INVESTIGATION OF *Leucaena leucocephala* LEAVE EXTRACTS AS ECO-FRIENDLY ANTI-FUNGAL AGENT FOR WOOD

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by

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DECLARATION

I declare that this thesis entitled "Investigation of *Leucaena leucocephala* Leave Extracts as 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 : ________________________________

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BNSL	bhilwan nut shell liquor
CO2	Carbon Dioxide
Cal/Kg	Calorie per Kilogram
CCA	Copper Chromium Arsenic
CCB	Copper Chrome Boron
CNSL	cashew nut shell liquor
FAO	Food of Agriculture Organization
FRIM	Forest Research Institution Malaysia
HSD	Honest Significant Difference
KPa	Kilopascal
MC	Moisture Content
MOR	Modulus of Rupture
PCP	Pentachlorophenol
PDA	Potato Dextrose Agar
RISDA	Rubber Industry Smallholder Development Authority
RTM	Room Temperature
FELCRA	Federal Land Consolidation and Rehabilitation Authority
FELDA	Federal Land Development Authority
UF	University of Florida
UTM	Universal Test Machine

LIST OF SYMBOLS

g	gram
h	hours
ha	hectare
m	metre
mm	Millimetre
N	Newton
O	Water Absorption
P	Bending Strength
λ	Thickness Swelling
±	Plus/Minus
°C	Degree Celcius
%	Percentage

Investigation of *Leucaena leucocephala* Leave Extracts as Eco-Friendly Anti-Fungal Agent for Wood

ABSTRACT

Commercial synthetic wood preservatives are widely used in wood industry as they are both fungicide and insecticide. Nevertheless, to investigate environmentally-benign wood preservative, this study was carried out with to investigate the antifungal potential of leaves of *Leucaena leucocephala*. Antifungal activity was determined by disc diffusion method and fungal decay test on Rubberwood. The antifungal activity of extracts (100, 250, 500, 1000 µg/ml) of leucaena were tested against white-rot fungal (*Trametes versicolor*) to determine the Minimum Inhibitory Concentration (MIC). The result showed that the remarkable inhibition of the fungal growth was shown at 500 µg/ml concentration. Fungal decay test was evaluated by comparison means of weight loss of wood samples, which comprises of treated and untreated Rubberwood. Wood samples was impregnated with the leave extracts and commercial preservative respectively and left for 1 month of period along with the untreated woods. Results shown that specimens treated with crude extracts are the most effective in exhibit fungal growth. Thickness swelling and water absorption of Rubberwood specimens treated with leave extracts, commercial preservative and blank as control were up to the EN Standards.

Kajian Penyelidikan Ekstrak Daun Petai Belalang sebagai Agen Anti-Kulat Mesra Alam terhadap Kayu Getah

ABSTRAK

Pengawet kayu sintetik komersial digunakan secara meluas dalam industri kayu sebagai racun kulat dan serangga. Walau bagaimanapun, untuk identifikasi pengawet kayu yang lebih mesra alam, kajian ini telah dijalankan dengan mengenalpasti potensi anti-kulat dari ekstrak daun Petai Belalang. Aktiviti anti-kulat telah dikenalpasti oleh kaedah cakera penyebaran dan ujian kerosakan kayu getah oleh kulat. Dalam kaedah cakera, ekstrak (100, 250, 500, 1000 μg / ml) daun Petai Belalang telah diuji terhadap kulat putih (*Trametes versicolor*) untuk menentukan kadar minimum kepekatan perencatan (MIC) bagi aktiviti kulat tersebut. Hasilnya, perencatan pertumbuhan kulat telah ditunjukkan pada 500 μg kepekatan / ml. Ujian kerosakan kayu dinilai melalui perbandingan antara berat sampel kayu, yang terdiri daripada kayu getah yang dirawat dan tidak dirawat. Sampel kayu telah dirawat dengan ekstrak daun dan pengawet komersial masing-masing dan ditinggalkan untuk satu bulan, begitu juga dengan kayu yang tidak dirawat. Keputusan menunjukkan bahawa spesimen kayu yang dirawat dengan ekstrak daun adalah yang paling berkesan dalam melindungi kayu dari pertumbuhan kulat. Pengenalpastian kadar pengembangan ketebalan kayu dan penyerapan air spesimen kayu getah yang dirawat dengan ekstrak daun, pengawet komersial dan kayu tanpa rawatan sebagai kawalan adalah mematuhi Standard EN.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Malaysia is a developing country with the rapid urbanization, increasing in the population of growth and the industrialization is lead to economic development. The manufacturing industry has been an important sector in the Malaysian economy for the past three decades. The Malaysian wood industry has contributed to the nation's economic growth.

Wood and wood products have played a critical role in the evolution of humankind. From the beginning, humans have used wood for survival and to improve the quality of life. In the twenty-first century, people continue to use wood for many of the same purposes that their most ancient ancestors did. Construction of shelter, furniture and paper production is one of the major uses of wood.

These wood and wood products are likely to face biological attack problem from the environment itself. Thus, people are attracted to treat them by using chemicals to against biological attack. The addition of preservative chemicals is necessary because most wood products are manufactured from non-durable species. Apart from that the addition of preservative chemicals causes safety and the environmental concerns (Yen *et al.*, 2007; Kotan *et al.*, 2008).

Because of this, research has been focused on using natural preservatives to overcome the problem. Derivatives from a range of plant taxa and various plant parts such as bark, wood, leaves, seeds and fruits have been examined for their wood protection properties in many studies and the information has been recently reviewed (Yang, 2009). For example, cinnamon extracts, linseed oil and extracts from certain fruits all have shown promising results. Extracts from cinnamon leaves have proved highly effective against wood decay fungi and termites and can potentially be developed into excellent organic preservatives (Wang *et al.*, 2005; Cheng *et al.*, 2006; Lin *et al.*, 2007; Maoz *et al.*, 2007). Essential oils from lemongrass, rosemary, tea tree and thyme are effective against mould on wood (Yang & Clausen, 2007).

Leucaena leucocephala is one of the plants that has been investigated for its uses since long before. For example, various parts of *Leucaena leucocephala* have been reported to have medicinal properties. The seed oil of *Leucaena leucocephala* was investigated for its antimicrobial activity and the pharmaceutical properties of its lotion formulation (Aderibigbe *et. al*, 2011). However, until today there is very limited information of bioassay on *Leucaena leucocephala* leaves extract against wood rotting fungi. In this research, determination of the potential of *Leucaena leucocephala* methanol leave extracts as wood preservatives from antifungal will be studied.

1.2 Problem Statement

Wood is an important raw material for many wood base industries which has diversified uses. But still it cannot be escaped from the biological attack from their environment itself. Wood-degrading fungi such as *Pycnoporus sanguineus*, *Lenzites palisotii* and *Ganoderma applanatum* rapidly destroy the wood (Teoh, 2013). This had led to the deterioration of wood and may cause economic loss.

