar *et al*., 2014). Biological control can also help to lower the contamination of aflatoxin in crops (Chang *et al*., 2014). Saleem *et al*. (2017) reported that the phenolic compositions of plant extracts are primarily responsible for their antioxidant and antimicrobial activities. Additionally, the phenolic compounds that suppressed aflatoxin B1 synthesis in plants are acetosyringone, syringaldehyde and sinapic acid, however the phenolic compounds that inhibit the production of *A. flavus* are thymol,

cinnamic acid, salicylic acid, vanillin and vanillyl acetone, by targeting on the oxidative mitochondrial stress as a defense mechanism.

## 2.2 Medicinal Plants

### 2.2.1 *Ziziphus mauritiana*

*Ziziphus mauritiana* is a family of *Rhamnaceae* and it is normally recognized as Indian jujube, Indian plum and Chinese date (Dahiru *et al*., 2006). This type of plant is a fast-growing plant that is practically evergreen however during dry season it is deciduous (Jain *et al*., 2012). Parmar *et al*. (2012) stated that the length of *Z. mauritiana* is up to 15 m tall and its diameter is up to 40 cm. Besides, it has elliptical and alternate leaves which are 2.5-3.2 cm long with three different veins. *Z. mauritiana* is extensively grown in both hemispheres in mild-temperate, relatively dry conditions, and is adaptable to warm climates (Dahiru *et al*., 2006). The flowers are small in size, bisexual, yellow or greenish in colour and produced in cluster of 2-3 at the leaf corners on short stalks (Parmar *et al*., 2012). The fruiting season for *Z. mauritiana* is from February to March and the fruit is red in colour and juicier similar to litchi (Rathore *et al*., 2012).

Figure 1. *Ziziphus mauritiana.* (Adapted from Sellers, 2021)

*Z. mauritiana* has been used for centuries for medical purposes. According to Al Ghasham *et al*. (2017), the fruits of *Z. mauritiana* are very high in fiber and have laxative properties which make them beneficial to human health. Besides that, the roots can be used to treat dyspepsia and decoction made from the roots has been used to treat fevers (Gunjal *et al*., 2021). The leaves of *Z. mauritiana* are used to treat a number of illnesses such as asthma, liver problem and fever (Al Ghasham *et al*., 2017).

### 2.2.2 *Persicaria odorata*

*Persicaria odorata* or typically known as Vietnamese coriander is classified under *Polygonaceae* family (Pawłowska *et al*., 2020). This plant thrives in warm and humid condition of tropical and subtropical regions (Ridzuan *et al*., 2013). According to Ridzuan & Wan Salleh (2019), in an optimum condition, this plant can reach a height of 15 to 30 cm. Besides, the plant has leaves which are dark green in colour and each leaf is attached to the stem. Shavandi *et al*. (2012) stated that *P. odorata* is believed to have a variety of therapeutic and other advantageous properties like restrain thirst and heat diabetes, treat feverish cough, heal stomach and lung injuries and sometimes used as anaphrodisiac. In addition, other advantages are antitumor-promoting activity, antimicrobial property, antioxidative property, anti-inflammatory activity as well as insect antifeedant activity.

Figure 3. *Persicaria odorata.* (Adapted from Devi Khwairakpam, 2019)

The phytochemical constitution of *P. odorata* essential oil has been widely studied and compounds like analogical alcohols, aliphatic aldehydes, monoterpenoids, sulfanyl derivatives and sesquiterpenoids are found by using GC analysis (Pawłowska *et al*., 2020). The leaves of *P. odorata* commonly used to treat fungal or bacterial skin diseases while in Traditional Chinese Medicine, the roots of this plant have been utilized for a range of medicinal applications (Ridzuan & Wan Salleh, 2019).

