# *Sesbania grandiflora* Leave Extract As The Eco-Friendly Anti-Fungal Agent For Wood

by

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A report submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science (Sustainable Science) with Honours

**FACULTY OF EARTH SCIENCE**
**UNIVERSITI MALAYSIA KELANTAN**

2017

**DECLARATION**

I declare that this thesis entitled "*Sesbania grandiflora* Leave Extract as the Eco-Friendly Anti-Fungal Agent for Wood" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature: _____

Name: _____

Date: _____

## ACKNOWLEDGEMENT

First at all, I would like to grateful to Allah S.W.T for making me able to explore His beautiful creation and also giving me the opportunity and strength to write this Final Year Project (FYP) thesis report. His blessing making me works harder to complete this task.

**"If There Is No Struggle, There Is No Progress"**

**Frederick Douglas**

**"It Does Not Matter How Slowly You Go As Long As You Do Not Stop"**

**Confucius**

Secondly, my sincere thanks to the Dean of Faculty of Earth Science (FSB), Prof Dr Aweng a/l Eh Rak for his support in completing this report.

Thirdly, I would like to express my deepest gratitude to my supervisor, Dr Boon Jia Geng for his excellent guidance from A until Z, constructive criticisms, caring, patience, motivation and encouragement during this study of FYP. He is giving a lot of effort and useful information in the *Sesbania grandiflora.* Besides that, he is also providing me a lot of reading material in order to help for the writing of thesis. He also provided me with the good experience and atmosphere to do the research.

Fourthly, I would like to thanks to the examiners which are Dr Jannatun Naim bt Jemali,Dr Norasyikin bt Mohd Fauzi, Dr Sitti Fatimah bt Mhd Ramlee and En Mohd Sukhairi b Mat Rasat for their constructive and useful comment in order to improve the quality of writing the FYP thesis.

Fifth, I would like to acknowledge my humble appreciation to my family, especially for my parent that give the fully support either directly or indirectly. They are also act as my backbone when I lose my motivation to continue this thesis research. They are very special to me because they are sacrifice a lot to make me success in life,

Lastly, I would like to extend my thanks to my fellow friends Liyana,Hidayah,Syahirah Atikah and Syawal for their helps either directly or indirectly. Without them, the data collection of the thesis is will not success. Thanks a lot

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| ACZA | Ammonium Copper Zinc Arsenate |
| ACA | Ammoniacal Copper Arsenate |
| CCA | Chromated Copper Arsenate |
| MIC | Minimum Inhibitor Concentration |
| PDA | Potato Dextrose Agar |
| PF | Pentachlorophenol |
| Psi | Pounds per square inch |

## LIST OF SYMBOL

| | |
|---|---|
| $^{o}C$ | Celsius |
| µl | Micro liter |
| cm | Centimeter |
| mm | Millimeter |
| min | minutes |
| % | Percentage |

***Sesbania grandiflora* leave extract as the eco-friendly anti-fungal agent for wood**

## ABSTRACT

The wood destroying fungal is one of the major problem in wood bio deteriorate. The chemical preservative has been used in order to preserve the wood. However, the chemical preservatives are lead to the environmental and human health issue. The study was conducted in order to substitute the uses of the chemical preservative. The *Sesbania grandiflora* leaves methanol extractive has high potential to use as the fungicide. The *Sesbania grandiflora* extractive is one of the organic agents that can inhibit the growth of the fungal**.** In this experiment were conducted three tests in order to give overview about the potential *Sesbania grandiflora* as the fungicide, which are the disk diffusion method to determine the MIC, the wood decaying test by the white rot and the physical properties such as thickness swelling and water absorption. In the study to determine MIC, the result indicated that the minimum inhibition concentration to inhibit the growth of *Tratemes versicolor* is 1000µg. While in decaying test, the rubberwood that treated with the *Sesbania grandiflora* extractive are show no difference with the rubberwood that treated with the commercial preservative in term of the percentage of weight loss, which is 6.28±0.31 and 6.33±0.5 respectively. The water absorption and thickness swelling of rubberwood were not affected by the treatment, the changes among the wood specimens showed insignificant different.

**Ekstrak daun *Sesbania grandiflora* sebagai agen anti-kulat mesra alam kepada kayu**

## Abstrak

Kulat yang memusnahkan kayu adalah salah satu masalah utama dalam biokemerosotan pada kayu getah. Pengawet kimia telah digunakan bagi memelihara kayu. Walau bagaimanapun, bahan pengawet kimia banyak membawa kepada isu alam sekitar dan kesihatan manusia. Kajian ini telah dijalankan untuk menggantikan penggunaan bahan pengawet kimia dengan sumber organik. Ekstraktif daripada *Sesbania grandiflora* mempunyai potensi yang tinggi untuk digunakan sebagai anti-kulat. Tiga ujian telah dijalankan dalam kajian ini untuk memberi gambaran keseluruhan tentang *sesbania grandiflora* yang berpotensi sebagai racun kulat, dimana keadah penyerapan pada kertas turas untuk menentukan kepekatan perencatan minimun . Disamping kaedah peruputan kayu oleh kulat putih dan ciri-ciri fizikal seperti peningkatan ketebalan dan penyerapan air juga dijalankan. Dalam kajian keadah penyerapan pada kertas turas untuk menentukan kepekatan perencatan minimun , kepekatan minimum yang diperlukan untuk menghalang pertumbuhan *Tratemes versicolor* adalah 1000µl. Dalam kaedah pereputan spesimen kayu, kayu getah yang dirawat dengan ekstraktif *Sesbania grandiflora* yang menunjukkan tiada perbezaan yang ketara dengan kayu getah yang dirawat dengan bahan pengawet komersial dari segi peratusan kehilangan berat kayu, dimana masing-masing ialah 6.28 ± 0.31 dan 6.33 ± 0.5. Penyerapan air dan peningkatan ketebalan kayu getah tidak terjejas oleh rawatan, perubahan antara spesimen kayu menunjukkan tiada perbezaan yang ketara.

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of study

Nowadays our mother earth is heading to a crisis that called as the "ecological credit crunch". This kind of crisis is more disaster than the current financial issue. It is due to the human are over-consuming the natural resources of the planet. The human are using 30% more resources than the world can replace in a year, for example are deforestation, degraded soil, polluted air, polluted water, and the declining of number fishes and other species. The problem is getting more serious when the number of the global population getting growth and the consumptions rate also keep growing faster than the research and technology could not find new ways to expanding what can be reproduced from the natural world. (Grauerholz *et al.*, 2015)

In order to conserve the uses of the natural resources, there many ways that used by the human to minimize the over-consumption, such as using chemical fungicide to maintain and preserve the condition of the wood. According to study of Roubhi (2010) the fungicides can be either contact, translaminar or systemic. For the contact fungicides, the mechanism is the fungicides not taken inside the tissue plant and they only protect the plant that spray is deposited. For the translaminar, the fungicides spread the fungicide from the upper to the bottom. Last but not least, systematic fungicides are taken up and spread through the xylem. The most common of fungicide are Sulphur. However, the over consumption of the chemical fungicide can lead the environmental and health issue. The uses of the natural preservative and fungicide will reduce such of the problem (Das *et al*, 2013).