However, it has led to the development of economical industrial scale treatment of Rubberwood using boron compounds which are particularly for indoor application to protect from insects, borers and fungi. Unfortunately, these compounds are odorless and pose a serious health hazard to workers performing treatment and processing of treated timber (Teoh, 2013). The uses of this compound also may cause residual of chemicals which is imposed adverse impact to the environment.

As wood-decaying fungi present a serious threat to the wood, conventional chemical control has been used to preserve the wood against stain and decay fungi growth. But, the effect of these chemicals is of concerns because they create problems for the environment and public health.

1.3 Objectives

- To investigate the potential of *Leucaena leucocephala* leave methanol extracts against wood rotting fungi.
- To evaluate the properties of wood that treated with *Leucaena leucocephala* leave methanol extracts.

CHAPTER 2

LITERATURE REVIEW

2.1 *Leucaena Leucocephala*

Leucaena leucocephala is widely known as 'Petai Belalang' in Malaysia. It is originated from tropical America and the lead tree is a shrub plant. It could grow over 16 feet in height with bipinnate leaves up to 10 inches long. Based on UF (2014), there are about 12 pairs of lanceolate shaped leaflets, each about 9-12 mm long, 2-3.5 mm wide with oppositely arranged. For this plant, the flowers blooms clustered on the end of branches. Singular blossoms are white, turning brown with maturity.

L. leucocephala is a productive seed maker. The dull brown seed pods are flat with around 20 seeds. Plus, the long is about 4 to 6 inches. The seeds are shiny brown in color, oval in shape, flattened, with 6 mm long. They are spread by birds, rodents and may also thru manure of cattle. Additionally, the lead tree is able to produces number of new shoots when cut back. Seed germination and vegetative regeneration from basal shoots will likewise happened taking after a fire. This species has a worldwide reputation as a 'miracle tree', because of their numerous potential of uses (UF, 2014).

From long before L. leucocephala has been focused on a lot of research and perceived as profoundly esteemed to the ecological remedy. It has found to be very convenient and helpful for soil conservation, reforestation and bioremediation. This is due to their capability in nitrogen fixation, rapid growth, and its deep root system.

It stifles nature's grasses and preserves the soils. At this point of view, the utilization of L. leucocephala in soil reclamation has been examined by a few analysts (Hove *et al*., 2001).

In tropical ranch crops L. leucocephala has been utilized as shade and act as a live support for climbing crops. Plus, it is also a helpful source of poles, timber and firewood. Nowadays, it has been recommended for support conservation of soil and fertility maintenance. This can be applied in contour planting with small-scale tropical farming systems. High seeding leaves of this plant is likely nutritious profoundly for ruminants and number of data have been publicized regarding animal production. These data could affirm the grain value of L. leucocephala (Duke, 1983). The types are a nuisance because due to the numerous seedlings that grow and contend with the crops and additionally attacking somewhere else that would remove the native greenery (Hove *et al.*, 2001).

Nonetheless, Malaysia that also has plenty of this plant generally utilized as fuelwood, food to livestock, material for reforestration, and green dung. Other than that, this plant has been broaden in many different of field including pole wood, pulpwood, production of gum, production of furniture, construction of timber, shade and backing plants in agroforestry systems. In Southeast Asia, enormous growing trees are utilized for shading coffee and cocoa ranch (NAS, 1979; Nazri *et al.*, 2011).

Furthermore, many studied had found that the strength of wood composite products resulted from the increase of strand length (Post, 1958; Brumbaugh, 1960; Barnes, 2000). In addition, the utilization of this fast growing plant might be alternative method for augmenting the source of wood in wood industry, as well as to protect natural assets from overexploitation. Since L. leucocephala that categorized from leguminous bush is a multifunction fast growing plant, it has played a critical role in the tropic region (Vietmeyer *et al*., 1977).

However, even L. leucocephala has been focused on many great deal of research, that studied on the leaves, seeds and roots, but no study has been done on leave extracts of this species to date, as an eco-friendly preservative. Hence, this study investigated the properties of leave from *Leucaena leucocephala* as wood preservatives. Figure 2.1 shows the Leucaena leucocephala leaves.

Figure 2.1: *Leucaena leucocephala* leaves
Source: (Hattaway, 2011)

2.2 Wood and Wood-Based Panel

Nowadays variety types of wood products which known as wood and wood based panel has taken place in our market. This development of technology has a remarkable range of engineering properties. With a regulative necessity and regularly developing of basic material situation, lead to continuous renovation and advancement of wood products and the process of manufacturing (Thoemen *et al*., 2010).

Besides, until today panel products are still be fabricated on account of men's general craving for improvement in building material. Since before wood has been chosen to be used as a building material due to its variability, strength, density and durability, which is the crucial aspects that should be considered, not just in appearance. However, as a building material, wood still has a considerable measure of points of disadvantages, such as its inconstancy, quality of changing over time or at different places (Thoemen *et al*., 2010).

Different advantages of wood composites originate from the way that their properties can be designed and created. Wood is restricted to an expansive degree by size, the width specifically. It is hard to acquire wood more extensive than 225 mm and thicker than 100 mm. The measurements of common boards differ from market to market, however their dimensions are normally 2–2.5 m long and 1–1.5 m wide, yet it is conceivable to purchase boards in much bigger sizes if essential (Thoemen *et al*., 2010).

Most houses that have been built today used particleboard as floor material, due to the fact that wooden floors are more costly to purchase and lay. Based on the globally standard of dimensions of particleboard floor panel, its large in size enable those to be laid down more faster as well as make it less "creaky" flatter compared to the traditional wood floor. Normally the size of dimensions is 2440 x 660 mm in the UK. This kind of wood composites could have special properties, like conductivity with low thermal, imperviousness to fire, advancement on their surface or better bio-resistance. These aspects could elevate for decorative purposes. In spite of that, these panels still cannot resists from the biological attack from its surrounding, such as bacteria, insects and fungi that could affect the wood because of wood decay activity.

This activity is likely to happen when it is moist (Thoemen *et al*., 2010). Wood panel product is shown in Figure 2.2.

Figure 2.2: Wood panel
Source: (Anonymous, 2017)

2.3 Rubberwood

Last of 19th century, rubber industry was originated in South and Southeast Asia. Asia presented the world largest rubber plantations that account for 95% and the countries that become the largest producer with more than 75% are Indonesia, Thailand and Malaysia. Other than that, China, Vietnam, India and Sri Lanka also have about 18% that involved in this industry, presenting Asia-Pacific producers. Overall the world has covered with 9 million ha with rubber plantations (Balsiger *et al*., 2000).