## 2.3 Antifungal Compounds

Seladi-Schulman (2019) stated that fungi can be found in a wide variety of habitats around the world and majority of fungi do not harm humans. However, certain species are able to cause infection and diseases to humans. According to McKeny *et al*. (2021), fungal infection or mycoses can range from minor to major infections such as 'jock itch' and cryptococcal meningitis respectively. On the other hand, every chemical compound, pharmacologic drugs as well as natural products which are used to heal mycoses are referred as ‘antifungal’.

There are four types of antifungal drugs that are currently used to treat infection of fungus such as azoles, polyenes, echinocandins and allylamines (Campoy & Adrio, 2017). Azoles antifungal agents act by inhibiting fungal enzyme lanosterol 14-α demethylase, a rate-limiting enzyme in ergosterol of fungi biosynthetic pathway (McKeny *et al*., 2021). Azoles disrupt the fungal membranes' stability by causing morphological changes and growth inhibition (Scorzoni *et al*., 2017). Azoles can be classified into two classes which are triazoles (fluconazole and itraconazole) and imidazoles (miconazole, ketoconazole and clotrimazole) (Campoy & Adrio, 2017). Polyenes antifungal agents act by binding to ergosterol hence forming channels within cell membrane of fungus (McKeny *et al*., 2021). Three polyenes that are used in clinical field are amphotericin B, nystatin and natamycin (Campoy & Adrio, 2017).

Echinocandins antifungal agents act by preventing the production of β-1,3 glucan, a polysaccharide cell wall that is important for fungi, which resulted in the cell wall disruption of fungi (Nett & Andes, 2016). The three classes of echinocandins are anidulafungin, caspofungin and micafungin (Seladi-Schulman, 2019). According to Campoy & Adrio (2017), allylamines antifungal agents act by inhibiting squalene epoxidase that responsible for generating precursors to ergosterol. Therefore, inhibition of this enzyme will cause squalene accumulation which can increase permeability that leads to interference of cellular organization. Two groups that belong to allylamines are naftifine and terbinafine (Scorzoni *et al*., 2017). Antifungal drugs are important components to treat mycoses in modern medical treatment because they represent large pharmacological group of drugs (McKeny *et al*., 2021). Antifungal drugs are also used to prevent phytopathogenic fungi from infecting crops (Dikhoba *et al*., 2019).

## 2.4 Plant Extraction Method

Nowadays, medicinal plants receive great attention due to their special characteristic as a valuable source of therapeutic phytochemicals which can contribute to establishment of novel medications (Dekebo, 2019). Two techniques such as pre-extraction and extraction are the initial steps in analysis of medicinal plants and these steps are crucial to process bioactive elements from plant materials (Azwanida, 2015). Plant extraction separates secondary metabolites of plant such as terpenes, alkaloids, saponins, glycosides, flavonoids and steroids from inactive components by using suitable solvent and standard procedure for extraction (Abubakar & Haque, 2020). Zhang *et al*. (2018) described four stages in the extraction of natural products. Firstly, the solvent will pass through the solid matrix. The solute then dissolves inside the solvent. Next, the solute will be dispersed out from the solid matrix. Finally, the solutes that have been extracted are collected.

Based on Abubakar & Haque (2020), several methods that are commonly used in plant extraction are maceration, decoction, infusion, percolation, digestion, soxhlet extraction, microwave-assisted extraction (MAE) and Ultrasound-assisted extraction (UAE). Furthermore, the selection of extraction method is based on the intended use of the extracts therefore five factors which have to be considered before selecting the method for extraction such as stability of plant material to heat, the nature or type of the solvent, the cost of plant material, the time taken for extraction and the final volume needed. Truong *et al*. (2019) stated that numerous solvents have been utilized in plant extraction to extract the bioactive compounds in plants such as water, ethanol, methanol and acetone. However, the type of solvent used is determined by the plant type, the section of plant which will be extracted, the structure of bioactive components and solvent accessibility.

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 Collection and preparation of plant materials

Two types of medicinal plants were used in this study such as *Z. mauritiana* and *P. odorata.* The fresh leaves from the two plants were collected from Kuching, Sarawak. All the leaves were washed under running tap water and were dried in the oven at 60 ℃ before keeping them in a clean bag at 4 ℃ for later use.