The plant has their special and unique characteristic that the characteristic are need by human need to use in their daily life. For example, *Sesbania grandiflora* is very important agroforestry species. This kind of species has pharmacologically vital parts, such as alkaloid, flavonoid, tannins, gums, mucilage, and anthraquinone glycoside that used by folks as the traditional remedy. There are two varieties of plant that grown in Malaysia, which are white flower and the red flower is more medicinal and very rare to be seen. The flower of the plant contains high of protein and has immunomodulatory action (Reeta *et al.,* 2013).

*Sesbania grandiflora* is a native plant from Asia and widespread to other neighbor country. For example Malaysia, Indonesia, Philippines, and India. The Malay names of this plant are turi and geti. All parts of *Sesbania grandiflora* have been used from long time ago for medical and traditional remedy uses (Hasan *et al.,* 2012).

The *Sesbania grandiflora's* juice from the leaves and flower are popular for nasal catarrh remedy and headache. Besides that, it also uses to epilepsy for older people. In Malaysia, the people are used the flower and young leave of *Sesbania grandiflora* as their vegetable to supplement meals (Kashyap *et al.,* 2012).

The leave extraction of *Sesbania grandiflora* commonly extracted in a soxhlet apparatus using solvent like petroleum ether, methanol, ethanol, chloroform or acetone. The results obtained from the different solvent vary between 13% to 25%. The maximum yield was obtained with the methanol extracts of *Sesbania grandiflora* and while the minimum was with the acetone (Das *et al.,* 2013).

From the extraction leave of *Sesbania grandiflora* content of mineral that was analyzed using AAS which revealed the presence of calcium, phosphorus, potassium,

copper and ferrous ions. Besides that, the dried leaves showed that they are rich in the protein, carbohydrate and lipid (Kashyap *et al.,* 2012).

Over along the decade ago, the fungal on the wood is the main problem to the human. Which is folk use the natural preservative to maintain the condition of the wood, however the preservative is not able to maintain a long of period time.

The application of the *Sesbania grandiflora* extract as the eco-friendly preservative directly will reduce the impact to the environment and depended on the artificial chemical preservative to preserve the wood.

## 1.2 Objective

i. To investigate the potential of *Sesbania grandiflora* leave methanol extracts against fungal.
ii. To evaluate methanol leave extracts of *Sesbania grandiflora* incorporating with the physical and mechanical properties of wood panel.

## 1.3 Problem Statement

The climate change cause the fungal in the wood as the major problem in our society, the dry and moisture weather will encourage the rapid growth of the fungal on the wood make it the wood easy to decay and not suitable use any longer (Striegel, & Mary, 2011). This situation may lead the uses of the artificial chemical preservative the preserve the wood to maintain the condition of particular wood. Without realizing, the chemical toxicity of the artificial preservation may lead to the harmful to environment and human health issue. For example, if the artificial preservation is run off to the environment it may lead to the pollution due to their toxicity characteristic. While it may

be dangerous to human due to potent chemical characteristic to human. However, today the uses of the natural preservation is still lacking because of cost and research and development. This study about the *Sesbania grandiflora* as the anti-fungal will help research and development about the eco-friendly preservative product.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 ***Sesbania grandiflora***

The *Sesbania grandiflora* is also called as agati, syn. *Aeschynomene grandiflora* that belong to the family *Fabaceae*. It is one of the vital vegetables famous and vital as the traditional medical plants by Indian. *Sesbania grandiflora* has been reported that have antibacterial, antifungal, antidiabetic, antioxidant and anti-tumorigenic activities (Das *et al.,* 2013.The bark of *Sesbania grandiflora* highly contained of tannins and gum. Saponin and Sesbanimide isolated from seeds it has reported as an important dietary nutrition sources and often planted for its edible flowers and pods in Southeast Asian countries. The *Sesbania grandiflora* is believed originated either in India or Southeast Asia and grows primarily in humid and hot tropical areas of the world. The other names of *Sesbania grandiflora* that commony people called are include agathi, agati sesbania, August flower, Australian corkwood tree, flamingo bill, sesban, swamp pea, tiger tongue, West Indian pea and white dragon tree (Sangeetha *et al.,* 2013).

*Sesbania grandiflora* is plants that can easy adapted to hot, humid environments and inhibit the growth in the subtopics particularly area with the minimum cool season of the temperature is below $10^0$C. Besides that, *Sesbania grandiflora* has ability to tolerate waterlogging and ideally suited to seasonally waterlogged or flooded environments. When flooded, they initiate floating adventitious roots and protect their stems, roots and nodules with spongy, aerenchyma tissue and another outstanding feature is its tolerance of both saline and alkaline soil conditions (Suttie, 2014).

## 2.2 Taxonomy of *Sesbania grandiflora.*

In the botanical classification the *Sesbania grandiflora* is belong to the kingdom of plantae and subkingdom of trachebionta. Below are the summary about the botanical classifation of *Sesbania grandiflora* (Table 2.1).

**Table 2.1:** Summary of Taxonomy of *Sesbania grandiflora*

| | |
|---|---|
| ***Kingdom*** | *Plantae* |
| ***Subkingdom*** | *Tracheobionta* |
| ***Superdivision*** | *Spermatophyta* |
| ***Division*** | *Magnoliophyta* |
| ***Class*** | *Magnoliapsida* |
| ***Family*** | *Leguminisae* |
| ***Genu*** | *Sesbania* |
| ***Species*** | *Sesbania Grandiflora* |

**Figure 2.1**: The *Sesbania grandiflora plants*

## 2.3 The Compositions *in Sesbania grandiflora* Leave.

The Sesbania *grandiflora* is a plant that has secondary plant metabolites or according to phytochemicals study previously the plants have been extensively investigated with unknown pharmacological activities as a source of medicinal agents. (Kumar *et al.*, 2015).

Plant that has secondary metabolites can be divided into three different chemical groups such as terpenes, phenolic and nitrogen containing compounds. Another plant defense response against infection is the synthesis of hydrolytic enzymes that attack the cell walls of pathogens. Miscellaneous glucanases, chitinases and other hydrolases affected by fungal attack. Perhaps the best studied plant responses to bacterial and fungal attack is Phytoalexins synthesis. This element can obtained from any plant parts such as bark, leaves , flowers, seeds and other knowledge of the chemical constituents of plants is desirable because it will be a great value for the synthesis of chemical substances that are complex property were carried out in leave extract Sesbania grandiflora (Kumar *et al.*, 2015).

The chemical constituents that can be found are galactommannans, linoleic acid, beta-sitosterol and carbohydrates. *Sesbania grandiflora* has major contributors of phenolic substances in the plants which is simple phenolics acids. Besides that, the other bioactive compounds reported contained in this plant, which are saponins. In generally, the people are used the stem of *Sesbania grandiflora* as astringent and used for treatment of small pox, ulcers in mouth and alimentary cannal, infantile disorders of stomach, scabies etc. (Al-Dawah *et al.*, 2015).

## 2.4 The Application of *Sesbania Grandiflora*

The *Sesbania gradiflora* is multipurpose plants that all the part of the tree, such as bark, root, leaf, seed, and stem have different used. First is as the green manure. The *Sesbania grandiflora* is growing nitrogen fixing leguminous plant species that capable degraded when it incorporated into soil serving as the green manure (Nigussie & Alemayehu, 2013). Besides that, they serve as forage source. The *Sesbania grandiflora* leave and tender branches have high of protein (20-25% of protein) and easily digested if eaten by animals (Nigussie & Alemayehu., 2013). In addition they as have vital role in the medical uses. *Sesbania grandiflora* is a remedy for bruises, catarrh, dysentery, fever, smallpox, sore, stomatitis, headache, inflammation, gout and rheumatism (Mukul *et al.*, 2012). Besides that, they have Antioxidant effect, example, the flower and petal of *Sesbania grandiflora* have value as the antioxidant sources that protect from the free radical by scavenging them and retard the effect of progress of many chronic diseases. (Nigussie & Alemayehu, 2013).