Meanwhile, in Malaysia there are agencies that engaged to organize the smallholdings. They are including Federal Land Consolidation and Rehabilitation Authority (FELCRA), Rubber Industry Smallholder Development Authority

(RISDA) and Federal Land Development Authority (FELDA). By 1990, these government units had regulate around 940 000 ha of smallholdings and around 240 000 ha were personally owned (Ismariah & Norini, 1999; Teoh *et al.*, 2011).

Conventionally, Rubberwood has been utilized for charcoal and fuelwood as a part of steel industries, processing of rubber, manufacturing of brick and tobacco manufacturing (Balsiger *et al.*, 2000). The alluring components of Rubberwood are its rich shading and great carpentry properties. It has caused many businesses to use Rubberwood in order to replace or ramin timber which is more costly (Killmann & Hong, 2000; Teoh *et al.*, 2011).

Literally, Rubberwood has engraved a specialty for itself and has turned into utilization of timber as a part of numerous wood items (Ho & Roslan, 1999; Teoh *et al.*, 2011). As indicated by Food of Agriculture Organization (FAO) in 2010, for every 25-30 years rubber trees are replanted. It is when the rubber is expensive for production of latex. The cut down or destroyed rubber trees were utilized as fuelwood before it was used for timber and production of timber-based products (Teoh *et al.*, 2011).

There are several factors that contribute to the founding of Rubberwood as an essential and extensive wood product including ready and ease accessibility, light shading, uncomplicated machining and its recoloring properties (Balsiger *et al.*, 2000). According to Hong (1999) and Teoh *et al*. (2011), since late 1970s Malaysia has begun the business that uses Rubberwood. For example, there are industries that use fuelwood for curing tobacco, making brick, and drying and smoking of sheet-rubber. Plus, there is block board and charcoal industries which also consume fuelwood.

These days, Rubberwood can be utilized for making an extensive variety of items, for example, panels from Rubberwood-based. It could comprises of plywood, particle board and medium-density fiberboard. Other than that, Rubberwood also can be used for making floor tiles and parquet, furniture and joinery products, and moldings (Zhou *et al.*, 2007; Siti, 2008; Zhao, 2008; Teoh *et al.*, 2011).

Aside from that, deprivation is still present which could be an obstacle to escalate the uses of Rubberwood, which is its susceptibility to the biological attack. This kind of problem that might come from their environment itself, such as insect and fungal attacks is able to lead to economic loss to rubber producers. Due to its likelihood of being harmed or influenced by insect and fungal attacks, Rubberwood has to be handled and processed soon after the trees are cut (Balsiger *et al.*, 2000).

Rubberwood is likewise exceptionally inclined to assaults by parasites and wood borers in green and dry conditions (Ho, 1999; Teoh et al., 2011). As indicated by George (1985), recoloring parasites can genuinely assault Rubberwood as soon as first day after it has been cutting down. A case is *Botryodiplodia theobromae*, which happens along with the surface mold, known as *Aspergillus sp.* and *Penicillium sp*. which lead to an extensive loss of quality in Rubberwood (Teoh *et al.*, 2011).

There are sorts of wood rotting fungi that able to cause Rubberwood damage. For example, *Lenzites palisotii* and *Ganoderma applanatum* which has the tendency to attack on carbohydrate in the wood itself, known as sugar and starch. Few scientists had reported that high carbohydrate holds kept in the parenchyma are main considerations that controlling the high rot activity in Rubberwood. In the perspective of the extreme decay problem, a treatment to preserve the wood to against the pests or bio-deteriorating organisms is needed (Azizol & Rahim, 1989; Wong, 1993; Teoh *et al.*, 2011).

Recently, application of artificial chemical preservative has become common practice in wood industry such as boron compounds. They are usually will be impregnated into the Rubberwood to act as fungicides, insecticides, and so on. However, organic or biological options which is more eco-friendly and has low mammalian harmfulness, or toxicity are required to substitute with the commercial chemical preservative. This alternative is needed to expand the market of Rubberwood which could comprise of variety of applications. This is including children's toys, food-related materials and areas that health and human wellbeing is of the best concern (Teoh *et al.*, 2011). Figure 2.3 shows sapstain (or blue stain) on Rubberwood.

Figure 2.3: Sapstain (or blue stain) on Rubberwood
Source: (Teoh *et al.*, 2011)

2.4 Wood Rotting Fungi

Wood is extremely prone and easily to be attack by many living things or microorganisms from their environment itself. They are decay fungi, mold and blue stain fungi, and microbes. As for wood decay fungi, they are generally divided into three different types, known as brown-rot fungal, white-rot fungal and soft-rot fungal. These fungal can caused deterioration of wood by assaulting the carbohydrates and lignin in the cell wall (Zabel & Morrell, 1992; Ritschkoff, 1996).

Fungal that attacks the carbohydrates in the cell wall known as brown-rot fungal, whilst fungal that attacks both carbohydrates and lignin in the cell wall known as white-rot fungal. As for soft-rot, it is caused by microfungal which is selectively assaulting the cell wall. On the other hand, other pests that could also attack the wood without deteriorate the strength properties of the wood is called mold and blue stain fungi. They only utilized the simple carbon compounds that are available in the wood (Zabel & Morrell, 1992; Ritschkoff, 1996).

Since decay fungal attack on the carbohydrates and lignin in the cell wall, they could possess a harmful effect to the strength properties of the wood. For instance, there are brown-rot fungal known as *Serpula lacrymans* and *Coniophora puteana* which recorded as the most harmful creature happen in wood used. In temperate regions such as Finland, this wood decay favor softwoods to hardwoods as their substrates (Campbell, 1952; Viitanen & Ritschkoff, 1991; Viitanen, 1994; Ritschkoff, 1996).

The chemical and physical structure of wood cell wall affect greatly to the type and intensity of decay (Jeffries, 1987). The natural durability of different wood species is very variable. In general, the Finnish wood species are relatively unendurable. The heartwood of *Pinus sylvestris* resists microbial attack better than the sapwood (Viitanen & Ritschkoff, 1991). It has shown that the component of wood from a living tree is more durable than the wood material in use. The natural durability of wood is affected by several factors, such as the permeability of moisture and the amount and composition of wood extractives (Findlay, 1951; Rudman, 1963; Ritschkoff, 1996).

Sufficient moisture content in woody materials is one of the most important factors promoting fungal growth and development. Studies has demonstrated that the necessary humidity for the development of brown-rot fungi must be in above 95-98% for a long period and the wood moisture content around the fiber saturation point, 25-30% water of the wood dry weight (Boddy, 1983, Cartwright & Findlay, 1946; Griffin, 1977; Viitanen, 1994). The formation of the free water layer in the wood cell lumen provides essential gas conditions for the growth of fungal cell (Deacon, 1984). The moisture requirements vary considerable depending on the fungal species (Ritschkoff, 1996).