## 3.2 Collection of fungal strains (*A. flavus*)

The fungi (*A. flavus*) were attained from collection of Molecular Biology laboratory, Faculty of Resources Science and Technology (FRST), UNIMAS. According to Zulhafeez (2017), the fungi were grown on Potato Dextrose Agar (PDA) plates which contained 50 mg/ml of ampicillin. The cultures were incubated for 5 days at ambient temperature.

## 3.3 Plant extraction

Maceration method was used in this study to extract the plant materials. Firstly, the plant leaves were chopped and ground into fine powder by using electronic blender (Abidin, 2012). Based on method used by Jeff-Agboola & Awe (2016) with a slight modification, 30 g of ground leave powder of each type of the plants was soaked in a conical flask containing 300 mL of ethanol for 72 hours at room temperature and was shaken periodically at 140 rpm. Then, Whatman No. 1 filter paper was used to filter the mixture solution and the filtrates were evaporated at 50 ℃ by using rotary evaporator

vacuum to attain the crude extracts of plant. The crude extracts for each plant were dissolved in dimethyl-sulfoxide (DMSO) to get 100 mg/ml of stock concentration. The stock solution was diluted in DMSO to get three different concentrations (10, 50, and 90 mg/ml).

**3.4 Antifungal activity assay**

Each plant extract was tested for antifungal activity against *A. flavus* at three different concentrations by using the agar well diffusion method. Based on method described by Balouiri (2016) with some modifications, 6 mm in diameter of a sterile pipette tip was used to punch 3 holes in the PDA agar. Then, 60 µL of 10 mg/ml of plant extract were placed into one of the wells of the PDA agar. This step was repeated for the other two plant extract concentrations (50 and 90 mg/ml). Subsequently, a plug of *A. flavus* mycelia from a 5-day-old culture was introduced at the centre of the Petri dish. The agar plates were then incubated at room temperature for 3 days. The antifungal agent diffuses through the agar medium inhibiting the *A. flavus.* All the treatments were done in triplicates. As a positive control of this study, the plant extract was replaced with 70 % ethanol while for the negative control, the media were not treated with any extracts. According to Jeff-Agboola & Awe (2016), the observation of clear regions (halo zone) on the plates was interpreted as an indication of growth inhibition of *A. flavus.* The diameter of the clear regions was measured and the percent of inhibition was calculated and recorded. The inhibitory percentage was calculated according to calculation by Khalid et al. (2017).

$$\text{Percentage of inhibition} = \frac{\text{Average inhibition zone in cm of treatment}}{\text{Average inhibition zone in cm of positive control}} \times 100$$

# CHAPTER 4

# RESULTS

The antifungal assay results of *Z. mauritiana* and *P. odorata* extracts showed that these medicinal plants had indicated antifungal effect against *A. flavus* at different concentrations. The area of clear zone and the percentage of inhibition of *A. flavus* are calculated and recorded. The results obtained from the antifungal assay on plant extracts showed that the effectiveness of antifungal activity in each extract of plants fluctuates with different concentrations.

## 4.1 The results of agar well diffusion method for negative control

Figure 4 shows results of negative control from this study where there was no inhibition zone on the agar plates from the three replicates.