### 2.4.1 The other uses of *Sesbania grandiflora*.

The all part of *Sesbania grandiflora* were studied as the antimicrobial activity. The researcher were use the *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* by method of disc diffusion to evaluated the microbial growth inhibitory (Minimum inhibition concentration) property of the extracts. However, the stem was prove that the extraction of bark have better antimicrobial activity than the extraction of leave (Kashyap *et al.,* 2012).

## 2.5 Anti-Fungal

The Anti-fungal are compound that can kill or inbibit the fungal and fungal spore. The fungal can cause the serious problem either for plant or animals. (Kumar *et al.,* 2015) In the market, there are many artificial chemical fungicides to reduce and inhibit the fungal population. However, the fungal are undergo the mutation and may immune to the certain fungicide. The chemical fungicide such as anilopyrimidine, benzimidazoles, demethylation and dicarboximide are example that their effectiveness is losses to against the fungal (Kumar *et al.,* 2015).

In the previous research, the anti-fungal activity was also determined by Agar cup method. The PDA (Potato Dextrose Agar) plate was prepared and overnight growth isolated of fungi such as *Candida albicans* and *Aspergillus niger* were swabbed on the PDA (Kumar *et al.,* 2015).

Well were bored using cutter and extract of difference dosage concentrations were added and the zones of inhibition were measured after overnight incubation and the zone of inhibitor compared with that of standard antibiotics.While Clotrimazole was used as the positive control (Kumar *et al,* 2015).

## 2.6 Wood

Woods are broadly used as the natural raw material in the construction, the furniture making industry and domestics uses. It's having distinguished physical properties and its warm properties compared to competitive material such as concrete, metal, and plastics. Natural wood is a more complex material compared with synthetic plastics or metals. Below is the example of wood (Figure 2.2). Depending on the wood

species, their dry mass is mainly composed by the three natural polymeric structures. Which are cellulose, lignin and hemicelluloses such as glucomannan, glucuronoxylan and other polysaccharides. The other elements consisted in extractable products like terpenes, waxes, tannins, and mineral salts. The cellulose and hemicelluloses are mainly build up the wood cells, where the lignin acts mainly as binder between the wood cells. (Pascal & Rogez, 2003). Wood is divided in two categories, which are hardwood and softwood. Ash, beech, oak, poplars are the example of hardwood. While fir, pine, spruce are example of softwood. Both of hardwood and softwood are mainly consist of various types of cells, such as vessel elements, tracheids, libriform fibres, axial parenchyma cells, ray parenchyma cells, epithelial cells. In wood cell, their wall and lumen have typical shapes and dimensions. The cell walls are usually consisted of primary and secondary wall layers which made up from basic natural polymers.(Reinprecht & Ladislav, 2010).

Woods is a natural material with means that they can be degraded by the biological agents, such as bacteria, fungi and insects. Wood-destroying fungal examples are brown-rot and white rot. The example of brown rot are *Serpula lacrymans, Coniophora puteana, Antrodia vaillantii, Gloeophyllum trabeum, Lentinus lepideus,* and while white-rot such as *Trametes versicolor, Trametes hirsuta, Schizophyllum commune, basidiomycetes,* or soft-rot *(Chaetomium globosum, Monodictys putredinis).* The polysaccharides (cellulose and hemicelluloses) that present in the cell walls are destroyed by the Ascomycetes (Reinprecht & Ladislav, 2010). The fungi degraded the depolymerization of the polysaccharides by producing vary types of hydrolytic enzymes (Reinprecht & Ladislav, 2010).

**Figure 2.2**: The example of the wood

## 2.7 Rubberwood

Rubberwoods are mainly for manufacturing of indoor furniture.. Rubber is cultivating as a plantation species mainly to obtain latex and also as a byproduct rubberwood is generated after certain period of time. Below is the figure of rubberwood (Figure 2.3). In early years there are no demanding for rubber wood for construction and manufacturing, the people only used rubber wood as the fuel for burning. The demanding of the rubber wood is increasing when the natural resource decreasing. Rubber is less durable compared with another timber product and the rubber wood also more susceptible for insect and microorganism attack. For the rubber wood, the main problem is insect and fungal infestations due to its high starch content and also, the moisture content of timer provides conductive condition for the entry and establishment of fungus (Udya, 2011).

**Figure 2.3**: The rubberwood

## 2.8 White Rot Fungal

The typical white rot are can be related with the hardwood decay and their pattern of decaying are differ. The white rot usually has bleached appearance. The white rot are grouped in the *Eumycota* (true filamentaous fungi), and more detail in the sub division of *Basidiomycetes Ascomycetes* fungal (Terry, 1974)

*Phanerochaete carnosa* is a one of the example of white rot fungi. Below is the example of white fungi that affected the Rubberwood (Figure 2.4). The fungi secreted the enzymes that secreted that capable degrading cellulose, hemicellulose and lignin (Mahajan & Sonam, 2011).

**Figure 2.4**: The white fungal

### 2.8.1 Nutritional Factors and Environmental for growth and survival of fungal.

The first factor that should be considered is moisture. The high relative humidities which 75% and above will lead a spore from mold to germinate. The moisture content of the plants is important condition that its favor to growth and survival. The hyphae are analogous to liquid-filled soda straw which means they are required a lot amount of water to transfer the nutrients from the plants subtrate to the mold and to remain turgid. With these liquified nutrients, the hyphae glucan that containing enzymes that will break down the substrate. While this process occurs, the mold mycelial mat grows and, in a few days, will be visible to the naked eye. The fungi are required moisture to grow and producing enzymes in order to obtain nutrients from substrate on the growing with. Commonly, the organic materials, for example paper, wood, and textile will take the moisture from its surrounding. Second factor that should be considered is the temperature. The temperature is also important for fungal growth. The highest temperature which is no growth occurred, and the optimum temperature is

the most favorable the growth of the fungal. Commonly, the favorable temperatures are range from 59 to 95$^{o}$F, but the optimum temperature is 86$^{0}$F. The temperature below than that are no growth occurred, it's because the temperature at which the potential for growth is destroyed. Fungi and fungal spores can survive long periods at sub-zero temperatures. Fungi are less tolerant of alternating below-freezing and above-freezing temperatures. The temperature above than 100$^{o}$F which no growth occurred, it's not relevant to growth since the temperature is very high. Besides that, temperature will make the fungal hydrated (Bertalan *et al.,* 1994).

Third factor that should be considered is the nutrient. The nutrient is one of most important needed by fungal. In natural the compound that can be utilized by fungi as their sources of the carbon and energy. For example of element are carbon, hydrogen, oxygen, nitrogen, Sulphur, potassium and magnesium. And the trace element such as Trace elements such as iron, zinc, copper, manganese, and in some cases, calcium may also be required. Certain vitamins may also be needed (Bertalan *et al.,* 1994).

The fourth is pH. The fungi are favorable in slightly acid medium to growth. The pH of 6 being near is optimum for the most species of fungal. The some prove that the pH in either high or low is inhibiting the growth of the fungal, but it depends on the other variables. Besides that, the pH of the substrate also will lead the fungal metabolic product (Bertalan *et al.,* 1994).