However, fungal water economics is affected by the fungal species, physiological status of the fungus and the properties of the wood material (Viitanen & Ritschkoff, 1991). According to some studies, most brown-rotters are fairly sensitive to moisture conditions especially at the initial state of the decay, which could be due to the limited amount of oxygen in the wood material (Ritschkoff, 1996).

For rot fungal activity, their temperate needed usually varies between 0-45°C, depending on the species of the fungal. It indicates the mesophilic characteristics of most fungal species (Viitanen, 1994). The growth of decay fungi can initiate at more than 0°C and the fungal metabolic activity and growth rate increase with increasing temperature (Boddy, 1983; Griffin, 1981). High temperature has been observed to act as a restricting factor for fungal growth. However, the thermotolerance of fungi is determined by the developmental stage and age of the fungal colony, and thus high temperature is endured better by old mycelia than by young ones (Ritschkoff, 1996).

Wood decay fungi vary in the optimal nutritional needs but the basic requirements are satisfied by the structural carbohydrates, certain inorganic compounds and vitamins in wood. In laboratory conditions the successful cultivation takes place on simple media such as malt extract or potato dextrose, and artificial media containing essential inorganic substances together with a suitable carbon source. Successful colonization of wood depends largely on the ability of fungi to spread rapidly by using nonstructural carbohydrates (Deacon, 1984; Ritschkoff, 1996).

Thiamine is the only essential vitamin for growth of most wood-decay fungi that present in wood. Thiamine is needed mainly because of the pyridine unit, which acts as a necessary precursor for the biosynthesis of thiazole compounds (Deacon, 1984; Ritschkoff, 1996). Mycelial fans of a fungus on decaying wood as shown in Figure 2.4.

Figure 2.4: Mycelial fans of a fungus on decaying wood
Source: (T.E.R:R.A.I.N, 2008-2014)

2.5 Brown Rot Fungi

The most serious kind of microbiological deterioration of wood is caused by fungi because they can cause rapid structural failure. Brown-rot decay is the most common and most destructive type of decay of wood in use (Cowling, 1961; Green & Highley, 1997). Decay by these fungi in the natural environment is typically initiated via the deposition of spores or mycelial fragments that are carried to wood or other lignocellulosic surfaces by the wind, water, insect or animal vectors; or because of mycelial growth into the wood in direct soil contact (Arantes & Goodell, 2014).

As with all wood-inhabiting fungi, germination of spores and the initiation of fungal growth into the wood will not occur until appropriate conditions of moisture and temperature are met. In general, many types of brown rot decay can be initiated when the wood moisture content is above the fiber saturation point, but the cell lumen void space is not saturated, and when the temperature is between 10 and 45°C.

The optimal temperatures for growth and degradative activity vary with fungal species. But still, there are many other factors that play a role in whether decay will initiate let alone grow into the wood, and then begin metabolite production for initiation of the decay process. Of the millions to billions of spores that can be generated by a single fungus, only a few survive to actually initiate decay in lignocellulose materials (Arantes & Goodell, 2014).

Brown-rot fungi utilize the hemicelluloses and cellulose of the cell wall, leaving the lignin essentially undigested, albeit modified by demethylation and oxidation. The wood darkens, shrinks, and breaks into brick-shaped pieces that crumble easily into a brown powder. Brown-rot fungal, rapidly depolymerize holocellulose, and the degradation products are produced faster than they are utilized (Cowling, 1961; Green & Highley, 1997).

The rapid depolymerization of the wood carbohydrates is reflected by the substantial increase in alkali solubility products and the rapid decrease in strength properties of brown- rotted wood. Chemical analysis of brown-rotted white pine (*Pinusmonticolu*) and hard maple (*Acer rubrum*) shows that the hemicellulose glucomannan is removed considerably faster than cellulose or xylan (Highley, 1987; Green & Highley, 1997).

Xylan is usually depleted faster than cellulose. Because hemicelluloses form an encrusting envelope around the cellulose microfibrils, further degradation and removal of depolymerized cellulose may depend on prior removal of the hemicelluloses. Thus, hemicellulose utilization may be a critical initial step in establishment of brown-rot fungi in wood (Green & Highley, 1997).

The traditional wood preservatives (e.g., chromated copper arsenate, pentachlorophenol creosote) are effective in controlling brown-rot decay, but the safety of these preservatives is a concern. The move to less toxic methods for the preservation of wood could be facilitated if the process was better understood. Then, it would be possible to devise natural screening procedures to find inhibitors of novel targets in the fungi (Green & Highley, 1997). Figure 2.5 shows part of a pine stump showing the characteristic brick-like decay by brown-rot fungi.

Figure 2.5: Part of a pine stump showing the characteristic brick-like decay by brown-rot fungi.
Source: (Deacon, 2005)

2.6 White Rot Fungi

White rot fungi are typically associated with hardwood decay and their wood decay patterns can take on different forms. White rotted wood normally has a bleached appearance and this may either occur uniformly, leaving the wood a spongy or stringy mass or it may appear as a selective decay or a pocket rot. White rot fungi possess both cellulolytic and lignin degrading enzymes and these fungi therefore have the potential to degrade (Barry Goodell, 2012).

White-rot fungi decay all structural cell wall constituents, although the rate at which they do this differs. Selective (or preferential) white-rots decay hemicellulose and lignin first, resulting in defibrillation through dissolution of the middle lamella. In contrast, non-selective (or simultaneous) white-rots remove lignin and structural carbohydrates at a similar rate, resulting in homogeneous cell wall decay. The influence of white-rot decay on wood chemistry has been investigated by various methods (Blanchette *et al.*, 1985; Perez *et al.*, 1993; Davis *et al.*, 1994; Blanchette, 1995; Worrall *et al.*, 1997; Enoki *et al.*, 1998; Pandey & Pitman, 2003). Two common Ascomycota that cause white rots is as shown in Figure 2.6.

Figure 2.6: Two common Ascomycota that cause white rots.
Source: (Deacon, 2005)

2.7 Artificial Chemical Preservatives

Wood preserving chemicals can be classified into three groups. The first one is creosote, that is toxic oil derived from coal and has been the original benchmark preservative for more than 100 years. Second is pentachlorophenol, which is a manufactured salt that soluble in oil and has been used since the 1950s. The third group consists of several water soluble salts, the most commonly used one being chromated copper arsenate (Robert, 1986).

Creosote is often called coal tar creosote because of its close relationship to toluene, benzene and tar. These materials are condensed from the distillation of coal as it is converted to coke. It is highly toxic to decay fungi and insects. It is relatively insoluble in water and its oily nature helps protect wood against weathering (Robert, 1986).

Freshly creosoted poles and timbers give off vapours which have a strong odour and may produce a sunburn-like rash on human skin. This happens more during warm summer days and will cease after a few years of service. Creosote soils clothing and is difficult to impossible to paint over satisfactorily. The odour of creosote is unpleasant to some people. Precipitation and groundwater will not leach creosote from the wood of utility poles and other outdoor applications. Permanence and excellent toxicity to decay fungi and insects have made creosote a favourite wood preservative for railroads and port authorities (Robert, 1986).