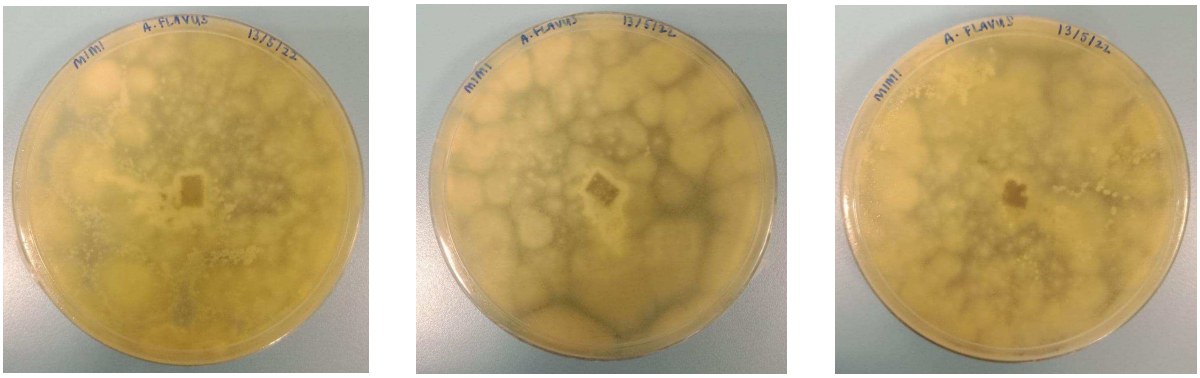


Figure 4: No zone of inhibition of *A. flavus* on three replicates of agar plates that serve as negative control.

* A indicates Replicate 1, B indicates Replicate 2 and C indicates Replicate 3.

### 4.2 The results of agar well diffusion method for positive control

Figure 5 shows results obtained from agar well diffusion method using 70 % ethanol as the positive control. There was inhibition zones of *A. flavus* observed at each replicate.

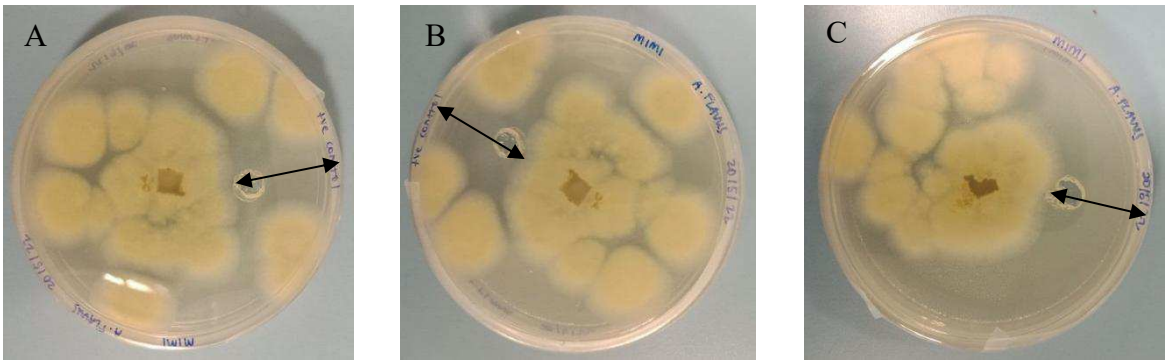


Figure 5: Zone of inhibition of *A. flavus* on three replicates of agar plates with 70 % ethanol used as positive control.

* A indicates Replicate 1, B indicates Replicate 2 and C indicates Replicate 3.

### 4.3 The results of agar well diffusion method for *Z. mauritiana* extract

Figure 6 shows results obtained from agar well diffusion method for *Z. mauritiana* at concentration of 10, 50, and 90 mg/ml. The three replicates exhibited different sizes of inhibition zones of *A. flavus.*