Last but not least, the light. The light is not well define influenced the growth of the fungal. Its due to fungal consist no chlorophyll. In this case, the light played as the minimal role for the growth of fungal. Some species of fungal are diurmal that means,

the light is actually will inhibit the growth during the day and growing fast during the night (Bertalan *et al.,* 1994).

### 2.8.2 Decomposition

Fungi are saprophytic species, which means that they are live on and take up the energy of the dead or decayed material. The mycelium that growth on the surface or within the substrate that obtained nutrients by osmosis through the hyphal walls, it will cause the decaying of the organic matter that they utilize. The fungi secrete enzymes to break down proteins into simplest form of amino acids, carbohydrates to sugars and fats to fatty acids and glycerins. The organic substrate is broken down by particular enzymes into the needed nutrient for growth (Bertalan *et al.,* 1994).

### 2.8.3 Staining

The staining effect produced by the mold growth or the dead colonies, it cause by the metabolic processes. For example, the acid that produced during the hydrolysis of the cellulose or other nutrient matter by the hydrolytic enzymes. Besides that, chemicals element produced while the digestive process and excreted by-product or simply by pigments present in the fungal structure itself (Bertalan *et al.,* 1994)

## 2.9 Chemical Preservation for wood protection.

There are three types of the chemical preservative of chemical for the protection of the wood. For example, tar oil based, oil-borne and water-borne preservation. For the tar oil preservative are commonly used Creosote or always called as coal tar creosote. This type of material is condensed from the distillation of coal and it's converted to

carbon. The creosote will penetrated deep into wood and remain inside that for long time. For example the wood that treated with the creosote is built for farm, fence, railroad and other outdoor building material. For the oil borne preservative, the pentachlorophenol (PF) is widely used to treat the wood. The PF-treated wood can be used for the commercial interior application except for the laminated beams or construction component. Lastly, for the water-borne preservative are commonly used arsenic and copper based preservative such as ammonium copper zinc arsenate (ACZA), ammoniacal copper arsenate (ACA), chromated copper arsenate (CCA) and copper naphthenate (Hiziroglu & Salim, 2014).

**2.10 Extraction**

The extraction process represent as the first step in order to get crude extract from the natural element. The main goals of the extraction process are important in production high yield. Besides that, they produce the high purify of product that means the product of extraction has low amount of the interfering from another elements. Third is they are produce high of sensitivity, it resulting the extraction allowed for different quantification that produce high slope in the calibration curve. Last but not least, low limit of detection. The element in the extraction can easily detected at the low level, it's because the low noise are obtained in the analytical system (Palma *et al.*, 2013).

### 2.10.1 Soxhlet extraction

Soxhlet extractor was inverted by Franz von Soxhlet in 1879, Soxhlet extractor is one of the laboratory apparatus that capable to extract the plants and others. Below is figure of Soxhlet extraction (Figure 2.5). The originality of design is for lipid extraction from the solid stage. However, today the function of the extractor is not only limited to lipid extraction, it also can be extract anything that in solid state of material to extract into liquid. In generally, the solid material that containing some of the desired compound is placed inside a thick filter paper or also known as thimble, and then placed into main chamber of the soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent and then attached with a condenser. The solvent is heated to reflux. While the process of extraction, the solvent vapor are travels up a distillation arm and floods into chamber housing the thimble of solid. The main function of the condenser is to ensure that any solvent vapor cools, and drips back down into the chamber housing the solid material. The solid material in the chamber is slowly filled by the warm solvent. Some of the desired compound will then dissolves in the warm solvent.. The chamber is automatically empty itself when it's almost full through the siphon arm and return back to distillation flask. This cycle repeated for many times, it may take the hours or days of period to over. Lastly, extraction process is over after the solvent is removed from the extraction process.(Harwood *et al.,* 2012).

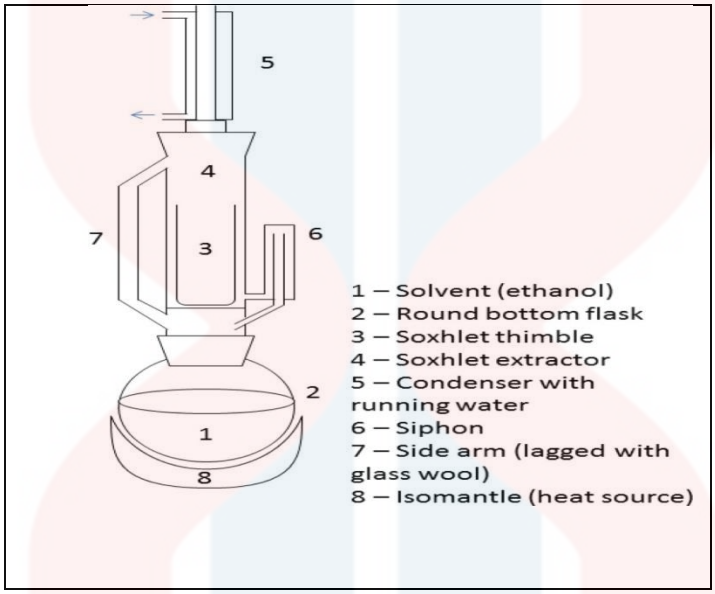



**Figure 2.5**: The illustration of soxhlet apparatus

The basic procedure in soxlet is called as solid sample that will be filled in thick paper or known as thimble and it will condense solvent to extract continuously. The basic component in the soxhlet apparatus are condenser, porous container or thimble and distilling pot. The condenser is functioning to cool the solvent vapor and cause it to condense. The thimble or other porous container is functioning to hold the solid sample and allow for condensed solvent to saturate and pass through the thereby extracting active material. This can be likened to a filter in the retains the insoluble components and allows whatever is dissolved to pass. Last but not least, the pot of distilling is to hold the solvent pool and serve as the reservoir to hold the concentrated material.

## 2.11 Solvent

The word of "solvent'' are commonly referring to the organic compound that has been use to dissolve solid material. In generally, the solvents can be made up from natural sources such as turpentine and citrus solvents, however, it mostly they are produced from petroleum or other synthetic sources. Solvent are broadly used due to their characteristic that can dissolve material like resins and plastics. Besides that, they are also quickly evaporate and cleanly. Commonly, all solvent are fall into various classes of chemicals. A class is a group of chemical with the similar molecular structure and chemical properties. However, the most important classes of group are aliphatic, aromatic, chlorinated hydrocarbon, alcohols, esters, and ketones. In generally, the solvent are used in the most paints, varnished, inks, and their thinner, in the aerosol, leather, textile dyes and extraction solvent (Rossol, 2006)

### 2.11.1 Methanol

For long generation ago, the methanol known as wood alcohol, they were initially produced from the distillation of wood. The proligneous liquor, from the heating of wood in the vacuum state, it contains 4 percent of methanol and 7 percent of acetic acid (James *et al.,* 1996). Methanol with chemical formula $CH_3OH$ is simplest alcohol. It a volatile, colorless, flammable and polar liquid in the room temperature, Below is the

flat molecular structure of methanol (Figure 2.6). The methanol alcohol boils at 64.96° C (148.93° F) and solidifies at -93.9° C (-137° F) (Institute, 2016).