Pentachlorophenol is manufactured salt that is usually called as penta. It is relatively insoluble in water but dissolves readily in several petroleum oils. Treating solutions accepted as equal to creosote are made by dissolving five percent penta salt in petroleum oil. It is also used in liquified petroleum gas and methylene chloride when the wood must have a natural look. Penta-petroleum treating solutions were popular during the post of World War II years but have been widely replaced by the waterborne salts in recent years. The age of environmentalism placed serious doubt in the minds of many regarding the health hazards of penta. Spectacular increases in the cost of petroleum oil also played an important role in the shift to waterborne salts (Robert, 1986).

This waterborne salt is a green colour and has several names. It is formulated from chromium trioxide, copper oxide and arsenic pentoxide. Three distinct mixtures of these ingredients are used by the treating industry and various trade names are applied to each. In all cases, the toxic salt is carried into the wood cells by means of water. Following impregnation, the chemicals react with the wood sugars and become insoluble. The water is dried from the wood, leaving the salt behind. These chemicals have become popular for the treatment of lumber and plywood for retail sale to the public. They also are used for utility poles, guard rail posts and landscape timbers (Robert, 1986).

Several other waterborne preservative salts have been used by the wood preserving industry. Almost all of the waterborne preservative treatments used today are of the chromated copper arsenate type. Acid copper chromate contains about 32% copper oxide and 68% chromic acid. Ammoniacal copper arsenate contains about 50% copper oxide and 50% arsenic pentoxide. Chromated zinc chloride contains 80% zinc oxide and 20% chromium trioxide. Fluorchromearsenate-phenol contains 22% fluoride, 37% chromium trioxide, 25% arsenic pentoxide and 16% dinitrophenol. Leaching by precipitation and groundwater is a problem with most of these materials (Robert, 1986).

Today, Boron and Copper Chromium Arsenic (CCA) have been reported as important compounds in Rubberwood preservation (Zaidon *et al.*, 2003; Hwang *et al.*, 2007; Teoh *et al.*, 2011). Boron compounds are odorless and relatively less toxic compared with some other preservatives (e.g., lindane) that can pose serious health hazards to the workers performing the treatment and processing of treated timber. CCA-treated Rubberwood is rarely used because of the unnatural color of the treated wood. However, if the timber will be used for construction or structural purposes, it is best to treat the wood with CCA to ensure resistance against bio-deteriorating organisms (Mohd Dahlan *et al.*, 1999; Teoh *et al.*, 2011).

But still, these compounds are becoming less popular nowadays because they are toxic and hazardous to humans (Zaidon *et al.*, 2008; Teoh *et al.*, 2011). In fact, the persistent use of this chemical is of environmental concern at present and has resulted in the need to search for an alternative approach to Rubberwood preservation, especially one utilizing natural resources (Teoh *et al.*, 2011). Figure 2.7 shows example of chemical preservative for wood.

Figure 2.7: Example of chemical preservative for wood
Source: (Anonymous, 2017)

2.8 Natural Preservatives

Most of the plantation that grown wood species and being likely non-durable in nature, easily to be attacked by wood destroying agencies. Therefore, in order to overcome this type of problem, preservative treatment becomes necessary. Over the past few years the use of artificial chemical preservatives such as Copper Chrome Aresenate (CCA), Pentachlorophenol (PCP), Copper Chrome Boron (CCB) and may more are under scrutiny due to environmental reasons (Onuorah, 2000).

Alternative means or eco-friendly method for pest control should be searched to minimize the use of synthetic chemicals. Therefore, much emphasis now is paid towards the development of eco-friendly formulations from natural resources such as plants (Tripathi *et al*., 2009). Plants are known to be the storehouse of various biologically active compounds that possess toxicity against microbes and insects (Tripathi *et al*., 1978; Rao, 1982; Kubo and Taninguchi, 1988; Tripathi *et al*., 2009).

The use of botanicals and biocontrol agents are a promising alternative to chemical control. Botanical pesticides possess an array of properties including insecticidal activity, repellency to pests, antifeedency, insect growth regulation, toxicity to agricultural pests (Prakash and Rao, 1986; Prakash and Rao, 1987; Prakash *et al*., 1987; Prakash *et al*., 1989; Prakash *et al*., 1990; Verma *et.al*, n.d.).

For example, is pest control. Plant derived natural products such as entomopathogenic fungi, nematodes and bacteria are some of the alternative methods of pest control such as termites (Verma *et al*., n.d.). Nematodes caused high mortality of *Reticulitermes flavipes* (Kollar) termites in laboratory tests (Trudeau, 1989). The entomopathogenic fungi play significant role in integrated pest management (Carrunthus *et al*., 1991; Verma *et al*., n.d.). *Beauveria bassiana* (Balsamo) Vuillemin has been shown to be highly pathogenic to many insect species in both temperate and tropical regions (Stranes *et al*., 1993; Verma *et al*., n.d.). A fungus *Metarhyzium aniospliae* (Bio Blast) is another biological termiticide that require special application and handling technique (Verma *et al*., n.d).

Furthermore, some of the eco-friendly formulations that have been developed and tested against wood destroying agencies are CNSL (cashew nut shell liquor), BNSL (bhilwan nut shell liquor) and Cu-lignin complexes (Shukla *et al*., 1972; Lepage & Delelis, 1980; Tripathi *et al*., 2003; Tripathi *et al*., 2009).

Working in the same direction of development of environmental safe eco-friendly formulation from natural resources, *Leucaena leucocephala* was chosen for this study. This plant will be used to prepare the wood preservative derived from the leave extracts to overcome the problem from biological attack from their environment, which is wood decay fungal.

CHAPTER 3

METHODOLOGY

3.1 Materials

The materials that been used in this experiment was healthy leaves of *Leucaena leucocephala* to extract it with methanol solvent; white-rot fungal (*Trametes versicolor)* for fungal decay and paper disc test; and commercial antifungal preservative.

3.2 Method

3.2.1 Sample Preparation

The experiment was conducted at Universiti Malaysia Kelantan (UMK). One kilogram of the healthy leaves of *Leucaena leucocephala* was collected in Jeli, Kelantan. The leaves were kept in a fridge to ensure the freshness of the leave until the experiment was conducted.

3.2.2 Extraction

The sample of *Leucaena leucocephala* leaves were washed thoroughly with running water and rinsed with distilled water. The leaves were powdered using pestle and mortar; then completely dried in an oven. Solvent extraction of this sample was performed as in Kawamura *et al.*, (2004) with some modification. The crude leaves were filled in thimble and extract with methanol solvent in Soxhlet extractor for 6 hours. The mass of the extracts was completely dried, weighed and yield determined. The yield of extracts was calculated based on oven dry weight and ground sample.