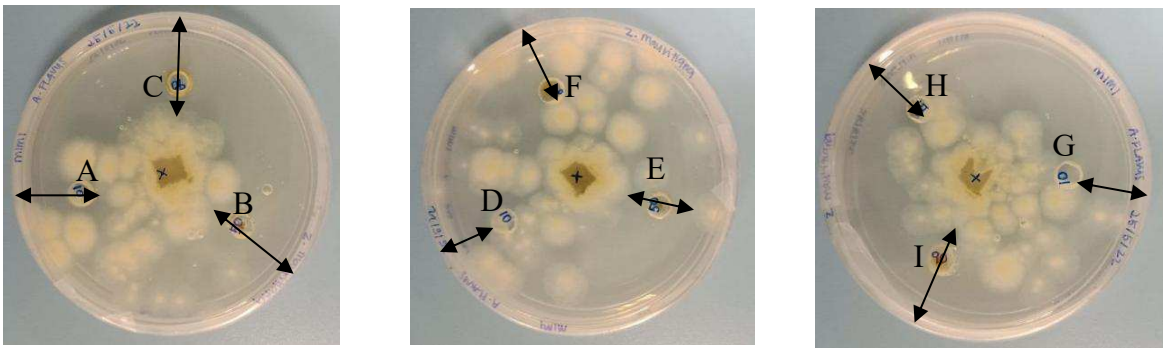


Figure 6: Zone of inhibition of *A. flavus* on three replicates of agar plates with different concentration of *Z. mauritiana* extract (10, 50, 90 mg/ml).

* A indicates 10 mg/ml, B indicates 50 mg/ml, and C indicates 90 mg/ml of Replicate 1.
* D indicates 10 mg/ml, E indicates 50 mg/ml, and F indicates 90 mg/ml of Replicate 2.
* G indicates 10 mg/ml, H indicates 50 mg/ml, and I indicates 90 mg/ml of Replicate 3.

### 4.4 The results of agar well diffusion method for *P. odorata* extract

Figure 7 shows results obtained from agar well diffusion method for *P. odorata* at concentration of 10, 50, and 90 mg/ml. The three replicates exhibited different sizes of inhibition zones of *A. flavus.*

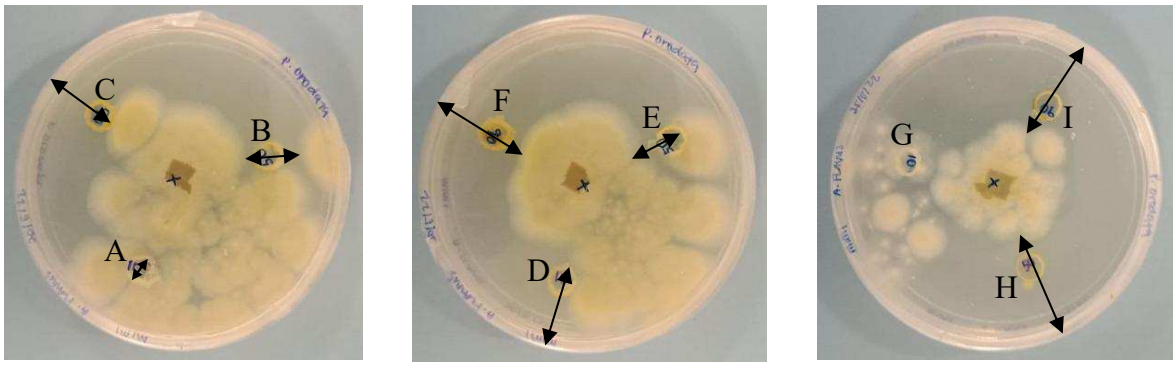


Figure 7: Zone of inhibition of *A. flavus* on three replicates of agar plates with different concentration of *P. odorata* extract (10, 50, 90 mg/ml).

* A indicates 10 mg/ml, B indicates 50 mg/ml, and C indicates 90 mg/ml of Replicate 1.
* D indicates 10 mg/ml, E indicates 50 mg/ml, and F indicates 90 mg/ml of Replicate 2.
* G indicates 10 mg/ml, H indicates 50 mg/ml, and I indicates 90 mg/ml of Replicate 3.

Based on Table 1, the average inhibition zone for positive control from this study is 2.8 cm.

Table 1: The average inhibition zone of negative and positive controls against *A. flavus.*

| Control | Inhibition zone (diameter in cm) | | | Average inhibition zone (cm) |
|---|---|---|---|---|
| | R1 | R2 | R3 | |
| Negative control | - | - | - | - |
| Positive control | 2.9 | 2.7 | 2.8 | 2.8 |