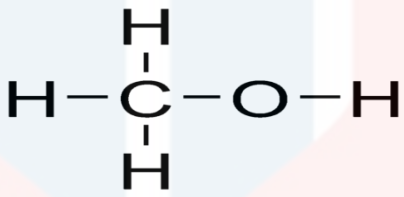


**Figure 2.6**: Chemical compound of methanol

## 2.12 The methanol extracts of *Sesbania grandiflora.*

The *Sesbania grandiflora* methanol extracts of the have many bioactive chemical compounds. Such as alkaloids, glycosides, steroid, terpenoid and tannin were identifying in the *Sesbania grandiflora* extract. Besides that, the studies also found that another compound in the extraction *Sesbania grandiflora* by methanol, which are 3,4,5-Trimethoxyphenol (2.5 %), Erucic acid (2.8 %), 2-Furancarboxaldehyde (2.8 %), Vitamin E acetate (3.13%), 4-methyloxazole (5 %), Palmticacid (11.8 %), 9-hexadecenol (9.0 %), Dioctyl ester (10.1 %) that are functioning in the medical field and antimicrobial. (Hussain & Kumaresan.,2014).

## 2.13 Eco friendly preservative

Protection of wood from the biotic decay is needed nowadays because the natural resources become more decreasing against time. The wood preservatives have to ensure the resistance of wood to biotic agents for long period time in their services. So that it very important that active ingredient to fixed permanently to the wood and cell wall (Palanti *et al.*, 2013).

The water repellent is one of the classical wood preservative that being used long time ago, besides that, the uses of eco-friendly preservative will reduce the effect on the environmental issues. The environmental friendlier water repellent such as extractives from trees and natural resins. The water repellency and dimensional stability of wood also has been studied by Borgin in 1961. Besides that, natural oil is also capable for preventing water to uptake the wood by their chemical and physical composition. The unsaturated oil such as drying oil can oxidize when it exposed to the open air, which is more protective layer on the wood surface. For example of natural oil is tall oil, the tall oil is by-product of pulp and paper industry. The colour of crude tall oil is dark. It is composed by triglycerides with mixture of fatty acids, rosin acids and unsaponifiable elements such as sterols, waxes and hydrocarbons. The treatment with the eco-friendly tall oil will reduce the capillary water uptake of pine sapwood (Hyvönen *et al.*, 2005)

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 Plant Collection of *Sesbania grandiflora*

The leaves of the *Sesbania grandiflora* were collected the Kelantan state area (Figure 3.1). Besides that, the leaves was pluck at the end of the branch with carefully. And After that, the leave was dried and grounded into powder. The dry powder was extracted by using Soxhlet extractor with the methanol as main solvents.

**Figure 3.1**: *Sesbania grandiflora* plant collection.

## 3.2 Diffusion disk to determine the MIC

The 3.9% of Potato Dextrose Agar (PDA) medium of was prepared and autoclaved at $120^0$C and at 15 psi ($0.10N/mm^2$) for 20min, and then about 15 ml of solution was dispensing into 9 cm petri dish to solidify. The white rot that keep incubator was inoculate at the center of the PDA medium, and the paper dish treating with the extract will be place on the dish about 3 cm away from the inoculated fungus. The filter paper

approximately 6mm was impregnated with the extractive, which is 1000 µg. The dilution is 0.1g/ml from crude extractive. After that, the sample was incubated in the dark room at 25± $2^0$C. The activity of the antifungal from each extract was evaluated at 24 h intervals for 7 days. The clear zone from the fungal with positive antifungal on $7^{th}$ day and will be defining as the minimum inhibitory amount (Hashim *et al.*, 2009).

### 3.3 Fungal Decay Test

The 3.9% Potato Dextrose Agar (PDA) medium was prepared and autoclaved at $120^0$C and at 15 psi (0.10N/$mm^2$) for 20min, and then about 15 ml of solution was dispensing into 9 cm petri dish to solidify. The white rot that keep incubator was inoculate at the center of the PDA medium, and the one of block rubberwood treated with the commercial preservative, one rubberwood block treated with *Sesbania grandiflora* leave methanol extract and one rubberwood block are treated with no treatment that place on the dish about 3 cm away from the inoculated fungus. The petri dish with the specimens is leave at the room the temperature to observe. The average percentage mass loss was determined from the conditioned mass before and after exposure to decay fungus. Three specimens was test for each treatment group.

### 3.4 Physical and Mechanical Properties Evaluation

#### 3.4.1 The determination of increasing mass by water absorption.

The wood sample was prepared at 100mm X 100mm X 100mm rubberwood thickness. The flat bottle was prepared in order to immerse the wood sample, so the size of the bottle is not less than 140mm deep and 130mm wide. The temperature was set up at 20 ± 2 °C in the flat bottomed of the container to

test the wood sample, besides that the test will be either for 2 hour or 24 hours. After that, the flat bottomed of the container will be refill with the water. The depth of the water above the wood sample was maintained between 25mm and 30mm. The test of the wood sample was done after the wood sample remove from the flat bottomed bottle after the period either 2 hour or 24 hours. After removed the wood sample from the bottle, remove the access water by using a cloth. After that, immediately weight each of the tests of the wood sample and record the mass.

The water absorption O can be interpreted as the percentage after immersion for 1 hours or 24 hours. It can be calculated from a formula of percentage of absorption.

$$O = \frac{(M2-M1)}{M1} \text{ X } 100\% \text{.....(equation 3.1)}$$

Where

$M_1$ is the mass of rubberwood sample before soak

$M_2$ is the the mass of rubberwood sample after soak

### 3.4.2 Determination of Thickness Swelling

**The determination thickness swelling by water absorption.**

The wood sample was prepared at 100mm X 100mm X 100mm rubberwood thickness. The flat bottle was prepared in order to immerse the wood sample, so the size of the bottle is not less than 140mm deep and 130mm wide. Besides that the test will be either for 2 hour or 24 hours. After that, the flat

bottomed of the container will be refill with the water. The depth of the water above the wood sample was maintained between 25mm and 30mm. The test of the wood sample was done after the wood sample remove from the flat bottomed bottle after the period either 2 hour or 24 hours. After removed the wood sample from the bottle, remove the access water by using a cloth. After that, immediately measured each of the tests of the rubberwood sample and record the thickness with the Vanier caliper.

The thickness swelling O can be expressed as the percentage after immersion for 2 hours or 24 hours. It can be calculated from a formula of percentage of thickness swelling.