3.2.3 Antifungal Assay

The antifungal activities of methanol leaves extracts was evaluated with the paper disc assay based on Kawamura *et al.* (2004) with some modification. The plant extracts was diluted with respective solvent. This diluted extracts was then pipetted out to 6mmWhatman Paper AA disc as in the bio-assay. The disc was left uncovered on a clean bench for 3 days to let the solvent evaporate leaving residual pure extract on the paper disc. The amount of this extract in mg on the disc was used as the weight of the extract.

Potato Dextrose Agar (PDA) medium of 3.9% was prepared and autoclaved at 120˚C at 15 psi (0.10 N/mm2) for 20 min, and approximately 15 ml was dispensed into 9 cm petri dishes to solidify. Fungus selected, *Trametes versicolor* that was kept in an incubator was inoculated at the center of the PDA medium. The paper disc treated with the extracts was placed on the dish about 3 cm away from the inoculated fungus. The sample was incubated in the dark at 25±2˚C. The antifungal activities of the extracts were evaluated at 24 h intervals for 7 days. The lowest weight of extract in microgram with positive antifungal activities on 7th day was defined as the minimum inhibitory amount.

3.2.4 Wood Treatment

Rubberwood was used as wood sample in the test, along with the fungal species which is the white-rot fungus, *Trametes versicolor*. The samples were exposed to the white-rot fungus. Each treatment which is leaves methanol extracts, commercial preservative and blank preservative were had 3 replicated for the fungal species.

3.2.5 Fungal Decay

Samples of Rubberwood were tested according to Kawamura *et al.* (2004) with some modification using a representative wood-attacking fungal, white-rot (*Trametes versicolor).* The Rubberwood samples were oven-dried at 40˚C to reach constant weight (W1), which is the initial weight before the decay test. The number of blocks replications per test condition was 9 specimens measuring 1 x 1 x 1 cm in size.

Three different treatments which are leaves methanol extracts, commercial preservative and blank preservative were had 3 replicated for the fungal species. The leaves extracts and commercial preservative were diluted to 1% of dry weighed by using methanol and distilled water respectively. Both solutions were applied to their respectively three samples of wood then were leave to dry.

Potato Dextrose Agar (PDA) medium of 3.9% was prepared and autoclaved at 120˚C at 15 psi (0.10 N/mm2) for 20 min, and approximately 15 ml was dispensed into 9 cm petri dishes to solidify. Fungus selected, *Trametes versicolor* that was kept in an incubator was inoculated at the center of the PDA medium. The untreated and treated wood samples was placed on the dish about 3 cm away from the inoculated fungus. All the dishes were placed in a control condition chamber at 27˚C and a relative humidity 80% for a month.

After decay resistance testing, all test samples were removed from the petri dish. Each specimen was carefully clean to remove the mycelium form at the sample surface. It was stored at room temperature for 24 hours and oven-dried at 40˚C to reach a constant weight (W2). The weight loss of each sample was calculated.

3.2.6 Physical Properties Evaluation

The physical properties including thickness swelling and water absorption of wood specimens were determined in accordance with EN 317 (1993) and EN 317 (1993) respectively, based on European Standard.

3.2.6.1 Determination of increase in mass (water absorption)

The difference in mass of the test specimen before and after immersion in water for a period of 2h and 24h was determined. The same test specimens were used for determination of both water absorption and swelling. Test specimens were 100 mm × 100mm × board thickness.

Each test specimen was weighed and recorded the mass to the nearest 0.1g. The test specimens was immersed in water at 20 ± 2 °C in the flat bottomed container for 2h and 24h with their 100mm faces vertical. The depth of water above the test specimens was maintained between 25mm and 30mm. The test specimens were removed from the water after 24h and excess water was removed with a cloth.

Each test specimen was weighed immediately and the mass to the nearest 0.1g was recorded.

For calculation and expression of results, the water absorption, O, expressed as a percentage after 2h and 24h, was calculated from the equation;

$$O = \frac{(M_2 - M_1)}{M_1}(100)$$

Where;

M_1 is the mass of the test specimen before immersion (in g);

M_2 is the mass of the test specimen after immersion for 2h and 24h (in g).

The result was expressed to the nearest 0.1%.

3.2.6.2 Determination of increase in thickness (swelling)

The increase in thickness due to general absorption of water was determined from the differences in thickness of the test specimen before and after immersion in water for 2h and 24h. Test specimens were in accordance with 3.2.6.1.

The four measuring points on each test specimen was marked and the mean thickness was recorded. The test specimens were immersed in water in the flat bottomed container in accordance with 3.2.6.1. The test specimens were removed from the water after 2h and 24h and excess water was removed with a cloth. The mean thickness was immediately re-measured and recorded using exactly the same points as before.

Calculation and expression of results of the swelling of the test specimen λ, expressed as a percentage, after 2h and 24h, was calculated from the equation;

$$\lambda = \frac{(T_2 - T_1)}{T_1}(100)$$

Where;

T_1 is the mean thickness of the test specimen before immersion (in mm);

T_2 is the mean thickness of the test specimen after immersion for 2h and 24h (in mm).

The result was expressed to the nearest 0.1 %.

3.2.7 Statistical Analysis

The results of each wood were analyzed of variance ANOVA at 5% and the comparison of the means was performed with HSD Tukey test.

CHAPTER 4

RESULT & DISCUSSION

4.1 Determination of Plant Yield

Table 1 showed the properties of methanolic crude extracts of *Leucaena leucocephala* leaves, the yield was found at 35.88%. The crude extract of *Leucaena leucocephala* was dark green in color with a foetid smell.

Several parameters could influence the yield and composition of plant extract which include extraction method, period of the extraction process, temperature, as well as the solvent selected.

Table 4.1: The yield, color and odor of *Leucaena leucocephala* leave extracts.

Plant Extract	Percentage Yield (%)	Color	Odor
***L. leucocephala* leaves**	35.88	Dark Green	Foetid smell

4.2 Antifungal Assay

The fungal assay was conducted to test Minimum Inhibitory Concentration (MIC) of extracts with different concentration on paper disc, which are 100 µg, 250 µg, 500 µg and 1000 µg. The experiment was tested against *Trametes versicolor* and the result is shown in Table 4.2.

Table 4.2: The zone of inhibition of *Leucaena leucocephala* extracts.

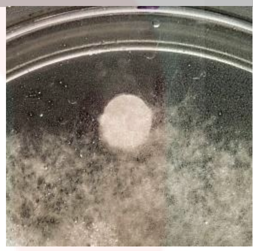
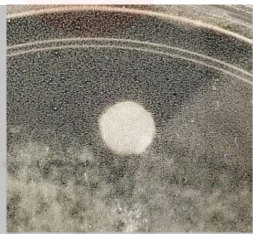
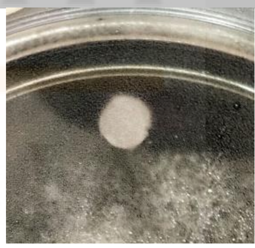
Concentration (µg/ml)	Observation
100	
250	
500	
1000	

Table 4.3: MIC of *Leucaena leucocephala* leaves extracts

Concentration (µg/ml)	Fungal activity
100	+
250	+
500	-
1000	-

Note: '+': Growth; '-': No growth

The result presented in Table 4.3 shows that the most efficient MIC value for the plant *Leucaena leucocephala* was higher than 500µg/ml for *Trametes versicolor*. Thus, this lead to an assumption that explain the lowest concentration of *Leucaena leucocephala* extracts that inhibits the growth of fungal is more than 500 µg/ml.

The inhibitory effects of leave extracts and its components on the white-rot decay fungus shows that *Leucaena leucocephala* leave extracts and methanol exhibit both fungistatic and fungicidal activities at 500 µg/ml against *Trametes versicolor*. Higher concentration of leave extracts (500 µg/ml) was required to inhibit the growth of *Trametes versicolor*. The extracts proved to be highly inhibitory to molds with the MIC value of 1000 µg/ml against the white-rot fungal.

It has been reported that *Leucaena leucocephala* leaves has positive result in presence of mimosine, an alkaloid (Wee & Wang, 1987; Soedarjo & Borthakur, 1998). Furthermore, the presence of tannins, vitamin E, ascorbic acid, carotenes, xanthophylls and phenolics in *Leucaena leucocephala* also were known to be antioxidative substances, but may also possessed antibacterial properties (Chanwitheesuk *et al*., 2001).

In general, the plant antifungal substances appears to be more inhibitory to *Trametes versicolor* with larger amount of concentration (500 µg/ml and above) rather than lower amount. It may be remembered that the crude extracts may contained some or less components of phytochemicals with biological activity. With the presence of tannin and phenolic compounds, it might be the crucial reason to possess antifungal activities against a number of fungal.

4.3 Fungal Decay Test on Rubberwood

Table 4.4: Weight loss (%) of Rubberwood impregnated with different treatment.

Treatment	Weight loss (%)
Commercial preservative	9.09 ± 1.12 a
Extract	5.98 ± 5.10 b
Blank	14.04 ± 1.10 c

*Different letter within same column showed significant different at alpha value 0.05

The mean percentage weight losses for samples exposed to *Trametes versicolor* for 1 month are given in Table 4.4. From the result, mean weight losses of control samples that decayed by *Trametes versicolor* was 14.04%. It recorded highest weight loss after 4 weeks. While wood samples that treated with commercial preservative recorded higher mean percentage of weight loss compared to wood samples that treated with *Leucaena leucocephala* leave extracts. This indicates that extractives derived from *Leucaena leucocephala* provide highest protective effect to the wood samples.

These results showed that there is an improvement in the durability of the test sample after treatment with extract derived from leaves of *Leucaena leucocephala*. The weight loss of the samples treated with the leaves extracts was the lowest than those treated with commercial antifungal preservative, with only 5.98% weight loss. Whilst the samples treated with commercial preservative recorded an intermediate weight loss with 9.09%. Weight loss for untreated wood samples that also was exposed to *Trametes versicolor* was 14.04% that was recorded as the highest amount of weight losses. This weight loss indicates an aggressive decay environment and positive fungal viability, thus indicating that the untreated wood samples had no decay resistance.

It seems that there is moderate difference between commercially treated woods and woods treated with extracts from *Leucaena leucocephala*. However, these results showed that there is an improvement in the durability of the test sample after treatment with extracts from *Leucaena leucocephala.*

Based on Tawata et al., (2008), mimosine has two potential purposes, which are to defend itself from invaders, and to develop and expand the species. The presence of mimosine in leucaena protects the plant from extinction. Mimosine has ability to forms DHP by the action mimosinase when the leave of leucaena is attacked by fungus. Thus, antifungal properties of DHP are responsible for the phytoalexin of leucaena has been confirmed. Plus, mimosine also responsible for allelopathy of leucaena.

4.4 Physical Properties Evaluation

The means and statistical comparisons of physical properties of Rubberwood were listed in Table 4.4 and Table 4.5 The values of water absorption and thickness swelling were very homogenous and showed no significant difference according to the ANOVA test ($\alpha = 0.05$).

4.5 Water absorption of specimens with different treatment

Table 4.5: Weight difference (%) of wood samples with different treatment

Treatment	2 h duration (%)	24 h duration (%)
Commercial preservative	74.57 ± 21.28 a	107.36 ± 10.66 a
Extracts	65.40 ± 21.76 a	98.25 ± 18.57 a
Blank	46.04 ± 6.96 a	91.97 ± 4.38 a

*Means value of 9 specimens

*Different letter within same column showed significant different at alpha value 0.05

This test is to study the effect of water absorption on mechanical properties of Rubberwood, treated with commercial preservative and crude extracts; also untreated Rubberwood after immersion in water with different period of time.

According to the results obtained in this study, the values of water absorption undergo an increment gradually over time. Wood samples treated with commercial preservative has the highest percentage of water absorption whilst wood samples treated with leave extracts ranked second in water absorption. Untreated wood samples seemed to be most water-resistant in the first 2 h and the next 22 h.

The values of water absorption after 2 h ranged from 46.04% to 74.57%. After 24 h these limits increased to 91.97% to 107.36% for Rubberwood of blank and commercial preservative, respectively. During the first 2 h the water absorption was 62.00% in average and after the last 22 h increased to 99.19%.

4.6 Thickness swelling of specimens with different treatment

Table 4.6: Thickness difference (%) of wood samples with different treatment

Treatment	2 h duration (%)	24 h duration (%)
Commercial preservative	5.88 ± 3.64 a	6.71 ± 3.09 a
Extracts	2.77 ± 0.84 a	4.14 ± 0.80 a
Blank	4.26 ± 2.59 a	4.87 ± 3.55 a

*Means value of 9 specimens

*Different letter within same column showed significant different at alpha value 0.05

The values of thickness swelling ranged from 2.77% to 5.88% after 2 h of water immersion and from 4.14% to 6.71% after 24 h. During the first 2 h the thickness swelling was 4.30% in average, and after the last 22 h it increased to 5.24%.

The thickness swelling of wood samples that impregnated with commercial preservative ranged from 5.88-6.71% and 2.77-4.14% for wood samples impregnated with *Leucaena leucocephala* leave extracts.

Based on Kojima *et al.*, (2009), thickness swelling provides a measure of the Rubberwood in dimensional stability which the lower thickness swelling values, the stable the Rubberwood is. There are several factors that can affect thickness swelling such as wood species, element geometry, density of board, resin level, blending efficiency and pressing conditions.