$$O = \frac{(T2-T1)}{T1} \text{ X } 100\% \text{.....(equation 3.2)}$$

Where

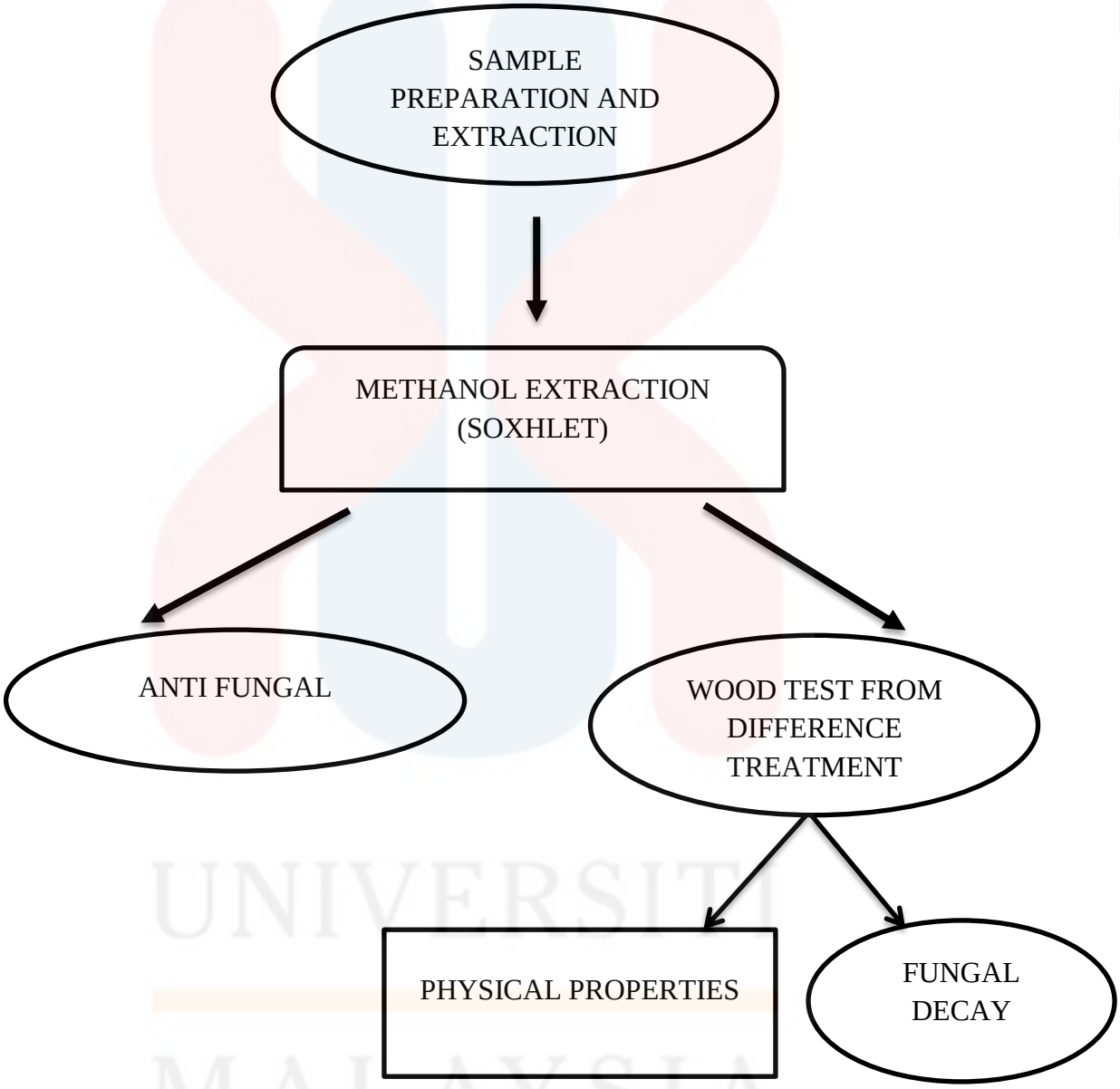
$T_1$ is the thickness of rubberwood sample before soak

$M_2$ is the thickness of rubberwood sample after soak

### 3.5 Statistical Analysis

Data for each test were analyzed with statistically. The analysis of one-way variance were use ($\alpha<0.05$) to test the significant difference between factors. While the ANOVA indicated a significant difference among factors, the comparison values were evaluated with the post hoc tukey test in order to determine whether the differences have any significant level.

**FLOWCHART OF THE EXPERIMENT**



# CHAPTER 4

# RESULT AND DISCUSSION

## 4.1 The yield of *Sesbania grandiflora* leaves methanol extract

In this experiment, by the hot extraction method using Soxhlet apparatus the grounded leaves were extracted with the methanol as the solvent. According to Ahmad *et al.* (2009), in approximately by using the methanol as the solvent in the extraction process is high of yield extraction by weight. It may be influenced by the polarities of the solvent. (Luque de Castro, 1994 & Romdhane, M., 2002). In this experiment from that method we are able to collect about yield is about 47.41%.

## 4.2 Diffusion disk method to determine MIC


**Figure 4.1**: Diffusion disk method to determine MIC

Based on the Figure 4.1, the result obtained from the experiment are only able to detect the inhibition zone on the filter papers that impregnated with 1000 µg crude extracts (inside the red circle). The inhibitor of the white rot very minimal by the

extractive it could be the concentration of active compound obtain from the extraction lowest than expected

The previous study conducted by Hussain & Kumaresan,(2014) found that there are many bioactive chemical elements in the *Sesbania grandiflora* leaves extractive. Which are alkaloid, glycosides, tannin and phenolic compounds, this kind of active compound are has anti-bacteria and antifungal activities In the study by Mathiventhan *et al.* (2013) the total phenolic contain in the *Sesbania grandiflora* is about 102.7 ± 41.9 mg/100g WW. So that, the concentration might be low produce in order to against the growth of white rot.

According to Zovko *et al,*. (2012) the effect of the crude leaf extraction and Nanosized leaf extraction also played as the important role against the microorganism. The crude leaf extraction has irregular size particle and it not contact directly with the microbes. So that, their efficiency will decreases compare with the Nanosized leaf of *Sesbania grandiflora.* Besides that,the white rots fungal have more resistance to the preservative if compared to the brown rot due to their enzymes secrete by them.(Zovko *et al.*,2012)

**4.3 Wood decay test**

The purpose of this study was to determine the potential *Sesbania grandiflora* leave extraction as the anti-fungal agent to the rubberwood compared with the chemical preservative. The identification of the potential is by the percentage of weight loss from the decay to rubberwood.

The experiment conducted to compare the effectiveness between *Sesbania grandiflora* extractive as the anti-fungal agent and commercial preservative.

**Table 4.1:** Weight loss of specimen with different treatment after exposed to *Trametes versicolor*

| Treatment | Weight loss of specimen (%) |
|---|---|
| Commercial Preservative | 6.28±0.31a |
| *Sesbania grandiflora* leave extractive | 6.33±0.5a |
| Blank | 11.2±2.88b |

(different letter within same column are significantly different at alpha value 0.05)

The table 4.1 showed that the means of percentage of weight loss of rubberwood specimen against the treatment. The highest percentage of the weight loss is the rubberwood with no treated with any preservative which is 11.2±2.88, followed by the rubberwood that treated with the *Sesbania grandiflora* leave extraction preservative which is 6.33±0.5 and the lowest of the percentage of the weight loss is the rubberwood that treated with the commercial preservative, which is 6.28±0.31.

The highest of weight loss is the rubberwood that not treated with any preservative. It is because the white rot tendency to attack unprotected rubberwood, that rubberwood has no shield with any active element to protect rubberwood. If compared the mean result of the rubberwood that treated with the commercial preservative and treated *Sesbania grandiflora* leave extractive there are only not significant different between their percentage, which are 6.28% and 6.33%. It could due to be, in 1ml of commercial preservative there are 100% of the active compound that can inhibit the growth of the white rot, and while in the 1 g of *Sesbania grandiflora* leave extraction are

contained may be less than 100% of phytochemical active compound. It will one of the reason why there are slightly different between them.