For this study, the thickness swelling value was found to decrease with impregnation of *Leucaena leucocephala* leave extracts. However, there is still no significant increase in Rubberwood between the treated and untreated wood samples that were conditioned for 24 h. Generally, leave extracts treatment cause reduction in thickness swelling as they indirectly gave an impact to the internal bonds. It has been reported that there is a direct relationship between internal bond and thickness swelling (Febrianto *et al.*, 2010).

The presence of crude extracts on Rubberwood prevented strong linkage between fibres as the resin is not able to have direct contact with the surface of fibres. Thus, it will reduce the internal bond that consequently will cause reduction in thickness swelling. As crude extracts is resistant to leaching by water, this preservative can be acceptable for outdoor use which is more exposed to high humidity. This water-resistant characteristic could also reduce moisture uptake that resulting dimensionally more stable Rubberwood.

CHAPTER 5

CONCLUSION & RECOMMENDATION

5.1 Conclusion

In conclusion, from the preliminary evaluation of the *Leucaena leucocephala* leave extracts as an antifungal agent, by the method of paper disc diffusion and antifungal efficacy test, this present work has proved that the antifungal efficiency of the crude extracts is sufficient to use as an antifungal agents for Rubberwood. Antifungal activity of *Leucaena leucocephala* leave extracts was investigated against white-rot decay fungus, which is *Trametes versicolor*.

In this study, methanol was used as a solvent which is an effective solvent for extraction process. It gave high yield of chemical constituents, that helpful in getting good results. The results indicated that the phytochemicals and active compounds contents that present in *Leucaena leucocephala* leave extracts play an important role in exhibit fungal activity by reducing activity mechanism.

Leucaena dominates a numeral of promising compounds, which may be investigated and exploited for multi-purposes such as the development in various field such as pesticides, cosmetics, and medicines. Additionally, this plant is widely grown in the subtropics and tropics. Thus, it can provide a low-cost source of plant biomass. Not to mention, leucaena is an essential species for the establishment of integrated eco-friendly wood preservative.

5.2 Recommendation

Summarizing, my findings showed *Leucaena leucocephala* has the potential to be explored further to identify the antifungal compounds in this plant. The present results will form the basis for selection of potential plant species for further investigation in the potential discovery of new natural bioactive compounds.

Determination of potential effectiveness of the leave extracts as the antifungal agent, the threshold concentration of extracts to prevent significant decay and leaching characteristics of crude extracts components from wood panel can be the key areas to be explored in the future.

On top of that, isolation and structure elucidation of antifungal active constituents from the plant and comparison of the properties with a wider range of commercial preservatives is recommended. Further work with this ideology could lead to environmental-friendly wood preservative.

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APPENDIX A

Figure A1: Preparation of leaves of leucaena

Figure A2: Extraction process by using Soxhlet

Figure A3: Crude extracts of leucaena leaves

Figure A4: Preparation of wood specimens

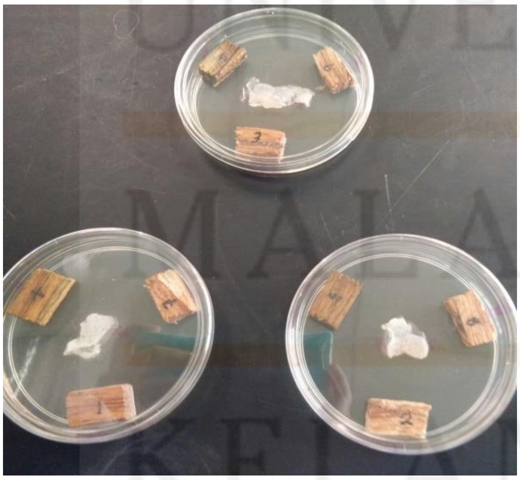

Figure A5: Wood decay fungal test

Figure A6: Wood decay fungal test after 1 month

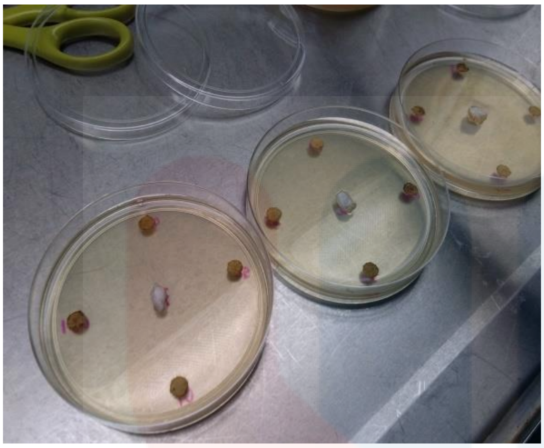

Figure A7: MIC test

Figure A8: MIC test after 3 days

Figure A9: Wood treatment process

Figure A10: Physical properties evaluation

APPENDIX B

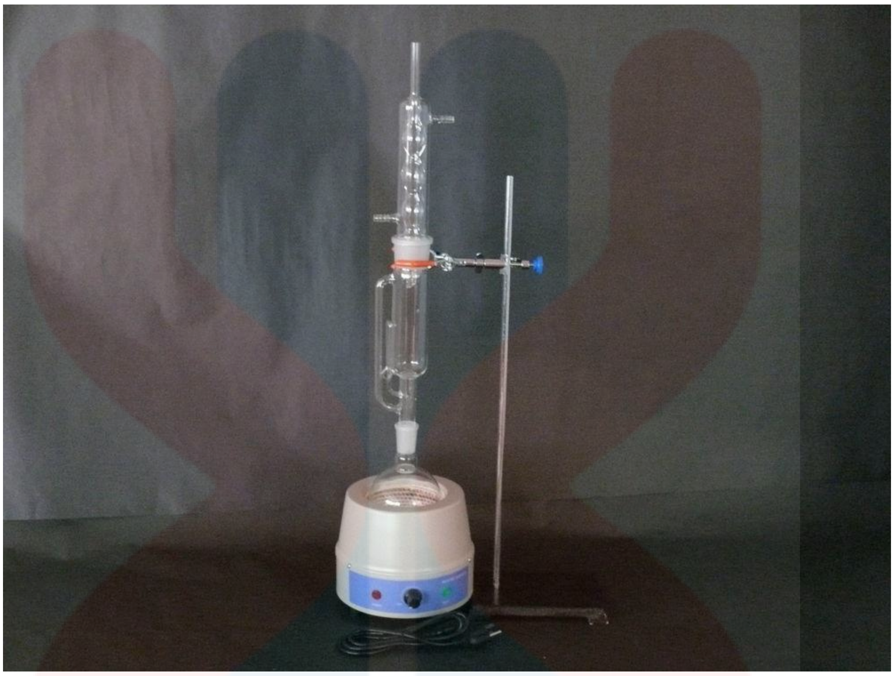

Figure B1: Soxhlet extractor

Figure B2: Rotary evaporator