In addition, there are slightly different between commercial preservative and *Sesbania grandiflora* in the percentage of weight loss of rubberwood because in the chemical preservative there are might be have stable chemical compound compared with *Sesbania grandiflora* leave extraction. The stable chemical can inhibit more the white rot compared unstable chemical compound in the leave extraction. The unstable chemical compound will bind with the enzymes that secreted by the white rot fungal, it might be can act as nutrient for the growth of the white rot. (Terry, 1974)

In addition, the particle size of the active compound is play as the important role of the effectiveness of the fungicidal performance. The formulation of the fungicide with the small particle active compound is very toxicity in the laboratory compared into the outdoor, its due to it cannot withstand with the weathering. Besides that, there theory which is the fungicidal effectiveness activity is increase as the particle is decrease. The active compound chlorothalonil has very small sized which is < 1 µm. So that, with the greater surface area of rubberwoods it more quickly to dissolve into the rubberwood. (Backman *et al*.,1976).

The previous studies conducted by Hussain & Kumaresan (2014) state that the *Sesbania grandiflora* leave methanol extractive study by the GC-MS analysis are mainly composed from the oxygenated hydrocarbon and predominantly phenolic hydrocarbon. These kinds of elements are very important to various pharmacological actions, for

example antimicrobial. *Sesbania grandiflora* leave methanol extract contained bioactive chemical compound, which are Alkaloid, glycosides, steroid, terpenoid and tannin.

### 4.4 Physical properties of rubberwood for water absorption and thickness swelling.

The physical properties of the rubberwood such as water absorption and thickness swelling are used as the indicator of the effeteness to the physical properties from the treatment of the preservative to the wood.

**Table 4.2:** Water absorption of wood specimens with different treatment

| **Treatment** | **Water absorption (%)** | |
|---|---|---|
| | **2Hours** | **24 Hours** |
| Commercial Preservative | 74.42±0.28a | 103.33±0.26a |
| Extractive Preservative | 79.72±0.19a | 101.43±0.16a |
| Blanks | 73.28±0.16a | 106.29±0.15a |

(different letter within same column are significantly different at alpha value 0.05)

Table 4.2 showed that the result of the water absorption (WA) of the rubberwood sample against immersion time in the water bath. The table above proves that there are two pattern of water absorption by the rubberwood. During the first two hours of immersion, the rubberwood samples are absorb the water more than half of the final absorbed water occurred. After that, they are occurred a little bit slow and consistent water absorption. In two hour of the soaking time the higher intake of water is by the rubberwood that treated with the extractive preservative which is 79.72±0.19 followed by commercial preservative which is 74.42±0.28 and lastly is rubberwood that no treatment, 73.28±0.16. For the 24 hours of submission the highest water intake by the

rubberwood that no treated with the preservative, followed by the commercial preservative and lastly the rubberwood that treated with the extractive preservative. The higher water intake rate can be explained by diffusion phenomena, like the water particle move inside the rubberwood sample, where the water spread inside the rubberwood sample trough the cellular wall, capillaries and vessel of the rubberwood. Besides that, there also two form of water up take present, which are interstitial and bound water. For the interstitial water in already available in the cellular cavities and while, the water bound is retained in the cellular walls. The main reason water absorption is depended on the differences of saturation water content and water content at a particular time, it also called as driving force. The moisture diffuse inside the rubberwood occurred because the moisture gradient factor between the surface and center are difference. if there movement of water inside, so that the water content are increasing in the rubberwood, So that, it will decrease the driving force. However, the interstitial water in the rubberwood is relatively weaker than, bound water, so that the water will move from move concentrated medium into the less concentrated medium which is water bath.

Besides that, the size and shape of the rubberwood play as important role and its very significant factor that will influence the water movement inside the rubberwood sample. The water absorption rate will increase if the smaller particle thickness. It is because the maximum over pressure is high in the center of the rubberwood and also the length of the distance transportation water from the surface is halved compared to the biggest size. The ability of the rubberwood to absorb water is the indicator that they are having porosity

**Table 4.3**: Thickness swelling of wood specimens with different treatment

| Treatment | Thickness Swelling (%) | |
|---|---|---|
| | 2Hours | 24 Hours |
| Commercial Preservative | 3.36±0.01a | 3.96±0.01a |
| Extractive Preservative | 3.87±0.02a | 3.94±0.01a |
| Blanks | 3.90±0.02a | 4.48±0.04a |

(different letter within same column are significantly different at alpha value 0.05)

The Table 4.3 showed the result of Thickness swelling that affected by the weight fraction, the result showed that a decreasing of the weight fraction will be effect the thickness swelling. Based on the result the of the thickness swelling against soaking period, the trend is drastically increase in period of 2 hours and increasing with the consistent after two hours. The thickness swelling is measure the stability of the rubberwood sample, the lowest percentage of the value thickness swelling will indicated the more stability of the rubberwood. The result showed that, the rubberwood treated with the commercial preservative has lowest thickness swelling after two hour which is 3.36±0.01 and while extractive is 3.87±0.02 and highest is the rubberwood that no treated with any preservative which is 3.90±0.02. For the twenty four hours of soaking time in the water bath the result showed that the trend of highest thickness swelling is 4.48±0.04 followed by the commercial preservative and the lowest is rubberwood treated with the extractive, which are 3.96±0.01 3.94±0.01. In generally, after 24 hour of submission in the water bath the rubberwood with the commercial preservative are the rubberwood that treated extractive preservative are not affected the physical properties of the rubberwood. It could be the distilled water and methanol are the polar subtances and the cell wall of rubberwood is tendency to attract and bind with the polar element.

According to Hyvönen *et al.*(2008), the cell of thewood wall is mainly composed by polymers with hydroxyl and other oxygen containing group that have high tendency to bind with the any moisture through the hydrogen bonding. In the wood, there are cell that responsible for water intake, which are hemicelluloses and cellulose. Besides that, in the wood there are three form of water already exist in the cell, which are, free water, bound water and water vapor. The free water is the liquid that fills the lumen. The action of water absorption is by the force of capillary action. While the water bound is already inside the cell wall. This water is bind to the hydroxyl group through the monocellular or polymolecullar adsorption. The polycellular is stronger adsorption compared with the monocellular. Because of the water adsorbed into the cell wall, thus the volume of the cell wall increase with proportionally to the water added until the water is saturated. So that, any water that beyond the saturated point will remain as the free water in the wood structure and does not affect the swelling.

# CHAPTER 5

## CONCLUSION AND RECOMMENDATION

As conclusion, the methanolic crude extracts fo *Sesbania grandiflora* leaves showed potential to develops as wood preservatives against wood rotting fungal.

The experimental results in this research were revealing that the *Sesbania grandiflora* leave methanol extract has potential in the fungicides activities against the white rot which is *Tratemes versicolor*. In this experiment, the diffusion dish method is conducted in order to determine the minimum inhibitor concentration (MIC) to inhibit the growth of the white rot. The results showed that the MIC for *Trametes versicolor* is 1000µg of extractives.

The research result in this experiment for the wood decaying of rubberwood showed that, the chemical preservative have no different with the rubberwood that treated with S*esbania grandiflora* leave extractive, their performance is compactible. With the statistical evaluation with ANOVA post hoc tukey test there are only few different of numberical means of percentage of the weight loss, which is only 6.28±0.31 and 6.33±0.5.In addition, the properties of physical rubberwood when impregnated with the S*esbania grandiflora,* such as the thickness swelling and water absorption. While the physical properties such as water absorption and thickness swelling are generally showed that no different between the rubberwood treated with the commercial preservative, rubberwood treated with the extractive and the rubberwood with no treatment.

Recommendation

In the recommendation, the active compounds in *Sesbania grandiflora* against wood rotten fungal should be conducted. Besides that, it is recommended to conduct successive extraction to the *Sebania grandiflora* leave with the different solvent. In order to determine how much the yield of percentage active compound that will be produce.

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## Appendix A:

**Figure A1: Sample preparation of *Sesbania grandiflora***

**Figure A2: Extraction of *Sesbania grandiflora* leaves using soxhlet**

**Figure A3: Collection of *Tratemes versicolor***

## Appendix B

**Figure B1: Preparation of rubberwood sample**

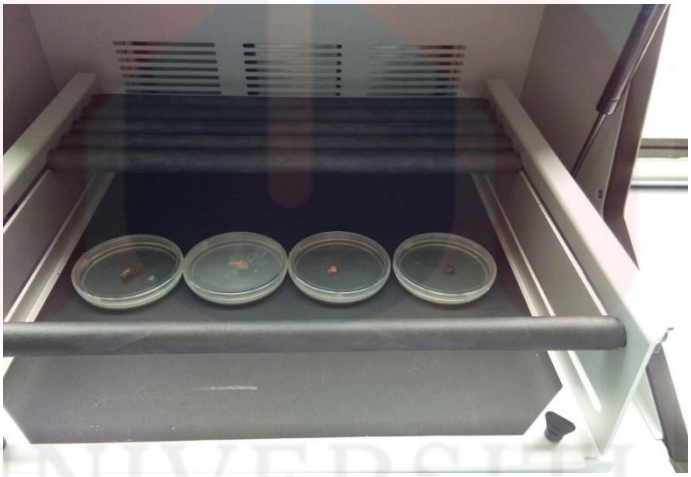

**Figure B2: Preparation of PDA**

## Appendix C

**Figure C1: Wood decay test**

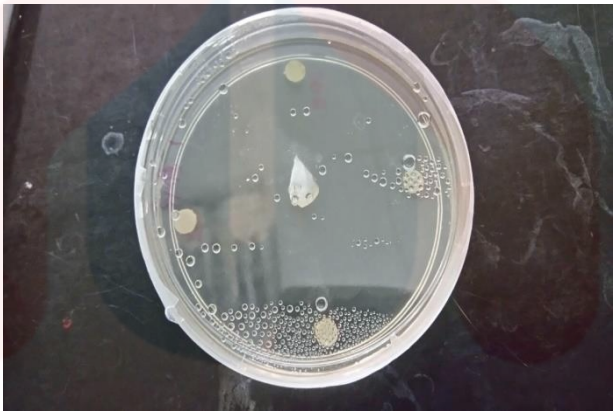

**Figure C2: Diffusion disk to determine MIC**

## Appendix D

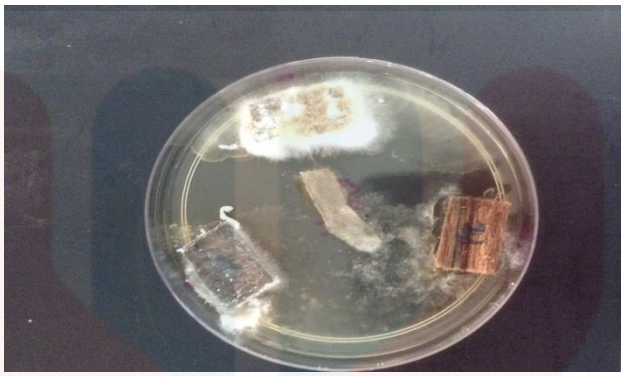

**Figure D: Wood decay test after one month**


**Figure D2: Diffusion disk to determine MIC**

## Appendix E

### Table E1: Statistical ANOVA post Hoc tukey test for wood decay

### Multiple Comparisons

Dependent Variable: Value

Tukey HSD

| (I) treatment | (J) treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Commercial Preservative | Extractive preservative | .07000 | 1.40016 | .999 | -4.2261 | 4.3661 |
| | Blank | -4.69000* | 1.40016 | .036 | -8.9861 | -.3939 |
| Extractive preservative | Commercial Preservative | -.07000 | 1.40016 | .999 | -4.3661 | 4.2261 |
| | Blank | -4.76000* | 1.40016 | .033 | -9.0561 | -.4639 |
| Blank | Commercial Preservative | 4.69000* | 1.40016 | .036 | .3939 | 8.9861 |
| | Extractive preservative | 4.76000* | 1.40016 | .033 | .4639 | 9.0561 |

*. The mean difference is significant at the 0.05 level.

**Table E2: : Statistical ANOVA post Hoc tukey test for thickness swelling**

**Multiple Comparisons**

Tukey HSD

| Dependent Variable | (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| Two Hours | Commercial Preservative | Extractive preservative | -.51333 | 1.40807 | .930 | -4.8337 | 3.8070 |
| | | Blank | -.53667 | 1.40807 | .924 | -4.8570 | 3.7837 |
| | Extractive preservative | Commercial Preservative | .51333 | 1.40807 | .930 | -3.8070 | 4.8337 |
| | | Blank | -.02333 | 1.40807 | 1.000 | -4.3437 | 4.2970 |
| | Blank | Commercial Preservative | .53667 | 1.40807 | .924 | -3.7837 | 4.8570 |
| | | Extractive preservative | .02333 | 1.40807 | 1.000 | -4.2970 | 4.3437 |
| Twenty Four Hours | Commercial Preservative | Extractive preservative | -.31000 | 1.79648 | .984 | -5.8221 | 5.2021 |
| | | Blank | -1.21000 | 1.79648 | .787 | -6.7221 | 4.3021 |
| | Extractive preservative | Commercial Preservative | .31000 | 1.79648 | .984 | -5.2021 | 5.8221 |
| | | Blank | -.90000 | 1.79648 | .874 | -6.4121 | 4.6121 |
| | Blank | Commercial Preservative | 1.21000 | 1.79648 | .787 | -4.3021 | 6.7221 |
| | | Extractive preservative | .90000 | 1.79648 | .874 | -4.6121 | 6.4121 |

**Table E3 : Statistical ANOVA post Hoc tukey test for water absorption**

**Multiple Comparisons**

Tukey HSD

| Dependent Variable | (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| Two Hours | Commercial Preservative | Extractive preservative | -.51333 | 1.40807 | .930 | -4.8337 | 3.8070 |
| | | Blank | -.53667 | 1.40807 | .924 | -4.8570 | 3.7837 |
| | Extractive preservative | Commercial Preservative | .51333 | 1.40807 | .930 | -3.8070 | 4.8337 |
| | | Blank | -.02333 | 1.40807 | 1.000 | -4.3437 | 4.2970 |
| | Blank | Commercial Preservative | .53667 | 1.40807 | .924 | -3.7837 | 4.8570 |
| | | Extractive preservative | .02333 | 1.40807 | 1.000 | -4.2970 | 4.3437 |
| Twenty Four Hours | Commercial Preservative | Extractive preservative | -.31000 | 1.79648 | .984 | -5.8221 | 5.2021 |
| | | Blank | -1.21000 | 1.79648 | .787 | -6.7221 | 4.3021 |
| | Extractive preservative | Commercial Preservative | .31000 | 1.79648 | .984 | -5.2021 | 5.8221 |
| | | Blank | -.90000 | 1.79648 | .874 | -6.4121 | 4.6121 |
| | Blank | Commercial Preservative | 1.21000 | 1.79648 | .787 | -4.3021 | 6.7221 |
| | | Extractive preservative | .90000 | 1.79648 | .874 | -4.6121 | 6.4121 |